Saurashtra University

Re – Accredited Grade 'B' by NAAC
(CGPA 2.93)

Chauhan, Sanjaykumar P., 2010, *"Phytochemical and pharmacological screening of fruit of Opuntia Elatior Mill"*, thesis PhD, Saurashtra University

http://etheses.saurashtrauniversity.edu/id/eprint/782

PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF FRUIT OF *OPUNTIA ELATIOR* MILL.

A THESIS

SUBMITTED TO

SAURASHTRA UNIVERSITY

RAJKOT

FOR

The Award of

Doctor of Philosophy (Ph. D.)

Under the

FACULTY OF MEDICINE (PHARMACY)

By

Mr. Sanjaykumar Prakashbhai Chauhan
(M. Pharm)

Smt. R. B. Patel Mahila Pharmacy College
Kailashnagar, Bhavnagar road,
At: Atkot, Ta: Jasdan, Dist: Rajkot
Gujarat

RESEARCH GUIDE

Dr. N. R. SHETH
M. Pharm, Ph. D, L.L.B., D.B.M.
Professor & Head,
Department Of Pharmaceutical Sciences,
Saurashtra University,
Rajkot

December, 2010

PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF FRUIT OF *OPUNTIA ELATIOR* MILL.

A THESIS

SUBMITTED TO

SAURASHTRA UNIVERSITY

RAJKOT

FOR

The Award of

Doctor of Philosophy (Ph. D.)

Under the

FACULTY OF MEDICINE (PHARMACY)

By

Mr. Sanjaykumar Prakashbhai Chauhan
(M. Pharm)

Smt. R. B. Patel Mahila Pharmacy College
Kailashnagar, Bhavnagar road,
At: Atkot, Ta: Jasdan, Dist: Rajkot
Gujarat

RESEARCH GUIDE

Dr. N. R. SHETH
M. Pharm, Ph. D, L.L.B., D.B.M.
Professor & Head,
Department Of Pharmaceutical Sciences,
Saurashtra University,
Rajkot

December, 2010

Certificate

This is to certify that the research work embodied in this thesis entitle, "**Phytochemical and Pharmacological screening of fruit of Opuntia elatior Mill.**" was carried out by Mr. Sanjaykumar Prakashbhai Chauhan at Smt. R. B. Patel Mahila Pharmacy College, Atkot under my guidance and supervision. This work is up to my satisfaction. The work embodied in this thesis is original and no part of the thesis has been submitted previously to this university or to any other university for the award of Ph. D. or any other degree or diploma.

Guided by

Dr. N. R. Sheth

M. Pharm., Ph. D., L.L.B., D.B.M.
Professor & Head,
Department of Pharmaceutical Sciences,
Saurashtra University,
Rajkot.

DECLARATION

I, hereby declare that the thesis entitled **"Phytochemical and Pharmacological screening of fruit of *Opuntia elatior* Mill."** is genuine record of research work carried out by me under the guidance and supervision of **Dr. N. R. Sheth** Professor & Head, Department of Pharmaceutical Sciences, Saurashtra University, Rajkot. No part of thesis has been submitted to any University or Institution for the award of any degree or diploma.

Rajkot
December, 2010

Mr. Sanjaykumar Prakashbhai Chauhan
M. Pharm.
Smt. R. B. Patel Mahila Pharmacy College, Atkot.

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the Saurashtra University, Rajkot shall have the right to preserve, use and disseminate this dissertation / thesis in print or electronic format for academic / research purpose.

Mr. Sanjay P. Chauhan

Ph. D. Reg. No. – 3627

Department of Pharmaceutical Sciences,

Saurashtra University,

Rajkot.

Date:

Place: Rajkot

ACKNOWLEDGEMENTS

Dr. Bhagirath for continuous prayers, inspiration and selfless help, who suffered a lot to keep me free from domestic responsibilities. My entire family members are the actual driving force that enabled me to reach this stage.

Last but not least, I owe gratitude and thankfulness to all who were involved directly or indirectly, knowingly or unknowingly to reach in the venture of mine.

Date:

Place: **Sanjaykumar Prakashbhai Chauhan**

Dedicated
to
My Family,
Hetal, Yajat
and
My Guru

Content

Chapter	Title	Page No.

Abbreviations

AAS: Atomic Absorption Spectrophotometer

ALP: Alkaline phosphatase

BAW: *n*-Butanol:Acetic acid:Water

CSF: Colony-stimulating factor

DAD: Diode array detector

EPO: Erythropoietin

FLT-3 ligand: FMS-like tyrosine kinase 3 ligand

G-CSF: Granulocyte-colony stimulating factor

GM-CSF: Granulocyte Macrophage-colony stimulating factor

Hb: Haemoglobin

HgCl$_2$: Mercuric chloride

HPLC: High Performance Liquid Chromatographic

HPTLC: High Performance Thin Layer Chromatographic

i.p.: Intraperitoneal

IDA: Iron Deficiency Anaemia

IgE: Immunoglobulin E

IL: Interleukin

LC-MS: Liquid Chromatography – Mass Spectroscopy

LTC$_4$: Leukotriane C$_4$

LT-Rc: leukotriene receptor

MCH: Mean Cell Haemoglobin

MCHC: Mean Cell Haemoglobin Concentration

MCP-2, 3, 4: Monocyte chemotactic protein-2, 3, 4

M-CSF: Macrophage-colony stimulating factor

MCV: Mean Cell Volume

MDC: Macrophage-derived chemokine

MIP-1α: Macrophage inflammatory protein-1α

MPV: Mean Platelet Volume

MS: Mass spectrometry

NK: Natural killer

OFJ: *Opuntia elatior* Mill. Fruit Juice

p.o.: per oral

PAD: Photodiode-array detector

PAF: Platelet Activating Factor

PCV: Packed Cell Volume / Haematocrite

PDE: Phosphodiesterase

PDW: Platelet Distribution Width

PGD$_2$: Prostaglandin D$_2$

PHZ: Phenylhydrazine

RBC: Red Blood Cell

RDW: Red blood cell Distribution Width

rhEPO: recombinant hyman erythropeietin

rhIL-2: recombinant human interleukin-2

rhTPO: recombinant human thrombopoietic

ROS: Reactive Oxygen Species

SCF: Stem Cell Factor

SDF-1: Stromal cell-derived factor-1

TGF: Tissue Growth Factor

TL: Total lipids

TLC: Thin Layer Chromatographic

TNF: Tumour Necrosis Factor

TPO: Thrombopoietin

TXA$_2$: Thromboxane A$_2$

WBC: White Blood Cell

WHO: World Health Organization

List of Table

List of Figures

ABSTRACT

Anaemia is defined as a reduction of haemoglobin concentration in the blood. Two billion people suffer from anemia worldwide and most of them having iron deficiency and haemolytic anaemia due to toxicants and oxidants. Bronchial asthma is characterized by increased airway reactivity to spasmogens and release of inflammatory mediators which results in acute bronchoconstriction, airway hyperresponsiveness and bronchial airway inflammation. Asthma is thought to affect about 3% of the population in most countries. About 70 – 80% of the world populations, particularly in the developing countries, rely on non-conventional medicine in their primary healthcare as reported by the WHO. There has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants.

Opuntia species (Cactaceae) are a well known and important plant widely used in several indigenous system of medicine for the treatment of various ailments, *viz.* asthma, inflammatory diseases, ulcer and diabetes. Betalains and phenolic compounds have been reported as the major phytoconstituents of this species. Different pharmacological experiments in a number of *in vitro* and *in vivo* models have convincingly demonstrated the ability of *Opuntia* species to exhibit various pharmacological activities, leading support to the rational behind several of its traditional uses. Due to remarkable biological activity of *Opuntia* and its constituents, it will be appropriate to develop them as a medicine. The literature study reveals that still today there is no record of phytochemical composition and pharmacological study of *Opuntia elatior* Mill. fruits in support of traditional and folkloric use. So present study aiming to study phytochemical and pharmacological screening of fruits of *Opuntia elatior* Mill., commonly known as "*Hathlo Thor*" belongs to the family Cactaceae. Various animal models and experimental protocols were used in the present study to evaluate haematinic, analgesic and anti-asthmatic activity of fruit of *Opuntia elatior* Mill.

The authenticity of the freshly collected plant was confirmed by comparing their morphological characters with the description mentioned in different standard texts and floras. The phytochemical analysis was carried out for standardization of fruit juice contained carbohydrates, flavonoids, phenolics and betalains. The fruits were preliminary evaluated by estimation of proximate analysis. The average weight of fruit was 24.568 ± 7.134 g/unit and among percentage of peel and seed was very low compared to the edible portion.

Phytochemical analysis indicates the presence of color pigment betacyanin as an active principle and sugar content in high amount and low acidity of fruit which make it very sweet and delicious. Presence of betacyanin was confirmed by spectrophotometric, HPLC and LC-MS techniques. The total betacyanin content (47.10 mg/100 ml) equivalent to betanin obtained from fruits of *Opuntia elatior* Mill. was higher compare to *Opuntia ficus-indica* and *Opuntia undulata* Griff. while lower compare to *Opuntia stricta* Haw.

The fruit juice (20 ml/kg) showed no significant change in the various autonomic and behavioral responses of rat compared to the control animals in acute toxicity study. Based on acute toxicity study we have selected three dose of fruit juice low (5 ml/kg), medium (10 ml/kg) and high (15 ml/kg).

Haematinic activity of fruit juice was evaluated by mercuric chloride and phenylhydrazine induced anaemia. Fruit juice at the dose of 10 ml/kg and 15 ml/kg showed good percentage of recovering in haemoglobin, 32.99 % and 38.18 %, respectively, which was higher than standard treated group (29.8 %) indicating correction of anaemia induced by mercuric chloride after 30 days treatment. Treatment with fruit juice (5, 10, 15 ml/kg) for 30 day showed significant increase in RBC ($p < 0.001$) compared to positive control at day 30 and it equivalent to standard in mercuric chloride induced anaemia.

Phenylhydrazine altered the haematological parameters by haemolysis characterized by decrease in haemoglobin concentration, total RBC counts and PCV on day 3. However, the haematological parameters were restored to normal range after treatment with fruit juice of *Opuntia elatior* Mill. for 12 days. The

speedy and progressive recovery of anaemic rats responding to treatement of *Opuntia elatior* Mill. fruits may be due to increased erythropoiesis and/or antioxidant property of betacyanin.

The potential antinociceptive as central analgesic by using tail immersion test and peripheral analgesic by using acetic acid induced writhing test of the fruits of *Opuntia elatior* Mill. was investigated. Fruits of *Opuntia elatior* Mill. is endowed with central and peripheral analgesic properties might be due to presence of phenolics and betanin content.

Anti-asthmatic activity was characterized using spasmolytic, mast cell stabilizing and anti-inflammatory models. Bronchodilating effect of fruit juice was dose dependant against spasm induced by acetylcholine and histamine. *Opuntia elatior* Mill. fruits possess a significant inhibitory effect on rat and guinea pig ileum contraction via antihistaminic and antimuscarinic action. Fruit juice was also found to inhibit the degranulation of mast cells induced by an immunological and a non-immunological stimulus. Fruit juice was found to have a potent anti-inflammatory activity against carrageenan induced pedal edema in rats and significantly reduced neutrophil adhesion. Fruits of *Opuntia elatior* Mill. has potential anti-asthmatic activity that may be due to its bronchodilator, mast cell stabilization, anti-inflammatory and reduction of neutrophil adhesion property.

The peel extracts of fruit exhibited antimicrobial actions in a dose dependant manner against both test bacteria and fungi. Antimicrobial activity of the peel extracts is directly concerning with the components that they contain. Petroleum ether, benzene and methanol extracts showed maximum inhibitory action against gram positive bacteria, gram negative bacteria and fungi, respectively.

These findings on haematinic, analgesic and anti-asthmatic effects of fruit juice, further add value to the nutritional characteristics of the fruits of *Opuntia elatior* Mill.

1. Introduction

1. Introduction

The haematological disorders include various types of anaemia where the patient having deficiency or reduction of haemoglobin content. Anaemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. It occurs at all stages of the life cycle, but is more prevalent in pregnant women and young children. In 2002, iron deficiency anaemia (IDA) was considered to be among the most important contributing factors to the global burden of disease. Anemia is a decrease in the number of red blood cells (RBCs), a decrease in the amount of hemoglobin, or both a decrease in the number of RBCs and hemoglobin. When there is an insufficient amount of hemoglobin to deliver oxygen to the tissues, anemia exists. Among the other causes of anaemia, heavy blood loss as a result of menstruation, or parasite infections such as hookworms, ascaris, and schistosomiasis can lower blood haemoglobin (Hb) concentration. Acute and chronic infections, including malaria, cancer, tuberculosis and HIV can also lower blood Hb concentration. The presence of other micronutrient deficiencies, including vitamins like A, B_{12}, folic acid, riboflavin, and trace elements like iron, copper, zinc can increase the risk of anaemia. Furthermore, the impact of haemoglobinopathies on anaemia prevalence needs to be considered within some populations. Globally, the most significant contributor to the onset of anaemia is iron deficiency so that IDA and anaemia are often used synonymously, and the prevalence of anaemia has often been used as a proxy for IDA. It is generally assumed that 50% of the cases of anaemia are due to iron deficiency, but the proportion may vary among population groups and in different areas according to the local condition. The main risk factors for IDA include a low intake of iron, poor absorption of iron from diets having high concentration of phytate or phenolic compounds, and period of life when iron requirements are especially high (i.e. growth and pregnancy) (Rang and Dale, 2003; Williams and Lemake, 2002; Watkins and Renau, 2003; Karimi et al., 2004; Malhotra et al., 2004; McLean et al., 2008; Seth & Seth, 2009).

Anemia is one of the most common health problems in India. The problem is much more in rural than the urban area. The high-risk groups for anemia are pregnant and lactating females and children. Prevalence in this subgroup has been found to vary from 50 - 90% in different parts of India. Almost all interventions at national and local level have focused predominantly on these groups. Reliable data on the prevalence of anemia in adult population (non-pregnant females and adult males) is not available. The prevalence of anemia in 16 - 70 years age group was 47.9%. The prevalence of anemia was higher among females (50%) than males (44.3%). The prevalence of mild anemia was higher (males 29.3%; females 32%) than moderate and severe anemia in this population. Prevalence of anemia was maximum (52.8%) in the age group of more than 45 years among males whereas among female subgroup, younger females (<30 years) had higher prevalence of anemia (55%). Both males and females, who were uneducated, smokers, belonging to low socioeconomic status and having low or normal body mass index had higher prevalence of anemia (Malhotra et al., 2004).

According to a UNICEF report, two billion people suffer from anemia worldwide and most of them having IDA, especially in underdeveloped/developing countries, where 40-50% of children under age five are suffering from IDA (Karimi et al., 2004). The highest prevalence is in preschool-age children (47.4%) and the lowest prevalence is in men (12.7%). However, the population group with the greatest number of individuals affected is non-pregnant women (468.4 million). WHO regional estimates generated for preschool-age children and pregnant and non-pregnant women indicate that the highest proportion of individuals affected are in Africa (47.5–67.6%), while the greatest number affected are in South-East Asia where 315 million individuals in these three population groups are affected (Table 1.1). The level of anaemia as the public health problem across countries is illustrated by maps for preschool-age children and pregnant and non-pregnant women in Figure 1.1 (Bruno de Benoist et al., 2008).

Table 1.1: Anaemia prevalence affected in preschool – age children, pregnant women, and non – pregnant women in each region as per WHO[#].

WHO region	Prevalence (%)		
	Preschool-age children	**Pregnant women**	**Non-pregnant women**
Africa	64.3 – 71.0	52.8 – 61.3	43.4 – 51.6
Americas	26.8 – 31.9	17.3 – 30.8	12.9 – 22.7
South–East Asia	61.0 – 70.0	43.9 – 52.5	41.9 – 49.4
Europe	15.4 – 28.0	18.6 – 31.6	14.7 – 23.3
Eastern Mediterranean	42.2 – 51.2	38.2 – 50.3	29.2 -35.6
Western Pacific	21.9 – 24.4	28.8 – 32.7	20.8 – 22.2
Global	**45.7 – 49.1**	**39.9 – 43.8**	**28.7 – 31.6**

[#]Bruno de Benoist et al., 2008.
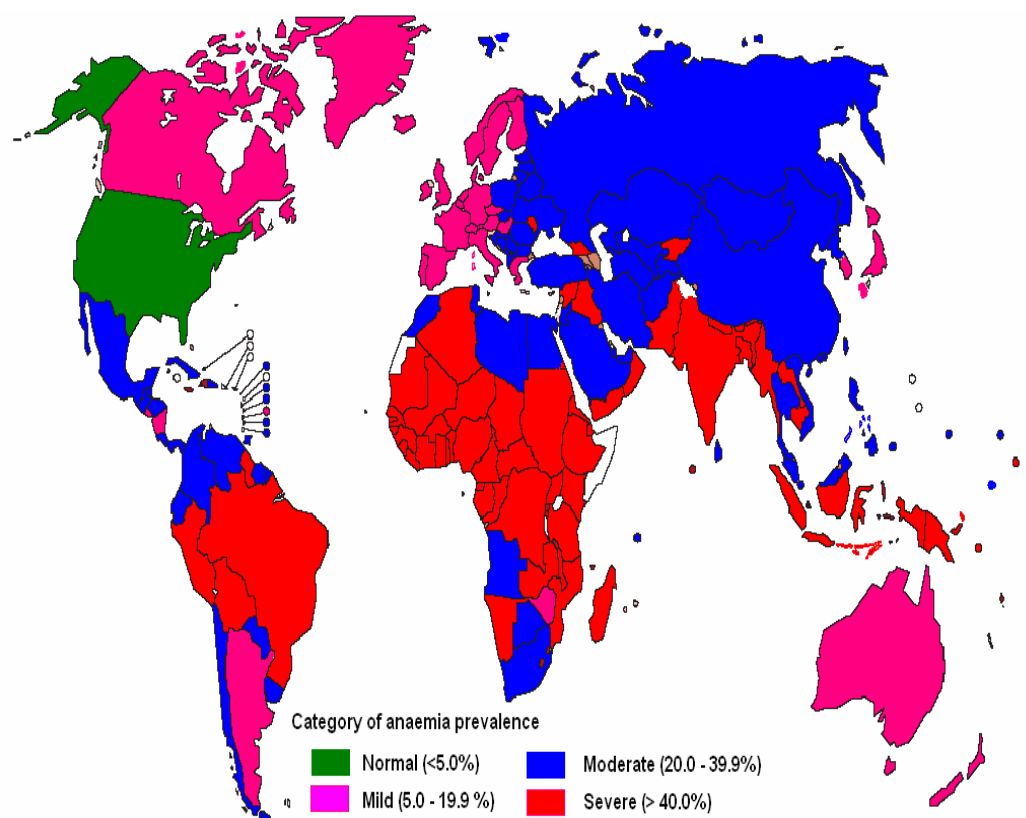
Figure 1.1a: Anaemia as a public health problem by country: Preschool – age children (Bruno de Benoist et al., 2008).
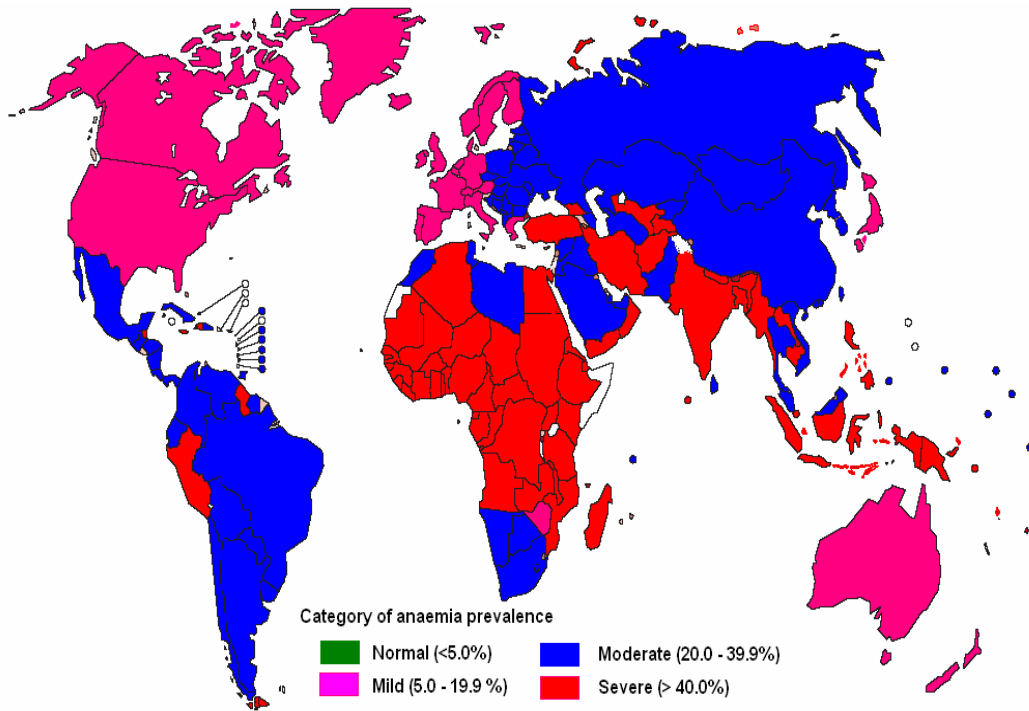
Figure 1.1b: Anaemia as a public health problem by country: Pregnant women (Bruno de Benoist et al., 2008).
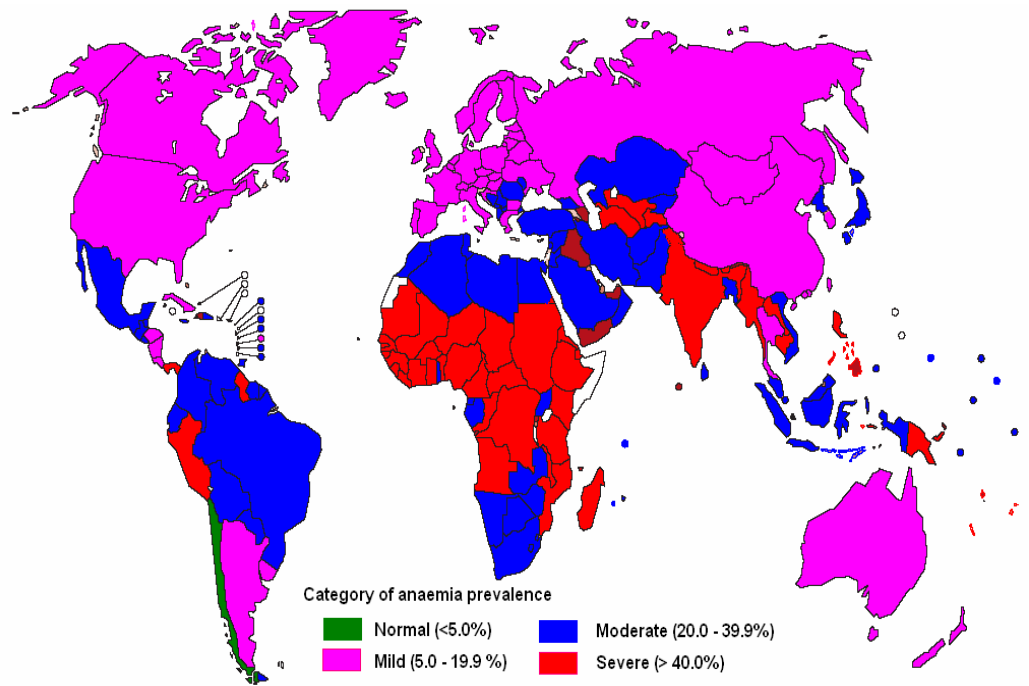
Figure 1.1c: Anaemia as a public health problem by country: Non – pregnant women (Bruno de Benoist et al., 2008).

Asthma is a Greek word which means 'breathless' or 'to breathe with open mouth'. The Global Strategy for Asthma Management and Prevention Guidelines define asthma as 'a chronic inflammatory disorder of the airways associated with increased airway hyper-responsiveness, recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night/early morning. Airway inflammation produces airflow limitation through acute bronchoconstriction, chronic mucus plug formation and airway wall swelling or remodelling. These symptoms may be relieved either spontaneously or after treatment. Asthma can occur at any age (Murthy and Sastry, 2005). Generic susceptibility and personal/family history of atopy along with environmental exposures produce the clinical symptomatology of asthma. These signs and symptoms are highly variable in severity and duration (Seth & Seth, 2009). Acute asthma attacks are triggered by a variety of stimuli, including exposure to allergens or cold air, exercise, and upper respiratory tract infections. Recently, a number of genetic polymorphisms have been associated with an increased risk of developing asthma. Thus, genetic factors probably contribute to the exaggerated response of the asthmatic airway to various environmental challenges. The most severe exacerbation of asthma, s*tatus asthmaticus,* is a life-threatening condition that requires hospitalization and must be treated aggressively. Unlike most exacerbations of the disease, *status asthmaticus* is by definition unresponsive to standard therapy (Torphy and Douglas, 2008).

Asthma has become more common in both children and adults around the world in recent decades. The increase in the prevalence of asthma has been associated with an increase in atopic sensitization, and is paralleled by similar increases in other allergic disorders such as eczema and rhinitis. The rate of asthma increases as communities adopt western lifestyles and become urbanized. With the projected increase in the proportion of the world's population that is urban from 45% to 59% in 2025, there is likely to be a marked increase in the number of asthmatics worldwide over the next two decades. It is estimated that there may be an additional 100 million persons with asthma by 2025 (Masoli et al., 2003). Asthma is thought to affect about 3% of the population in most countries. The highest prevalence (almost 30%) is found in New Zealand. The prevalence in a number of countries falls in the

range of 10%–17% (Murthy and Sastry, 2005). Figure 1.2 shows the prevalence of asthma in the world.
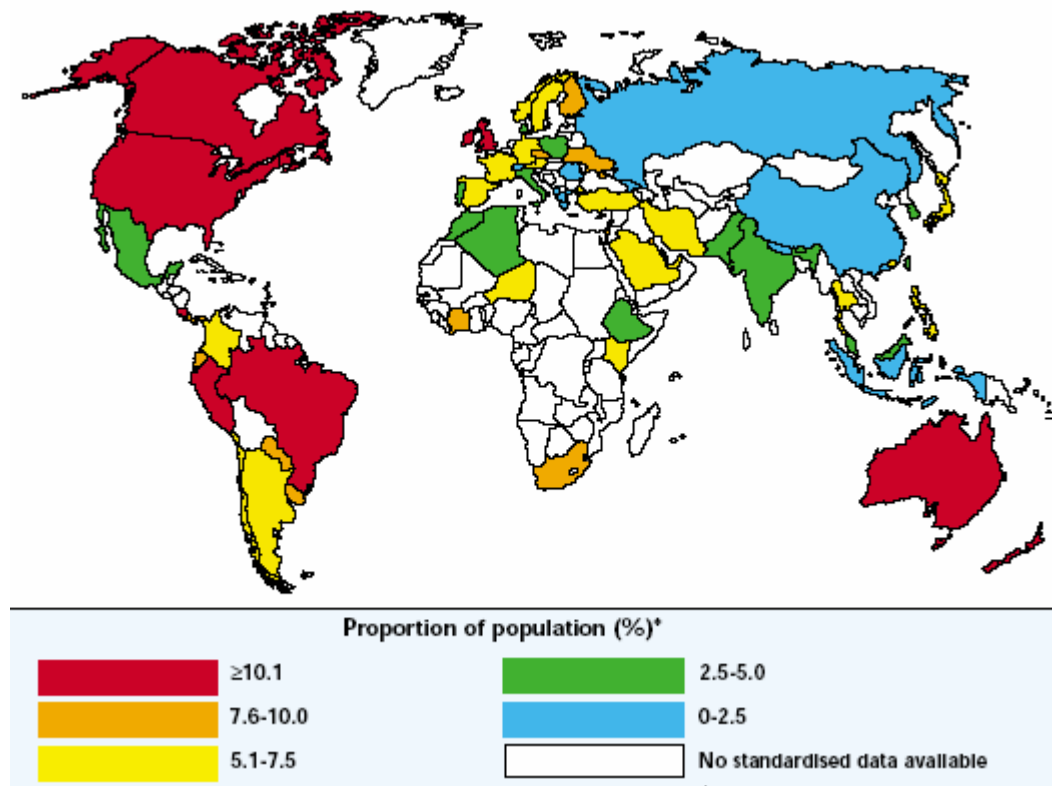
Figure 1.2: The prevalence of asthma in the world (Masoli et al., 2003).

There are only a few studies from India on field epidemiology of asthma. In a study conducted more than 30 years ago, prevalence of asthma was reported as 2.78% in an urban population aged 30-49 years. It was also reported in the same study that the prevalence in morbidity surveys of Government employees and their families in Delhi was 1.8% (Aggarwal et al., 2006). According to the National Family Health Survey-2 (NFHS-2) report the estimated prevalence of asthma in India is 2468 per 100,000 persons. The prevalence was higher in rural than in urban areas. The prevalence among males was slightly higher than among females. Among those below 15 years of age, asthma was seen in 950 per 100,000 persons. The prevalence of asthma in adult males (18 years and above) during 1995–97 was 3.94% in urban and 3.99% in rural areas. In females of the same age group, the prevalence was 1.27% in urban as well as rural areas. Increasing in prevalence is associated

with spreading urbanization, exposure to domestic mites, vehicle exhausts, smoking, allergens and family history (Murthy and Sastry, 2005).

Drugs so far invented for the treatment includes β_2 agonists, anticholinergics, corticosteroids, phosphodiesterase inhibitors, leukotriene modifiers, PAF inhibitor, TXA_2 inhibitor etc. Still newer molecules are being investigated on the basis of the new mechanisms involved. Specific ß$_2$ agonists and xanthine derivatives like theophylline or their combination are most commonly used by majority of asthmatic patients from mild to severe asthma in the tablet form. However, it has been reported that their prolong treatment produce variable adverse effects. Muscle tremor and hypokalemia are major adverse effects of ß$_2$ agonists. Theophylline has narrow therapeutic index and requires monitoring of drug levels. Glucocorticoids are used as an anti-inflammatory drug but they have no immediate effect on the early bronchoconstriction response to allergen or exercise. Steroids reverse tolerance to ß-adrenergic agonists and increase the density of ß-adrenergic receptors. Glucocorticoids cause adrenal suppression and variable adverse effects on prolong therapy. The growing environmental pollution with rapid and extensive industrialization is also responsible for aggravation of this disease. The alarming rise in the incidence of this disease in metropolitan cities has posed a serious problem (Williams and Lemake, 2002; Watkins and Renau, 2003; Rang and Dale, 2003; Undem, 2006).

About 70 – 80% of the world populations, particularly in the developing countries, rely on non-conventional medicine in their primary healthcare as reported by the WHO. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants. This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be ineffective (e.g. side effects and ineffective therapy), abusive and/or incorrect use of synthetic drugs results in side effects and other problems, a large percentage of the world's population does not have access to conventional pharmacological treatment, and folk medicine and ecological awareness suggest that "natural" products are harmless. However, the use of these substances is not always authorized by legal authorities dealing with efficacy and safely procedures,

and many published papers point to the lack of quality in the production, trade and prescription of phytomedicinal products. About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the WHO, 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursor (Raghavendra et al. 2009).

For centuries people have used plants for healing. Plant products – as parts of foods or botanical portions and powders – have been used with varying success to cure and prevent diseases throughout history. Uses of indigenous drugs from plant origin are the major sources as an alternative system of medicine or traditional system of medicine since ancient (Joshi et al., 2004). Traditional system of medicine involves the use of herbs, animal parts and minerals. It also includes acupuncture, manual therapy and spiritual medicines. As per the report of the Inter Regional Workshop on Intellectual Property Rights, the world market for traditional systems of medicine including herbal products and the raw materials has been estimated to have an annual growth rate of 5 to 15% and the total global herbal market may reach to five trillion US dolors by 2050. As per statistical data the Indian medicinal plant based industry is growing at the rate of 7 to 15% per annum. The value of botanicals related trade is about US $10 billion per annum with annual export of US $1.1 billion in India, while China's annual herbal drug production is worth US $48 billion with export of US $3.6 billion. Presently, the United States is the largest market for Indian botanical products accounting for about 50% of the total exports. Global trend leading to increasing demand of medicinal plants for pharmaceuticals, phytochemicals, neutraceuticals, cosmetics and other products is an opportunity sector for Indian trade and commerce (Singh et al., 2003, Raghavendra et al. 2009).

WHO define the traditional system of medicine as diverse health practice, approach, knowledge and belief incorporating plant, animal and / or mineral based medicine, spiritual therapy, manual technique and exercise applied singularly or in combination to maintain well being as well as to treat, diagnose or prevent illness. This system aims to promote healthy and enhance quality of life. The concept of constitutional uniqueness of human individuals

leading to prescription of suitable drugs and specific diet is a remarkable feature of traditional medicines (Patwardhan et al., 2006).

The R & D thrust in the pharmaceutical sector is focused on development of new drugs, innovative or indigenous processes for known drugs and development of plant based drugs through investigation of leads from traditional systems of medicine. Neutraceuticals and cosmoceuticals are of great importance as a reservoir of chemical diversity aimed at new drug discovery and are explored for antimicrobial, cardiovascular, immunosuppressant and anticancer drugs (Patwardhan et al., 2004). Natural products including plants, animal and minerals have been the basis of treatment of human diseases. Numerous molecules have come out of experimental base; examples include rauwolfia alkaloids for hypertension, holarrhena alkaloids in amoebiasis, guggulosterones as hypolipidemic agents, piperidine as bioavailability enhancer, curcumines in inflammation and picrosides in hepatic protection (Patwardhan and Hooper, 1992).

Drug discovery is no longer a game of chance or just limited to the availability of new technology but it is a better understanding of various approaches and key learning from the past with the appropriate strategy for the future is essential to make a significant difference (Schmid and Smith, 2004). During the past few years a large number of approved new drug applications have originated from the biotechnology industry and analysts expect a continuation of pharmaceutical-biotechnology alliances to help expand pipelines (Hughes, 2009). Similarly, natural products have contributed nearly half of all small molecules approved in this decade. It has been suggested that the current drug discovery approach of finding 'new entity drugs', if shifted to 'combining existing agents' may be helpful. Therefore natural product drug discovery based on ethnopharmacology and traditional medicines may also be considered as attractive strategic options (Kong et al., 2009).

The social expectation about drug safety and efficacy are rising while R & D productivity in the pharmaceutical industry is not met the requirements. The critical path initiative of FDA was intended to modernize drug development by incorporating recent scientific advances, shows a proactive policy approach to enhance innovation opportunities in a public/private partnership model (Woodcock and Woosley, 2008). It is suggested that traditional medicine may

offer better routes to the discovery, development and delivery of new drugs with enhanced performance in terms of cost, safety and efficacy. To this end, it is also believed that the basic principles, experiential wisdom, holistic approach and systematic database of Ayurveda may offer useful bio prospecting tools and an efficient discovery engine (Patwardhan et al., 2004).

The mass screening of plants in the development of new leads or drugs are tremendous expensive and inefficient. But the traditional knowledge based on bio prospecting offered better leads for the treatment of AIDS and cancer. About 60% of anticancer and 75% of anti-infective drugs approved from 1981 to 2002 was developed from natural origins (Gupta et al., 2005). A multidisciplinary approach combining natural product diversity with total, combinatorial synthetic and biosynthesis may provide a useful solution to the current innovation quandary (Newman and Cragg, 2007).

Earlier, the term reverse pharmacology has been used in relation to ligand-independent orphan functions that can modulate well-defined drug targets, but this review does not cover such aspects (Angelique and Ralf, 2008). Reverse pharmacology is defined as a rigorous scientific approach of integrating documented clinical experiences and experimental observation into lead by transdisciplinary exploratory studies and further developing these into drug candidates or formulations through robust preclinical and clinical research (Vaidya and Devasagayam, 2007). In this process 'safety' remains the most important starting point and the efficacy becomes a matter of validation. The novelty of this approach is the combination of living traditional knowledge such as Ayurveda and the application of modern technology and processes to provide better and safer leads.

It is suggested that drug discovery need not be always confined to the discovery of a single molecule. Many analysts believe that the current 'one drug fits all' approach may be unsustainable in the future. The growing interest in polypill concept is indicative of the need to collectively address multiple targets, risk factors or symptoms (Kumar et al., 2008). In the management of polygenic syndromes and conditions there is renewed interest in multi-ingredient synergistic formulations (Zimmermann et al., 2007). The rationally designed polyherbal formulations also could be explored as an option for multitarget therapeutic and prophylactic applications. Both

traditional medicines and Ayurveda, over thousands of years have been developed various practical theories to create polyherbal formulations in which multiple agents contained in one formula act synergistically (Hong-Fang Ji et al., 2009).

Development of standardized, synergistic, safe and effective traditional herbal formulations with robust scientific evidence can also offer faster and more economical alternatives. Ayurvedic texts include thousands of single or polyherbal formulations (Anonymous, 2003). These have been rationally designed and have been in therapeutic use for many years. Sufficient pharmacoepidemiological evidence, based on actual clinical use, can be generated to support their safety and efficacy (Vaidya et al., 2003). Systematic data mining of the existing formulations' huge database can certainly help the drug discovery processes to identify safe, effective and synergistic formulations.

The World Health Organization's Commission on Intellectual Property and Innovation in Public Health has also recognized the promise and role of traditional medicine in drug development for affordable health solutions (Patwardhan, 2005). India, China, Korea, Malaysia, Brazil, South Africa, Australia and number of other countries are becoming increasingly aware of the value of their traditional knowledge. On the other hand, the global pharmaceutical industry is looking for innovative solutions to expedite the discovery process. Therefore, innovative approaches inspired by traditional knowledge like Ayurveda and folkloric uses may aptly occupy this niche strategy to expedite drug discovery and development process especially in the existing global economic environment (Patwardhan and Mashelkar, 2009).

Ayurvedic physicians suggest leafy green vegetables for the treatment of haematological disorders as a source of iron and other minerals. Various herbs used in anaemia are *Ageratum conyzoides, Boerhavia diffusa, Centella asiatica, Hemidesmus indicus, Ichnocarpus frutescens, Momordica charantia, Moringa oleifera, Phyllunthus amarus, Phyllunthus emblica, Punica granatum, Ocimum tenuiflorum, Solanum americanum* (Silja et al., 2008)*, Adenia gummifera, Allophylus rubifolius, Albizia versicolor, Brackenridgea zanguebarica, Bridelia cathartica, Comniphora africana, Hibiscus sabdariffa,*

Lannea stuhlmanni, Sorgum bicolor, Theobroma cacao, Triumfetta rhomboidea etc. (Omolo el al. 1997; Falade et al. 2005; Oladiji et al. 2007).

An ancient system of Indian medicine has also recommended a number of drugs from indigenous plant sourced for the treatment of bronchial asthma and allergic disorders (Charaka Samhita, 1949). Various herbs used in asthma *are Achyranthes aspera, Adhatoda vasika, Albizzia lebbeck, Artemisia caerulenscens, Boswellia serrata, Calotropis gigantea, Calotropis procera, Cedrus deodara, Clerodendron serratum, Curcuma longa, Eugenia caryophylis, Eleocarpus spharicus, Inula racemosa, Ocimum sanctum, Picrorrhiza kurroa, Piper longum, Sarcostemma brevistigma, Solanum xanthocarpum, Tephrosia purpurea, Tinospora cordifolia, Tylophora asthmatica, Vitex negundo* etc. (Gokhale & Saraf, 2002).

The fruit of *Opuntia* is considered a refrigerant, and is said to be useful in gonorrhea. The baked fruit is said to be given in whooping cough and syrup of the fruit is said to increase the secretion of bile and control spasmodic cough and expectoration (Kirtikar and Basu, 1999; The Wealth of India, 2001). The fruits of *Opuntia elatior* Mill., commonly known as *"Hathlo Thor"* belongs to family Cactaceae, are use as haematinic, anti-asthmatic and spasmolytic action by tribal people of Saurashtra region of Gujarat state, and have been successfully controlled the disease as well. Although the fruits have haematinic, anti-asthmatic and spasmolytic activity, systematic study which can identify possible mechanism and phytoconstituents of fruits of *Opuntia elatior* Mill. for such action is not yet to be found.

In the light of the above, the objectives of present study were
   (i)   To carry out qualitative and quantitative phytochemical analysis of fruits of *Opuntia elatior* Mill.
   (ii)   To screen and evaluate ethanopharmacological use of fruits of *Opuntia elatior* Mill. as haematinic, anti-asthmatic and spasmolytic agent using various animal model.
   (iii)   To screen and evaluate antimicrobial action of fruit peel of *Opuntia elatior* Mill.

2. Review of Literature

2. Review of Literature

2. Review of Literature

2.1 Anaemia

Anaemia is defined as a reduction of haemoglobin concentration in the blood. It may give rise to fatigue but, especially if it is chronic, is often surprisingly asymptomatic. The commonest cause is blood loss related to menstruation and child bearing, but there are several different types of anaemia, and several different diagnostic levels. Determining indices of red cell size and haemoglobin content and microscopical examination of a stained blood smear of blood allows characterization into:

- Hypochromic, microcytic anaemia (small red cells with low haemoglobin; caused by iron deficiency)
- Macrocytic anaemia (large red cells, also decrease in RBC)
- Normochromic normocytic anaemia (fewer normal-sized red cells, each with a normal haemoglobin content)
- Mixed pictures.

Further evaluation may include determination of concentrations of ferritin, iron, vitamin B_{12} and folic acid in serum, and microscopic examination of smears of bone marrow. This leads to more precise diagnosis of anaemias. There are various causes of anaemias like:

(I) Deficiency of nutrients necessary for haemopoiesis, most importantly:

- Iron
- Folic acid and vitamin B_{12} (Megaloblastic anaemia)
- Pyridoxine, vitamin C

(II) Depression of the bone marrow, caused by:

- Toxins (e.g. drugs used in chemotherapy)
- Radiation therapy
- Diseases of the bone marrow of unknown origin (e.g. idiopathic aplastic anaemia, leukaemias)

- Reduced production of, or responsiveness to, erythropoietin (e.g. chronic renal failure, rheumatoid arthritis, acquired immunodeficiency disease (AIDS))

(III) Excessive destruction of red blood cells (i.e. haemolytic anaemia); this has many causes including haemoglobinopathies (such as sickle cell anaemia), adverse reactions to drugs and inappropriate immune reactions (Rang and Dale, 2003; Dawson, 2007; Ritter et al., 2008; Pazdernik and Kerecsen, 2009; Seth and Seth, 2009).

2.1.1 Haematopoiesis

Haematopoiesis (from Ancient Greek: *haima* blood; *poiesis* to make) is the formation of blood cells. All cellular blood components are derived from haematopoietic stem cells. In developing embryos, blood formation occurs in aggregation of red blood cells in the yolk sac, called blood islands. As development progresses, blood formation occurs in the spleen, liver and lymph nodes. When bone marrow develops, it eventually assumes the task of forming most of the blood cells for the entire organism. However, maturation, activation, and some proliferation of lymphoid cells occur in secondary lymphoid organs (spleen, thymus, and lymph nodes). In children, haematopoiesis occurs in the bone marrow of the long bones such as the femur and tibia. In adults, it occurs mainly in the pelvis, cranium, vertebrae, and sternum (Greenburg, 1996; Mercadante et al., 2000; Aster, 2007; Hall, 2007; Hodges et al., 2007; Ritter et al., 2008; Wagner et al., 2008).

All blood cells are divided into three lineages.

- Erythroid cells are the oxygen carrying red blood cells. Both reticulocytes and erythrocytes are functional and are released into the blood. In fact, a reticulocyte count estimates the rate of erythropoiesis.
- Lymphocytes are the cornerstone of the adaptive immune system. They are derived from common lymphoid progenitors. The lymphoid lineage is primarily composed of T-cells and B-cells (types of white blood cells). This is lymphopoiesis.
- Myelocytes, which include granulocytes, megakaryocytes and macrophages and are derived from common myeloid progenitors, are

involved in such diverse roles as innate immunity, adaptive immunity, and blood clotting. This is myelopoiesis. Granulopoiesis (or granulocytopoiesis) is haematopoiesis of granulocytes. Megakaryocytopoiesis is haematopoiesis of megakaryocytes.

The cells of the haematopoietic system are functionally diverse (Table 2.1). Red blood cells, or erythrocytes, carry oxygen; many types of white blood cells, from granulocytes and macrophages to lymphocytes, fight against infection and help to protect against cancer etc.; and platelets help to control bleeding. Nonetheless, these cells all have one feature in common: they will produce from a common cell in the bone marrow called the pluripotent haemtopoietic stem cell (Figure 2.1). Haematopoietic stem cells are induced to differentiate along committed lineages into red blood cells, white blood cells, or platelets though interactions with glycoproteins called haematopoietic growth factors.

Red and white blood cell production is regulated with great precision in healthy humans, and the production of granulocytes is rapidly increased during infection. The role of various growth factors in haematopoiesis is shown in figure 2.2. The proliferation and self-renewal of these cells depend on stem cell factor (SCF). Glycoprotein growth factors regulate the proliferation and maturation of the cells that enter the blood from the bone marrow, and cause cells in one or more committed cell lines to proliferate and mature. Three more factors that stimulate the production of committed stem cells are called colony-stimulating factors (CSFs) and include granulocyte-macrophage CSF (GM-CSF), granulocyte CSF (G-CSF) and macrophage CSF (M-CSF). These stimulate much granulocyte formation and are active on either progenitor cells or end product cells. Erythropoietin is required for a myeloid progenitor cell to become an erythrocyte. On the other hand, thrombopoietin makes myeloid progenitor cells differentiate to megakaryocytes (thrombocyte-forming cells) (Greenburg, 1996; Mercadante et al., 2000; Aster, 2007; Hall, 2007; Hodges et al., 2007; Ritter et al., 2008; Wagner et al., 2008).

Table 2.1: Haematopoietic cells, Growth factors and its analogues[#].

Cell type	Major functions	Lineage specific growth factor	Deficiency state	Therapeutic agents
RBC (erythrocyte)	Oxygen transport	Erythropoietin (EPO)	Anaemia	rhEPO, darbepoetic
Platelet (thrombocyte)	Hemostasis	Thrombopoietin (TPO)	Thrombocytopenia	rhTPO, IL-11, PEG-rHuMGDF (TPO analogue)
Monocyte / macrophage	Phagocytosis of bacteria and cellular & chemical debris, stimulation of T lymphocytes	M-CSF	____	___
Neutrophil	Phagocytosis of bacteria, immune stimulation	G-CSF	Neutropenia	Filgrastim, sargramostim
Eosinophil	Control of parasites	IL – 5	___	___
Basophil	Phagocytosis of bacteria	___	___	Filgrastim, sargramostim
B lymphocytes	Production of antibody, stimulation of T lymphocytes	Specific interleukins	Various immunodeficiency syndromes	___
T lymphocytes	Killing of virus and bacteria infected cells, control of immune response	Specific interleukins	Various immunodeficiency syndromes	rhIL – 2
NK cells	Killing of cancer cells	___	___	___

[#] Aster, 2007; Ritter et al., 2008; Wagner et al., 2008
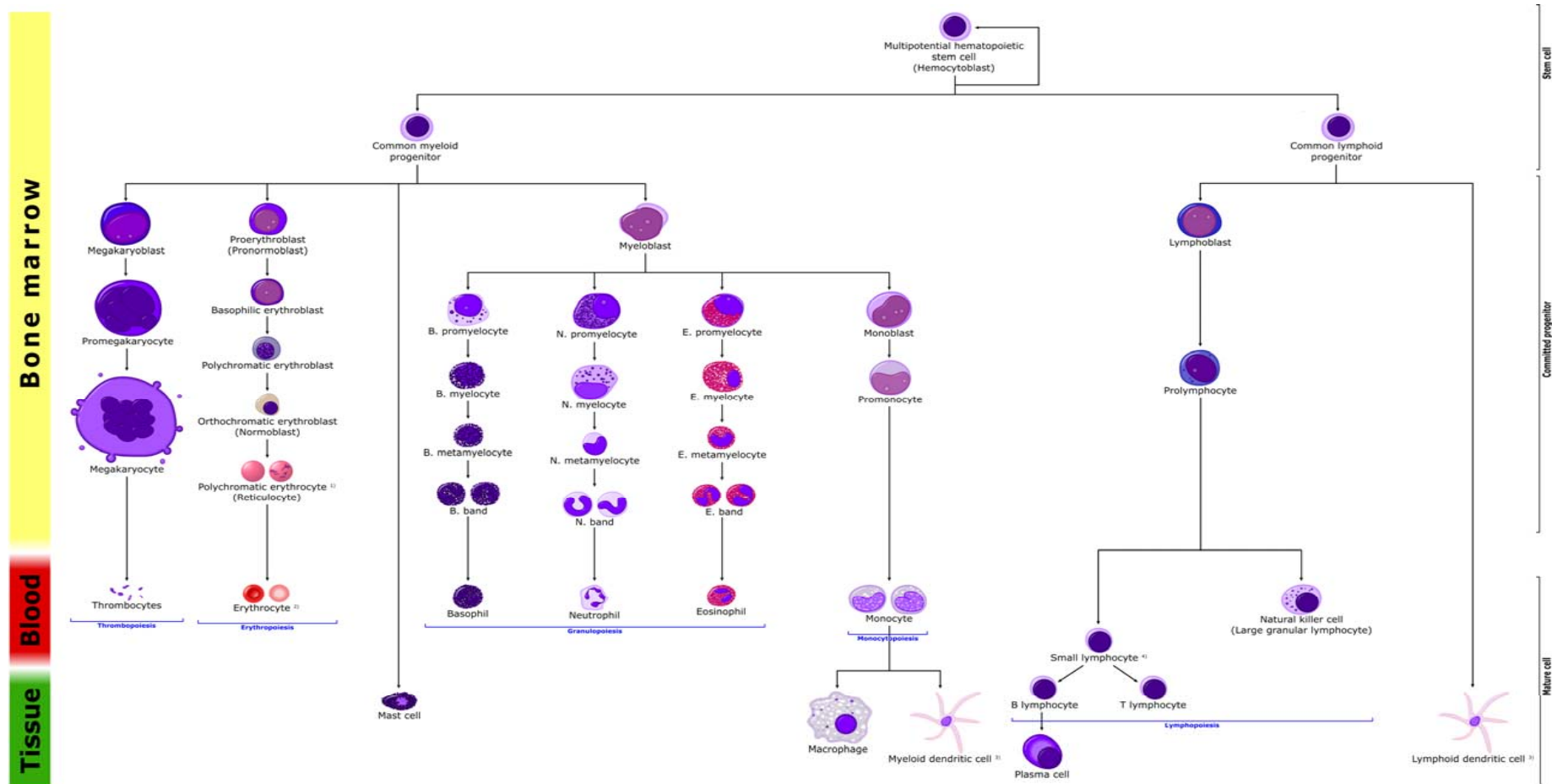
Figure 2.1: Development of cells of the Haematopoietic system (Aster, 2007; Ritter et al., 2008; Wagner et al., 2008).
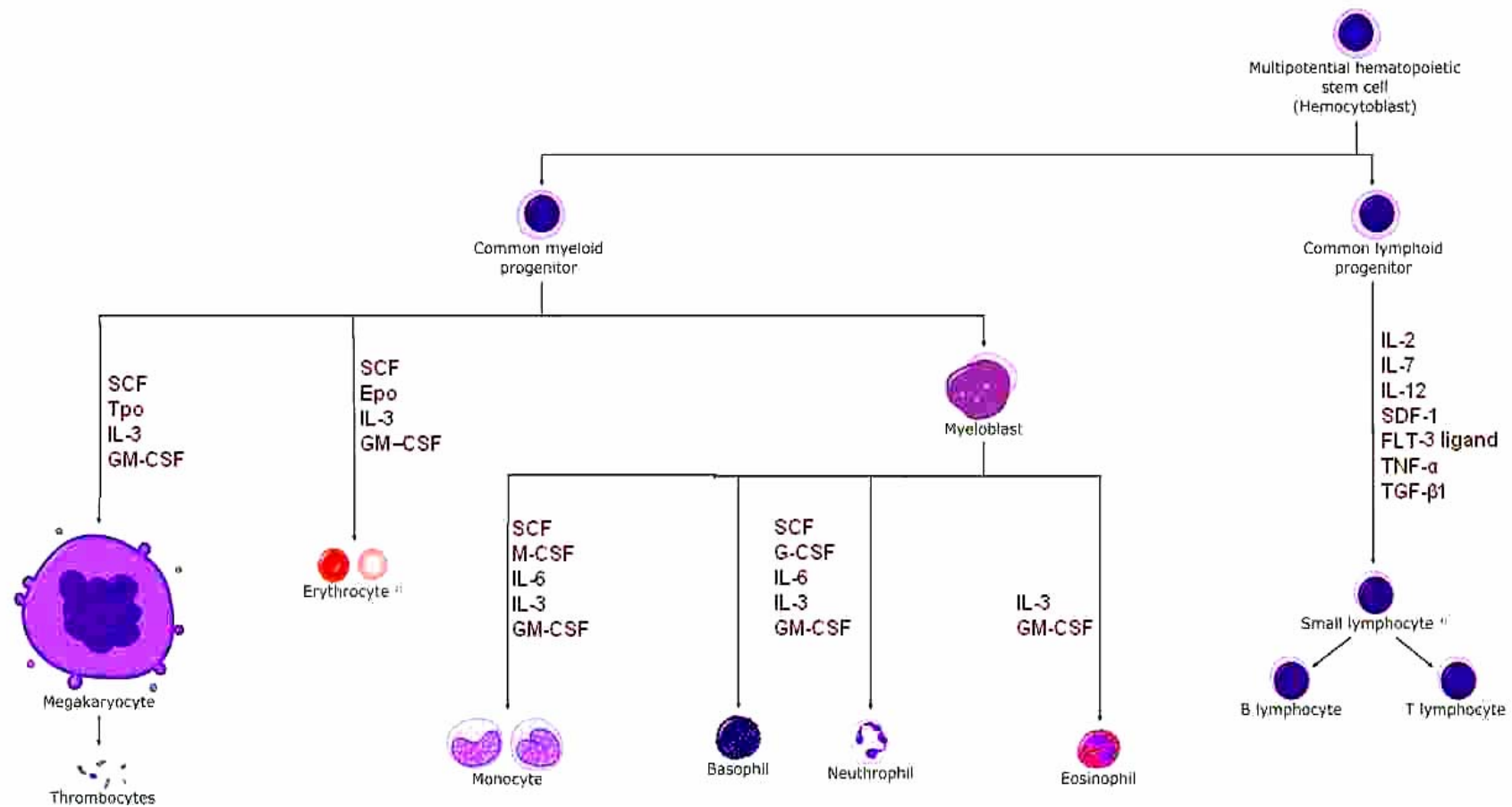
Figure 2.2: Haematopoietic growth factor development (Aster, 2007; Ritter et al., 2008; Wagner et al., 2008).

2.1.2 Pathophysiology of Anaemia

The function of red cells is to transport oxygen to peripheral tissues. The reduction of oxygen-carrying capacity of blood usually results from a deficiency of red cells, or anaemia, defined as a reduction below normal limits of the total circulating red cell mass. Measurement of red cell mass is not easy; however, in routine practice anaemia is defined as a reduction below normal in the volume of packed red cells, as measured by haematocrit, or a reduction in the haemoglobin concentration of the blood. Occasionally, fluid retention can expand plasma volume and dehydration can contract plasma volume, creating spurious abnormalities in these values (Greenburg, 1996; Mercadante et al., 2000; Aster, 2007; Hall, 2007; Hodges et al., 2007; Ritter et al., 2008; Wagner et al., 2008). There are innumerable classifications of anaemia. An acceptable one based on underlying mechanisms is presented as follows:

Blood Loss

Acute: Troma

Chronic: Lesions of gastrointestinal tract, gynecological disturbances

Increased Rate of Destruction (Haemolytic anaemias)

Intrinsic (intracorpuscular) abnormalities of red cells

Hereditary

Red cell membrane disorders

Disorders of membrane cytoskeleton: sperocytosis, elliptocytosis

Disorder of lipid synthesis: selective increase in membrane lecithin

Red cell enzyme deficiencies

Glycolytic enzymes: pyruvate kinase deficiency, hexokinase deficiency

Enzymes of hexose monophosphate shunt: G6PD, glutathione synthetase

Disorders of haemoglobin synthesis

Deficient globin synthesis: thalassemia syndromes

Structurally abnormal globin synthesis (haemoglobinopathies): sickle cell anaemia, unstable haemoglobins

Acquired

Membrane defect: paroxysomal nocturnal haemoglobinuria

Extrinsic (extra corpuscular) abnormalities

Antibody mediated

Isohaemagglutinins: transfusion reactions, erythroblastosis fatalist

Auto antibodies: idiopatic, drug-associated, systemic lupus

erythematosus, malignant neoplasm, mycoplasmal infection

Mechanical trauma to red cells

Microangiopathic hemolytic anaemias: thrombotic thrombocytopenic

purpura, disseminated intravascular coagulation

Cardiac traumatic hemolytic anaemia

Infections: Malaria, Hookworm

Chemical injury: lead and mercury poisoning

Sequestration in mononuclear phagocyte system: hypersplenism

Impaired Red Cell production

Disturbance of proliferation and differentiation of stem cells: aplastic anaemia, pure

red cell aplasia, anaemia of renal failure, anaemia of endocrine disorders.

Disturbance of proliferation and maturation of erythroblasts

Defective DNA synthesis: deficiency or impaired use of vitamin B_{12} and folic

acid (megaloblastic anaemias)

Defective haemoglobin synthesis

Deficient heme synthesis: Iron deficiency

Deficient globin synthesis: thalassemias

Unknown or multiple mechanisms: sideroblastic anaemia, anaemia of chronic

infections, myelophthisic anaemias due to marrow infiltrations.

The breakdown of an RBC is in part a recycling process (Figure 2.3), and understanding this physiological process makes understanding haemolysis easy. The haemoglobin is broken down into haem and globin. The haem is broken down into iron, which is bound

and transferred by transferrin to the marrow erythroblasts (primitive RBCs growing in the bone marrow) and protoporphyrin, which is broken down mainly into bilirubin with some carbon monoxide (which is expired via the lungs). Remembering what happens to the bilirubin is essential in understanding how to investigate haemolysis. Bilirubin is normally circulated to the liver where it is conjugated to bilirubin glucuronides, which are excreted into the gut via the bile and converted to stercobilionogen and stercobilin, which are excreted in the faeces. Some stercobiliogen and stercobilin is reabsorbed and excreted in the urine as urobilinogen and urobilin. The globin chains are degraded into amino acids, which are reused in protein synthesis around the body (Hall, 2007).
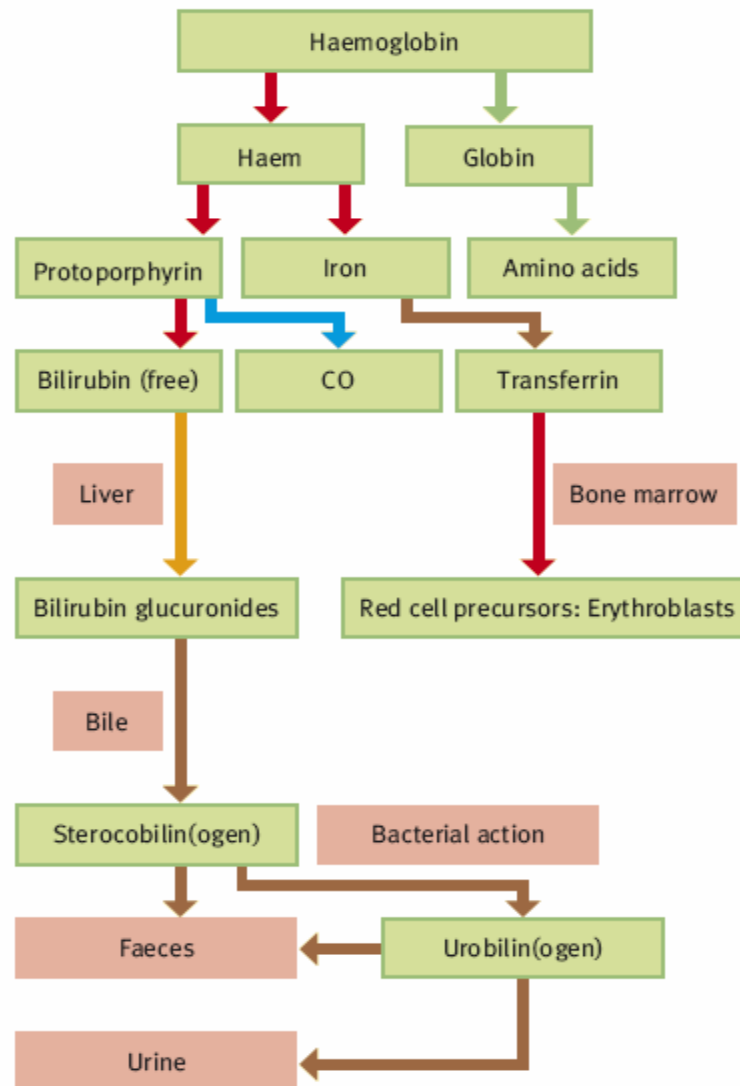
Figure 2.3: Normal breakdown of Haemoglobin (Hall, 2007).

A second useful approach classifies anaemia according to alterations in red cell morphology, which often correlates with the cause of red cell deficiency. Morphologic characteristics providing etiologic clues include red cell size (normocytic, microcytic, or macrocytic); degree of haemoglobinization, reflected in the color of red cells (normochromic or hypochromic); and other special features, such as shape. These red cell indices are often judges qualitatively by phycians, but precise quantitation is done in clinical laboratories using special instrumentation.

Haematopoiesis also requires an adequate supply of minerals (*e.g.,* iron, cobalt, and copper), vitamins (*e.g.,* folic acid, vitamin B_{12}, pyridoxine, ascorbic acid, and riboflavin) and growth factors in the various diseased conditions.

2.1.3 Mercuric chloride –induced anaemia

Heavy metals are non biodegradable environmental pollutants and their levels in different environmental compartments (air, water, and food) are gradually increasing due to industrial and agricultural practices. Growing pollution of the environment with metals contributes to various disorders, including cancer, hematotoxicity, allergic disease, and immunotoxicity. Anemia is a common finding in animals after exposure to certain heavy metals, such as lead, cadmium, arsenic, and mercury, and immunodeficiency is a consequence of long-term anemia and hypoxia (Dieter et al., 1983; Lund et al., 1991; Lecavalier et al., 1994; Jadhav et al., 2007). Mercury and its compound have had a long history in medicine. While not as important in modern medicine today, certain mercury salts are still used widely in ayurvedic system of medicine. Metallic mercury is relatively non-toxic. The mercurous (Hg^+) and mercuric (Hg^{++}) cations are toxic. Mercury vapor, however, is toxic. Mercury poisoning from inhaling mercury vapour is believed to have occurred in scientists working with mercury, in industrial situations and in people living near industrial plants emitting mercury vapour in the air. The classic example used to illustrate mercury poisoning is that of the fishermen and their families living around Mina Mata Bay in Japan. Mercury induced haematological effect among occupationally or accidentally exposed human beings are well established (Sauder et al., 1988) and its effects on experimental animals are also well documented (Rathore and Vaghese, 1994).

Acute inhalation exposure to mercury vapour may be followed by chest pains, dyspnoea, coughing, haemoptysis, and some times interstitial pneumonitis leading to death. The ingestion of mercuric compounds, in particular mercuric chloride, has caused ulcerative gastroenteritis and acute tubular necrosis causing death from anuria. Effects of inorganic mercury on experimental animals and *in vitro* test systems are well documented in EHC-118 (1991). Accumulation of mercury in the blood of mice has already been proved using inorganic radio mercury (Mehra and Kanwar, 1979). Mercury induced anemia was also reported in mice exposed to 0.1 mM and 0.5 mM of $HgCl_2$ for 100 and 30 days respectively via drinking water (Varghese et al., 1997).

Various mechanisms have been proposed to explain the biological toxicity of mercuric chloride ($HgCl_2$), including oxidative stress. Hg^{2+} reacts with thiol groups (-SH), thus depleting intracellular thiols, especially glutathione, and causing cellular oxidative stress or predisposing cells to it (Gstraunthaler et al., 1983). Other antioxidants, including ascorbic acid and vitamin E, have been reported to be depleted in $HgCl_2$-treated rats (Fukino et al., 1984). Many experiments suggest that oxidative stress can be involved in cellular damage and that it can be implicated in the toxicity of many xenobiotics (Gutierrez et al., 2006). If animals are pretreated with superoxide dismutase (Cu, Zn-SOD) before acute intoxication is induced, histological changes are prevented (Girardi and Elias, 1995).

Renal mercury content, urinary mercury excretion and renal function were studied in rats with acute renal failure-induced by subcutaneous injection of 2, 3, 6, or 10 mg/kg $HgCl_2$ and protected against acute renal failure by continuous intravenous infusion of furosemide and saline (Brunner et al., 1985). Gradual alterations of testicular tissues were noted in rats treated with mercuric chloride at dosages of 0.05 mg/kg and 0.10 mg/kg body weight (i.p.) over a period of 90 days (Chowdhury et al., 1986). Effects of methyl mercuric chloride (24 mg/kg, i.p.) on the blood parameters of Swiss mice were studied and found significant decreases in haemoglobin content, red blood cell (RBC) count and haematocrit value compared to the control (Shaw et al., 1991). Rathore and Siddiqui (2000) investigated the effect of homoeopathic drug in mice against mercuric chloride (10 μg/ml) induced anemia. Sarkar et al. (2007) evaluated the haematinic effect of two

ayurvedic preparations, *Lauha Bhasma* and *Mandura Bhasma* (11 mg/kg), on mercuric chloride (9 mg/kg) –induced anemia in rats.

2.1.4 Phenylhydrazine –induced anaemia

Phenylhydrazine (PHZ) was the first hydrazine derivative characterized by Hermann Emil Fischer in 1875. This compound is used worldwide mainly as a chemical intermediate in the pharmaceutical, agrochemical, and chemical industries (Berger, 2007). PHZ is a strong oxidant agent, which is extensively used in industry, laboratory and therapeutic settings. Indeed, the ability of PHZ to cause removal of erythrocytes from circulation was the basis of its former use as a therapeutic agent for polycythemia vera, a disorder in which increased red cell mass in the circulatory system is one symptom (Shetlar, & Hill, 1985). A variety of toxic effects of PHZ have been described, including hemolytic anemia, hypoxia, inflammation, alterations in the liver, kidney, central nervous system, autoimmune disturbances and cancer (Goldberg and Stern, 1977; Parodi et al., 1981; Nassberger et al., 1991; Brugnara and De Franceschi, 1993; Nicolas et al., 2002;). A large amount of research effort has been devoted to trying to understand the processes that occur in erythrocytes, or with oxyhemoglobin, on exposure to PHZ and related compounds and how PHZ-induced changes in erythrocytes lead to hemolytic anemia. PHZ is known to shorten life-span of red blood cells (RBCs) resulting in severe hemolytic anemia, enhanced erythropoietic activity, increased iron absorption and tissue iron overload. Oxyhemoglobin forms methemoglobin by PHZ-induced processes and the reduction to methemoglobin to deoxyhemoglobin in anaerobic systems or the formation of oxyhemoglobin in aerobic environments can also be induced to occur. These reactions of hemoglobin, promoted by PHZ, do not proceed without accompanying irreversible degradative reactions. Hemoglobin, whether free in solution or within erythrocytes, reacts with PHZ to yield "green hemoglobin", a form in which the heme group is modified. Processes induced by PHZ also cause destabilization of the globin portion of hemoglobin, leading to denaturation and precipitation (Beaven & White, 1954). The auto-oxidation of PHZ leads to generation of reactive oxygen species (ROS) and a complex array of PHZ-derived radicals, such as phenylhydrazyl radical, phenyldiazene and benzenediazonium ions (Misra and Fridovich, 1976). Not only ROS, PHZ metabolites can also react with

plasma membrane to cause lipid peroxidation and protein oxidation resulting in the destruction of RBCs and hemolytic anemia (Chakrabarti et al., 1995).

The exposure too many chemicals including the administration of some drugs has been associated with red blood cell destruction (Beutler, 2001), and haemolytic anaemia is a part of the clinical syndrome associated with intoxication. Chemicals can cause haemolysis by interacting with sulfhydryl groups, the inhibition of various enzymes, immune mechanisms, and the fragmentation of erythrocytes as they pass through the platelet-fibrin mesh or by unknown or poorly defined mechanisms. In haemolytic anaemia, erythrocytes have a shortened life-span. Yeshoda (1942) induced anaemia in rats following a single phenylhydrazine intraperitoneal administration at a dose of 20 mg/kg b.w. (aqueous solution): erythrocyte concentration lowered to about 50% and haemoglobin level to about 60% of normal values in the course of 4 days. Phenylhydrazine is used for the induction of haemolytic anaemia and the study of its mechanism in many species: rabbit (Nakanishi, 2003; Xie, 2003), rat (Yeshoda, 1942), mouse (Golab et al., 2002), calf (Sharma et al., 1991), chicken (Datta et al., 1990), and *in vitro* also in both rat and human erythrocytes (Pokhrel and Lau-Cam, 2000; Claro, 2006).

Previous study demonstrated that rats treated with PHZ (125 mg/kg i.p.), a dose lower than the LD50, consistently exhibited severe hemolytic anemia, vascular dysfunction, oxidative stress and hypotension in rats within 48 h suggesting an involvement of inflammatory mediators (Luangaram et al., 2007). Manis & Schachter (1966) studied the effects of erythropoiesis in the rat on iron transport across averted duodenal gut sacs *in vitro* by phenylhydrazine hydrochloride (100 mg/kg) subcutaneously and Flanagan & Lessler (1970) studied reticulocytosis in rat by intraperitoneal injection of phenylhydrazine hydrochloride (40 mg/kg) every other day of a nine-day experimental period. PHZ (15 mg/kg, i. p.) induced oxidative damage to cellular membranes reduced by melatonin and ascorbic acid (Karbownik et al., 2000). The effect of Haptoglobin on renal oxidative tissue damage, renal functions, hemoglobin precipitation in renal tissues, and general tissue damage was determined in phenylhydrazine- (200 mg/kg) induced haemolysis in mice (Lim et al, 2000). The mechanisms of regulation of erythropoiesis

were studied during hemolytic anemia induced by phenylhydrazine. Blood hypoxia was induced by intraperitoneal injection of phenylhydrazine hydrochloride in single dose of 30 and 150 mg/kg (Zyuz'kov et al., 2004). The haematinic activity of an orally administered aqueous extract of *Hibiscus cannabinus* leaves was evaluated on phenylhydrazine (10 mg/kg, p.o) –induced anemia for a period of 8 days (Agbor et al., 2005). Rokushima et al. (2007) analyzed gene expression profiles in the spleen by phenylhydrazine- (20 & 80 mg/kg/day, i.p.) and phenacetin- (500 & 1000 mg/kg/day, p.o.) induced hemolytic anemia. The extract of *Tectona grandis* leaves was evaluated on anaemia model of rat induced by intraperitoneal injection of phenylhydrazine at 40 mg/kg for 2 days (Diallo et al., 2008).

2.2 Asthma

Asthma is a reversible obstructive disease of the lower airway. With asthma there is increasing airway obstruction caused by bronchospasm and bronchoconstriction, inflammation and edema of the lining of the bronchioles, and the production of thick mucus that can plug the airway.

There are three types of asthma:

1. Extrinsic (also referred to as allergic asthma and caused in response to an allergen such as pollen, dust, and animal dander).

2. Intrinsic asthma (also called non-allergic asthma and caused by chronic or recurrent respiratory infections, emotional upset, and exercise).

3. Mixed asthma (caused by both intrinsic and extrinsic factors).

Figure 2.4 identifies the asthmatic pathway from both intrinsic and extrinsic stimulus. Extrinsic or allergic asthma causes the IgE inflammatory response. With exposure, the IgE antibodies are produced and attach to mast cells in the lung. Re-exposure to the antigen causes them to bind to the IgE antibody, releasing histamine and other mast cell products. The release of these products causes bronchospasm, mucous membrane swelling, and excessive mucous production. Gas exchange is impaired, causing carbon dioxide to be trapped in the alveoli so that oxygen is unable to enter (Rang and Dale, 2003; Dawson, 2007; Gibbs, and Cripps, 2007; Hussain and Kumar, 2007; Galanter and Lazarus, 2008; Ritter et al., 2008; Pazdernik and Kerecsen, 2009; Seth and Seth, 2009).
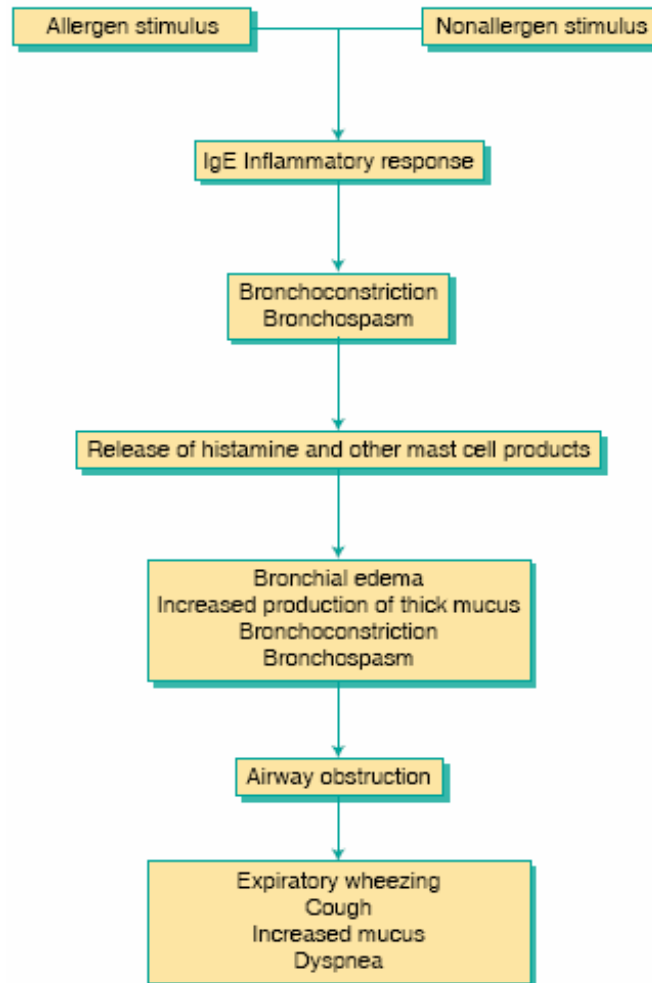
Figure 2.4: Asthmatic pathway from intrinsic and extrinsic stimulus (Pazdernik and Kerecsen, 2009).

2.2.1 Pathophysiology of asthma

In asthma, smooth muscle that surrounds the bronchi is hyper responsive to stimuli, and underlying inflammatory changes are present in the airways. Asthmatic stimuli include inhaled allergens, occupational allergens, and drugs or non-specific stimuli such as cold air, exercise, stress and pollution. The stimuli cause asthmatic changes through several complex pathways (Figure 2.5). The possible mechanisms of these pathways include the following:

- Immune reactions (type 1 hypersensitivity) and release of inflammatory mediators – the cross-linking of IgE by allergens causes mast cell degranulation, and release of histamine and powerful eosinophil and neutrophil chemotactic factors. The mediators, *viz.* histamine, tryptase, LTC_4 and D_4, and PGD_2, when released enter through airway mucosa and stimulate mucosa and stimulate muscle contraction and vascular leakage, i.e. early asthmatic response. Re-exposure to allergen causes the synthesis and release of a variety of cytokines, *viz.* interleukin-4 (IL_4) and IL_5, granulocyte – macrophase colony-stimulating factor (GM-CSF), tumour necrosis factor (TNF), and tissue growth factor (TGF) from T cell and mast cells. These cytokines attract and activate eosinophils and neutrophils, which re-create eosinophil cationic protein, proteases, and platelet activating factor (PAF). These mediators cause edema, mucous hyper secretion, bronchoconstriction, and increase in bronchial activity associated with late asthmatic response.

- An imbalance in airway smooth muscle tone involving the parasympathetic nerves (vagus), non-adrenergic non-cholinergic nerves and circulating noradrenalin that acts under normal circumstances to control airway diameter.

- Abnormal calcium flux across cell membranes, increasing smooth muscle contraction and must cell degranulation.

- Leaky tight junctions between bronchial epithelial cells allowing allergen access.

The above result in symptoms of wheezing, breathlessness and sometimes cough. In many people the asthmatic attack consists of two phases – an early-phase response and a late phase response (Figure 2.6).

Early – phase response

An early – phase response occurs on exposure to the eliciting stimulus. The response consists mainly of bronchospasm. Bronchodilators are effective in this phase.

Late – phase response

Several hours later, the late-phase response occurs. This consists of bronchospasm, vasodilatation, edema and mucus secretion caused by inflammatory mediators released from eosinophils, platelets and other cells, and neuropeptides released by axon reflexes. Anti-inflammatory drug action is necessary for the prevention and/or treatment of this phase (Woodruff and Fahy, 2002; Boyce, 2003; Rang and Dale, 2003; Wenzel, 2003; Puxeddu et al., 2005; Bradding et al., 2006; Dawson, 2007; Gibbs and Cripps, 2007; Hussain and Kumar, 2007; Galanter and Lazarus, 2008; Ritter et al., 2008; Seth and Seth, 2009; Pazdernik and Kerecsen, 2009).
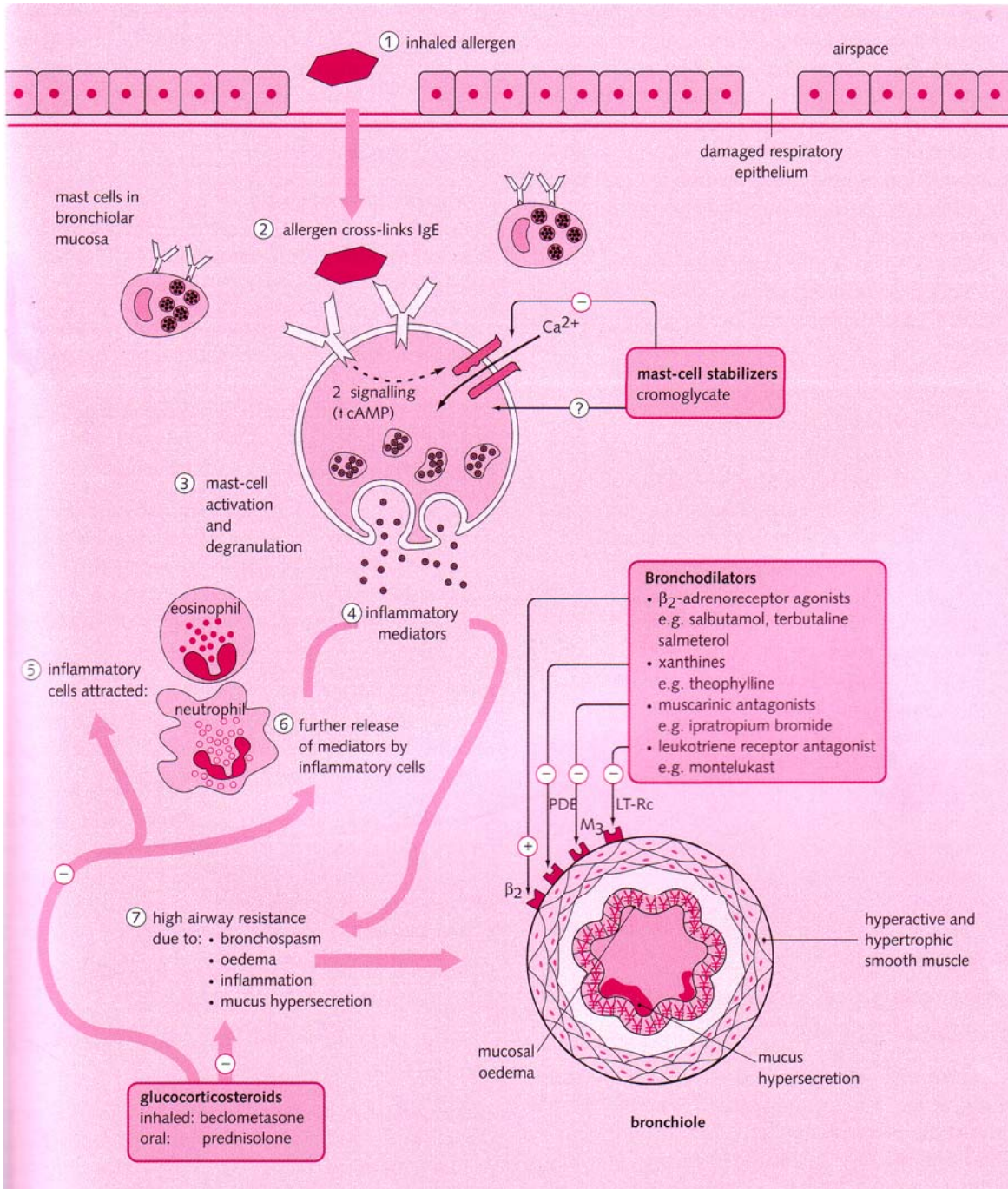
Figure 2.5: Pathogenesis and drug action in asthma (Dawson, 2007).
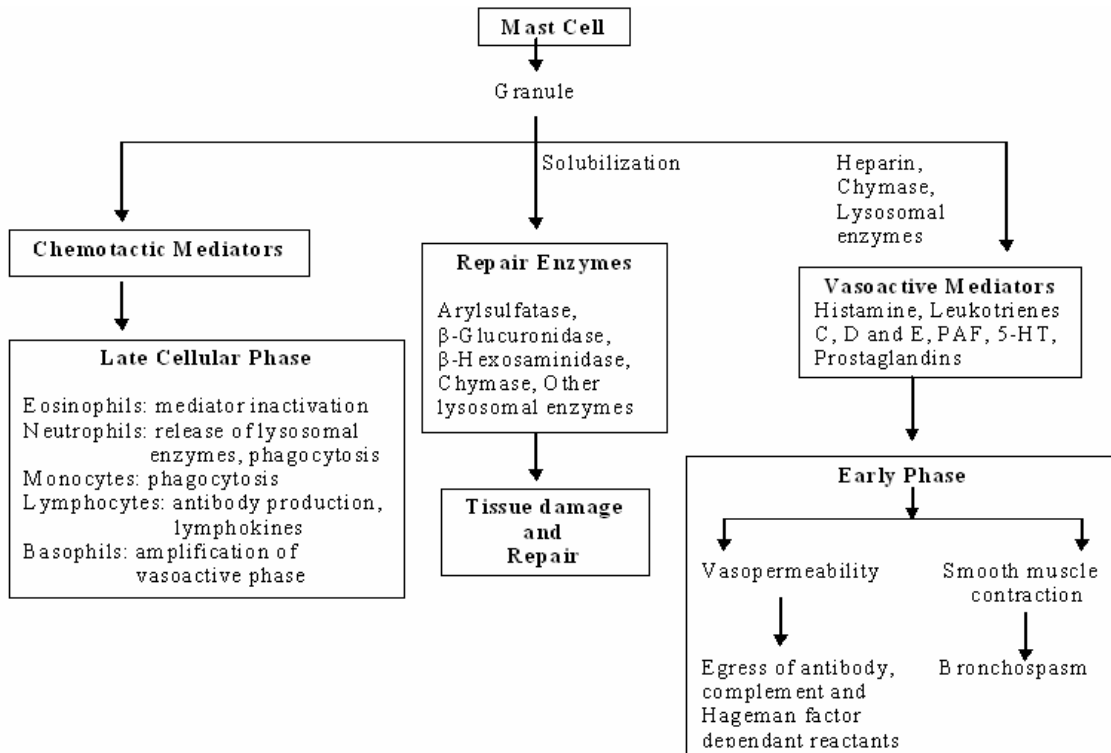
Figure 2.6: Early-phase and Late-phase responses in asthma (Hussain and Kumar, 2007).

Role of Mediators

Seven hours after allergen challenge during the late phase response, eosinophils increase in sputum samples of asthmatics, and this is associated with the appearance of eosinophil-basophil progenitors, and eosinophilia in peripheral blood. Progenitor CD 34+ cells bear the IL-5 receptor (IL-5R) with increased responsiveness to IL-5 suggesting they are primed toward the development of eosinophils. IL-5 generated in the inflamed lung tissues in asthma acts hormonally on the bone marrow to increase the production of eosinophils. The presence of eosinophil progenitors and eosinophil growth factors IL-3, IL-5 and GM-CSF within the asthmatic lung indicates the potential of local eosinophil differentiation. The migration of eosinophils into the airways is initiated by local chemo attractant factors. Many chemotactic substances act on eosinophils, including lipid mediators (LTB_4 and PAF), anaphylatoxins and chemokines (Macrophage inflammatory protein-1α MIP-1α, macrophage-derived chemokine MDC, monocyte chemotactic protein-2 MCP-2, MCP-3, MCP-4, IL-8 and IL-16). The increased number of eosinophils

in asthmatic patients is the combination of increased eosinophilopoiesis and rate of egress from the bone marrow. The eosinophil recruitment results from the complex mechanisms that involve interaction of adhesion molecules on the eosinophils with counter ligands on endothelial cells, extracellular matrix proteins and other tissue structures. Among these mechanisms are tethering and rolling on the endothelial surface, firm adhesion and transendothelial migration. The initial reversible tethering and rolling of eosinophils on the endothelium involve the formation of numerous weak reversible bonds between P-selectin and P-selectin glycoprotein ligand-1 and very late activation antigen-4 with vascular cell adhesion molecule-1. Preformed P-selectin is stored intracellularly in the Weibel-Palade bodies, from where it is mobilized to the endothelial surface by histamine and PAF. The tethering and rolling of eosinophils on the endothelium is followed by the activation step mediated by chemo attractants. Chemo attractants direct the migration of the tethered cells, involving crawling along the endothelium where chemokines are deposited in a solid phase, activation, diapedesis, and immigration into the tissue along a gradient of chemotactic signals. The activation results in up- regulation of β_2 - integrins and β_1- integrin. β_2 – integrins bind to intracellular adhesion molecule-1 on endothelium whereas β_1 – integrin binds to vascular cell adhesion molecule – 1 resulting in the firm arrest that is critical for transmigration. RANTES induces transient activation of very late activation antigen – 4 increasing their adhesiveness to vascular cell adhesion molecule – 1, whereas MCP-3 stimulation results in conformational change of Mac-1 leading to increased ICAM-1 adhesion. IL-4 and IL-13 induce expression of VCAM-1, whereas TNF-α and IL-1 induce expression of intracellular adhesion molecule-1 on the surface of endothelial cells. Binding of the chemokines (eotaxin, eotaxin 2, RANTES and MCP-3) to their G-protein-coupled receptors activates Ca^{2+} flux- induced polymerization and breakdown of actin leads to the formation and retraction of lamellipodia, which function like arms and legs of the migrating cells. Transendothelial migration also requires the function of matrix metalloprotease-9 that degrades type IV collagen, entactin, proteoglycans, and elastin, permitting eosinophil penetration through basement membrane. Eosinophils are richly endowed with matrix metalloprotease-9 in its precursor, with enzyme activation occurring when eosinophils adhere either to endothelial or epithelial cells. The extensive secretion of this enzyme with its capacity to

degrade epithelial adhesion molecules, epithelial basement membrane collagen and proteoglycans acts as a component of the airways remodelling. After migration through the endothelium, eosinophils come into contact with extracellular matrix proteins that are likely to play important roles in the regulation of eosinophil activation (Thomas and Warner, 1996; Filipović and Cekić, 2001; Foster et al., 2002; Berry, 2004, 2005).

Pathogenetic role of mediators in asthma

The recruitment of eosinophils into bronchial mucosa in which allergic inflammation occurs is a critical contributor to the late asthmatic reaction of congestion and mucus hyper secretion (Figure 2.7). When these cells arrive they degranulate and perpetuate underlying airway inflammation. Eosinophils are a rich source of cytotoxic proteins, lipid mediators, oxygen free radicals and cytokines. In asthmatic patients, after transendothelial migration, eosinophils transmigrate and adhere to bronchial epithelium where they degranulate and release substances (eosinophil cationic protein, major basic protein, eosinophil peroxidase and superoxide) which are toxic for epithelial cells. Damage and desquamation of cells, cilliostasis, and epithelial secretion manifest the toxicity to airway epithelium. Major basic protein is a selective, allosteric antagonist for M_2 muscarinic receptors (auto receptors). The loss of M_2 muscarinic receptor function results in increased airway tone due to increased release of acetylcholine and potentiation of vagally mediated reflex bronchoconstriction and bronchial hyperresponsivenss. Major basic protein also stimulates histamine release from basophils and mast cells. Lipid bodies (intracellular lipid rich domains) are induced to be developed in the activated eosinophils, and are the sites for enhanced synthesis of both lypoxygenase and cyclooxygenase-derived eicosanoids. Eosinophils are capable of producing significant quantities of cysteinyl leukotrienes (especially LTC-4). Cysteinyl leukotrienes contract airway smooth muscle (100-1000 fold more potent bronchoconstrictors than histamine), increase vascular permeability, stimulate mucus secretion, decrease mucocilliary clearance, stimulate eosinophil and neutrophil recruitment into the airways, stimulate smooth airway muscle proliferation and cause neuronal dysfunction. Eosinophils have the potential to synthetize and release a number of cytokines and chemokines. Cytokines produced by eosinophils include the autocrine-eosinophil active growth factors (IL-3, IL-

5, GM-CSF), immunoregulatory cytokines (IL-2, IL-4, IL-1, TGF-β, IFN-γ), proinflammatory cytokines (IL-1, IL-6, TNF-α, IL-16) and chemokines (IL-8, MIP-1α, RANTES). Transforming growth factor-β (TGF-β) is an immunoregulatory factor with a direct effect on growth of some cell types (stimulation on fibroblast growth and inhibition of epithelial cell growth) and up regulation of the synthesis of ECM proteins, inflammatory mediators and cytokines, making it an important factor in the remodelling process (Thomas and Warner, 1996; Filipović and Cekić, 2001; Foster et al., 2002; Woodruff and Fahy, 2002; Boyce, 2003; Wenzel, 2003; Barry, 2004, 2005; Puxeddu et al., 2005; Bradding et al., 2006).
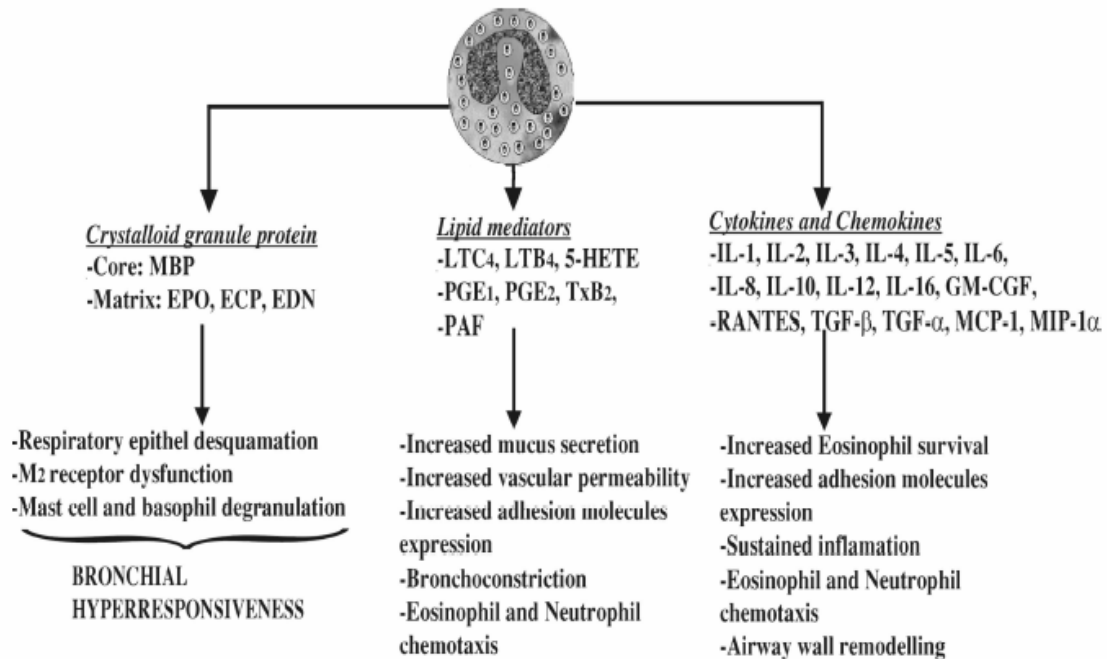
Figure 2.7: Role of eosinophils in the late asthmatic reaction (Puxeddu et al., 2005).

2.3 Opuntia species – A Phytochemical and Ethanopharmacological Review

Opuntia is a large genus of succulent shrubs, native of the new world, now widely grown in the warmer parts of the world, on account of their unique appearance and attractive flowers. They are commonly known as Prickly pears, because of their edible fruits. The prickly pears are said to have been accidentally introduced into India and other eastern countries by early European travelers, who used to carry these plants for use as vegetable to prevent scurvy during their long voyages. In India, as well as in other countries, they spread with rapidity and soon become noxious weeds, monopolizing large areas of forest and cultivated lands (The Wealth of India, 2001). The scientific classification of plant as follows (Robinson, 1974; Datta, 1988; Datta, 2003; Pinkava, 2002; Evans, 2005).

> Kingdom: Plantae
>
> Division: Magnoliophyta (Angiosperms)
>
> Class: Magnoliopsida (Dicotyledons)
>
> Subclass: Archichlamydeae
>
> Order: Caryophyllales (Cactales)
>
> Family: Cactaceae
>
> Subfamily: *Cereoideae, Opuntioideae, Pereskioideae*
>
> Tribe: *Opuntieae*
>
> Genus: *Opuntia*
>
> Species: *Opuntia elatior* Mill.

The genus *Opuntia* producing about 250 species and is mainly growing in arid and semiarid zones. It was found that cacti in India did not all belong to one species, *O. dillenii* was assumed, but to three or four species distributed over different regions in India. *O. dillenii* Haw. was found mainly in the southern parts of the India while *O. vulgaris* Mill (Syn *O. monocantha* Haw.) was distributed mainly in the northern parts; *O. elatior* Mill. was found in western India (The Wealth of India, 2001).

Species: *Opuntia elatior* Mill.

Synonyms: *O. nigricans* Haw.; *O. burgeriana*; Cactus tuna var. *elatior*; *C. elatior*

Vernacular names (Kirtikar and Basu, 1999):

Arabic:	Jhakawoon
Bengal:	Negphana, Phenimama
Burma:	Kalzaw, Shasounglitwa
Canarese:	Chappatigalli, Dabbugalli, Mullugalli, Nagadali, Papasakalli, Papasukattale, Sivaramakalli
Deccan:	Chappal, Chappalsend, Nagphansi
English:	Prickly pear, Slipper Thorn,
French:	Raquette,
Gujarati:	Chorhathalo, Zhorhatheylo
Hindi:	Haththathoira, Nagphana, Nagphani
Malayalam:	Nagamullu, Nagatali, Palakakkalli
Marathi:	Chapal, Nagaphana Samar
Porebunder:	Hathalo
Portuguese:	Palmatoria d'inferno
Sanskrit:	Bahudugdhika, Bahushala, Dondavrikshaka, Guda, Gula, Kandarohaka, Kandashakha, Krishnakhara, Kubshadruma, Mahavriksha, Nagadru, Nagaphana, Netrari, Nistrinshapatrika, Samantadugdha, Shakhakanta, Shihunda, Sihunds, Sinhatunda, Snuha, Snuhi, Snuka, Snusha, Sudha, Vajra, Vajradruma, Vajrakantaka, Vajri, Vidara, Visvasakara
Sinhalese:	Kodugaha
Tamil:	Kalli, Manjarnagadali, Mullukkalli, Nagadali, Nagakkalli, Palagaikkalli, Pattanadugalli, Sappattu, Sappattukkalli, Sapattumul
Telugu:	Nagadali, Nagajemudu, Nagamullu
Tulu:	Kalli
Urdu:	Nagaphani, Thuar
Uriya:	Nagophenia, Nagopheni, Poturiyasiju

2.3.1 Botanical Description

(i) *Opuntia* genus

A general characterization of each of the varieties is given followed by a particular descriptor (Ochoa, 2003).

➢ Plant descriptors

  ▪ Plant Size

    • Small (height < 1.5 m)

    • Medium (1.6 – 2.0 m)

    • Large (> 2.1 m)

  ▪ Plant Shape (Figure 2.8)

    • Flat

    • Round

    • Elongate (width < height)
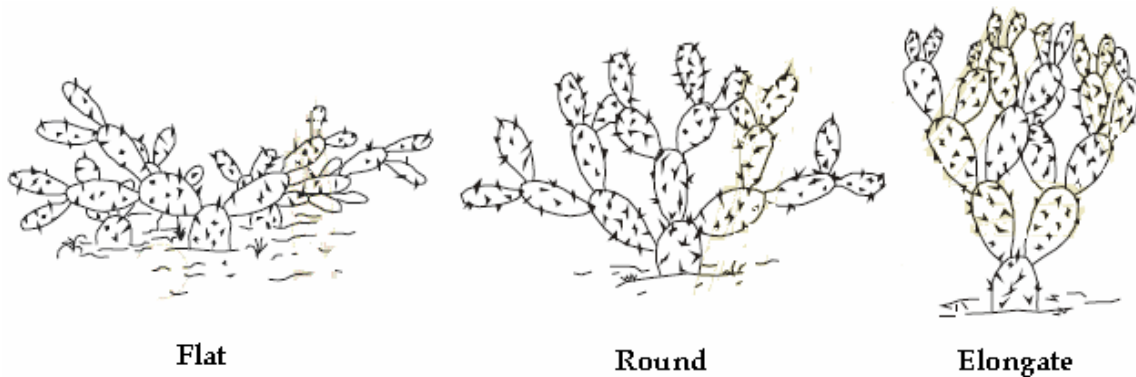
Figure 2.8: The plant shape of *Opuntia* spp.

➢ Habitus (Figure 2.9)

  ▪ Upright

  ▪ Medium

  ▪ Spreading

  ▪ Prostrate

  ▪ Shrubby

  ▪ Arborescent
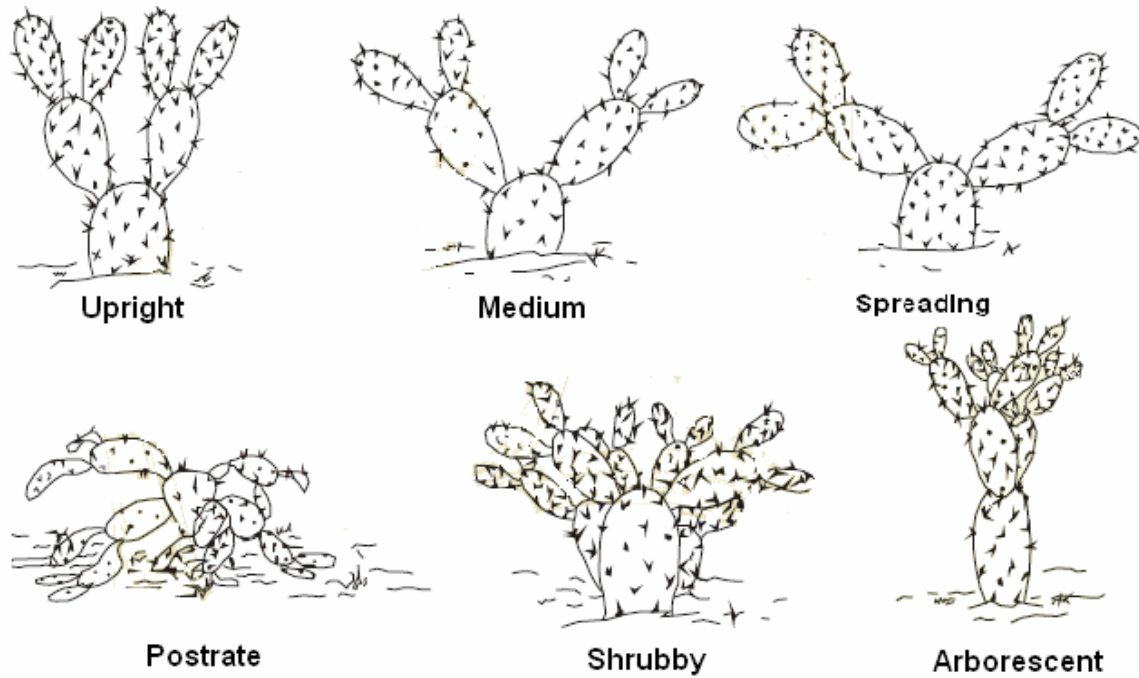
Figure 2.9: Habitus of *Opuntia* spp.

➢ Phylloclades descriptors

▪ Cladodes Shape (Figure 2.10)

• Ovate

• Round

• Elliptic
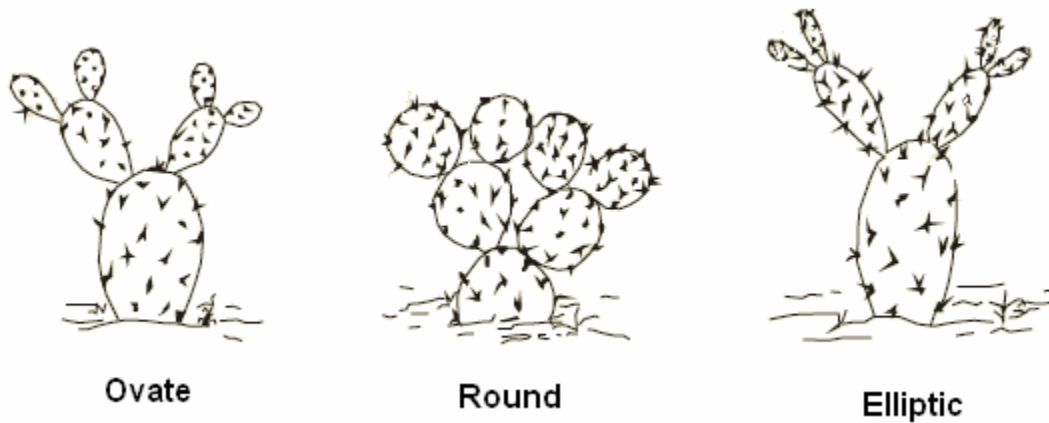
Figure 2.10: Phylloclades Shape.

- Spines

  - Absent

  - Few

  - Intermediate

  - Few

- Glochides: They are very little thorns that shoot up from the areoles of a dense fascicule having their front free end some what rised so that they act as a hook penetrating the skin easily thought it is hard to take them out.

  - Absent

  - Few

  - Intermediate

  - Many

➢ Fruit descriptor

- Shape (Figure 2.11)

  - Ovoid

  - Round

  - Elliptic

  - Oblong
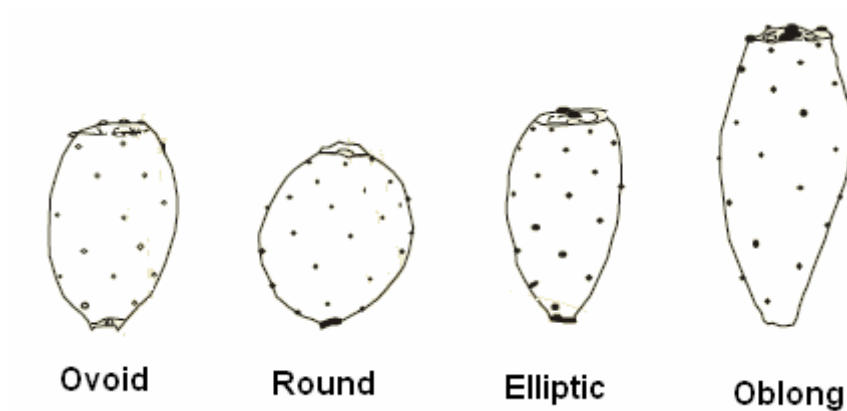
Figure 2.11: Shape of Fruit of *Opuntia* spp.

- Recepticular Scar Position (Figure 2.12): This characteristic is included because its importance in the spines removal process.

  - Elevated
  - Flattened
  - Sunken

Elevated   Flattened   Sunken

Figure 2.12: Recepticular Scar Position in Fruit of *Opuntia* spp.

- Fruit Color

  (a) Green   (b) White   (c) Light Yellow

  (d) Yellow   (e) Orange   (f) Pink

  (g) Red   (h) Purple

(ii)   *Opuntia elatior* Mill.

Subarborescent or shrubby, 3 meter high or more. Leaves 7.5 mm long, subulate, recurved, reddish at the tips. Joints variable in size, about 18-30 cm in height by 10-18 cm in width, obovate or elliptic, rather thin, not undulate, dull bluish green. Areoles bearing about 4-5 cm increasing up to 10 cm, rather slender straight prickles which are grey and opaque except when quite young, the largest 3-5 cm. long; glochidia inconspicuous, almost hidden amongst woolly hairs, rusty-brown. Flowers 5 cm. across, yellow or orange. Perianth rotate, the outer segments short, ovate, acute, red in the centre, yellow at the edges, the inner spathulate, acute. Stamens a little shorter than the perianth. Style exceeding the stamens; stigmas 6 in number. Berry pyriform, angular or more or less warty, bearing tufts of glochidia and occasionally a few prickles, reddish purple when ripe (Kirtikar and Basu, 1999).

2.3.2 Traditional Uses of *Opuntia* species

The plant is bitter, hot; laxative, stomachic, carminative, diuretic, antipyretic, alexiteric; cures biliousness, burning, leucoderma, "vata", urinary complaints, tumors, ascites, loss of consciousness, piles, inflammations, vesicular calculi, anaemia, ulcers, cures bronchitis of children, ophthalmia, liver complaints lumbago and enlargement of the spleen. The cladodes are very tasty, stomachic; cure inflammations, ascites, tumors, pains. They mashed up and applied as a poultice are said to allay heat and inflammation. The hot cladode applied to boils hastens suppuration; it made into a pulp is applied to the eyes in cases of ophthalmia. In South Africa and in Australia a decoction of the stem has been used as a diabetes remedy. A wineglassful of a strong decoction, to which sodium bicarbonate is often added, is taken thrice daily. It must be freshly prepared each day. It is reported from Australia to relieve the symptoms and to lower the blood sugar level in diabetes. A second method of preparation is to cover the minced stem with sodium bicarbonate over night. A black treacly liquid exudes, which is used as a diabetes remedy. The flowers cure bronchitis and asthma. The fruit is considered a refrigerant, and is said to be useful in gonorrhea. The baked fruit is said to be given in whooping cough and syrup of the fruit is said to increase the secretion of bile and control spasmodic cough and expectoration (Kirtikar and Basu, 1999; The Wealth of India, 2001). In addition to food, Indian fig is used to treat whooping cough, diabetes, prostate problems, rheumatism, nosebleed, and in dentistry in central Mexico (Duke and Vasquez, 1994). Sicilians use the fruits as Mexicans do, boiling the juice into syrup and also producing a jam. A tea is made from the flowers and drunk for kidney problems. Dried flowers are also ground into a paste and applied to the skin for measles (Galt and Galt, 1978). The Sicilians do not eat the stem joints, however, which Mexicans call nopales and nopalitos. Instead, stem joints are fed to livestock on occasion because of their high water content (Barbera et al., 1992). Many species of cactus are found growing either as wild plants in arid and semiarid regions of India or an ornamental plant in urban homes and gardens. Generally, these species are used as live fences to protect agricultural fields from human and animal encroachments with few exceptions; there has been no attempt to cultivate this plant as a horticultural or fodder crop in India. In countries such as Mexico, USA, Spain, Italy and northern Africa, where the crop is commonly known, it already forms an integral part of

the people's dietary requirement. In addition to the excellent quality and favor of the fresh fruit, the young phylloclades serve both as a vegetable and salad dish and the immature fruit is used to make mock gherkins (Gurbachan singh, 2003). Although traditionally appreciated for its pharmacological properties by the Native Americans, cactus pear is still hardly recognized because of insufficient scientific information (Feugang et al., 2006).

2.3.3 Phytochemical Compositions

The *Opuntia* cladodes and fruits serve as a source of varied number of phytoconstituents. The composition varies depending on the edaphic factors at the cultivation site, climate and the age of the plant (Retamal et al., 1987; Rodriguez-Felix & Cantwell, 1988; Batista et al., 2003).

2.3.3.1 Phylloclades

The weight and length of harvested cladodes may vary depending on the species, generally from 40–100 gm and 11–20 cm respectively (Cantwell et al., 1992, 1995; Nerd et al., 1997). The respective chemical constituents vary among species and should not be taken as absolute values. A wide class of compounds like minerals, sugars, organic acids, amino acids, lipids, terpenes, vitamins, carotenoids, chlorophyllus and phenolic constituents are observed (Table 2.2).

Table 2.2: Total phytochemical constituents of *Opuntia spp.* Cladodes.*

Constituents	Dry Weight Basis (g/100 g)	Fresh Weight Basis (g/100 g)
Water	NE	88 – 95
Minerals	0.1 – 5.6	NE
Vitamins	NE	0.00014 – 0.022
Protein	4 -10	0.5 – 1
Sugars	64 – 71	3 – 7
Hydrocolloids	18	1 -2
Organic acids	NE	35 – 985
Lipids	1 – 4	0.2
Polyphenols	NE	0.008 – 0.009
Ash	19 – 23	1- 2

*Modify form (Stintzing & Carle, 2005; Feugang et al., 2006); NE: Not Estimated

Minerals, Vitamins and Amino acids

Opuntia cladodes are rich in potassium followed by calcium and magnesium whereas other elements are in typical range (Munoz de Chavez et al., 1995; Batista et al., 2003; McConn & Nakata, 2004; Ben Salem et al., 2005) also a good source of vitamin C while niacine, riboflavine, thiamine and β – carotene are investigated (Rodriguez-Felix & Cantwell, 1988; Pimienta-Barrios, 1993; Guevara et al., 2001). Teles et al. (1997) reported the crude protein to be reached upto 11 g/100g on a fresh or 0.5 g/100g on a dry weight basis respectively and 77 – 112 mg/g dry weight was found by Ratamal et al. (1987). Glutamine was reported in greater amount followed by glutamic acid and proline. Table 2.3 shows their minerals, vitamins and amino acids content in *Opuntia spp.* cladodes.

Table 2.3: Minerals, Vitamins and Amino acids content in *Opuntia spp.* Cladodes.*

Components	mg/100 g
Minerals	
Calcium	18 – 57
Copper	0.8 – 0.9
Iron	5.9 – 6.6
Magnesium	11 – 17
Manganese	6.2 – 10.3
Potassium	50 – 55
Sodium	2 – 10
Zinc	2.2 – 2.7
Vitamins	
Vitamin C	7 – 22
Niacine	0.46
Riboflavine	0.60
Thiamine	0.14
β – Carotene	0.011 – 0.053
Amino acids	
Alanine	0.6 – 7.7
Arginine	2.4 – 5.5
Asparagine	1.5 – 4.0
Asparaginic acid	2.1 – 10.6
Cysteine	0.8 – 1.0
Glutamic acid	2.6 – 13
Glutamine	15.2 – 18.2
Glycine	0.5 – 4.8
Histidine	2 – 2.3
Isoleucine	1.9 – 5.2
Leucine	1.3 – 8.3
Lycine	2.5 – 5.9

Methionine	1.4 – 2.1
Phenylalanine	1.7 – 5.1
Proline	6.5 – 8.7
Serine	3.2 – 4.3
Theonine	2 – 4.3
Tryptophane	0.5 – 1
Tyrosine	0.7 – 4.1
Valine	3.7 – 7.0

*According to (Tales et al., 1997, 2005; Lee et al., 1999, 2005; Wahren, 2002; Bruckner & Westhauser, 2003; Stintzing & Carle, 2005)

Sugars, Hydrocolloids & Organic acids

Munoz de Chavez et al. (1995) reported free sugar content (0.32 g/100g fresh weight) while Rodriguez-Felix & Cantwell (1988) reported the reducing sugar fraction (0.64 – 0.88 g/100g dry weight). According to Sepulved et al. (2007) average mucilage yield after drying was 1.48% based on fresh weight and 19.4% based on dry weight and the dried mucilage had moisture (5.6%); protein (7.3%); ash (37.3%); nitrogen (1.14%); calcium (9.86%) and potassium (1.55%). According to Nobel et al. (1992), the average sugar composition of mucilage from *O. ficus indica* cladodes was arabinose (42%), xylose (22%), galactose (21%), galacturonic acid (8%) and rhamnose (7%).

The starch content, also addressed as glucan, from *O. ficus indica* cladodes fluctuated with seasons and reached mean value of 85 – 171 mg/g dry weight. The hydrocolloids comprised up to 36% of the cladode volume and water storage was reached upto 50% of their total weight due to their high swelling capacity (Sutton et al., 1981; Retamal et al., 1987;). Ben Thlija (2002) and Malainine et al. (2003) reported cellulose (11–21.6%), hemicellulose (8%) and lignin (3.6–3.9%) in the *Opuntia spp.* cladodes. The occurrence of pectins and comparison in various eight *Opuntia spp.* from Mexico is shown in table 2.4. The yield of soluble pectin in these samples was within a wide range of 0.13% to 2.64% in wet basis and 1.00% to 23.87% in dry-weight basis (Goycoolea & Cardenas, 2003).

Table 2.4: Pectin Content in *Opuntia spp.* phylloclades.

Species	Total Pectin (%)		Protopectin (%)		Soluble Pectin (%)	
	Wet Weight	Dry Weight	Wet Weight	Dry Weight	Wet Weight	Dry Weight
O. ficus-indica var I	1.91	13.84	0.097	3.56	1.418	10.28
O. ficus-indica var II	1.10	8.39	0.622	4.74	0.478	3.65
O. spp. (Blanca I)	0.95	7.6	0.448	3.58	0.482	4.02
O. spp. (Blanca II)	0.84	7.05	0.721	6.05	0.129	1.00
O. amylacea	1.40	9.58	0.685	4.69	0.715	4.89
O. megacantha	0.80	5.06	0.586	3.43	0.279	1.63
O. steptracantha	0.97	6.59	0.605	4.38	0.365	2.21
O. robusta	3.30	26.61	0.653	5.26	2.64	23.87

The organic acids content of *Opuntia* cladodes have been reviewed and found that malic acid was in greater amount followed by citric acid and other acids. Changes in tritratable acidity of 10 variants of "nopalito" with commercial value in response to time of the day of harvest were evaluated and differences in acidity among the nopalito variants harvested at 6:00 h (between 0.28 and 0.76%) and at 13:00 h (between 0.21 and 0.36%) were reported by Joel Corrales-Garia et al. (2004). The sugars, hydrocolloids and organic acids content are summarized in table 2.5.

Table 2.5: Sugars, Hydrocolloids and Organic acids content in *Opuntia spp.* phylloclades.*

Components	g/100 g
Sugars and Hydrocolloids	
Total Sugars	10.41
Polysaccharide	8.49
Cellulose	11 – 21.6
Hemicellulose	8
Lignin	3.6 – 3.9

Disaccharide	1.55 – 1.66
Monosaccharide	0.26 – 0.32
Arabinose	15 – 42
Xylose	9.1 – 22
Galactose	11 – 21
Galacturonic acid	8 – 46.3
Rhmanose	7 – 53.7
Mannose	1.5 – 1.9
Glucose	1.5 – 1.9
Organic acids	
Oxalic acids	35
Malic acid	985
Citric acid	178
Malonic acid	36
Succinic acid	Trace
Tartaric acid	Trace
Phorbic acid	Not quantified
Poscidic acid	Not quantified
Eucomic acid	Not quantified

*According to (Talese et al., 1984, 1994, 1994a; Nordal et al., 1965; Jianqin et al. 2002)

Lipids

Salt et al. (1987) reported the presence of cholesterol (4.4–5.0%), 24-ζ-methylcholesterol (8.0–8.8%) and sitosterol (86.7–87.0%) in *O. humifusa* and *O. comonduensis*, while Munoz de Chavez et al. (1995) reported high content of ω-3-fatty acids in the lipid fraction. Jianqin Jiang et al. (2002 & 2006) identified methyl-oleate (ω-9) and methyl-linoleate (ω-6) from *O. vulgaris* cladodes and two novel C_{29}-5β-sterols opuntisterol and opuntisteroside (Fig. 2.13) together with nine known compounds β-sitosterol, taraxerol, friedelin, methyl linoleate, 7-oxositosterol, 6β-hydroxystigmast-4-ene-3-one, daucosterol, methyleucomate and eucomic acid from *Opuntia dillenii* cladodes.
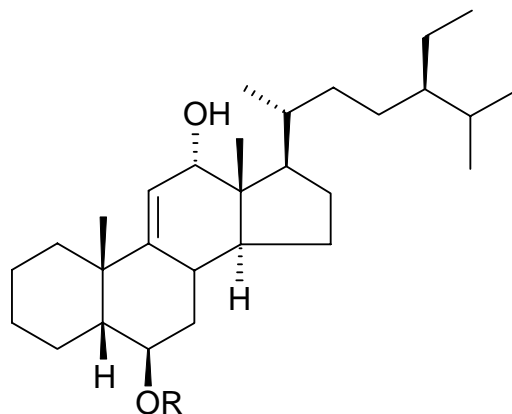
Opuntisterol   R = H

Opuntisteroside   R = ß - D - glucopyranosyl-

Figure 2.13: Structures of Opuntisterol & Opuntisteroside.

Polyphenols

The total phenolic content in *Opuntia spp.* cladodes was reported to be 8 – 9 mg/100g fresh weight (Rodriguez-Felix, 2002). Scientists reported various substituted Polyphenols, aromadendrin, kaempferol, taxifolin, quercetin, isorhmnetin, myricetin, vitexin, orientin, rutin and pyrone derivatives, 4-ethoxy-6-hydroxymethyl-α-pyrone, opuntiol and opuntioside from cladodes of *Opuntia spp.* (Fig. 2.14) (Gangulay et al., 1965; Telang, 1973; Richardson, 1978; Teramura, 1983; Gupta et al., 2002; Qiu et al., 2002, 2003; Eun Ha Lee et al., 2003).

4-Ethoxy-6-hydroxymethyl-alpha-pyrone

Opuntiol

Opuntioside

Aromadendrin

Taxifolin

Isorhamnetin

Vitexin

Kaempferol

Quercetin

Orientin
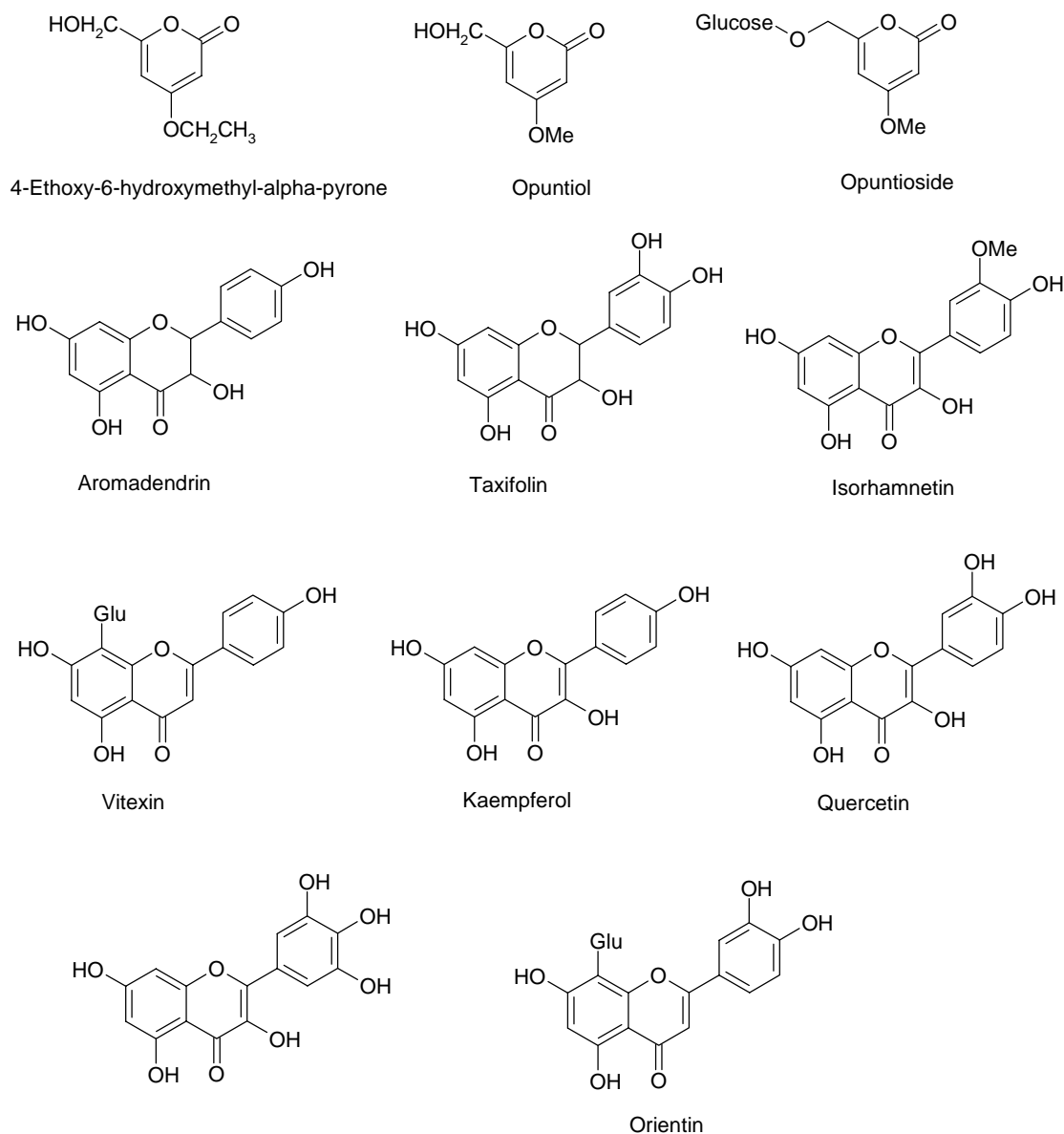
Figure 2.14: Phenolic compounds from cladodes of *Opuntia spp.*

2.3.3.2 Fruit

The cactus pear fruit is an oval, elongated berry with a thick pericarp and a juicy pulp and many hard seeds. The large variability in percentage of chemical composition depends on cultivar, cultural practices, fecundated and aborted seed number, fruit load, lighting period, elimate and harvesting season. The ripe fruits of *Opuntia spp.* are 30 – 220 g in weight contain pulp (43–67%), seeds (2–10%) and peel (33–55%). The pH range of the pulp is 5.3 – 7.1. The fairly high sugar content and low acidity of the fruit make it very

sweet and delicious (Piga, 2004; Moßhammer et al., 2006). The prickly pear may be divided into three fractions: peel, pulp and seed contain chief chemical constituents as summarized in table 2.6.

Table 2.6: Chief chemical constituents in fruits of *Opuntia spp.**

Parameters	Peel	Pulp	Seed
% of fresh Weight	33 – 55	43 – 67	2 – 10
Color	green, orange, red, purple	white, yellow – orange, red, purple	Not Available
Mineral	Potassium & Calcium	Potassium, Calcium & Magnesium	Potassium & Calcium
Vitamin	Vitamin E (in oil)	Vitamin C	Not Available
Amino acid	Not Available	Proline & Taurine	Not Available
Sugar	Glucose	Glucose & Fructose	Not Available
Hydrocolloids	Cellulose & Pectin	Pectin, Complext mixture of rhamnogalacturonan and at least 50% nonpectic substances	Cellulose, Arabinans, Rhamnogalacturonans
Organic acids	Not Available	Citric acid	Not Available
Lipid	γ – linolenic acid & α – linolenic acid	Linoleic acid, Palmitic acid,	Linoleic acid, Palmitic acid, Oleic acid
Sterols	β – sitosterol, Campesterol	β – sitosterol, Campesterol	β – sitosterol, Campesterol
Phenolic	Not Available	Quercetin, Kaempferol, Isorhamnetin	Not Available
Pigments	Betacyanin, Betaxanthins		Not Available

*According to (Moßhammer et al., 2006; Kossori et al., 1998)

2.3.3.2.1 Peel

The peel is more acidic compared to pulp having pH range of 5.4 – 5.8 (Moßhammer et al., 2006). The peel of *Opuntia spp.* fruit contains ash (11.5%), fat & wax (11%), Lignin (2.4%), Protein (8.6%), mucilage (4.1%), polysaccharides (35%) and cellulose (27%) on dry weight basis (Habibi et al., 2004).

Minerals, Vitamins and Amino acids

Kossori et al. (1998) reported high amount of calcium, potassium, magnesium and manganese from skin of prickly pear fruit of *Opuntia ficus indica* (Table 2.7). Vitamin E level was extremely high in the peel lipids and α-tocopherol constituted *ca.* 80.5% of the total vitamin E (21.8 ± 1.98 g/kg), the rest being β-tocopherol (*ca.* 10.2%), γ-tocopherol (*ca.* 8.00%) and δ-tocopherol (*ca.* 1.20%). Also a substantial amount of vitamin K_1 (1.09 g/kg) was estimated in peel lipids of *Opuntia ficus-indica* fruits (Ramadan & Morsel, 2003a). Since about amino acids in peel of *Opuntia spp.* fruit is not known, future studies may provide more knowledge.

Table 2.7: Mineral composition of prickly pear fruit peel.*

Minerals	mg/100g, dry matter
Ca	2090
Mg	322
Na	< 0.85
K	3430
P	0.064
Fe	8.31
Cu	< 0.85
Zn	1.70
Mn	72.9
Mb	< 0.34

*Adopted form (Kossori et al., 1998)

Sugars and Hydrocolloids

The peel of fruit contains sugar constituents, polysaccharides and pectin with high and medium degree of esterification of galacturonic acid residue (Moßhammer et al., 2006). Habibi et al. (2005) reported isolation and structural characterization of protopectin from the skin of *Opuntia ficus indica* prickly pear fruits. Dilute HCl extraction yielded series of soluble pectic polysaccharides which were de-esterified and separated into five fractions by anion exchange chromatography. Neutral fraction consisted of linear β-(1→4)-galactan and acid fraction consisted about 40 – 62% of galacturonic acid. Habibi et al. (2004) reported the cold water extract from the skin of *Opuntia ficus indica* fruits consisted of a polysaccharide composed of galactose and arabinose residue in the ratio 6.3:3.3 with traces of rhamnose, xylose and glucose but no uronic acid. Habibi et al. (2004a) extracted pectic polysaccharides from water and ethylene diamine tetra acetate (EDTA) solution and found 0.48 mol/mol and 0.36 mol/mol galacturonic acid residue in water and EDTA solution extracts respectively. Kossori et al. (1998) reported saccharose (2.36%), Glucose (21%), Fructose (2.89), hemicellulose (20.8 ±0.55%), cellusoe (71.4±1.99%), pectin (7.71 ± 1.45%) and lignin (0.06 ± 0.01%) from skin of prickly pear fruit (*Opuntia ficus indica*).

Lipids and sterols

The peel contained about 36.8 g/kg of total lipid on dry weight basis with presence of linoleic acid, palmitic acid, oleic acid, β-sitosterol and campesterol along with high amount of vitamin E (17.6 – 21.8 g/kg) in lipids extracted from *Opuntia ficus indica* (L.) Mill. fruit peel (Hassanien and Morsel, 2003; Ramdan & Morsel, 2003, 2003a) (Table 2.8). The peels of *Opuntia* fruit were rich in β – sitosterol followed by total vitamin E and campesterol.

Table 2.8: Lipids and sterols from peel of *Opuntia spp.* fruit.*

Compounds	g/kg of total lipids
Ergosterol	0.68 ± 0.22
Campestorl	8.76 ± 2.31
Sigmasterol	2.12 ± 0.42
Lanosterol	1.66 ± 0.32
β-Sitosterol	21.1 ± 2.55
Δ^5-Avenasterol	2.71 ± 0.33
Δ^7-Avenasterol	Not detected
Total Sterol content	37.0 ± 2.55
α – Tacopherol	17.6 ± 1.55
β – Tacopherol	2.22 ± 0.45
γ – Tacopherol	1.74 ± 0.31
δ – Tacopherol	0.26 ± 0.12
Total Vit E	21.8 ± 1.98
β – Carotene	2.54 ± 0.46
Vitamine K_1	1.09 ± 0.32

*Adopted form (Ramadan & Morsel, 2003a)

Polyphenol & Pigments

The peel of *Opuntia spp.* fruit may have orange, red and purple colored may be due to betacyanins and betaxanthins while green due to chlorophylls and carotenoids. Since little is known about polyphenols and pigments of the peel future studies may put forward our knowledge.

2.3.3.2.2 Pulp

The pulp is the edible part of the fruit and is composed of water, sugar, betacyanins, betaxanthins, minerals, vitamins and amino acids. Cassano et al. (2007) studied the potentiality of a membrane-based process for the clarification and the concentration of the cactus pear fruit juice. The juice quality was analysed in terms of total antioxidant activity (TAA), ascorbic, citric and glutamic acid, betalains and viscosity in order to

evaluate the effects of the membrane processes on the quality and composition of the juice. In table 2.9 the evaluation of total soluble solids (TSS), TAA and ascorbic acid, citric acid, glutamic acid, betaxanthins and betacyanins in various samples of *Opuntia ficus indica* (L.) Mill. fruit juice by ultrafiltration or osmotic distillation. Moßhammer et al. (2005) studied visual appearance, pigment stability and betalain content of fruit juice of *Opuntia ficus indica* (L.) Mill at pH values ranging from 3 to 7. Moßhammer et al. (2006) developed a process for the production of both juice concentrates and powders from *Opuntia ficus indica* fruit at laboratory and pilot plant-scale respectively and cross flow microfiltration and freeze drying processes reported due to thermolabile betalains for juice concentration and preservation.

Table 2.9: Evaluation of various samples of *Opuntia ficus indica* (L.) Mill. fruit juice obtained by ultrafiltration or osmotic distillation.

Parameters	Contents
TSS (ºBrix)	13.0 to 58.0
TAA (mM Trolox)	4.4 to 5.0
Ascorbic acid (mg/L)	30.0 to 43.0
Citric acid (mg/L)	365.0 to 427.4
Glutammic acid (g/L)	1.95 to 2.10
Betaxanthins (mg/L)	52.5 to 61.6
Betacyanins (mg/L)	11.0 to 19.9

Minerals, Vitamins & Amino acids

The mineral composition is characterized by high amounts of potassium, calcium and magnesium while other minerals are in the normal range of fruits (Table 2.10) (Dominguez-Lopez, 1995; Kossori et al., 1998; Stintzing et al., 2001; Piga, 2004; Feugang et al., 2006).

Table 2.10: The Mineral composition of cactus pear pulp.

Minerals	mg/100gm
Potassium (K)	90 – 217
Calcium (Ca)	12.8 – 59
Magnesium (Mg)	16.1 – 98.4
Phosphorus (P as PO_4)	15 – 32.8
Sodium (Na)	0.6 – 1.1
Iron (Fe)	0.4 – 1.5

Diaz Medina et al. (2007) reported mineral compositions in fruits belonging to two species of prickly pear *Opuntia ficus indica* and *Opuntia dillenii*, differentiating green and orange colour of pulp in *O. ficus indica* from Tenerife Island (Table 2.11).

Table 2.11: Mineral composition from *O. dillenii* and *O. ficus indica*.*

Minerals	*O. dillenii*	*O. ficus indica*		
	Total (mg/100g; Mean ± SD)	Total (mg/100g; Mean ± SD)	Green pulp (mg/100g; Mean ± SD)	Orange pulp (mg/100g; Mean ± SD)
K	90.8 ± 25.1	158.3 ± 32.8	159 ± 30.5	156 ± 36.2
Ca	53.5 ± 18.7	26.3 ± 7.6	24.4 ± 7.3	28.8 ± 7.5
Mg	45.4 ± 10.2	25.1 ± 5.7	26.7 ± 5.5	23.1 ± 5.4
Na	15.3 ± 16.2	0.625 ± 0.822	0.524 ± 0.709	0.758 ± 0.949
Fe	0.153 ± 0.031	0.198 ± 0.057	0.2 ± 0.05	0.195 ± 0.067
Cu	0.0334 ± 0.005	0.0389 ± 0.009	0.0384 ± 0.001	0.0396 ± 0.008
Zn	0.129 ± 0.049	0.205 ± 0.005	0.0204 ± 0.053	0.0207 ± 0.049
Mn	0.509 ± 0.380	0.303 ± 0.158	0.301 ± 0.156	0.306 ± 0.165
Ni	0.002 ± 0.008	0.0285 ± 0.01	0.0298 ± 0.012	0.0268 ± 0.007
Cr	0.0144 ± 0.003	0.0109 ± 0.003	0.0115 ± 0.004	0.0102 ± 0.004

* Diaz Medina et al., 2007.

Cactus pear is a good source of ascorbic acid (1 – 81 mg/100 g fresh fruit) along with trace amounts of niacin, riboflavin, thiamine, carotenoids, vitamin E and K_1. Various free amino acids were found in the cactus pear with extraordinarily high level of proline and taurine (Table 2.12) (Stintzing et al., 2001; Piga, 2004; Feugang et al., 2006).

Table 2.12: Amino acid contents in fruit pulp of *Opuntia spp.**

Amino acids	mg/100 g
Total Amino acids	257.24
Alanine	8.72 – 9.66
Arginine	3.05
Asparagine	4.16
Asparaginic acid	Not Valid
Glutamin acid	6.61 – 8.3
Glutamine	34.62 – 57.46
Glycine	1.13
Histidine	4.52
Isoleucine	3.12
Leucine	2.06
Lysine	1.74 – 5.33
Methionine	5.52 – 7.69
Phenylalanine	2.33
Serine	17.45 – 21.75
Threonine	1.33
Tyrosine	1.23
Tryptophane	1.26
Valine	3.94
Alpha-aminobutyric acid	0.11
Carnosine	0.59
Citrulline	1.63
Proline	126.52 – 176.87
Taurine	43.43 – 57.21

* Stintzing et al., 2001; Piga, 2004; Feugang et al., 2006

Sugars, Hydrocolloids & Organic acids

Total sugars range from 12 – 17 ºBrix and are mainly of the reducing type with glucose being the predominant sugar and fructose being the second sugar thus the fruit pulp is very sweet (Piga, 2003). Directly absorbable high glucose concentrations in cactus fruits represent an instantly available energy source for brain and nerve cells while fructose being sweeter may enhance the fruit's flavor (Feugang et al., 2006). Some authors have also reported the occurrence of galactose and maltose (Stintzing et al., 2001). The high sugar content of the pulp results in sugar:acid ratios within the range of 90:1 to 490:1 which is responsible for the bland taste and therefore far from a sensory pleasant ratio of 10 to 18 (Moßhmmer et al., 2006).

Extraction of peeled fruits of *Opuntia ficus indica* afforded with 3.8% yield mucilage, which contained 23.4% of galacturonic acid. Total hydrolysis of a mucilage and gas–liquid chromatographic analysis of the derived alditol acetates indicated the presence of arabinose, rhamnose, xylose and galactose in the molar ratio 1.0:1.7:2.5:4.1. Gel permeation chromatography on Sepharose CL-4B showed the polysaccharide to be composed of at least five fractions. Treatment with cetrimide allowed the separation of an insoluble fraction (44.3% yield) which contained 28.0% of uronic acid. This fraction contained xylose, rhamnose and galactose in the molar ratio 1.0:2.5:2.8. The soluble fraction in cetrimide (15.6% yields) contained uronic acid (16.0%) while arabinose and galactose in the molar ratio of 1:2.2. It is composed of two main subfractions as shown by gel permeation chromatography. These results indicated that the mucilage from fruits *O. ficus indica* is a complex mixture of polysaccharides less than 50% corresponding to a pectin-like polysaccharide (Betty, 2006).

Arabinose (33.1%), Galactose (20.3%), Glucose (1.0 %), Rhamnnose (6.9 %), Xylose (18.7 %) reported by Muller, (2001). Kossori et al. (1998) reported carbohydrates and fiber composition in the fruit pulp of *Opuntia ficus indica* (Table 2.13).

Table 2.13: Sugars (% of dry matter) and fiber (% of total fiber) composition in prickly pear fruit pulp.*

Sugars	%
Saccharose	0.22
Glucose	35
Fructose	29.6
Fibers	**% mean ± SD**
Hemicellulose	15.5 ± 0.45
Cellulose	14.2 ± 1.07
Pectin	70.3 ± 1.30
Lignin	0.01 ± 0.01

* Kossori et al., 1998.

The high pH values (5.6 – 6.5) and a low acidity (about 0.05% to 0.18% citric acid) of ripe fruits of cactus pears serves as a low acid food (pH > 4.5). Whereas citric acid (62 mg/100 g fruit weight) is the major organic acid in cactus pear followed by malic acid (23.3 mg/100 g), quinic (19.1 mg/100 g), shikimic (2.8 mg/100 g) and also oxalic acids were found while isocitric, fumaric, glycolic, and succinic acids were only found in traces. Additionally minor acids such as phorbic acid and piscidic acid have been detected in *Opuntia* leaves (Fig 2.15) (Stintzing et al., 2001; Moßhammer et al., 2006).
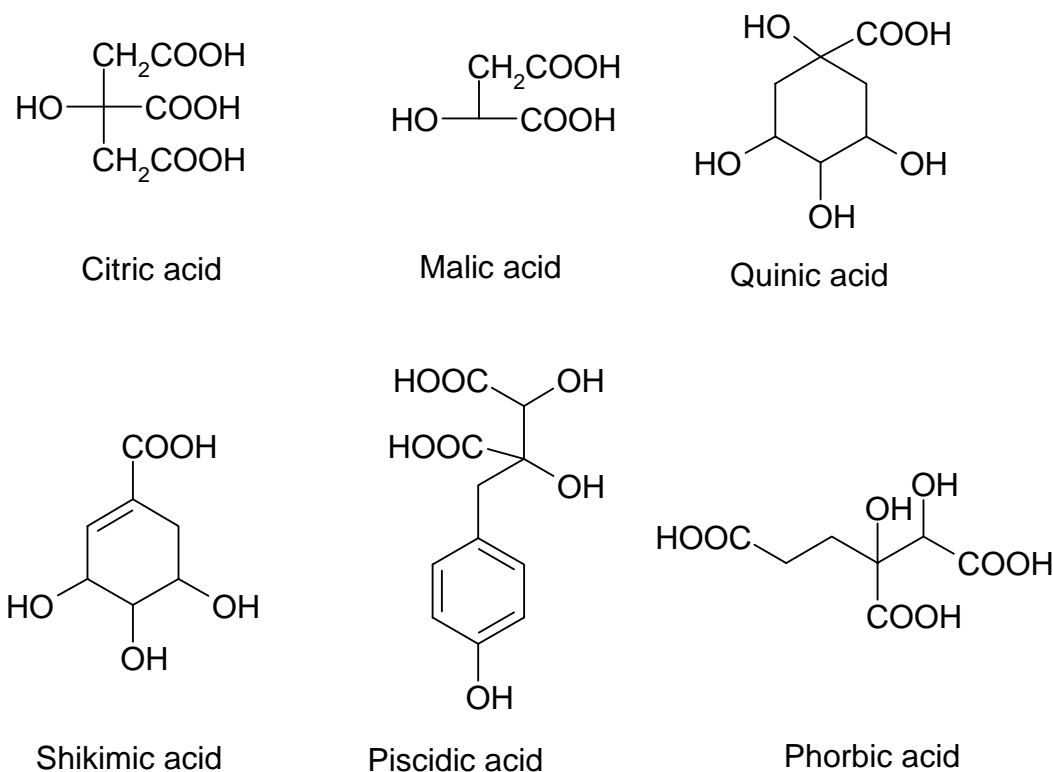
Figure 2.15: Chemical structures of organic acids in cactus pear fruit and phylloclades.

Lipids

It is well known that pulp of fruits generally contain very low levels of lipids ranging from 0.1 to 1.0%. In prickly pear pulp oil dominating fatty acid (linoleic acid) was reported along with palmitic acid and oleic acid also polyunsaturated fatty acids like γ – linolenic and α – linolenic acids were detected in good amounts. In pulp oil about 90% of the total sterol portion constituted by β – sitosterol followed by campeterol. Interestingly δ–tocopherol was the predominant vitamin E homologue followed by α-, β-, γ-tocopherols in far less amounts (Moßhammer et al., 2006). Seeds and pulp of cactus pear (*Opuntia ficus indica* L.) were compared in terms of fatty acids, lipid classes, sterols, fat-soluble vitamins and b-carotene. Total lipids (TL) in lyophilized seeds and pulp were 98.8 g/kg (dry weight) and 8.70 g/kg respectively. High amounts of neutral lipids were found (87.0% of TL) in seed oil while glycolipids and phospholipids occurred in high amount in pulp oil (52.9% of TL). In both oils linoleic acid was the dominating fatty acid followed by palmitic and oleic acids respectively. Trienes, γ-and α-linolenic acids were

estimated in higher amounts in pulp oil while α-linolenic acid was detected in fewer amounts in seed oil. The sterol marker, β-sitosterol, accounted 72% and 49% of the total sterol content in seed and pulp oils respectively. Vitamin E and β-carotene level was higher in the pulp oil than in the seed oil, whereas γ-tocopherol was the predominant component in seed oil and δ-tocopherol was the main constituent in pulp oil. Oils under investigation resembled each other in the level of vitamin K_1 (0.05% of TL) (Ramadan & Morsel, 2003). Information provided above is of importance for further chemical investigation of cactus pear oil and industrial utilization of the fruit as a raw material of oils and functional foods.

Polyphenols

Phenolics comprise a wide variety of compounds divided into several classes such as hydroxybenzoic acid, hydroxycinnamic acids, anthocyanins, proanthocyanidins, flavonols, flavones, flavanols, flavanones, isoflavones, stilbenes and lignans those occur in a great number of fruits and vegetables (Feugang et al., 2006). Su Feng Chang et al. (2008) reported total phenolics (91.5 ± 1.5) and flavonoids (29.2 ± 1.5) along with gallic acid (4 ± 0.6), catechin (22.7 ± 0.7) and epicatechin (10.9 ± 0.2) as mg/100 g fresh sample of *Opuntia dillenii* Haw fruits. The phenolic acid composition of the peel and pulp of the fruits of *Opuntia megacantha* (L.) Mill. were analyses and total phenolics, flavonoids and condensed tannin levels varied in their amounts (Ndhlala et al., 2007). In fruits belonging to two species of prickly pear *Opuntia ficus indica* and *Opuntia dillenii* contained 117 ± 10 and 45.2 ± 7.4 mg/100 g of total phenolics respectively (Diaz Media et al., 2007).

Conjugated flavonoids (quercetin, kaempferol and isorhamnetin), ascorbic acid and carotenoids were estimated from the fruit extracts of *O. ficus indica* (green-skinned), *O. lindheimeri* (purple-skinned), *O. streptacantha* (red-skinned) and *O. stricta var. stricta* (yellow-skinned). Quercetin was the most abundant in all varieties whereas kaempferol was found in green-skinned, purple-skinned and red-skinned varieties and isorhamnetin in green-skinned and purple-skinned varieties. Flavonols, total flavonoids, ascorbic acid and carotenoids content of four species are summarized in (Table 2.14) (Kuti, 2004).

Table 2.14: Flavonols, total flavonoids, ascorbic acid and total carotenoids content (μg/g fresh weight) in fruits of different *Opuntia spp.*

Opuntia spp.	Flavonol content			Total	Ascorbic	Total
	Quercetin	Kaempferol	Isorhamnetin	Flavonoids	acid	Carotenoids
O. ficus-indica	43.2 ± 2.5	2.2 ± 0.3	24.1 ± 1	69.5 ± 3.8	458	2.9
O. lindheimeri	90.5 ± 11.5	1.1 ± 0.4	1.9 ± 0.5	93.5 ± 12.4	121	6.7
O. streptacantha	51.0 ± 4.6	3.8 ± 0.5	ND	54.8 ± 5.1	815	14.6
O. stricta var. stricta	9.8 ± 3.0	ND	ND	9.8 ± 3.0	437	23.7

ND = Not Detectable

Eun Ha Lee et al. (2003) isolated and identified eight flavonoids namely kaempferol, quercetin, kaempferol 3-methyl ether, quercetin 3-methyl ether, narcissin, aromadendrin, toxifolin and eriodictyol by means of chemical and spectroscopic method for the first time from the fruits of *O. ficus indica var. saboten*. The flavonoids quercetin, (1)-dihydroquercetin and quercetin 3-methyl ether were isolated from the ethyl acetate fractions of the fruits and stems of *Opuntia ficus-indica var. saboten* and evaluated their protective effects against oxidative neuronal injuries induced in primary cultured rat cortical cells and their antioxidant activities by using three different cell-free bioassays (Jungsook Cho et al., 2003).

Pigments

The most common connotation with pigmented flower petals and fruits is the attraction of animals both for pollination and seed dispersal. Anthocyanins mask the chlorophyll containing organelles and thereby protect chloroplasts against high light intensities to prevent photo inhibition (Stintzing & Carle, 2004). Chalker-Scott (1999) suggested, three functions of anthocyanins in plants, namely as absorbers of harmful radiation, as transport vehicles for monosaccharides and as osmotic adjusters during periods of drought and low temperature. The anthocyanins are a subgroup within the flavonoids characterized by a C_6-C_3-C_6 skeleton. Different aglycones and anthocyanins with

structures and absorption maxima in acidified methanol are summarized in table 2.15 (Stintzing & Carle, 2004).

Table 2.15: Basic structure of anthocyanins and their absorption maxima.

Anthocyanin	R_3	$R_{3'}$	$R_{5'}$	λ_{max} (nm)
Pelargonidin	H	H	H	520
Cyanidin	H	OH	H	535
Delphinidin	H	OH	OH	546
Peonidin	H	OCH_3	H	532
Petunidin	H	OCH_3	OH	543
Malvidin	H	OCH_3	OCH_3	542
Pelargonidin-3-glycoside	Glucose	H	H	516
Cyanidin-3-glycoside	Glucose	OH	H	530
Delphinidin-3-glycoside	Glucose	OH	OH	543
Peonidin-3-glycoside	Glucose	OCH_3	H	536
Petunidin-3-glycoside	Glucose	OCH_3	OH	546
Malvidin-3-glycoside	Glucose	OCH_3	OCH_3	546

Betalains are of great taxonomic significance in higher plants. The presence of betalains in members of the order Caryophyllales has been an important criterion for their classification. The presence of betalains and anthocyanins is mutually exclusive in the angiosperms. Betalains are water soluble nitrogenous chromoalkaloids and can be divided into two major structural groups, (i) The red to red-violet betacyanin (Latin *Beta*, beet and Greek *kyanos*; blue color) and (ii) The yellow betaxanthins (Latin *Beta* and Greek *xanthos*; yellow color). Betalains may function as osmolytes to uphold

physiological processes, stabilize subcellular structures, reduce nitrogen toxicity and be an excellent radical scavenger. Structurally, betacyanins are characterized by a cyclo – Dopa structure with additional substitutions through varying glycosylation and acylation patterns at C_5 or C_6 whereas the betaxanthins are condensation products of betalamic acid and various amino compounds. Betacyanins can be further classified by their chemical structures into four types: betanin-type, amaranthin-type, gomphrenin-type and bougainvillein-type (Stintzing & Carle, 2004; Yi-Zhong Cai, 2005). Structures of betacyanins and betaxanthins found in the fruits of different *Opuntia spp.* are summarized in figure 2.16.

The biosynthetic steps involved in betalain biosynthesis are summarized in figure 2.17. While some 'early' and 'late' reactions are enzymatically catalysed, the intermediate steps (cyclizations, X–XIII; aldimine formation, XIV–XVIII) are assumed to proceed spontaneously, i.e. formation of cyclo-dopa via dopaquinone, betalamic acid via 4,5-seco-dopa, muscaflavin via 2,3-seco-dopa and the condensations of betalamic acid with cyclo-dopa (betanidin formation) or amino acids/amines (betaxanthin formation). Early reactions are catalysed by the bifunctional tyrosinase (EIA, EIB) and the dopa 4,5- or 2,3-dioxygenase (EII, EIII), and late reactions by glucosyl-(EIV, EV), hydroxycinnamoyl-(EVI) and malonyltransferases (EVII). In addition, there are two rare enzymatic steps (decarboxylation and methylation, EVIII, EIX) leading to dopamine-derived betalains (Strack et al., 2003).

Betacyanins

	R$_5$
Betanin	ß-Glucose
Phyllocactin	6'-O-(Malonyl)-ß-glucose

	R$_5$
Isobetanin	ß-Glucose

	R$_5$
Neobetanin	ß-Glucose

Betaxanthins

Betalamic acid

Compounds	R	R'
Indicaxanthin		Proline
Miraxanthin - II	H	Aspartic acid
Vulgaxanthin - I	H	Glutamine
Vulgaxanthin - II	H	Glutamic acid
Vulgaxanthin - IV	H	Leucine
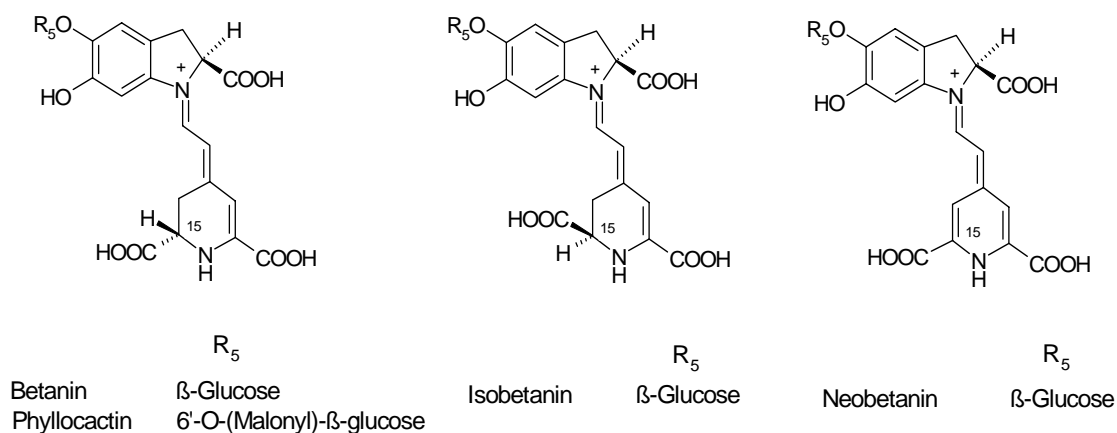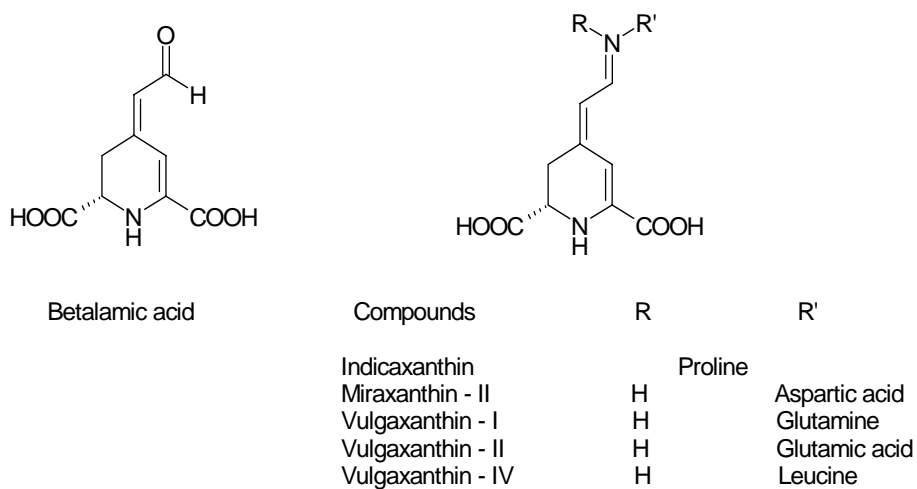
Figure 2.16: Structures of betacyanins and betaxanthins found in prickly pear.

64
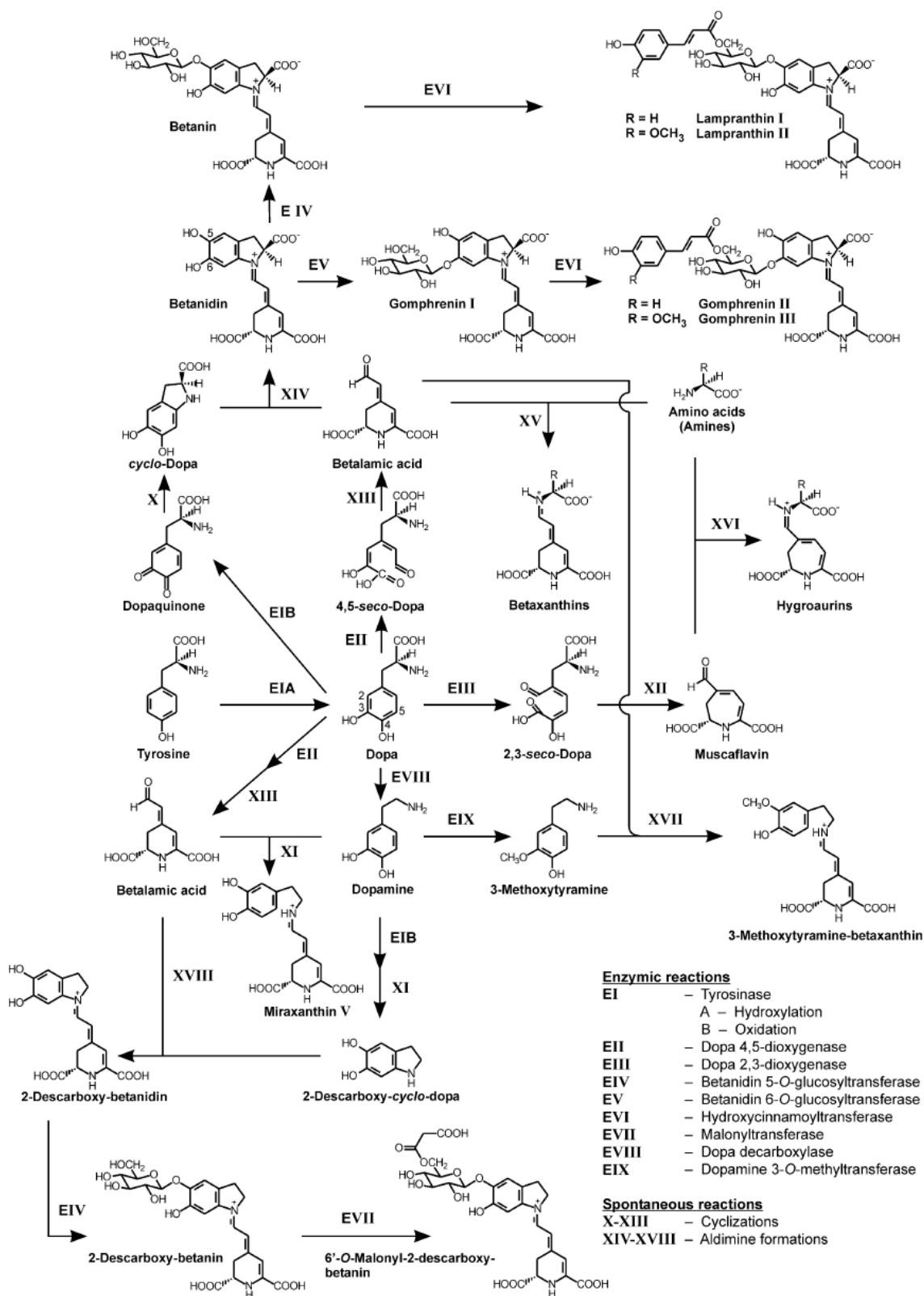
Figure 2.17: Biosynthetic scheme of betacyanin and betaxanthin formation.

Numerous analytical methods have been designed and developed for the qualitative and quantitative determination of betalains in fruits of *Opuntia spp.* and are reviewed as follows.

Qualitative Analysis

Chemical Tests: Harborne (2007) reported chemical tests for the identification of betacyanins. Red color of betacyanin vanishes upon heating with 2M HCl for 5 min at 100 ºC and color changes to yellow by adding 2M NaOH drop wise, indicate presence of betacyanins.

Spectrophotometric: Harborne (2007) reported visible spectrum of betacyanin in methanol-HCl give maximum absorbance in the range of 532 – 554 nm. Viloria-Matos et al. (2001) reported visible spectra of fruits of *Opuntia boldinghii* Br. et R., maximum absorbance at 537 nm at pH 6.1 which is similar to the earlier reported value of betacyanin (Bilyk, 1979, 1981; Delgado-Vargas et al., 2000). Farnandez-Lopez & Almela (2001) extracted pigments from the prickly pear fruits (*Opuntia ficus indica*) of reddish purple and yellow color, by homogenization of fruit flesh in methanol, with a ratio mass fruit (g) / solvent (ml) of 1:5 and two main pigments were obtained, which were identified as indicaxanthin (λ_{max} 484 nm) and betanin (λ_{max} 535 nm). The spectrophotometric analysis suggests that the external color of prickly pear fruits depends on the relative concentration of betacyanins (red pigments with maximum absorbance at around 535 nm) and betaxanthins (yellow pigments with maximum absorbance at around 480 nm) (Schliemann et al., 1996, 2000, 2001; Cai & Corke, 1999; Wybraniec et al., 2001; Fernandez-Lopez & Almela, 2001; Stintzing et al., 2003, 2005).

Chromatographic Method

Thin Layer Chromatographic method (TLC): Harborne (2007) reported chromatography in 1% aqueous HCl and *n*-butanol:acetic acid:water (BAW; 4:1:5) give high and very low R_f value respectively.

*High Performance Thin Layer Chromatographic method(HPTLC)***:** Viloria-Matos et al. (2001) isolated & identified betacyanin from fruits of *Opuntia boldinghii* Br. et R. by HPTLC using two solvent systems (System I: isopropanol:ethanol:water:acetic acid 55:20:20:5; System II: isopropanol:ethanol:water:acetic acid 30:35:30:5) in one dimension. Results showed a major red fraction with a maximum absorbance at 537 nm which is similar to the reported value for betacyanin.

*High Performance Liquid Chromatographic method***:** HPLC is an excellent means in the analysis of betalains. The most common support is C_{18}-derivatized silica providing adequate efficiency and retention of betacyanins as well as their sufficient resolution on conventional stationary phases. Because betacyanins exist in aqueous solution in different ionized forms at varying pH values, the use of typical acidic eluents with or without buffers is a useful factor governing their separation (Schliemann et al., 1996, 2000, 2001; Wybraniec et al., 2001, 2006).

Fernandez-Lopez & Almela, (2001) separated and identified betalain pigments from methanolic extract of two cultivars of prickly pear (*Opuntia ficus indica*) fruits using reversed-phase high performance liquid chromatography and photodiode array detector. The chromatographic separation program consisted of a 30 min linear gradient elution from solvent A (1% acetic acid in water) to 12 % solvent B (1% acetic acid in acetonitrile) with a flow of 1 ml/min. The chromatographic pattern of the methanolic extract showed two major peaks with a retention time of 16.2 min at 484 nm and 17.4 min at 535 nm, identified as indicaxanthin and betanin, respectively. Fernandez-Lopez et al. (2002) also analyzed presence of betalains using method proposed by Fernandez-Lopez & Almela, (2001) from the fruits of *Opuntia stricta, Opuntia undulata* and *Opuntia ficus-indica* and found HPLC patterns of betalains with retention time at 16.8 min (λ_{max} 484 nm), 19.6 min, and 22.8 min (λ_{max} 537 nm) assigned to indicaxanthin, betanin and isobetanin, respectively.

Stintzing et al. (2003) separated betalains from *Opuntia ficus-indica* cv. '*Rossa*' and cv. '*Gialla*' using aqueous 0.2% trifluoroacetic acid and 10% formic acid solutions at a ratio of 65/35 (v/v) as eluent A, and a mixture of 100% acetonitrile and 10% aqueous formic acid (80/20,v/v) as eluent B. After 15 min of isocratic elution with 100% A, a linear

gradient was followed from 0% B to 20% B in 60 min. Betaxanthins were monitored at 470 nm and betacyanins at 538 nm, respectively. Stintzing et al (2006) developed a process for the production of both juice concentrates and powders from *Opuntia ficus indica* fruits of the cultivar '*Gialla*' at laboratory and pilot plant – scale, respectively. Since betalains are regarded as thermolabile compounds, alternative processes for juice concentration and preservation, including cross-flow microfiltration and freeze drying, considered. HPLC – diode array detector (DAD) peak separation was achieved using mobile phase A (1% v/v formic acid in water) and B (Aqueous MeCN, 80:20 MeCN/H_2O v/v). Starting isocratically with 100% A for 2 min, a linear gradient was followed from 0 to 20% B in 60 min and then from 20% to 100% B in 5 min. Pigment retentions of the major betaxanthins and betacyanins were determined at 10.4 min (Histidine-betaxanthin), 16.3 min (Glutamine-betaxanthin), 29.2 min (GABA-betaxanthin), 29.9 min (Isoproline-betaxanthin), 31.2 min (Proline-betaxanthin) at 470 nm and 36.8 min (betanin) and 40.7 min (isobetanin) at 538 nm.

Wybraniec (2006, 2008) reported the effect of tetraalkylammonium salts on retention of betacyanins and decarboxylated betacyanins in ion-pair reversed-phase HPLC and investigated chromatographic acyl migration in betacyanin and their decarboxylated derivatives. Identification of betalains from the fruits of 10 Mexican prickly pear cultivars by HPLC-DAD analysis was performed by Yahia & Castellanos-Santiago (2008) using water (eluent A) and methanol (eluent B) mixture at a flow rate of 1 mL/min. Betalains were separated starting isocratically with 100% A in 10 min followed by a linear gradient from 0% B to 30% B in 30 min, and finally a linear gradient from 30% B to 100% B in 20 min, before re-equilibration to the starting conditions. Betaxanthins and betacyanins were monitored at 482 and 535 nm, respectively. Several solvent systems were used for betalain analysis; the best results were obtained in water/methanol system than other methods, acetic acid in water/acetic acid in acetonitrile or phosphoric acid solution buffer. Table 2.16 shows the qualitative data of betalains from fruits of *Opuntia spp.*

Table 2.16: Qualitative analysis of betalains by HPLC.

Solvent system	Chromatographic Separation Tech.	R_t (min)	λ_{max} (nm)	Compound	Reference:
A (1% acetic acid in H$_2$O) B (1% acetic acid in acetonitrile)	30 min linear gradient elution from solvent A to 12 % solvent B with a flow of 1 mL/min	16.2	484	Indicaxanthin	Fernandez-Lopez & Almela, (2001)
		17.4	535	Betanin	
		16.8	484	Indicaxanthin	Fernandez-Lopez et al., (2002)
		19.6	537	Betanin	
		22.8	537	Isobetanin	
A (1% v/v of formic acid in H$_2$O) B (Aq. MeCN, 80:20 MeCN/H$_2$O, v/v)	Isocratically with 100% A for 2 min, a linear gradient was followed from 0 to 20% B in 60 min and then from 20 to 100% B in 5 min.	10.4	470	Histidine-betaxanthin	Stintzing et al., (2006)
		16.3	470	Glutamine-betaxanthin	
		29.2	470	GABA-betaxanthin	
		29.9	470	Isoproline-betaxanthin	
		31.2	470	Proline-betaxanthin	
		36.8	538	Betanin	
		40.7	538	Isobetanin	

*Liquid Chromatography – Mass Spectroscopy***:** The use of mass spectrometry (MS) coupled with HPLC complements the use of photodiode-array detectors (PAD) and permits immediate identification of components of a mixture and characterization of an extract in terms of its chemical composition. MS provides molecular weight and structural information of the chromatographic bands so that fully-resolved peaks are not required, thus shortening chromatographic runs and reducing sample preparation while ensuring high sensitivity and selectivity. This technique is commonly used in investigations on betalain pigments (Schliemann et al., 1996, 2000, 2001; Wybraniec et al., 2001). Ferndndez-Lopez et al. (2002) screened the presence of betalain pigments in fruits of *Opuntia stricta, Opuntia undulata* and *Opuntia ficus-indica,* also Yahia & Castellanos-Santiago (2008) identified betalains from the fruits of 10 Mexican prickly pear cultivars by HPLC and ESI-MS, qualitative data summarized in Table 2.17.

Table 2.17: Qualitative data of betalains in prickly pear (Opuntia spp.) fruit by HPLC-ESI-MS.

Solvent system	Chromatographic Separation Tech.	R_t (min)	λ_{max} (nm)	$[M+H]^+$ m/z	Daughter ions	Compounds	Reference
A (88 mM acetic acid in H₂O) B (88 mM acetic acid in acetonitrile	Linear gradient from 100% Solvent A to 12% solvent B for 30 min.	16.8	484	309	263, 217	Indicaxanthin	Ferndndez-Lopez et al., (2002)
		19.6	537	551		Betanin	
		22.8	537	551		Isobetanin	
A (1% Formic acid in Water) B (Methanol)	Start isocratically with 100% A, followed by a linear gradient from 0% to 10% B in 20 min, and then a linear gradient from 30% to 100% B in 5 min.	1.6	438	325	309	Portulacaxanthin I	Yahia & Castellanos-Santiago, (2008)
		1.8	470	269	225	Portulacaxanthin III	
		5.1	474	326	295, 149	Vulgaxanthin III	
		5.2	478	349	215, 124	Muscaaurin	
		6.5	478	305	172, 149	Unknown	
		7.3	472	299	268, 136	Unknown	
		9.4	475	340	323	Vulgaxanthin I	
		14.5	474	341	325, 149	Vulgaxanthin II	
		18.9	535	713	551, 389	Betanidin-5-O-β – sophoroside	
		20.1	470	297	253, 149	Unknown	
		21.0	483	309	263, 188	Unknown	
		22.0	483	309	263, 219	Indicaxanthin	
		27.2	478	329	295, 297	Unknown	
		27.3	538	551	389, 149	Betanin	
		27.3	540	389	345, 150	Betanidin	
		28.5	538	551	389, 149	Isobetanin	
		30.2	472	311	175, 137	Unknown	

		30.3	470	311	299, 137	Unknown	
		32.0	475	398	353, 311	Unknown	
		33.5	480	549	387	*neo*-betanin	
		33.9	472	325	308, 219	Unknown	
		34.1	473	325	209	Vulgaxanthin IV	
		34.1	535	459	443, 413	Unknown	
		34.4	467	359	312, 225	Unknown	
		36.0	475	315	270	Unknown	

*Nuclear Magnetic Resonance***:** Unambiguous betalain structures can only be elucidated by nuclear magnetic resonance (NMR) measurements, required tedious isolation and solid experimental set up (Strack et al., 2003; Stintzing & Carle, 2007).

Stintzing et al. (2004) analyzed betacyanin pigments by LC – NMR and 2D NMR spectroscopy from red-purple pitaya (*Hylocereus polyrhizus* (Weber) Britton & Rose) and Wybraniec et al. (2006) & (2007) elucidated betacyanins of purple pitaya (*Hylocereus spp.*) fruits by ^{1}H and ^{13}C NMR spectroscopy.

Quantitative Analysis

*Spectrophotometric method***:** The most convenient way to quantify betalains is spectrophotometric method. First, Nelsson (1970) established a method to quantify pigments in beetroot. The total contents of betacyanins and betaxanthins were determined using the formula reported by Nelsson, (1970); Fernandez-Lopez & Almela, (2001); Ferndndez-Lopez et al., (2002); Cai et al., (2005); Chethana, (2007).

Their molar absorptivity (ε) values were 5.66 X 10^4 (amaranthin, E1%1 cm 536 nm =779), 6.16 X 10^4 (betanin, $E_{(1\%,1cm)}$ 536 nm= 1120), and 5.06 X 10_4 (gomphrenin I, $E_{(1\%,1cm)}$ 540 nm= 920). The mean molar absorptivity (ε) value for betaxanthins is 4.80 X 10^4.

Cai & Corke (1999) and Stintzing et al. (2003 & 2005) described another formula for determination of betalain content: [BLC (mg/L) = (A · DF · MW · 1000)/(e · 1)], where A is the absorption value at the absorption maximum, DF the dilution factor and 1 the pathlength (1 cm) of the cuvette. For quantification of betacyanins and betaxanthins, the

molecular weights (MW) and molar extinction coefficients (ε) of betanin (MW=550 g/mol; ε =60,000 L/mol cm in H_2O; λ =538 nm) and indicaxanthin (MW=308 g/mol; ε =48,000 L/mol cm in H_2O; λ =480 nm) were applied, respectively. Stintzing et al. (2005 & 2006) & Cassano et al. (2007) developed a process for production of cactus pear juice and fruit powders. Quantitative and qualitative color changes during processing were monitored by analysing juice samples after each processing step in terms of CIE*L*C*hº* and betalain contents. Table 2.18 summarizes spectrophotometric quantification of betalains.

Table 2.18: Spectrophotometric quantification of betalains in prickly pear.

Opuntia spp.	Betaxanthin	Betacyanin	Reference
Opuntia ficus indica (reddish purple)	30 mg/100g	19 mg/100g	Fernandez-Lopez & Almela, (2001)
Opuntia ficus indica (yellow)	not detected	25 mg/100g	
Opuntia ficus indica (L.) Mill.		14.5 mg/100g	Ferndndez-Lopez et al., (2002)
Opuntia stricta Haw.		70 mg/100g	
Opuntia undulata Griff.		18.5 mg/100g	
Opuntia ficus indica (L.) Mill. cv. 'Rossa' (red)	4.8 – 49.6 mg/L	66.5 – 80.4 mg/L	Stintzing et al., (2003) (betalains quantified at different pH and using different methods)
Opuntia ficus indica (L.) Mill. cv. 'Gialla' (orange-yellow)	10.5 – 53.7 mg/L	5.4 – 19.6	

Yahia & Castellanos-Santiago (2008) extracted the pigments using two solvents, McIlvaine buffer (pH 6.5, citrate-phosphate) and water from the fruits of 10 Mexican prickly pear Cultivars. The betalain content (BC) was calculated according to literature with a slight modification; BC [mg/g]) [(*A*(DF)(MW)*Vd / εLWd*)], where *A* is the absorption value at the absorption maximum of 535 and 483 nm for betacyanins and

betaxanthins, respectively, DF is the dilution factor, *Vd* is the dried pulp solution volume (mL), *Wd* is the dried pulp weight (g), and *L* is the path-length (1 cm) of the cuvette. In all cases, water extracted the highest level of pigments. Spectrophotometric quantification of betalains summarized in table 2.19.

Table 2.19: Spectrophotometric quantification of betalains in the fruits of 10 Mexican prickly pear cultivars.

Cultivar	Betacyanin content (mg/g dry pulp)		Betaxanthins content (mg/g dry pulp)		Total betalains (mg/g dry pulp)	
	Water	Buffer	Water	Buffer	Water	Buffer
Camuesa	5.29±0.35	5.01±0.60	2.86±0.24	2.56±0.42	8.15	7.57
Roja Pelota	2.06±0.06	1.86±0.28	0.99±0.03	0.84±0.12	3.04	2.71
Cardona	2.04±0.20	1.83±0.00	1.04±0.09	0.80±0.00	3.08	2.63
2142	0.71±0.04	0.66±0.01	0.44±0.03	0.38±0.01	1.16	1.04
Liria	0.39±0.03	0.34±0.02	0.14±0.01	0.11±0.00	0.53	0.45
Roja Lisa	0.27±0.01	0.22±0.02	0.23±0.02	0.18±0.00	0.50	0.40
Naranjona	0.065±0.01	0.04±0.01	0.16±0.02	0.12±0.00	0.23	0.16
2651	0.072±0.00	0.04±0.01	0.14±0.02	0.09±0.01	0.21	0.13
21441	0.071±0.00	0.05±0.01	0.41±0.02	0.35±0.04	0.48	0.40
Reyna	0.05±0.02	0.03±0.03	0.12±0.01	0.23±0.20	0.17	0.26
Red beet	5.41±0.02	4.98±0.00	3.21±0.01	3.12±0.00	8.60	8.10

2.3.3.2.3 Seed

Minerals, Vitamins & Amino acids

Kossori et al. (1998) reported mineral composition of seeds (Table 2.20) of *Opuntia ficus indica* along with protein content (11.8%).

Table 2.20: Mineral composition of seeds of *Opuntia ficus indica*.*

Minerals	mg/100g dry matter
Ca	258
Mg	208
Na	<0.83
K	275
P	110
Fe	12.1
Cu	<0.83
Zn	4.16
Mn	<0.83
Mb	<0.33

* Kossori et al., 1998.

Nassar (2008) studied amino acids composition of prickly pear seed flour and its protein concentrate are presented in table 2.21. Glutamic acid was the most predominant amino acid followed by aspartic acid, leucine, lycine and arginine. The values of amino acids showed that cystine and methionine were in the lowest amounts in prickly pear seed flour and protein concentrated. On the other side essential amino acids were reported 28.68 and 30.46% while nonessential amino acids were reported 43.81 and 45.88%. Total essential:non essential amino acid ratio was 0.65 and 0.66 for prickly pear seed flour and protein concentrate respectively.

Table 2.21: Amino acids composition of prickly pear seed flour and protein concentrate.*

Amino acids	Prickly pear seed flour	Flour protein concentrate
Leucine	7.21	7.82
Isoleucine	4.50	4.76
Methionine	0.51	0.47
Phenylalanine	3.81	3.96
Lysine	4.93	4.98
Therionine	1.11	1.46
Tyrosine	2.24	2.38
Valine	4.37	4.63
Aspartic	7.56	7.79
Glutamic	15.73	15.58
Serine	6.14	6.77
Glycine	3.67	3.89
Alanine	3.45	3.71
Histidine	2.26	2.87
Arginine	4.81	5.09
Cystine	0.27	0.18

* Nassar, 2008.

Sugars and Hydrocolloids

Vignon et al. (2005) isolated hemicellulosic polysaccharides from depectinated cell wall material of seed endosperm of *Opuntia ficus indica* fruit by alkaline extraction. Two xylans were isolated, fractionated and characterized. The structural investigations were achieved by sugar and methylation analysis, and were confirmed by ^{1}H and ^{13}C NMR. Vignon et al. (2005a) studied reserve storage polysaccharide of the endosperm seed of *Opuntia ficus indica* fruit after removal of starch. Cell wall material was extracted successively by boiling water called water soluble fraction (WSF), hot calcium chelating agent solution (CSF) and cold mild alkaline solution (CASF). All polysaccharides extracted were fractionated by ion-exchange chromatography into five fractions. The

resulted major fractions were purified by size-exclusion chromatography and analyzed by sugar composition and glycosyl linkage analyses. The investigations were also supported by ^{1}H and ^{13}C NMR spectroscopy analysis. The results showed that the major fraction of WSF consisted of an arabinan. The backbone contained α-(1→5)-linked arabinofuranosyl residues with high percentage of arabinose units substituted at O-2. The predominant fractions from CSF and CASF were related to rhamnogalacturonan type I which consisted of a disaccharide repeating unit→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→backbone with α-(1→5)-linked arabinan side-chains attached to O-4 of the rhamnosyl residues. Kossori et al. (1998) reported fiber composition of prickly pear fruit hemicellulose (9.95±0.58), cellulose (83.2±0.25), pectin (6.69±0.46) and lignin (0.19±0.04) as percentage of total fiber.

Lipids

Ramadan & Morsel (2003) compared seeds and pulp of cactus pear (*Opuntia ficus indica* L.) in terms of fatty acids, lipid classes, sterols, fat-soluble vitamins and β-carotene. Total lipids (TL) in lyophilized seeds and pulp were 98.8 (dry weight) and 8.70 g/kg respectively. High amounts of neutral lipids were found (87.0% of TL) in seed oil while glycolipids and phospholipids occurred at high levels in pulp oil (52.9% of TL).

Ennouri et al. (2005) investigated fatty acid composition and physicochemical parameters of the seed oil from *Opuntia ficus indica* and *Opuntia stricta* fruits. The main fatty acids of prickly pear seed oil were C16:0, C18:0, C18:1, C18:2. With an exceptional level of linoleic acid up to 70% the content of unsaturated fatty acids was high, at 88.5% and 88.0% for *O. ficus indica* and *O. stricta* respectively. Wei Liu et al. (2009) investigated supercritical carbon dioxide extraction of seed oil from *Opuntia dillenii* Haw. and its antioxidant activity. The maximum extraction yield of 6.65% was achieved at 46.96 MPa, 46.51ºC, 2.79 h and 10 kg/h of pressure, temperature, time and CO_2 flow rate respectively. The chemical composition of the seed oil was analysed by GC–MS. The main fatty acids were found linolenic acid (66.56%), palmitic acid (19.78%), stearic acid (9.01%) and linoleic acid (2.65%). The antioxidant activity of seed oil was assessed by means of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and β-carotene bleaching test. Both methods demonstrated notable antioxidant activity of seed oil which

was nearly comparable to the references ascorbic acid and butylated hydroxytoluene. The antioxidant activity of the seed oil was also found to be concentration dependent.

2.3.4 Ethanopharmacological Action

Opuntia species has been used by humans for thousands of years. Besides being consumed as food or beverages, most portions of the plants have been used as medicine and in modern times have also been prepared as juice, jam, flour, frozen fruit, juice concentrate, and spray-dried juice powder (Smith, 1967; Stintzing & Carle, 2005, 2006; Feugang et al., 2006;). A remarkable number of cacti are used by indigenous people of the New World for healing. According to Parmar and Kaushal (1982), Kirtikar & Basu (1999) and Patil et al. (2008), the plant is bitter, laxative; stomachic, carminative, antipyretic. Cures biliousness, burning, leucoderma, urinary complains, tumours, loss of consciousness, piles, inflammations, anaemia, ulcers, respiratory disorders like asthma and the enlargement of the spleen. The flowers cure bronchitis and asthma. Medically related used of some species are discussed here. The Shoshoni make a poultice from the inner part of the stem of *Opuntia basilaris* and apply it to cuts and wounds for pain (Moerman, 1998). Grenand et al. (1987) report that *Opuntia cochenillifera* is widely used in Mexico and Central America as an antifungal agent. People throughout Asia employ *Opuntia dillenii* for a variety of purposes. In India, it is used to treat sores, pimples, even syphilis (Jain and Tarafder, 1970). Curtin (1949) reports that the Pima apply the heated stem segment of *Opuntia engelmannii* and *Opuntia phaeacantha* to a new mother's breasts to stimulate the flow of milk. The Okanagan-Colville and the Shuswap make a poultice from the stem of O. fragilis and use it to treat sores, including sore throat. They also eat the stems as a diuretic (Moermann 1998). Moore (1989) reports that various species are used as drawing poultices, for gum infections and mouth sores, as an analgesics for such problems as painful urination, even that prickly pear juice reduces the discomfort of honeymoon cystitis. Dried flowers are useful in treating ailments characterized by inflamed mucous membranes such as chronic colitis, asthma, vaginitis, and diverticulosis. Some species are effective in reducing the adverse consequences of adult-onset or insulin-independent diabetes. This may result from the presence of

saponins in these species. The extracts of *O. ficus-indica* were effective in treating abdominal cancer (Cruse, 1973).

1. *Analgesic & Anti-inflammatory*

Park et al. (2001) studied the various fractionation of the methanol extract of stems of *Opuntia ficus-indica* Mill. for anti-inflammatory action using adjuvant-induced pouch granuloma model in mice and identified β-sitosterol as an active anti-inflammatory compound. Lyophilized aqueous extract (100–400 mg/kg, i.p.) of the fruits of *Opuntia dillenii* (Ker-Gawl) Haw was evaluated for analgesic activity using writhing and hot plate test in mice and rat, respectively and also anti-inflammatory activity using carrageenan-induced paw edema in rats, the results exhibited dose dependent action (Loro et al., 1999).

2. *Anticancer*

Most recent studies suggests that the cactus pear fruit extract (i) inhibits the proliferation of cervical, ovarian and bladder cancer cell lines *in vitro,* and (ii) suppresses tumor growth in the nude mice ovarian cancer model *in vivo*. These experiments showed that inhibition was dose- (1, 5, 10 and 25% cactus pear extract) and time- (1, 3 or 5 day treatment) dependent on *in vitro*-cultured cancer cells. The intra-peritoneal administration of cactus extract solution into mice did not affect the animal body weight, which indicated that cactus did not have a significant toxic effect in animals. Growth inhibition of cultured-cancer cells was associated with an increase in apoptotic cells and the cell cycle arrest at the G1-phase. Moreover, the induced growth inhibition seems dependent on the P53 pathway, which is the major tumor suppressor. Annexin IV was increased and the VEGF decreased in the tumor tissue obtained from animals having received the cactus solution. The antiproliferative effect of betanin, isolated from the fruits of *Opuntia ficus indica*, was evaluated on human chronic myeloid leukemia cell line (K562). The results show dose and time dependent decrease in the proliferation of K562 cells treated with betanin with an IC_{50} of 40 μM. Further studies involving scanning and transmission electron microscopy revealed the apoptotic characteristics such as chromatin condensation, cell shrinkage and membrane blebbing. Agarose electrophoresis of

genomic DNA of cells treated with betanin showed fragmentation pattern typical for apoptotic cells. Flow cytometric analysis of cells treated with 40 mM betanin showed 28.4% of cells in sub G0/G1 phase. Betanin treatment to the cells also induced the release of cytochrome *c* into the cytosol, PARP cleavage, down regulation Bcl-2, and reduction in the membrane potentials. These studies demonstrate that betanin induces apoptosis in K562 cells through the intrinsic pathway and is mediated by the release of cytochrome *c* from mitochondria into the cytosol, and PARP cleavage. The mechanisms responsible for executing the antiproliferative effects include: (i) induction of alterations in the cell differentiation pattern, which plays a vital role in the invasiveness and metastatic progression of the tumors, (ii) blockade of pre neoplastic cell expansion or induction of apoptosis, and (iii) intervention of metabolic activation of carcinogens by scavenging ROS (Sreekanth et al., 2007).

3. *Antidiabetic*

The prickly pear cactus stems have been used traditionally to treat diabetes in Mexico (Domínguez López, 1995). Nowadays, *Opuntia* species is amongst the majority of products recommended by Italian herbalists that may be efficacious in reducing glycemia (Cicero et al., 2004). The hypoglycemic activity of broiled stem of *Opuntia streptacantha* Lemaire was demonstrated using different extract preparation and dosed in diabetic and non-diabetic human volunteers by Meckes-Lozyoa and Roman-Ramos (1986), Frati et al. (1989, 1989a, 1990, 1991), and Roman-Romas et al (1991). Some studies have demonstrated the hypoglycemic activity of the prickly pear cactus extract on non-diabetics and diabetic-induced rats or diabetic humans (Ibanez-Camacho et al., 1979, 1983; Frati et al., 1988, 1990a; Trejo-González et al., 1996). The anti-hyperglycemic effect of 12 edible plants was studied on rabbits, submitted weekly to subcutaneous glucose tolerance tests after gastric administration of a juice of stems of *Opuntia streptacantha* (dose, 4 ml/kg) which decrease significantly the area under the glucose tolerance curve and the hyperglycemic peak (Roman-Ramos et al., 1995). The hypoglycemic activity of a purified extract from stems of *Opuntia fuliginosa* Griffiths was evaluated on Streptozotocin-induced diabetic rats. Blood glucose and glycated hemoglobin levels were reduced to normal values by a combined treatment of insulin and

Opuntia extract. When insulin was withdrawn from the combined treatment, the prickly pear extracts alone maintained normoglycemic state in the diabetic rats. The magnitude of the glucose control by the small amount of *Opuntia* extract required (1 mg/kg body weight per day) to control diabetes contrast with the high quantities of insulin required for an equivalent effect (Gonzfilez et al., 1996). Plasma glucose concentrations in Streptozotocin-induced diabetic and non-diabetic rats were reduced by the orally administration of *O. megacantha* leaf extracts (20 mg/100 g body weight). The results suggest that leaf extracts not only reduce blood glucose levels, but may be toxic to the kidney as shown by the elevation in plasma urea and creatinine concentrations and the reduction of plasma Na^+ concentration (Bwititi et al., 2000). The seed oil from fruits of *Opuntia ficus-indica* is rich in polyunsaturated fatty acids with an exceptional level of linoleic acid (700g/kg). In this study, evaluated the effect of seed oil supplemented diet on rats, the results indicated a significant decrease in serum glucose concentration (22%) over the control group and an increase in the concentration of glycogen in liver and muscle. Blood cholesterol and low density lipoprotein-cholesterol decreased in the treated group and high density lipoprotein-cholesterol concentration increased during the treatment. These findings support the nutritional value of cactus pear as a natural source of edible oil containing essential fatty acids (Ennouri et al., 2006, 2006a).

4. Anti-hyperlipidemic & - Hypercholesterolemic

Experimental evidence suggested that cactus pear reduces cholesterol levels in human blood and modify low density lipoprotein composition (Fernandez et al., 1992; Frati, 1992; Gurbachan & Felker, 1998). Galati et al. (2003) have found that the cholesterol, low density lipoprotein and triglyceride plasma levels of rats were strongly reduced after 30 days of a daily administration (1 g/kg) of lyophilized cladodes of *Opuntia ficus-indica* L. Mill. Sterols which comprise the bulk of the unsaponifiables in many oils are of interest due to their ability to lower blood low density lipoprotein-cholesterol by approximately 10–15% as part of a healthy diet (Jones et al., 2000). Ennouri et al. (2006, 2006a, 2007) investigated the effects of diets enriched with cactus pear oil and seeds on serum and liver parameters, the results indicated a significantly decreased blood

cholesterol and low density lipoprotein-cholesterol and increased high density lipoprotein-cholesterol.

5. *Antioxidant*

The antioxidative action is one of many mechanisms by which fruit and vegetable substances might exert their beneficial health effects. The presence of several antioxidants (ascorbic acid, carotenoids, reduced glutathione, cysteine, taurine and flavonoids such as quercetin, kaempferol and isorhamnetin) has been detected in the fruits and vegetables of different varieties of cactus prickly pear. More recently, the antioxidant properties of the most frequent cactus pear betalains (betanin and indicaxanthin) have been revealed (Tesoriere et al., 2002, 2003, 2004, 2005, 2005a; Stintzing et al., 2005). Numerous *in vitro* studies have demonstrated the beneficial effect of phenolics and betalains. These are generally attributed to the ability of antioxidants to neutralize reactive oxygen species such as singlet oxygen, hydrogen peroxide or H_2O_2, or suppression of the xanthine/xanthineoxidase system, all of which may induce oxidative injury, i.e. lipid peroxidation. Regular ingestion of prickly pear (*Opuntia robusta*) is able to significantly reduce in-vivo oxidation injury in young patients suffering from familial isolated hypercholesterolemia and oxidation injury determined via 8-epi-PGF$_{2\alpha}$ in plasma, serum and urine. The findings on a decrease of 8-epi-PGF$_{2\alpha}$ were more pronounced in females than in males, the highest significance being found in urine, while, in contrast, the effects on total- and low density lipoprotein-cholesterol were more pronounced in males. Thus, this may have a significant cardiovascular benefit (Budinsky et al., 2001). Kuti (2004) investigated antioxidant compounds in extracts from four *Opuntia* species (*O. ficus-indica, O. lindheimeri, O. streptacantha, O. stricta var. stricta*) fruit. ZEN is one of the most widely distributed fusarial mycotoxins which are encountered at high incidence in many foodstuffs. In this study, the effect of a single dose of ZEN (40 mg/kg b.w.) alone and with extract of cactus cladodes (25, 50 and 100 mg/kg b.w.) on the induction of oxidative stress was monitored in kidney and liver by measuring the MDA level, the protein carbonyls generation, the catalase activity and the expression of the heat shock proteins (Hsp). The results clearly showed that ZEN induced significant alterations in all tested oxidative stress markers, while the combined treatment of ZEN

with the lowest tested dose of cactus extracts (25 mg/kg b.w.) showed a total reduction of ZEN induced oxidative damage for all tested markers (Zourgui et al., 2008).

Su-Feng Chang et al. (2008) investigated the antioxidant activity and inhibitory effect of extracts from *Opuntia dillenii* Haw fruit on low-density lipoprotein peroxidation. The results indicated that the antioxidant activity of methanolic extracts in Trolox equivalent antioxidant capacity and oxygen-radical absorbance capacity assays were in the order of seed > peel > pulp. Among the extracts, seed extracts 10 µg/ml) possessed the highest inhibitory effect on the formation of thiobarbituric acid reactive substances and relative electrophoretic mobility and contained the highest amounts of polyphenols and flavonoids (212.8 and 144.1 mg/100 g fresh seed), respectively.

6. Antiulcer

In Sicily folk medicine, *Opuntia ficus-indica* (L.) Mill. cladodes are used for the treatment of gastric ulcer and cicatrisant action. Galati et al. (2001, 2002a) studied the effect of lyophilized cladodes (1 g/kg) using ethanol-induced ulcer model in rat. In this study, the ultra structural changes were observed by transmission electronic microscopy confirming the protective effect exercised by administration of lyophilized cladodes. Probably, the mucilage of *Opuntia ficus-indica* is involved.

7. Antiviral

An interesting study by Ahmad et al. (1996) demonstrated that administration of a cactus stem extract (*Opuntia streptacantha*) to mice, horses, and humans inhibits intracellular replication of a number of DNA- and RNA-viruses such as Herpes simplex virus Type 2, Equine herpes virus, pseudorabies virus, influenza virus, respiratory syncitial disease virus and HIV-1. An inactivation of extra-cellular viruses was also reported by the same authors. However, the active inhibitory component(s) of the cactus extract used in this study was not investigated, and as of yet, no further study dealt with this specific topic. Mtambo et al. (1999) evaluated the efficacy of the crude extract of *Opuntia vulgaris* against Newcastle virus disease in domestic fowl in Tanzania.

8. Diuretics

Galati et al. (2002) studied the diuretic activity of *Opuntia ficus-indica* (L.) Mill. waste matter in rat. Acute and chronic diuretic activity of 15% infusion of cladodes, flowers and fruits were assayed. Natriuresis, kaliuresis and the activity on fructose-induced hyperuricemia was also studied. The results show that *O. ficus-indica* cladode, fruit and flower infusions significantly increase diuresis. This effect is more marked with the fruit infusion and it is particularly significant during the chronic treatment. The fruit infusion shows also antiuric effect. In this study, cladode, flower and fruit infusions showed a modest but not significant increase in natriuresis and kaliuresis.

9. Immunomodulatory

Schepetkin et al. (2008) provide a molecular basis to explain a portion of the beneficial therapeutic properties of extracts from *O. polyacantha* on human and murine macrophages demonstrated that all four fractions had potent immunomodulatory activity, inducing production of reactive oxygen species, nitric oxide, TNFα, and interleukin 6. Modulation of macrophage function by *Opuntia* polysaccharides was mediated through activation of nuclear factor κB.

10. Improve platelet function

Prickly pear is traditionally used by Pima Indians as a dietary nutrient against diabetes mellitus. Wolfram et al. (2003) examined the effect of daily consumption of 250g in 8 healthy volunteers and 8 patients with mild familial heterozygous hypercholesterolemia on various parameters of platelet function. Beside its action on lipids and lipoproteins, prickly pear consumption significantly reduced the platelet proteins (platelet factor 4 and β-thromboglobulin), ADP-induced platelet aggregation and improved platelet sensitivity (against PGI_2 and PGE_1) in volunteers as well as in patients. Also plasma 11-DH-TXB_2 and the WU-test showed a significant improvement in both patients and volunteers. In contrast, collagen-induced platelet aggregation and the number of circulating endothelial cells showed a significant response in patients only. Prickly pear may induce at least part of its beneficial actions on the cardiovascular system via decreasing platelet activity and thereby improving haemostatic balance.

11. Neuroprotective

Jungsook Cho et al. (2003) isolated the flavonoids quercetin, (+)-dihydroquercetin, and quercetin 3-methyl ether from the ethyl acetate fractions of the fruits and stems of *Opuntia ficus-indica var. saboten* and evaluated their protective effects against oxidative neuronal injuries induced in primary cultured rat cortical cells and their antioxidant activities by using lipid peroxidation, 1,1-diphenyl-2-picrylhydrazyl, and xanthine oxidase bioassays. Quercetin was found to inhibit H_2O_2 - or xanthine / xanthine oxidase-induced oxidative neuronal cell injury, with an estimated IC_{50} of 4–5 µg/ ml and no more protection at concentrations of 30µg/ml and above while (+)-dihydroquercetin concentration-dependently inhibited oxidative neuronal injuries, but it was less potent than quercetin. On the other hand, quercetin 3-methyl ether potently and dramatically inhibited H_2O_2 - and xanthine / xanthine oxidase-induced neuronal injuries, with IC_{50} values of 0.6 and 0.7 µg/ ml, respectively. In addition, quercetin and quercetin 3-methyl ether were shown to inhibit xanthine oxidase activity *in vitro*, with respective IC_{50} values of 10.67 and 42.01 µg/ ml and quercetin-3-methyl ether appears to be the most potent neuroprotectant of the three flavonoids isolated from this plant.

Jung-Hoon Kima et al. (2006) examined the methanol extract of *Opuntia ficus-indica* (MEOF) as a neuroprotective action against *N*-methyl-d-aspartate (NMDA)-, kainate (KA)-, and oxygen–glucose deprivation (OGD)-induced neuronal injury in cultured mouse cortical cells and also evaluated the protective effect in the hippocampal CA1 region against neuronal damage evoked by global ischemia in gerbils. Treatment of neuronal cultures with MEOF (30, 300, and 1000 µg/ml) inhibited NMDA (25 µM)-, KA (30 µM)-, and OGD (50 min)-induced neurotoxicity dose-dependently. The butanol fraction of *Opuntia ficus indica* (300 µg/ml) significantly reduced NMDA (20 µM)-induced delayed neurotoxicity by 27%. Gerbils were treated with MEOF every 24 h for 3 days (0.1, 1.0, and 4.0 g/kg, p.o.) or for 4 weeks (0.1 and 1.0 g/kg, p.o.), and ischemic injury was induced after the last dose. Neuronal cell damage in the hippocampal CA1 region was evaluated quantitatively at 5 days after the ischemic injury. When gerbils were given doses of 4.0 g/kg (3 days) and 1.0 g/kg (4 weeks), the neuronal damage in the hippocampal region was reduced by 32 and 36%, respectively. These results suggested

that the preventive administration of *Opuntia ficus-indica* extracts may be helpful in alleviating the excitotoxic neuronal damage induced by global ischemia.

12. Antispermatogenic

A methanolic extract from *O. dillenii* Haw. defatted with chloroform and petroleum ether exerted antispermatogenic effects in animal tests on rats. According to (Gupta et al., 2002), the flavone derivatives vitexin and myricetin were found to be the active principles. When 250 mg extract per kg body weight was applied, the weight of testis, epididymis, seminal vesicle, and ventral prostate were reasonably, that of Sertoli cells, Leydig cells, and gametes considerably reduced. The motility of the sperms was also diminished.

13. Wound healing

In traditional medicine extracts of polysaccharide-containing plants are widely employed for the treatment of skin and epithelium wounds and of mucous membrane irritation. The extracts of *Opuntia ficus-indica* cladodes are used in folk medicine for their antiulcer and wound-healing activities. The methanolic extract of *Opuntia ficus-indica* stems and its hexane, ethyl acetate, *n*-butanol and aqueous fractions (100 mg/site) exhibited wound healing activity in rats by measuring the tensile strength of skin strips from the wound segments. The extract and less polar fractions showed significant effects (Park & Chun, 2001).

Trombetta et al. (2006) described the wound-healing potential of two lyophilized polysaccharide extracts obtained from *O. ficus-indica* (L.) cladodes applied on large full-thickness wounds in the rat. The wound-healing effect is more marked for polysaccharides with a molecular weight ranging 10^4–10^6 Da than for those with molecular weight$>10^6$ Da, author supposed that the fine structure of these polysaccharides and their particular hygroscopic, rheologic and viscoelastic properties may be essential for the wound-healing promoter action.

14. Monoamino-oxidase inhibition

Besides catecholmethyltransferases, the monoamino-oxidases (MAOs) are usually involved in the catabolism of catecholamines, thus regulating the overall amine pool. In cladodes and fruits from the Korean *O. ficus-indica var. saboten* Makino, methyl esters derived from organic acids were identified as MAO inhibitors. The aqueous extracts showed least inhibitory activity, followed by the n-butanol fraction and the hexane extract whereas the ethyl acetate fraction exerted the highest inhibitory action. The active agents were identified as 1-methyl malate, 1-monomethyl citrate, 1,3-dimethylcitrate, and 1,2,3-trimethylcitrate. The purified components showed MAO-A inhibitory action with increasing number of methyl substituents, whilst the MAO-B inhibitory action was superior for 1-methylmalate compared to the mono- and dimethylcitrates. However, 1,2,3-trimethylcitrate exerted the strongest inhibition on both MAOs. When citrate was compared with its corresponding methyl derivatives, the methoxy moiety proved to be the effective moiety (Han et al., 2001).

15. Nutritional important

Cacti have long been considered an important nutritional source in Latin America (bread of the poor) among which *Opuntia* has gained highest economic importance worldwide. It is cultivated in several countries such as Mexico, Argentina, Brazil, Tunisia, Italy, Israel and China. Both fruit and stems have been regarded to be safe for food consumption. The constantly increasing demand for nutraceuticals is paralleled by a more pronounced request for natural ingredients and health-promoting foods. The multiple functional properties of cactus pear fit well this trend. Recent data revealed the high content of some chemical constituents, which can give added value to this fruit on a nutritional and technological functionality basis. High levels of betalains, taurine, calcium, magnesium, and antioxidants are noteworthy (Piga, 2004; Stintzing & Carle, 2005; Feugang et al., 2006).

The *Opuntia* species cladodes and fruits serve as a source of varied number of phytoconstituents mainly sugar, phenolics and pigments. Total betalains are well reported with their qualitative and quantitative analytical methods. Though various analytical

methods are reported, but still some focus is required towards HPTLC with marker's evidence. Although the reported evidences provide the effectiveness of *Opuntia* species, but active constituents, bioavailability, pharmacokinetics and physiological pathways for various biological actions are not well known with sufficient detail or confidence. Ethnopharmacological actions may be due to presence of phenolics and pigments. Still more attention is required towards the development of simple, feasible and cost effective pharmaceutical preparations of *Opuntia spp.* cladodes and fruit juice as well as the ethnopharmacological approach, if combined with mechanism of action, biochemical and physiological methods, would provide useful pharmacological leads.

2.4 Research envisage

Present study aiming to study phytochemical and pharmacological screening of fruits of *Opuntia elatior* Mill., in Gujarat, commonly known as "***Hathlo Thor***" belongs to sub-family Opuntioideae of the family Cactaceae. The literature study reveals that still today there is no record of phytochemical composition and pharmacological study of *Opuntia elatior* Mill. fruits in support of traditional and folkloric use.

The Present Project Deals with the Following study:

1. Collection of fresh plant (*Opuntia elatior* Mill.) from field and study morphology.
2. To authenticate the plant (*Opuntia elatior* Mill.) by the Government Herbarium Authority.
3. To study morphology of different parts of the plant.
4. To evaluate physical parameters of fruit juice.
5. To prepare different extracts of fruit peel and juice of fruit pulp, and screen to detect different types of phytoconstituents using chemical tests and thin layer chromatography.
6. To estimate different types of phytoconstituents using various instrumental methods.
7. To screen fruit juice for antiasthmatic and haematinic activity using different experimental models.

8. To screen different extracts of fruit peel for antimicrobial activity.

9. Estimation of various haematological parameters like haemoglobin content, total red blood cells (RBC), total white blood cells (WBC), differential white blood cells counts, haematocrit, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelet count, mean platelet volume (MPV), platelet distribution width (PDW) and red blood cell distribution width (RDW) and biochemical parameters like blood sugar, creatinine, urea, alkaline phosphatase, bilirubin, total Protein, total cholesterol, and triglycerides during pharmacological screening.

10. Histopathological study to conform pharmacological activities.

The Promising Aspect of the Project:

The *Opuntia elatior* Mill. is xerophytic wild plant. The plant can grow automatically in the desert area and virgin soil without any extra efforts. It does not require any maintenance to survive. Raw material of this plant is highly cheaper and easily available without any extra burden; in short it is highly economical. Even though easy, wide and cheap availability of *Opuntia elatior* Mill., it is not used in the medicine because therapeutic efficacy of this plant is not checked still today. Our aim is to investigate therapeutic worth of *Opuntia elatior* Mill. so that local community and common man can explore benefits of this plant. Ultimate our aim is

1. To generate pharmacological data of *Opuntia elatior* Mill. in the support of traditional and folkloric use.

2. This study can inspire poor people to use easily available cheaper plant as a medicine.

3. To generate morphological, physicochemical and phytochemical data to know the identity, purity and quality of the plant.

3. Materials and Methods

3. Materials and Methods

Sr No.	Title	Page No.
3.1	**Apparatus**	**89**
3.2	**Chemicals**	**90**
3.3	**Pharmacognostical studies**	**90**
3.3.1	Collection and authentication of plant	**90**
3.3.2	Macroscopic examinations	**90**
3.3.3	Proximate analysis	**91**
3.3.3.1	Determination of average weight, % of peel, pulp and seeds of fruit	**91**
3.3.3.2	Preparation of fruit juice of *Opuntia elatior* Mill. (OFJ)	**91**
3.3.3.3	Determination of pH of OFJ	**91**
3.3.3.4	Determination of Moisture content and Total solids	**91**
3.3.3.5	Determination of Ash value	**92**
3.3.3.6	Determination of Density of OFJ	**92**
3.3.3.7	Determination of Viscosity of OFJ	**92**
3.4	**Phytochemical studies**	**93**
3.4.1	Preparation of fruit peel extracts	**93**
3.4.2	Qualitative evaluation of peel extracts and OFJ	**94**
3.4.3	TLC profile of OFJ	**94**
3.4.4	Qualitative analysis of betalain	**94**
3.4.4.1	Spectrophotometric analysis	**94**
3.4.4.2	High performance liquid chromatographic (HPLC) analysis	**95**
3.4.4.3	Liquid chromatography – mass spectroscopic (LC-MS) analysis	**95**
3.4.5	Quantitative estimation of OFJ	**96**
3.4.5.1	Total sugar content	**96**

3. Materials and Methods

3.1 Apparatus

- A double beam atomic absorption spectrophotometer (AAS) (AA-6300, Schimadzu).

- ACCULAB digital balance, (Model No. ALC-310.3, Sartorius Mechatronics India Pvt. Ltd., Bangalore, India).

- Automated fluorescence flow cytometry 5-part different analysers (Sysmex XS800i, Japan).

- Blender (Boss appliances, Daman, India).

- Brookfield viscometer (Model DV-II+ Pro viscometer).

- Compufuge cooling centrifuge (Remi Instrument, Mumbai, India).

- Density bottle (Borosil Glass Works Ltd., Mumbai, India).

- Digital pH meter (model-EQ-610, Equip-Tronics, Ahmedabad, India).

- Double beam THERMO UV-visible spectrophotometer (Thermo Spectronic, Cambridge, UK) equipped with VisionPro software V 4.10.

- Glass filter G_4 (Borosil Glass Works Ltd., Mumbai, India).

- High performance liquid chromatography (Shimadzu).

- Liquid chromatography – Mass spectroscopy (TSQ Quantum Ultra, Thermo Scientific, USA).

- Muffle furnace (Janki Impex, Ahmedabad, India).

- Sahli's haemoglobinometer (Janki Impex, Ahmedabad, India).

- Silica gel 60 F 254 precoated plates (Alugram$^®$ SIL G/UV$_{254}$, Macherey – Nagel, Germany).

3.2 Chemicals

- Acetylcholine, carrageenan, compound 48/80, cresyl blue, gallic acid, histamine, mercuric chloride, phenylhydrazine hydrochloride were procured from Sigma Aldrich, Mumbai, India.

- Acetic acid, acetonitrile, anhydrous citric acid, anthrone reagent, benzene, chloroform, concentrated sulfuric acid, deionized water, distilled water, Folin-Ciocalteu's reagent, glucose, hydrochloric acid, methanol, nylon fibers, petroleum ether (60-80^{0}C), sodium carbonate, sodium hydroxide, toluidine blue were procured from S.D Fine Chemicals Mumbai, India.

- Muller Hilton agar, Potato Dextrose agar were purchased from Himedia Lab., India.

- Diagnostic kits used for estimation of blood sugar (Bayer diagnostics, Ahmedabad, India), serum urea, creatinine, total cholesterol, triglyceride (Nicholas India Pvt. Ltd., Ahmedabad, India), alkaline phosphate, bilirubin (Erba diagnostic Germany Ltd., Baroda, India) and total protein (Span diagnostics India Pvt. Ltd., Ahmedabad, India).

3.3 Pharmacognostical studies

3.3.1 Collection and authentication of plant

The fruits of *Opuntia elatior* Mill. were collected from road side weed near Atkot, Ta: Jasdan, Dist: Rajkot, Gujarat, India at Latitude (22^0 1' 48" N), Longitude (71^0 12' 0" E) and Elevation 193 M (633 ft) and authenticated by Dr. H. B. Singh, Scientist and Head, Raw Materials Herbarium and Museum, National Institute of Science and Communication and Information Resources, New Delhi (NISCAIR) and preserved the herbarium (specimen voucher No.: rbpmpc/museum/herbarium/07-08/01) in the museum of Dept. of Pharmacognosy, Smt. R. B. Patel Mahila Pharmacy College, Atkot.

3.3.2 Macroscopic examinations

The whole plant of *Opuntia elatior* Mill. was subjected to macroscopic examinations using reported methods in standard text (Datta, 2003) and the results were compared with the reported monographs (Kirtikar and Basu, 1999; The Wealth of India, 2001).

3.3.3 Proximate analysis

3.3.3.1 Determination of average weight, % of peel, pulp and seeds of fruit

Mature fruits (50 units) of *Opuntia elatior* Mill. were collected and taken immediately to the laboratory where they were weighed before and after manually peeled out, subjected to homogenization, and separated seeds were again weigh using ACCULAB digital balance, Model No. ALC-310.3 (Sartorius Mechatronics India Pvt. Ltd., Bangalore).

3.3.3.2 Preparation of fruit juice of *Opuntia elatior* Mill. (OFJ)

Mature fruits of *Opuntia elatior* Mill. were collected and immediately taken to the laboratory. Spines and glochides were removed from fruits by just heating on the burner and then washed with water. The peel of the fruits was removed manually and pulp subjected to homogenization for 5 minute using boss portable blender (Boss appliances, Daman). After homogenization, fruits juice was filtered though muslin cloth and filtered juice was used for various estimation and biological studies.

3.3.3.3 Determination of pH of OFJ

The pH of OFJ was determined five times using pH meter (Digital pH meter model-EQ-610, Equip-Tronics, India) (Anonymous, 1996).

3.3.3.4 Determination of Moisture content and Total solids

Estimation of moisture content and total solids was carried out five times as per Anonymous (1989). Fruit pulp (10 g) placed in a tarred evaporating dish and dried at 105 ºC in an oven at constant weight. The moisture content and total solids were determined using following equation.

% Moisture content = [(initial weight – dried weight)/initial weight] X 100

% Total Solids = (Dried weight / Initial weight) X 100

3.3.3.5 Determination of Ash value

Total ash, acid-insoluble ash and water-soluble ash values were determined five times as per Anonymous (1989) and WHO (2002).

Total ash: 3g of accurately weighed fruit pulp was taken in a tarred silica crucible and incinerated at a temperature not exceeding 450 ℃ until free from carbon and constant weight, cooled and weighed.

Acid-insoluble ash: Total ash obtained was boiled for five minutes with 25 ml of dilute Hydrochloric acid. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited, cooled and weighed.

Water-soluble ash: Total ash obtained was boiled for five minutes with 25 ml of distilled water, cooled and collect the insoluble matter on an ash-less filter paper, washed with hot water and ignited for 15 minutes at temperature not exceeding 450 ℃.

3.3.3.6 Determination of Density of OFJ

Density of OFJ was determined five times using density bottle at room temperature against water as reference compound (Gaud and Gupta, 2006).

3.3.3.7 Determination of Viscosity of OFJ

Viscosity of OFJ was determined five times using spindle S61 of Brookfield viscometer (Model DV-II+ Pro viscometer) at 100 rpm (Gaud & Gupta, 2006).

3.4 Phytochemical studies

3.4.1 Preparation of fruit peel extracts

Manually removed peel of fruits was subjected to air drying at room temperature. Air dried peel was pulverized and passed through 10 # sieve. A finely peel powder was extracted successively with petroleum ether (60-80^{0}C), benzene, chloroform, methanol and water in Soxhlet extractor for 24 hours (Scheme – I).

Powdered peel (100 gm)

Extracted with petroleum ether (60-80^{0}C)
(750 ml) in Soxhlet apparatus for 24 hours

Petroluem ether extract   Residue

(Air-dried, reweighed, repacked)

Benzene (750 ml)

Benzene extract   Residue

(Air-dried, reweighed, repacked)

Chloroform (750 ml)

Chloroform extract   Residue

(Air-dried, reweighed, repacked)

Methanol (750 ml)

Methanol extract   Residue

(Air-dried, reweighed, repacked)

Water (750 ml)

Water extract   Residue

(Discarded)

Scheme – I: Successive extraction of fruit peel of *Opuntia elatior* Mill.

3.4.2 Qualitative evaluation of peel extracts and OFJ

The successive peel extracts and OFJ were subjected to various qualitative chemical tests to determine the presence of alkaloids, carbohydrate, fats and fixed oil, flavonoids, glycosides, phenolics, steroids and terpenoids, wax, saponin and betalains phytoconstituents (Kokate, 1996; Evans, 1996; Harborne, 2007).

3.4.3 TLC profile of OFJ

The OFJ was subjected to thin layer chromatography studies using silica gel 60 F 254 precoated plates (Alugram® SIL G/UV$_{254}$, Macherey – Nagel, Germany) to confirm presence of carbohydrates with reference to standard using solvent system chloroform:methanol (6:4) (Egon, 2007) and betalains using solvent system n-Butanol:Acetic acid:Water (BAW) (4:1:5) and 1 % Aqueous Hydrochloric acid (Harbone, 2007). The R$_f$ values and color of spot were observed and compared.

3.4.4 Qualitative analysis of betalain

3.4.4.1 Spectrophotometric analysis

Betalain from the fruits of *Opuntia elatior* Mill. was extracted using methanol, deionized water and methanol containing 1% conc. hydrochloric acid (HCl), with a ratio mass fruit (g)/solvent (ml) 1:5. Freshly cut fruit pulp was homogenized in these solvents for 1 min and centrifuged at 3000 g at 15 ºC for 10 min in a Compufuge cooling centrifuge (Remi Instrument, Mumbai). Supernatants were filtered through a glass filter G$_4$ (Borosil Glass Works Ltd., Mumbai), and the filtrate obtained were analyzed using spectrophotometer. The spectrum (400 to 650 nm) of methanol, deionized water and methanol HCl extracts was recorded on double beam UV-visible spectrophotometer (Thermo Spectronic, Cambridge, UK) equipped with VisionPro software V 4.10, spectral bandwidth of 2.0 nm, wavelength accuracy of ± 0.5 nm and a pair of 1 cm matched quartz cells. The identity of absorption spectra was confirmed by observed λ$_{max}$ (Farnandez-Lopez & Almela, 2001; Yahia & Castellanos-Santiago, 2008).

3.4.4.2 High performance liquid chromatographic (HPLC) analysis

Identification of betalains by HPLC-DAD analysis was performed in a Shimadzu HPLC-PDA system comprising of two LC-20 AD liquid pumps, automatic rinsing kit for LC-20 AD, Gradient mixer, 7725i manual injector, fixed –loop manual kit, 25 μl syringe, injector adapter, SPD-M20A photo diode array detector, and LC solution Multi PDA workstation software. Analyses were performed using an analytical scale of 25 cm x 4.6 mm i.d. and a C18 column with a particle size of 5 μm (Merck), operating at a temperature of 25 ºC. The program consisted of a 30 min linear gradient elution from solvent A (1% acetic acid in water) to 12 % solvent B (1% acetic acid in acetonitrile) with a flow of 1 ml/min. In each analysis, 20 μl of the methanolic extract was directly injected onto the chromatographic column. The betaxanthin and betacyanin composition of methanolic extract was confirmed by their visible spectral characteristics in comparison to literature and retention times of chromatographic peaks (Farnandez-Lopez & Almela, 2001).

3.4.4.3 Liquid chromatography – mass spectroscopic (LC-MS) analysis

HPLC – DAD was coupled with mass spectrometer (TSQ Quantum Ultra, Thermo Scientific, USA) equipped with an electrospray ionization source and LC-quan software (2.5.6.1) operating in the positive ionization mode. Nitrogen gas was used as the dry gas at a flow rate of 12 L/min with nebulizing (40 psi). The spectra was take to promote $[M+H]^+$ ion production (electrospray voltage 4 kV), and nebulizer temperature was set at 200 ºC. The program consisted of a 30 min linear gradient elution from solvent A (1% acetic acid in water) to 12 % solvent B (1% acetic acid in acetonitrile) with a flow of 1 ml/min. The injection volume for fruit extract sample was 20 μl. The identification of betanin was confirmed by mass spectrometry (Farnandez-Lopez & Almela, 2001).

3.4.5 Quantitative estimation of OFJ

3.4.5.1 Total sugar content

The total sugar content was determined by the anthrone reagent method (Plummer, 2006). The fruit juice was prepared as described in 3.3.3.2 and transferred 1.0 ml of filtered juice to the 10 ml volumetric flask, and diluted to 10.0 ml with distilled water, having strength 100µl/ml, labelled as stock solution. Stock solution (0.1 ml) was transferred into 10 ml volumetric flask, and diluted to 10 ml with distilled water, having strength 10µl/ml. The anthrone reagent was prepared right before analysis by dissolving 0.2 g of anthrone (0.2%) in 100 ml of concentrated sulfuric acid, protected from light and used within 12 h. Anthrone reagent (4.0 ml) was added cautiously to each tube containing 1.0 ml of standard solutions of glucose (10 – 100 µg/ml) and test solution (10µl/ml). Tubes were then placed 10 min at 5 ^{0}C. Subsequently, tubes were boiled 5 min on constant boiling water bath. After heating, allowed to cool at room temperature for 15 min. The absorbance of the colored solution was measured at 620 nm against reagent blank. Readings were taken in triplicate. Spectrophotometric response was compared to a standard calibration curve of glucose, and total sugar content was expressed as g/100 ml of glucose.

3.4.5.2 Total phenolic content

The amount of total phenolics in the extracts was determined according to a modification of the Folin-Ciocalteu method (Kujala et al., 2000). A 1.0 ml of fruit juice as described in 3.3.3.2 extracted in 10.0 ml methanol:water (50:50 v/v), having strength 100 µl/ml was introduced into test tube and mixed with 1.0 ml of 50% Folin-Ciocalteu's reagent. The mixture was allowed to stand for a 2 to 5 min period which was followed by the addition of 2.0 ml of 20% Na_2CO_3. After 10 min incubation at room temperature, the mixture was centrifuged for 8 min (150 g) and the absorbance of the supernatant was measured at 750 nm on a UV-visible spectrophotometer against reagent blank. Readings were taken in triplicate. Spectrophotometric response was compared to a standard calibration curve of gallic acid, and the total phenolic content was expressed as gallic acid equivalents in mg/100 ml.

3.4.5.3 Titratable acidity

The titratable acidity was determined by titration with $0.1N$ sodium hydroxide (NaOH) to pH 8.1 according to AOAC (1995). The glass electrode of calibrated pH meter was immersed in 100 ml of fruit juice as described in 3.3.3.2 in beaker. Initial pH values before titration with $0.1N$ NaOH was noted down. Stirred moderately and added alkali quite rapidly until near pH 6. Then add alkali slowly to pH 7. After pH 7 was reached, finish titration by adding $0.1N$ NaOH 4 drops at time, and record total volume and pH reading after each addition. Continue titration ≥4 drops beyond pH 8.1, and interpolate data for titration corresponding to pH 8.1. Readings were taken in triplicate. The titratable acidity was expresses in g of anhydrous citric acid/100 ml.

3.4.5.4 Total betacyanin content

Quantification of total betalain content was carried out according to Cai & Corke (1999) and Stintzing et al. (2003) nine times from pigment extracted in deionised water as described in 3.3.3.2 without pH adaptation applying the molar extinction coefficients of betacyanin (ε =60,000 L/mol cm in H_2O; λ=538 nm; MW=550 g/mol). The pigment extract was diluted with deionized water to obtain absorption values of $0.9 \leq A \leq 1.1$. The total betacyanin content was calculated using the equation: Betacyanin Contents [mg/l] = [(AxDFxMWx1000)/(εxL)], where A is the absorption at 538 nm for betacyanins. DF is the dilution factor and L the pathlength of the 1-cm cuvette. For MW and ε, the molecular weights and extinction coefficients of the representative compounds betanin have to be considered.

3.4.5.5 Elemental analysis

The metallic elements were analyzed by atomic absorption spectrometry (AAS) using air/acetylene flame (Walsh, 1955). Weigh about 30 ml of fruit juice as described in 3.3.3.2 accurately in a silica crucible. Dried and then ignited over a bunsen burner till it charred. Transferred to muffle furnace at 500 ± 50 °C for ashing till all carbonaceous matter burned and dull grey ash resulted. Cool and then treated with 25 ml $6N$ HCl to dissolve the ash. Heated it over steam bath for 15-20 min and transferred the ash solution quantitatively to 50 ml volumetric flask and make up the volume. The solution was filtered

to remove any acid-insoluble matter using Wattsman filter paper no. 14. The sample solution obtained was used for further estimation of metal elements *viz.* copper, iron, zinc, calcium, potassium and sodium. The atomic absorption spectroscopic analysis was carried out at Consumer Education and Research Centre (CERC), Ahmedabad.

Instrumental conditions:

A double beam Schimadzu (AA-6300) atomic absorption spectrophotometer (AAS) was used.

Table 3.1: Instrumental conditions for elemental analysis using AAS.

Instrumental conditions	Elements					
	Copper	**Iron**	**Zinc**	**Calcium**	**Potassium**	**Sodium**
Wavelength (nm)	324.8	248.3	213.9	422.7	766.5	589.0
Slit width (nm)	0.7	0.2	0.7	0.5	1.0	0.2
Flame type	Air – C_2H_2					
Gas flow rate (L/min)	1.6	2.2	2.0	1.7	1.7	1.8
Burner height (mm)	06	09	07	10	07	07

3.5 Pharmacological studies

3.5.1 Plant material

The fruit juice (OFJ) of *Opuntia elatior* Mill. was prepared as described in 3.3.3.2 and used for evaluation of haematinic, analgesic, anti-inflammatory and anti-asthmatic actions.

3.5.2 Animals

Albino wistar rats of either sex (180-250 g body weight) were used for this study. They were housed at ambient temperature (22 ± 1^0C), relative humidity ($55\pm5\%$) and 12h/12h light dark cycle. Animals had free access to Amrut brand rat pellet diet supplied by Pranav Agro Industry, Baroda, and water given *ad libitum*. The protocol of the experiment was approved by the Institutional Animal Ethical Committee (IAEC) as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, vide certificate no. IAEC/RBPMPC/09-10/01 dated 18/07/2009.

3.5.3 Acute toxicity study

Acute toxicity studies were performed for fruit juice according to the acute toxic classic method as per guidelines 423 prescribed by OECD (2001). Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water. Theses were divided into two groups of each containing five animals. Each of these groups was then administered with water and fruit juice as described in 3.3.3.2 of *Opuntia elatior* Mill. at the dose of 20 ml/kg p.o. The animals were observed for 30 min and then periodically for first 24 h special attention during first 4 h and thereafter daily for 14 days. The observations like sedation, convulsions, tremors, lethargy, death etc were systemically recorded with individual records of each animal.

3.5.4 Estimation of haematinic action

3.5.4.1 Mercuric chloride (HgCl₂) –induced anaemia

3.5.4.1.1 Experimental design

Haematinic action was evaluated by methods previously described by Rathore and Siddiqui (2000) and Sarkar et al. (2007) with some modification. Mercuric chloride (HgCl₂) dose was arrived at after carrying out initial pilot studies. The dose of OFJ was selected after carry out acute toxicity study. It was found that maximum dose (20 ml/kg, p.o.) was safe and based on that we had selected three different doses low (5 ml/kg), medium (10 ml/kg) and high (15 ml/kg) for this study. Animals were divided in seven groups (n = 6) as per followings and treated accordingly.

Group A: Negative control (saline solution for 60 days)

Group B: Positive control (HgCl₂ solution, 4 mg/kg, p.o., upto 30 days)

Group C: HgCl₂ + Standard ferrous sulphate containing drug Fefol®
            (0.0214 mg/kg, p.o., treatment started on day 31 upto day 60)
            (Pandit et al., 1999)

Group D5:   HgCl₂ + OFJ (5 ml/kg, p.o., treatment started on day 31 upto
            day 60)

Group D10:   HgCl₂ + OFJ (10 ml/kg, p.o., treatment started on day 31 upto
            day 60)

Group D15:   HgCl₂ + OFJ (15 ml/kg, p.o., treatment started on day 31 upto
            day 60)

Group E: OFJ (15 ml/kg, p.o., treatment started on day 31 upto day 60)

Mercuric chloride (4 mg/kg, p.o.) was given to each rat except to group A and E for 30 days to induce anemia. After mercuric chloride exposure, treatment was given for the next 30 days except in group A and B. Group B animals were allowed to recover naturally. Group E animals were treated with only fruit juice (15 ml/kg) for next 30 days. Haematological and biochemical parameters were estimated on 30th and 60th day. At the end of study, histopathological study of liver, kidney and spleen were evaluated.

3.5.4.1.2 Measurement of Body weight

The change of body weight in grams of each animal was recorded at 7-day intervals using ACCULAB digital balance, (Model No. ALC-310.3, Sartorius Mechatronics India Pvt. Ltd., Bangalore, India).

3.5.4.1.3 Measurement of Haematological Parameters

Blood samples were withdrawn from retro-orbital plexus under light ether anesthesia, collected in heparinized capillary tubes and analyzed for haematological parameters.

Haemoglobin (Hb) content (gm %) of each animal was estimated by Sahli's haemoglobinometer at 7-day intervals. When blood is added to 0.1 N hydrochloric acid, haemoglobin is converted to brown colored acid hematin. The resulting color after dilution is compared with standard brown glass reference blocks of a Sahli haemoglobinometer. By using a pasteur pipette added 0.1 N hydrochloric acid in the tube up to the lowest mark (20% mark). Blood was drawn up to 20 µl mark in the Hb-pipette. Blood column adjusted carefully without bubbles. Blood was transferred to the acid in the graduated tube; reaction mixture mixed and allowed the tube to stand for at least 10 minutes. The solution was diluted with distilled water by adding few drops at a time carefully and by mixing the reaction mixture, until the color matches with the glass plate in the comparator. The matching was done only against natural light. The level of the fluid was noted at its lower maniscus and the reading corresponding to this level on the scale was recorded in gm % of haemoglobin.

Haematological parameters like total red blood cells (RBC), total white blood cells (WBC), differential white blood cells, haematocrit, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelet count, mean platelet volume (MPV), platelet distribution width (PDW) and red blood cell distribution width (RDW) were estimated on fully automated fluorescence flow cytometry 5-part different analyzers (Sysmex XS800i, Japan).

3.5.4.1.4 Measurement of Biochemical Parameters

For collection of serum, blood samples were withdrawn from retro-orbital plexus under light ether anesthesia without any anticoagulant and allowed for 10 minutes to clot at room temperature. It was then centrifuged at 2500 g for 20 minutes. The serum obtained was kept at 4ºC until used.

3.5.4.1.4.1 Estimation of Blood Sugar

Serum glucose levels were determined by the glucose oxidase – peroxidase (GOD-POD) method using Bayer Diagnostics reagent kit (Ahmedabad, India).

Principal:

The analysis method is based on a principle that the glucose oxidase enzyme when added to serum sample and incubated at 37°C for 15 minutes, converts glucose to gluconic acid and hydrogen peroxide. The hydrogen peroxide is converted to water and oxygen by the enzyme peroxidase. 4-aminophenazone, an oxygen acceptor, takes up the oxygen and together with phenol forms a pink colored chromogen. The optical density (OD) at 530 nm of the color formed is directly proportional to the glucose level (Godkar & Godkar, 2004).

Procedure:

Pipette in the tubes labeled as follows;

	Blank	Standard	Sample
Working Reagent	2 ml	2 ml	2 ml
Distilled Water	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl

Mix and read the optical density (OD) after 10 minutes incubation. The final color is stable for at least 1 hour.

Calculations:

Serum Glucose (mg/dl) = Abs. of sample/ Abs. of Std. X 100

3.5.4.1.4.2 Kidney functions study

Estimation of serum urea and creatinine were carried out by using standard kit (Nicholas India Pvt. Ltd., Ahmedabad, India) with semi-auto analyzer (photometer 5010).

3.5.4.1.4.2.1 Estimation of Creatinine (Modified Jaffe Method)

In vitro quantitative determination of the activity of creatinine in serum was done using enzymatic kit (Nicholas India Pvt. Ltd., Ahmedabad, India).

Principle:

Creatinine forms a colored complex with picrate in alkaline medium. The rate of formation of the complex is measured.

Procedure:

➢ Prepare a 1:1 mixture of reagent 2 and reagent 3 (Mono reagent) at least 10 min. before starting assay. This mixture is stable for 5 days at 15^0C- 25^0C when stored in a dark bottle.

	Blank	Standard	Test
Distilled water	100 μl	-	-
Creatinine standard	-	100 μl	-
Test	-	-	100 μl
Monoreagent	1000 μl	1000 μl	1000 μl

➢ Mix and start stopwatch at the same time. After 30 sec. read absorbance (A_1) at 492 nm and exactly after another 120 sec. read absorbance (A_2).

A_{sample} as well as $A_{std} = A_2 - A_1$

Calculation: Creatinine (mg/dl) = 2 X A_{sample} / A_{std}

3.5.4.1.4.2.2 Estimation of Urea (Urease-Glutamate dehydrogenase: enzymatic UV test)

In vitro quantitative determination of the activity of urea in serum was done using enzymatic kit (Nicholas India Pvt. Ltd., Ahmedabad, India).

Procedure:

➢ Mix 4 parts of reagent 1 with 1 part of reagent 2 (Mono reagent). Leave the mono reagent for at least 30 min. at 15- 25^0C before use and protected from light.

	Blank	Standard	Test
Distilled water	10 μl	-	-
Urea standard	-	10 μl	-
Test	-	-	10 μl
Monoreagent	1000 μl	1000 μl	1000 μl

➢ Mix and start stopwatch at the same time. After 60 sec. read absorbance (A_1) at 340 nm and exactly after another 60 sec. read absorbance (A_2). A_{sample} as well as $A_{std} = A_2 - A_1$

Calculation: Urea (mg/dl) = A_{sample} / A_{std} X conc. of Std

3.5.4.1.4.3 Liver functions study

3.5.4.1.4.3.1 Estimation of Alkaline phosphatase (ALP)

In vitro quantitative determination of the activity of ALP in serum was done using enzymatic kit (Erba Diagnostic Germany Limited, Baroda, India).

Principle: Alkaline phosphatase (ALP) catalyses the hydrolysis of p-nitrophenyl phosphate at pH 10.4, liberating p-nitrophenol and phosphate, according to the following reaction:

p-Nitrophenyl Phosphate + H_2O $\xrightarrow{\text{ALP , Mg2+}}$ p-Nitrophenol + Phosphate.

The rate of p-nitrophenol formation, measured photometrically, is proportional to the catalytic concentration of alkaline phosphatase present in the sample.

Procedure:

Pipette	Volumes
Working reagent*	1000μl
Test (Serum)	20 μl

*Working reagent: add the amount of Aqua-4 indicated on the label to contents of each vial, swirl to dissolve. Mix well and allow standing for 60 seconds and reading the O.D. against purified water on Photometers at 405nm, take the 3 reading with the interval of 60 seconds.

Calculation: The general formula for converting absorbance change into International Units (IU) of activity is:

$$IU/L = \frac{(\Delta A/\min) \times T.V \times 10^3}{S.V \times \text{Absorptivity} \times P}$$

Where:

T.V   = Total reaction volume in µl

S.V   = Sample volume in µl

Absorptivity   = Milimolar Absorptivity of p- nitrophenyle phosphate at 405 nm=18.8

P   = Cuvette lightpath (cm) = 1 cm

3.5.4.1.4.3.2 Estimation of Bilirubin (Diazo method of Pearlman & Lee)

In vitro quantitative determination of the activity of Bilirubin (Total & Direct) in serum was done using enzymatic kit (Erba Diagnostic Germany Limited, Baroda, India).

Principle:

Bilirubin reacts with diazotized sulphanilic acid in acidic medium to form pink colored azobilirubin with absorbance directly proportional to Bilirubin concentration. Direct Bilirubin, being water soluble directly reacts in acidic medium. However indirect or unconjugated Bilirubin is solubilised using a surfactant and then it reacts similar to Direct Bilirubin.

Procedure:

Prepare Total Bilirubin reagent by adding 0.2ml of Sodium Nitrite Reagent to 10ml of Total Bilirubin. Direct Bilirubin reagent is prepared by adding 0.1ml of Sodium Nitrite Reagent to 10ml of Direct Bilirubin.

Blank Solution: To 500 µl of above working reagent add 25µl of distilled water.

Standard solution: To 500µl of above working reagent add 25µl of standard solution.

Test Solution: To 500 µl of above working reagent add 25µl of test solution of serum.

Mix well; incubate for 5 minutes at 37ºC. Read the absorbance at 546 nm for Total Bilirubin and 630 nm for Direct Bilirubin against reagent blank.

Calculation:

       Total Bilirubin (mg/dl): Abs. of Test $\times$ 23 (Factor)

       Direct Bilirubin (mg/dl): Abs. of Test $\times$ 17 (Factor)

       Indirect Bilirubin (mg/dl): TB – DB.

3.5.4.1.4.3.3 Estimation of Total Protein (Biuret method)

In vitro quantitative measurement of total protein concentration in serum was done by using kit (Span diagnostics India Pvt. Ltd., Ahmedabad, India).

Principle: Peptide bonds in protein react with cupric ion in alkaline solutions to form a colored chelate, the absorbance of which is measured at 578 nm. The Biuret reagent contains sodium-potassium tartarate to complex cupric ions and maintains their solubility at alkaline pH. Absorbance data is proportional to protein concentration.

Chemicals and reagents:

Biuret reagent: Copper sulphate, Sodium hydtroxide, Sodium-potassium tatarate, Surfactant.

Protein Standard: 6.5 mg/dl

Procedure: Prepare blank, test and standard solution as follow

Blank solution: 3ml of Biuret reagent

Standard solution: Add 30μl of Total Protein standard solution to 3 ml of Biuret reagent.

Test Solution: Add 30μl serum to 3ml of Biuret reagent.

Mix well and incubate at 37ºC or at RT for 5 minutes. Read the absorbance at 578 nm against reagent blank.

Calculations: Total Protein (gm/dl) = Abs. of Test/ Abs. of Std. X 6.5

3.5.4.1.4.4 Estimation of Total Cholesterol (CHOD-PAP method)

In vitro quantitative determination of the activity of cholesterol in serum was done using enzymatic kit (Nicholas India Pvt. Ltd., Ahmedabad, India).

Principle:

Cholesterol esterase (CHE) hydrolyses cholesterol ester. Free cholesterol is oxidized by the cholesterol oxidize (CHO) to choloest-4-ene-3-one and hydrogen peroxide. Hydrogen peroxide reacts with 4-aminoantipyrine and phenol in the presence of peroxidase (POD) to produce pink colored quinoneimine dye. The intensity of color produced is proportional to cholesterol concentration.

Procedure:

➢ Pipette into 3 test tubes labeled Blank (B), Standard (S) and Total Cholesterol (Tc) as shown below;

	Blank	Standard	Test
Distilled water	10 μl	-	-
Cholesterol standard	-	10 μl	-
Serum sample	-	-	10 μl
Reagent-1	1000 μl	1000 μl	1000 μl

➤ Mix, incubate for 5 min. at 37°C and Read the absorbance of standard, total cholesterol against Blank at 505 nm.

Calculations: Total Cholesterol (mg/dl) = Abs. of Test / Abs. of Std X 200

3.5.4.1.4.5 Estimation of Triglyceride (GPO Method)

In vitro quantitative measurement of triglyceride concentration in serum was done by using kit (Nicholas India Pvt. Ltd., Ahmedabad, India).

Principle:

Triglycerides in the sample are hydrolyzed by microbial lipase to glycerol and free fatty acid (FFA). Glycerol is phosphorylated by adenosine 5-triphosphate (ATP) to glycerol 3-phosphate (G-3-P) in reaction catalyzed by glycerol kinase (GK). G-3-P is oxidized to dihydroxy acetone phosphate in a reaction catalyzed by the enzyme glycerol phosphate oxidase (GPO). In this reaction hydrogen peroxide (H_2O_2) is produced in equimolar concentration to the level of triglycerides present in the sample. H_2O_2 reacts with 4-aminoantipyrine and ADPS in the reaction catalyzed by peroxidases (POD). The result of this oxidative coupling is a quinoneimine purple colored dye.

The absorbance of this dye in solution is proportional to the concentration of triglycerides in sample.

Procedure:

	Blank	Standard	Test
Distilled water	10 μl	-	-
Standard reagent	-	10 μl	-
Serum sample	-	-	10 μl
Reagent-1	1000 μl	1000 μl	1000 μl

> ➢ Mix, incubate for 5 min. and read the absorbance of test and standard against reagent blank at 546 nm.

Calculations: Triglycerides (mg/dl) = Abs. of Test/ Abs. of Std. X 50

3.5.4.1.5 Histopathology of Liver, Kidney and Spleen

On 60th day rats were sacrificed by spinal dislocation method under light ether anesthesia and livers, kidneys and spleens were collected for histopathological study. Tissues were fixed in 10% neutral-buffered formalin solution were embedded in paraffin and used for histopathological examination. Tissues sections (4 - 5 μm) were cut on a microtome and taken on glass slides coated with albumin. The hematoxyline-stained sections were stained with eosin for two minutes and quickly passed through ascending grades of alcohol, cleaned by xylene, and mounted on Canada Balsam. The stained sections were examined under an Olympus BX 40 photomicroscope and photographed. The samples were either coded to perform a blind study or expert guidance was sought from a veteran pathologist to determine histopathological changes. (Barnard et al., 1990; Godkar & Godkar, 2004; Benjamin et al., 2006).

3.5.4.2 Phenylhydrazine-induced anaemia
3.5.4.2.1 Experimental design

Anemia was induced by intraperitoneal (i.p.) injection of phenylhydrazine hydrochloride (PHZ) at 40 mg/kg for 2 days (Diallo et al., 2008). The dose of OFJ was selected as per our previous study. Animals were divided in seven groups (n=6) as per followings and treated accordingly.

Group A: Negative control (saline solution for 15 days)

Group B: Positive control (PHZ, 40 mg/kg, i.p., for 2 days)

Group C: PHZ + Standard ferrous sulphate containing drug Fefol[®]
　　　　　　(0.0214 mg/kg, p.o., treatment started on day 3 upto day 15)
　　　　　　(Pandit et al., 1999)

Group D5: PHZ +OFJ (5 ml/kg, p.o., treatment started on day 3 upto day 15)

Group D10: PHZ+OFJ (10 ml/kg, p.o., treatment started on day 3 upto day 15)

Group D15: PHZ+OFJ (15 ml/kg, p.o., treatment started on day 3 upto day 15)

Group E: OFJ (15 ml/kg, p.o., treatment started on day 3 upto day 15)

Phenylhydrazine hydrochloride solution was prepared in dimethyl sulfoxide and injected intraperitoneally for 2 days except group A and group E. The vehicle, standard drug and fruit juice were administered orally from day 2 to day 15 after phenylhydrazine administration. Group B animals were allowed to recover naturally. Group E animals treated only 15 ml/kg fruit juice as a high dose to check the effect of fruit juice in normal condition.

3.5.4.2.2 Measurement of Body weight

The change of body weight in grams of each animal was recorded at day 0, 3, 7, 10 and 15 using ACCULAB digital balance, (Model No. ALC-310.3, Sartorius Mechatronics India Pvt. Ltd., Bangalore, India).

3.5.4.2.3 Measurement of Haematological Parameters

Haematological parameters were estimated on day 0, 3, 7, 10 and 15 in phenylhydrazine –induced anaemia. Blood samples were withdrawn from retro-orbital plexus under light ether anesthesia, collected in heparinized capillary tubes and analyzed for haematological parameters.

Haematological parameters like haemoglobin (gm%), total red blood cells (RBC), total white blood cells (WBC), differential white blood cells, haematocrit, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelet count, mean platelet volume (MPV), platelet distribution width (PDW) and red blood cell distribution width (RDW) were estimated using methods described in section 3.5.4.1.3.

3.5.4.2.4 Study of the Reticulocytes

At the days 0, 3, 7, and 15, slides of blood cells were made and stained by cresyl blue and percentage of reticulocytes was determined on the whole red blood cells (Diallo, A. et al., 2008).

3.5.4.2.5 Measurement of Biochemical Parameters

Biochemical parameters were estimated on day 0, 3, 7, 10 and 15 in phenylhydrazine –induced anaemia. For collection of blood and serum separation, blood samples were withdrawn from retro-orbital plexus under light ether anesthesia without any anticoagulant and allowed for 10 minutes to clot at room temperature. It was centrifuged at 2500 g for 20 minutes. The serum obtained was kept at 4ºC until used.

Level of serum glucose, urea, creatinine, alkaline phosphatase (ALP), bilirubin, total protein, total cholesterol and triglycerides were estimated as per methods described in section 3.5.4.1.4.

3.5.4.2.6 Estimation of Ferritin (ELISA method)

In vitro serum ferritin was estimated on day 0, 3, 7, 10 and 15 using Enzyme Linked Immunosorbent Assay (ELISA) kit (Diagnostic Automation, Inc., Calabasas, CA 91302).

Principle:

The Ferritin Quantitative Test is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes one rabbit anti-ferritin antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 45-minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of Tetramethylbenzidine (TMB) is added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1 N HCl, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of ferritin is directly proportional to the color intensity of the test sample.

Kit content

1. Microtiter strips: Coated with rabbit anti-ferritin.
2. Standard: Containing human liver or spleen ferritin in bovine serum with preservatives.
3. Enzyme Conjugate Reagent: Mouse monoclonal anti-ferritin antibody, conjugated to horseradish peroxidase.
4. TMB Reagent: Contains 3, 3', 5, 5' tetramethylbenzidine, stabilized in buffer solution.
5. Stop Solution: Contains 1 *N* hydrochloric acid (HCl).

Procedure:

1. Secure the desired number of coated wells in the holder.

2. Dispense 20μl of standard, specimens, and controls into appropriate wells.

3. Dispense 100μl of Enzyme Conjugate Reagent into each well.

4. Thoroughly mix for 30 seconds. It is very important to have completed mixing in this setup.

5. Incubate at room temperature (18-22 ºC) for 60 minutes.

6. Remove the incubation mixture by flicking plate content into a waste container.

7. Rinse and flick the microtiter wells 5 times with washing buffer (1X).

8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

9. Dispense 100μl of TMB solution into each well. Gently mix for 5 seconds.

10. Incubate at room temperature in the dark for 20 minutes.

11. Stop the reaction by adding 100μl of Stop Solution to each well.

12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

13. Read optical density at 450nm with a microtiter reader within 30 minutes.

Calculation:

Calculate the mean absorbance value (A450) for each set of reference standards, controls and samples. Standard curve was constructed by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical axis and concentrations on the horizontal axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of Ferritin in ng/ml from the standard curve.

3.5.4.2.7 Histopathology of Liver, Kidney and Spleen

Histopathology of liver, kidney and spleen were performed at the end of the study as per method described in section 3.5.4.1.5.

3.5.5 Antinociceptive tests

3.5.5.1 Writhing test

Abdominal constriction induced by intraperitoneal injection of acetic acid (0.75%) was carried out according to the procedures described previously by Koster et al. (1959). The fruit juice (OFJ) was tested at dose 5, 10, and 15 ml/kg. Diclofenac sodium, a reference peripheral analgesic compound, was used at 10 mg/kg. OFJ and reference substance were injected intraperitoneally (i.p.) 30 min before the intraperitoneal administration of acetic acid (0.75%, 8 ml/kg). Control animals received saline solution under the same experimental conditions (8 ml/kg, i.p.). Each group was composed of six rats. Immediately after the injection of the algic compound, each animal was isolated in an individual box and the numbers of constrictions were cumulatively counted during a period of 20 min, after 5 min acetic acid injection. The number of writhings and stretchings was recorded and the percentage protection was calculated by following equation:

Percentage of protection = $\dfrac{\text{Control mean} - \text{Treated mean}}{\text{Control Mean}}$ X 100

3.5.5.2 Tail immersion test

Tail immersion test was conducted as described by Aydin et al. (1999). This involved immersing extreme 3 cm of the rat's tail in a water bath containing water at a temperature of 55±0.5 ℃. Within a few minutes, the rats reacted by withdrawing the tail. The reaction time was recorded with a stopwatch. Each animal served as its own control and two readings were obtained for the control at 0 and 10 min interval. The average of the two values was the initial reaction time (Tb). The test groups were given OFJ (5, 10 and 15 ml/kg, p.o.), tramadol (10 mg/kg, p.o.). The reaction time (Ta) for the test groups was taken at intervals 0.5, 1, 2, 4, 5 and 6 h after a latency period of 30 min following the administration of the OFJ and tramadol (Vogel and Vogel, 1997).

3.5.6 Anti-asthmatic action

3.5.6.1 Bronchospasm induced by Acetylcholine and Histamine in guinea pigs

To screen the sensitivity of guinea pigs, guinea pigs of both sex (350–400 g) were placed in a plexiglass chamber and sprayed with 0.25% histamine and 0.5% acetylcholine chloride under the average pressure of 45 ± 5 mmHg for 15 s. The time to onset of respiratory distress (preconvulsive time) during challenge with these agents was measured; the guinea pigs with preconvulsive time of more than 120 s were considered to be insensitive and discarded (Xiangping Chu et al., 2007).

The eligible guinea pigs were randomly divided into six groups each containing six animals. The OFJ and standard drugs were administered orally. The single dose treatments were given one and half an hour before the study. The following schedule of treatment was administered:

Group	Treatment	Dose (p.o.)
A	Distilled water	1 ml/kg
B	Ketotifen	1 mg/kg
C	Atropine sulfate	2 mg/kg
D	OFJ	5 ml/kg
E	OFJ	10 ml/kg
F	OFJ	15 ml/kg

The method of histamine challenge was same as those of screening the sensitive guinea pigs and time for preconvulsion state was noted for each animal. After about 15 days of wash out period, the same animals were given the above treatments and time for preconvulsion state was noted for 0.5% acetylcholine chloride aerosol spray (Shah and Parmar, 2003).

3.5.6.2 Anticholinergic action on isolated rat ileum

Albino wistar rats of either sex were sacrificed by a blow to the head followed by exsanguination. A portion of ileum was removed and placed in oxygenated tyrode solution at room temperature. The connective tissue was carefully

trimmed from the ileum tissue, suspended in tyrode solution at 37 ^{0}C and bubbled with 95% oxygen and 5% carbon dioxide. Intestinal segments of about 20 mm length were prepared from the terminal ileum of the rat. They were cleaned and suspended in organ bath containing tyrode solution, aerated with 95% oxygen and 5% carbon dioxide. Prior to the measurements, the tissue was allowed to stabilize for 30 min under a resting tension of 500 mg. The longitudinal contraction was measured according to the method of Sheth at al. (1972), the concentration – response curve of acetylcholine was recorded using graded dose of acetylcholine till the maximum response is obtained with contact time of 30 seconds. Then second concentration response curves were obtained, in the presence of the test substance in the organ bath. A 1 ml of different concentration of OFJ test solutions (10, 50, 100 µl/ml) were added directly to the organ bath 3 min before the addition of the acetylcholine.

3.5.6.3 Antihistaminic action of isolated Guinea pig ileum

Guinea pigs were fasted for 24 h and later were sacrificed and a piece of ileum was isolated. The tissues were quickly transferred to petri dishes containing tyrode solution. The ileum was mounted in an organ bath maintained at 37 °C and containing tyrode solution. A basal tension of 500 mg was applied to both the tissues throughout the experiment. After stabilization for 45 min the tissues were exposed to graded doses of histamine and contractions were recorded. The responses to these standard drugs were re-elicited after exposing the tissue to different concentration of OFJ test solutions (10, 50, 100 µl/ml) for 3 min. After eliciting the response the tissues were washed-out thoroughly before proceeding for next response (Sheth et al., 1972).

3.5.6.4 Egg albumin induced mast cell degranulation test

A modification of the method described by Kanemoto et al. (1993) was used for egg albumin mast cell degranulation. Albino rats were sensitized by administering egg albumin (1 ml, 10%w/v) intraperitoneally as well as subcutaneously on the first, third, fifth and on the twelfth day of first egg albumin administration. At the end of experiment, rats were sacrificed by spinal dislocation under light ether anesthesia. The peritoneal cavity was lavaged with 10 ml of tyrode solution. The lavaged fluid was collected and

centrifuged at 2000 g for 5 min. The pellet was separated, washed with tyrode solution and finally resuspended in 1 ml tyrode solution. A 0.1 ml of this lavage fluid was transferred to 10 test tubes. The lavage fluid was then subjected to following treatment schedule.

Test Tube No. 1:   Negative Control

Test Tube No. 2:   Positive Control

Test Tube No. 3:   0.1 ml (10 μg/ml) of Sodium Cromoglycate

Test Tube No. 4:   0.1 ml (10 μg/ml) of Ketotifen

Test Tube No. 5:   0.1 ml (10 μl/ml) of OFJ

Test Tube No. 6:   0.1 ml (20 μl/ml) of OFJ

Test Tube No. 7:   0.1 ml (40 μl/ml) of OFJ

Test Tube No. 8:   0.1 ml (60 μl/ml) of OFJ

Test Tube No. 9:   0.1 ml (80 μl/ml) of OFJ

Test Tube No. 10:   0.1 ml (100 μl/ml) of OFJ

Test Tube No. 11:   0.1 ml (200 μl/ml) of OFJ

Each test tubes were incubated for 10 min at 37 °C and then egg albumin (0.1 ml, 1 mg/ml) was added to each test tube except test tube no. 1. After further incubation for 10 min at 37 °C, 0.1 ml of 10% toluidine blue was added and examined under light microscope with 450 X magnification. A minimum of 100 cells was counted for intact and disrupted mast cells and from it percentage protection from degranulation was calculated.

3.5.6.5 Compound 48/80 induced mast cell degranulation

A modification of the method described by Kanemoto et al. (1993) was used for compound 48/80 induced mast cell degranulation. Albino wistar rats were sacrificed by spinal dislocation under light ether anesthesia. The peritoneal cavity was lavaged with 10 ml of Tyrode solution. The lavaged fluid was collected and centrifuged at 2000 g for 5 min. The pellet was separated, washed with tyrode solution and finally resuspended in 1 ml tyrode solution. A 0.1 ml of this lavage fluid was transferred to 10 test tubes. The lavage fluid was then subjected to following treatment schedule.

Test Tube No. 1:   Negative Control

Test Tube No. 2:   Positive Control

Test Tube No. 3:   0.1 ml (10 μg/ml) of Sodium Cromoglycate

Test Tube No. 4:   0.1 ml (10 μg/ml) of Ketotifen

Test Tube No. 5:   0.1 ml (10 μl/ml) of OFJ

Test Tube No. 6:   0.1 ml (20 μl/ml) of OFJ

Test Tube No. 7:   0.1 ml (40 μl/ml) of OFJ

Test Tube No. 8:   0.1 ml (60 μl/ml) of OFJ

Test Tube No. 9:   0.1 ml (80 μl/ml) of OFJ

Test Tube No. 10:   0.1 ml (100 μl/ml) of OFJ

Test Tube No. 11:   0.1 ml (200 μl/ml) of OFJ

Each test tubes were incubated for 10 min at 37 °C and then compound 48/80 (0.1 ml, 10 μg/ml) was added to each test tubes except test tube no. 1. After further incubation for 10 min at 37 °C, 0.1 ml of 10% toluidine blue was added and examined under light microscope with 450 X magnification. A minimum of 100 cells was counted for intact and disrupted mast cells and from it percentage protection from degranulation was calculated.

3.5.6.6 Carrageenan-induced rat paw edema

Pedal inflammation in rats was produced according to the method described by Winter et al. (1962). Following an overnight fast, OFJ (5, 10 and 15 ml/kg, p.o.) were administered to animals in different groups using an oral cannula. At the same time, animals in the reference standard group received diclofenac sodium (10 mg/kg, p.o.), while animals in the control group received saline solution (10 ml/kg, p.o.). One hour later, an injection of carrageenan (1%, 0.1 ml) was made into the right hind limb of each rat under the subplantar aponeurosis. Measurement of paw size in cm was done by wrapping as piece of cotton thread, round the paw of each rat and measuring the circumference on a meter rule (Hess & Milonig, 1972; Olajide et al., 1999). This was done immediately before, and at hourly intervals for 5 h after carrageenan injection. Inhibitory activity was calculated at every one hour interval following carrageenan injection using the formula:

Percentage Inhibition = $\dfrac{[(C_t - C_o)\ \text{control} - (C_t - C_o)\ \text{treated}]}{(C_t - C_o)\ \text{control}} \times 100$

Where C_t is paw size at time (t) after carrageenan injection and C_o is paw size before carrageenan injection.

3.5.6.7 Neutrophil adhesion test

The study was carried out as described by Wilkinson (1978) & Ghule et al., (2006). Albino wistar rats of either sex were used for the study. The animals were randomly divided in four groups (n =6) and treated accordingly.

Group A: Control (treated with vehicle, p.o., for 14 days)

Group B: OFJ (5 ml/kg, p.o., for 14 days)

Group C: OFJ (10 ml/kg, p.o., for 14 days)

Group D: OFJ (15 ml/kg, p.o., for 14 days)

On day 14, blood samples were collected from the retro orbital plexus into heparinized vials and analyzed for total leukocyte count (TLC). The differential leukocyte count (DLC) was performed by fixing the blood smears and staining with leucofine and percent neutrophils in each sample was determined. After the initial counts, blood samples were incubated with 80 mg/ml of nylon fibers for 10 min at 37ºC. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and percent neutrophils gave the neutrophil index of blood sample. Percent neutrophil adhesion was calculated from the following formula:

$$\text{Neutrophil adhesion (\%)} = \frac{NIu - NIt}{NIu} \times 100$$

Where NIu= Neutrophil index of untreated blood sample

NIt= Neutrophil index of treated blood sample

3.5.7 Statistical analysis

All the values are expressed as Mean ± SEM (standard error of mean). The data were analyzed by one way ANOVA followed by Turkey's multiple comparison tests. A level of $p < 0.05$ was considered as statistically significant. A level of significance was noted and interpreted accordingly. Regression analyses were used to calculate ED_{50} for antinociceptive test and carrageenan –induced paw edema.

3.6 Antimicrobial assay of peel extracts

3.6.1 Test microorganisms

Following test microorganisms including Gram-negative, Gram-positive bacteria and fungus were used to study antimicrobial action of peel extracts of fruits of *O. elatior* Mill.

Table 3.2: List of organisms used for antimicrobial assay of peel extracts.

Test Organisms	Type	ATCC No.	NCTC No.
Micrococcus luteus	Gram - positive	9341	-
Bacillus pumillus		14884	-
Pseudomonas aeruginosa	Gram - negative	25619	-
E. coli		9002	-
Salmonella abony		-	6017
Candida albicans	Fungi	-	-
Aspergillus niger		-	-
Saccharomyces cerevisiae		14884	-

3.6.2 Preparation of test organism suspension

Test organism was maintained on slants of medium containing 300 mg of manganese sulphate per liter and transferred to fresh slant once a week. Then the slants of bacteria and fungi were incubated at temperature 37°C and 28°C for 24 h, respectively. Fungi were then scraped by using 3 ml saline solution from agar slant on to a large agar surface of medium such as Roux bottle containing 250 ml agar and incubated for 24 h. The growth of bacteria from the nutrient surface was washed using 50 ml saline solution. Then organism was stored under refrigeration. Inoculum was adjusted at 530 nm, leading to transmission equivalent to 1×10^8 cells/ml (Jain et al., 2007; Anonymous, 1996).

3.6.3 Antimicrobial assay

Antimicrobial activity of the peel extracts was determined by using cup plate method (Anonymous, 1996). Muller Hinton agar was used for the growth of bacterial strains and Potato Dextrose agar was used for the growth of fungi. Peel extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10, 20, 40, 60, 80, 100 µg/ml. The standard antibacterial and antifungal solution containing 100 µg/ml amoxycillin, ceftrioxone and fluconazole were prepared. Each plate was inoculated with 20 µl microbial suspensions having a concentration of 1 x 10^8 cells/ml. An aliquot (0.1 ml) of peel extracts were added to each cup. The plates containing bacteria were incubated at 37°C for 24h and those containing fungi were incubated at 28°C for 7 days. The positive antimicrobial activity was observed based on growth inhibition zone and compared with the standard drugs.

4. Results

4. Results

Sr No.	Title	Page No.
4.1	**Pharmacognostical study**	**120**
4.1.1	Macroscopic examinations	120
4.1.2	Proximate analysis	122
4.2	**Phytochemical studies**	**122**
4.2.1	Fruit peel extracts	122
4.2.2	Qualitative Chemical test	123
4.2.3	TLC profile of OFJ	123
4.2.4	Qualitative analysis of betalains	127
4.2.4.1	Spectrophotometric analysis	127
4.2.4.2	High performance liquid chromatographic (HPLC) analysis	128
4.2.4.3	Liquid chromatography – mass spectroscopic analysis	128
4.2.5	Quantitative estimation of OFJ	130
4.2.5.1	Total sugar content	130
4.2.5.2	Total phenolic content	130
4.2.5.3	Titratable acidity	131
4.2.5.4	Total betalains content	131
4.2.5.5	Elemental analysis	131
4.3	**Pharmacological studies**	**132**
4.3.1	**Acute toxicity study**	**132**
4.3.2	**Haematinic study**	**132**
4.3.2.1	**Mercuric chloride –induced anaemia**	**132**
4.3.2.1.1	Effect on Body weight	132
4.3.2.1.2	Effect on Haemoglobin	134
4.3.2.1.3	Effect on Haematological parameters	136
4.3.2.1.4	Effect on Total and Differential White Blood Cell count	136
4.3.2.1.5	Effect on Platelets	141
4.3.2.1.6	Effect on Blood sugar, Cholesterol and triglyceride content	141

4. Results

4.1 Pharmacognostical study

4.1.1 Macroscopic examinations

Opuntia elatior Mill. is xerophytic subarborescent or shrubby cacti, 3 meter high or more with ovate – oblong limbs. Herbarium was prepared in 1 % v/v formalin and preserved in the museum (figure 4.1). Phylloclade is an aerial modification (metamorphoses) of stem, having dimension (Length: 20 – 25 cm; Width: 12 – 15 cm; Thickness: 1 – 1.5 cm), broadly ovate to oblong in shape and greenish in color. Areoles bearing about 4-5 increasing up to 10, rather slender straight prickles which are grey and yellow when young, the largest was 3-5 cm long. Mature prickles are 3 – 5 cm long and young are 0.5 to 1.5 cm long; glochidia inconspicuous, almost hidden amongst woolly hairs, rusty-brown. Flowers are 4 – 6 cm long arise from areoles initially yellow coloured, turns pink upon maturation. Mature flowers comprises of perianth with pink color in centre and yellowish at the edge. Perianth is circular and outer segment short, ovate, acute, inner spathulate and acute. Stamens a little shorter than the perianth. Style exceeding the stamens; stigma 6. Fruits are berry pyriform, angular bearing tufts of glochidia and occasionally a few prickles, reddish purple when ripe and greenish when unripe. It is indehiscent, many seeded pulpy fruit. Depressed at the apex having dimensions (Length: 4 – 6 cm; Width: 3.3 – 3.7 cm) (Figure 4.2).

Figure 4.1: Photographs of plant *Opuntia elatior* Mill. (A) and Herbarium (B).
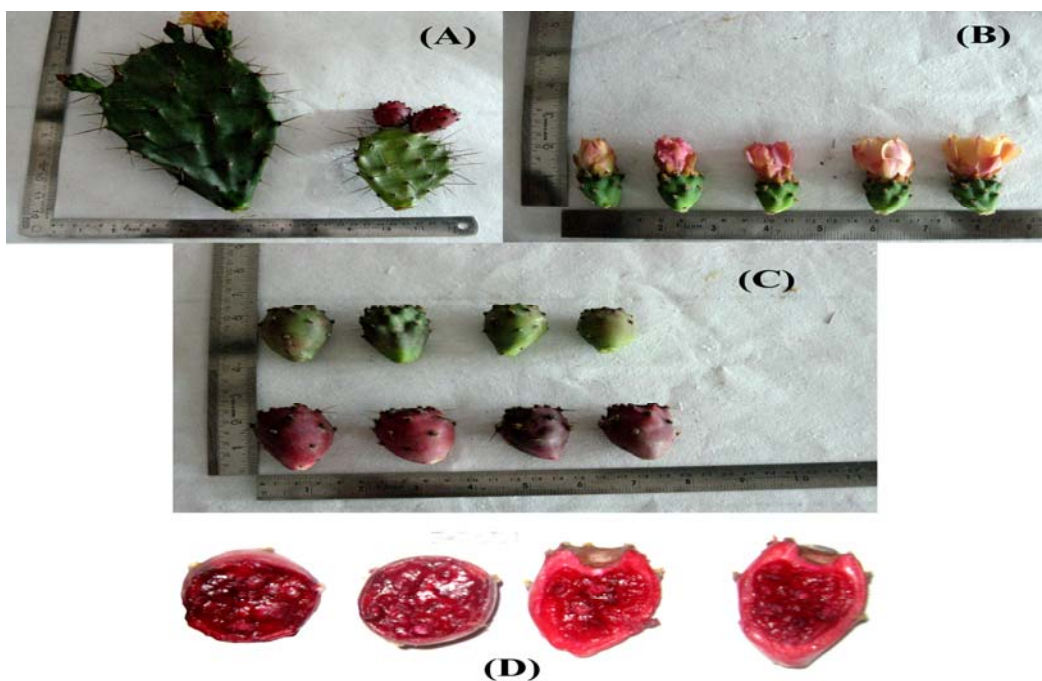
Figure 4.2: Macroscopic characters of phylloclade (A), flowers (B), ripe and unripe fruits (C) and transverse and longitudinal sections of fruit (D) of *Opuntia elatior* Mill.

4.1.2 Proximate analysis

Table 4.1 shows the results obtained from various determinations of proximate analysis. The average weight of fruit , percentage of peel, pulp and seed were found 24.568 ± 7.134 g/unit, 5.344 ± 2.150%, 94.655 ± 2.150%, 3.835 ± 0.828% (mean ± SD), respectively.

Table 4.1: Proximate analysis of fruit of *Opuntia elatior* Mill.

Parameters	Mean ± SD
Total ash	0.579 ± 0.164 % w/w
Acid insoluble ash	11.9 ± 3.4 % w/w
Water soluble ash	23.1 ± 1.43 % w/w
Moisture content	86.086 ± 1.23 % w/w
Total solids	13.913 ± 1.23 % w/w
pH	4.583 ± 0.0411
Density	1.10063816 ± 0.007565 g/cm^3
Viscosity	53.62 ± 0.402 centipoise

4.2 Phytochemical studies

4.2.1 Fruit peel extracts

The dried peel of fruits was subjected to successive solvent extraction using solvents in the order of increasing polarity. The average percentage yield and physical properties of the peel extracts so obtained are presented in table 4.2. Maximum percentage yield was obtained in methanolic peel extract (23.37 ± 4.58 %).

Table 4.2: Percentage yield and physical properties of the peel extracts.

Peel Extracts	Color & Consistency	Percentage Yield (% w/w) (Mean ± SD, n=5)
Petroleum ether (60 – 80 ºC)	Yellow sticky	4.42 ± 2.07
Benzene	Brown sticky	1.98 ± 0.90
Chloroform	Light brown sticky	2.13 ± 0.72
Methanol	Brown solid	23.37 ± 4.58
Water	Brown solid	18.16 ± 7.41

4.2.2 Qualitative Chemical test

Presence of phytoconstituents in peel extracts and OFJ are presented in table 4.3. Methanol and water extract of peel and OFJ contained carbohydrate, flavonoids and betalains in abundant amount.

Table 4.3: Presence of phytoconstituents in peel extracts and OFJ.

Class of compounds	Peel Extracts					OFJ
	Pet. Ether $(60^{0} - 80^{0}C)$	Benzene	Chloroform	Methanol	Water	
Alkaloids	-	-	-	-	-	-
Carbohydrate	-	-	-	+	+	+
Fats & fixed oil	+	-	+	-	-	-
Flavonoids	-	+	+	+	+	+
Glycosides	-	-	-	-	-	-
Phenolics/ Tannins	-	-	-	+	+	+
Steroids & Terpenoids	+	+	-	+	-	-
Waxes	+	+	+	-	-	-
Saponins	-	-	-	+	+	-
Betalains	-	-	-	+	+	+

- : Absent, +: Present

4.2.3 TLC profile of OFJ

The OFJ was subjected to identify the carbohydrates presence of standard compounds like D-Xylose (X), L-Arabinose (A), D-Fructose (F), D-Galactose (Gal), Maltose (M), Lactose (L), Glucose (Glu) and Sucrose (S). Results are summarized in table 4.4. R_f values of these compounds were compared with the OFJ (figure 4.3). A comparison of R_f values and spot color obtained in TLC of carbohydrates, it can be concluded that the fruit juice contained reducing hexose monosaccharides like galactose and glucose.

The presence of betalains was confirmed by TLC using solvent system *n*-Butanol:Acetic acid:Water (BAW) (4:1:5) and 1 % aqueous hydrochloric acid (figure 4.5). The R_f values found very low (0.081 ± 0.004) in BAW and high in 1% Aq. HCl (0.916 ± 0.030) which indicates presence of betacyanin. R_f values expressed in mean ± SD (n = 5).

Table 4.4: Identification of carbohydrates from OFJ.

Class of compounds	Solvent system	Detection reagent	Color of spot
Carbohydrates	Chloroform :Methanol (6:4)	α-Naphthol sulphuric acid	All spots are in blue color (figure 4.4 A)
		Aniline-diphenylamine-phosphoric acid	X & A (Bright blue), F (Red), L (Blue) others are in bluish violet in color (figure 4.4 B)
		Phenol-sulphuric acid	X & A (Red brown), F (Dark green), Gal (Grey yellow), L (Green to black), Glu (Bluish violet), S & M (Dark green), Juice (Greenish brown) in color (figure 4.4 C)
		Anthrone reagent	X (Light green), F & S (Dark greenish yellow) and others are trace of greenish yellow in color (figure 4.4 D)
		Thymol-sulphuric acid	All spots are pink in color (figure 4.4 E)
		p-Anisidine phthalate reagent	All spots are in brownish in color while juice showed tailing at the lower edge (figure 4.4 F)
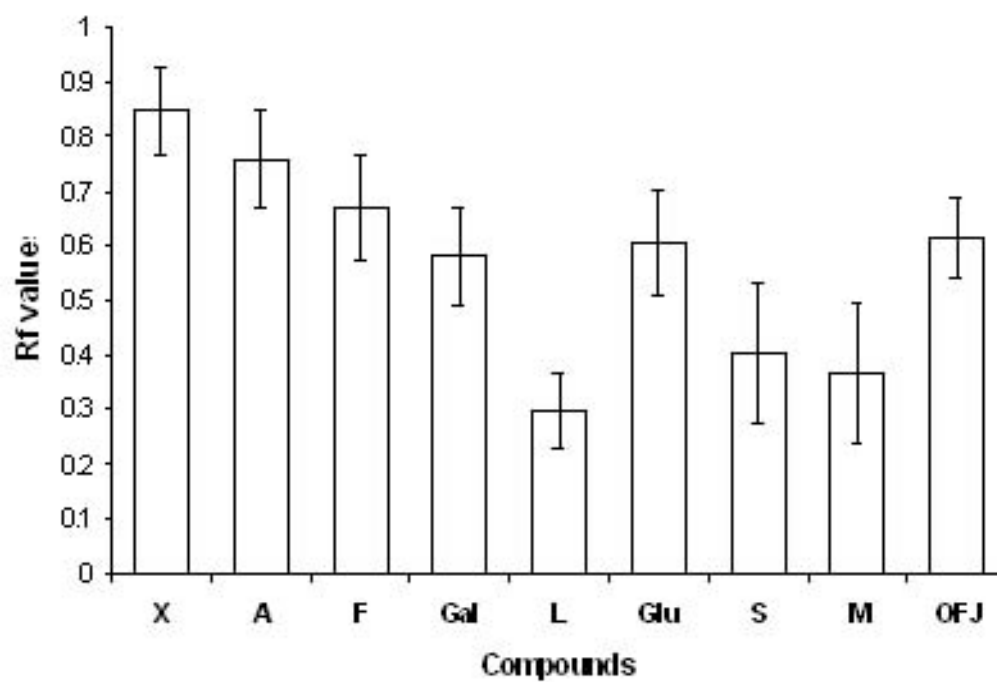
Figure 4.3: Comparison of R_f values of standard sugars and OFJ.
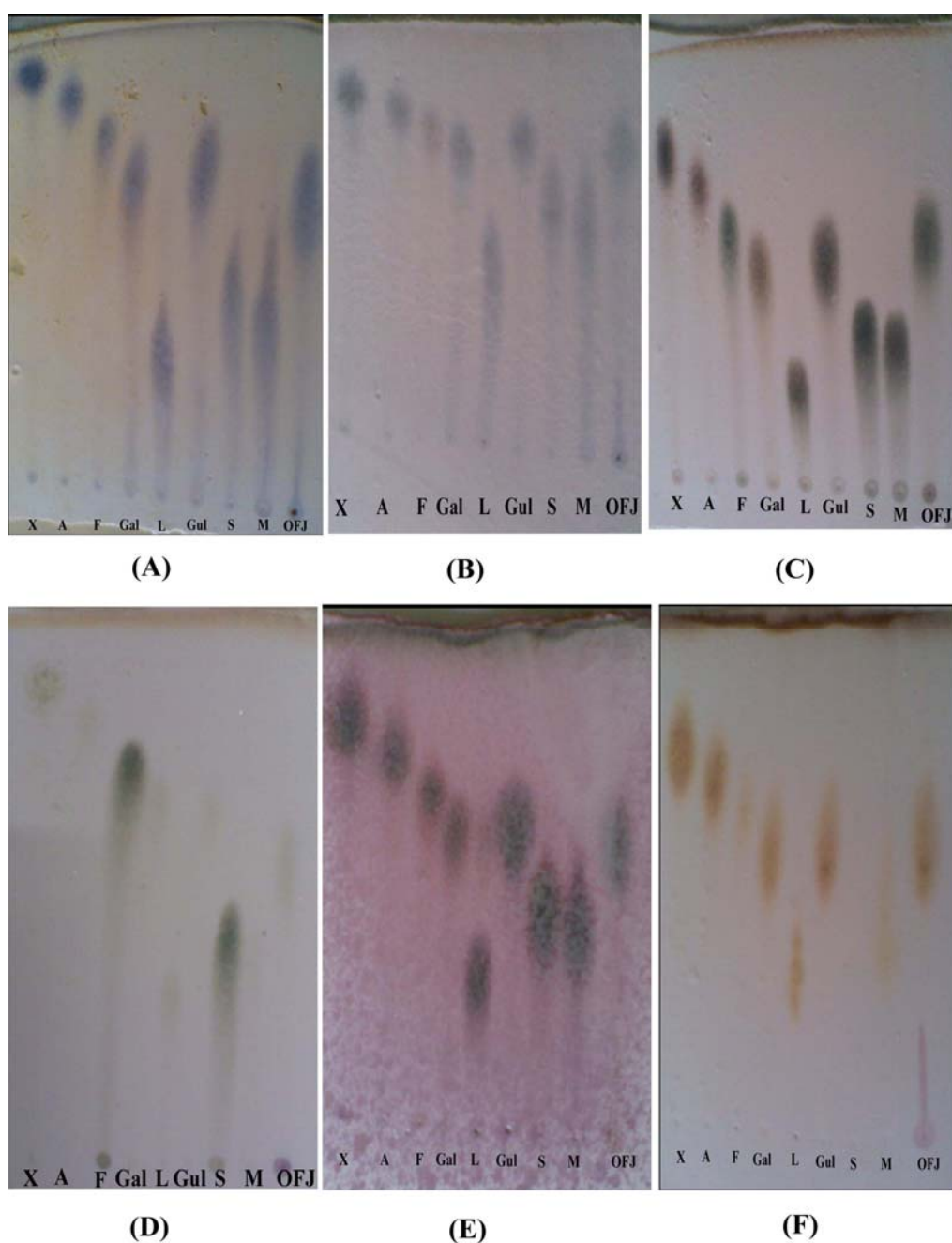
Figure 4.4: Identification of sugar from fruit juice using Thin Layer Chromatography (TLC). Detection reagent: α-Naphthol sulphuric acid (A), Aniline-diphenylamine-phosphoric acid (B), Phenol-sulphuric acid (C), Anthrone reagent (D), Thymol-sulphuric acid (E) and *p*-Anisidine phthalate reagent (F). TLC of OFJ was carried out in presence of standard compounds like D-Xylose (X), L-Arabinose (A), D-Fructose (F), D-Galactose (Gal), Lactose (L), Glucose (Glu), Sucrose (S) and Maltose (M).

126
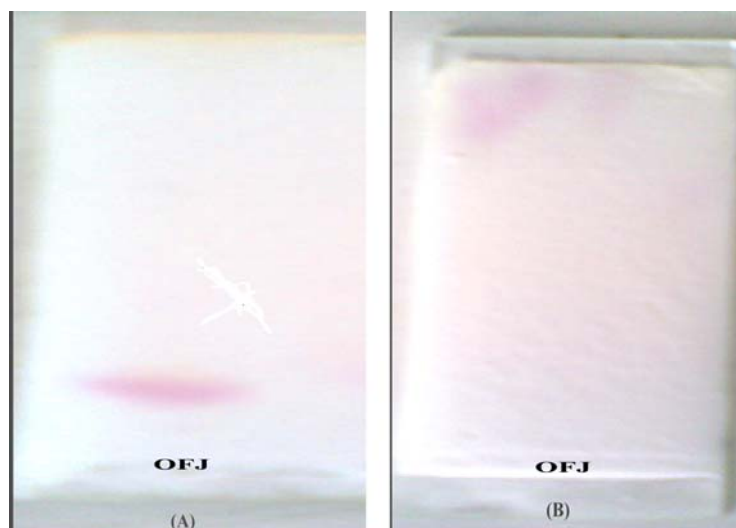
Figure 4.5: Photograph of thin layer chromatography (TLC) of OFJ in BAW (A) and 1% aqueous HCl (B). R_f value was found low in BAW and high in 1% aqueous HCl.

4.2.4 Qualitative analysis of betalains

4.2.4.1 Spectrophotometric analysis

Figure 4.6 shows the visible absorption spectra (400 – 650 nm) of water, methanol and methanol containing 1% conc. HCl extracts of the fruits of *Opuntia elatior* Mill. The spectrum of water and methanol extracts shows one peaks at 535 nm, while methanol containing 1% conc. HCl shows bathochromic shift at 545 nm.
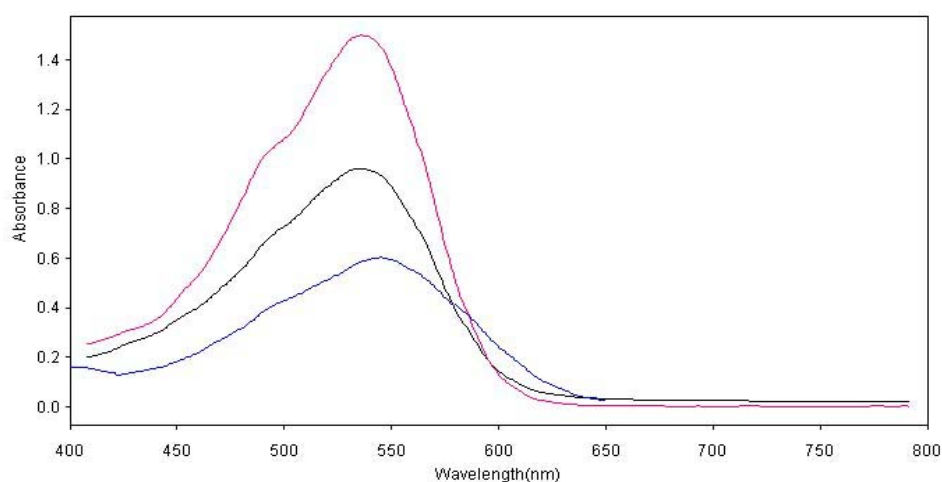
Figure 4.6: Visible light absorption spectra of water (red line), methanol (black line) and methanol HCl (blue line) extracts of fruits of *Opuntia elatior* Mill.

4.2.4.2 High performance liquid chromatographic (HPLC) analysis

Figure 4.7 shows the chromatographic pattern of the methanolic extracts of fruits of *Opuntia elatior* Mill. At 484 nm, one peak can be observed eluding at 21.76 min (peak 1). When the same extract is monitored at 535 nm, a large peak with a retention time of 22.67 min (peak 2) is observed. Peak 1 showed maximum absorbance (λ_{max}) at 484 nm and peak 2 at 535 nm. From the respective retention times, peak 1 with retention time 21.76 min at 484 nm was identified as indicaxanthin which might be present in very low amount and peak 2 with retention time 22.76 min at 535 nm as betanin which might be present in high amount due to high absorbance.
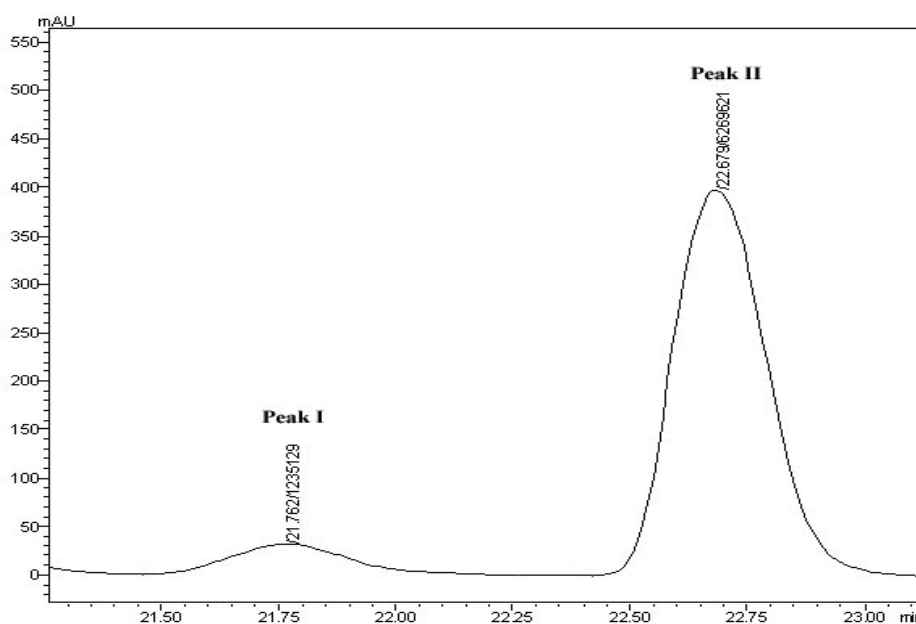
Figure 4.7: HPLC chromatogram of methanolic extract of fruits of
***Opuntia elatior* Mill.**

4.2.4.3 Liquid chromatography – mass spectroscopic (LC-MS) analysis

The use of MS coupled with HPLC complements the use of PAD and permits immediate identification of components of a mixture and characterization of an extract in terms of its chemical composition. The maximum absorbance (535 nm) and molecular ion ([M + H]$^+$ at m/z551) (Figure 4.8 A) suggested that this peak should correspond to a betacyanin structure very close to betanin and isobetanin. All betacyanins produced a daughter ion at m/z390, corresponding to [betanidin+ H]$^+$ (Figure 4.8 B).
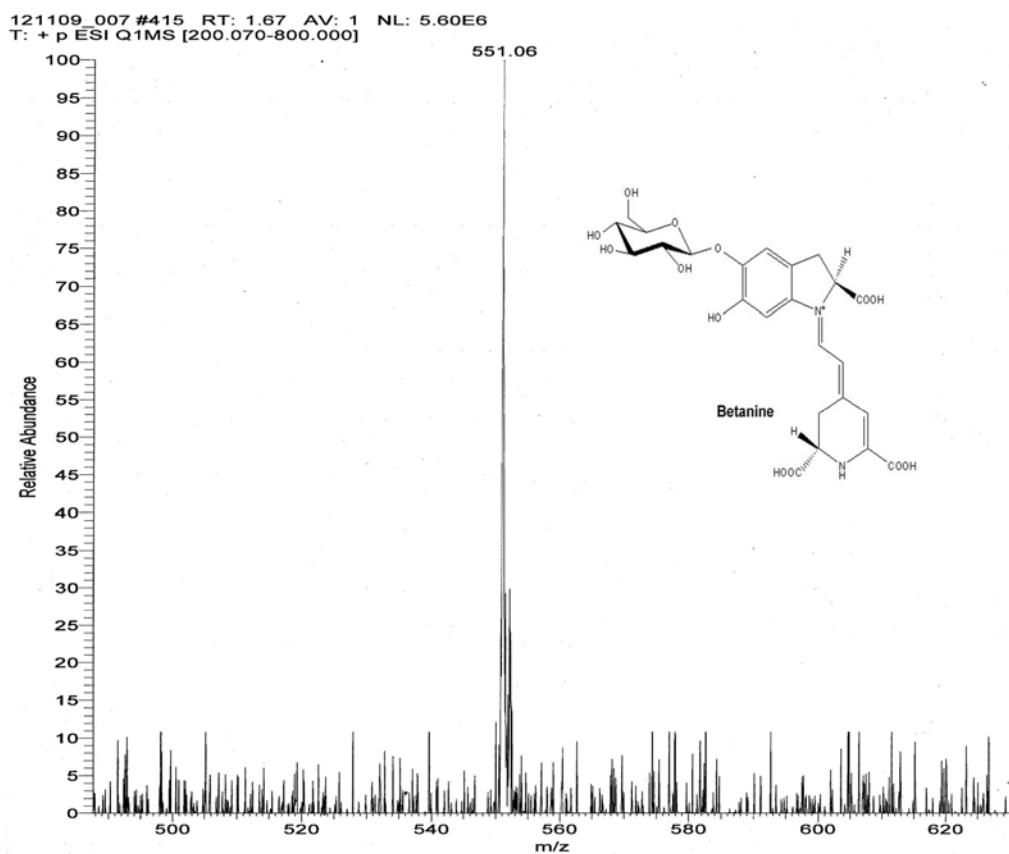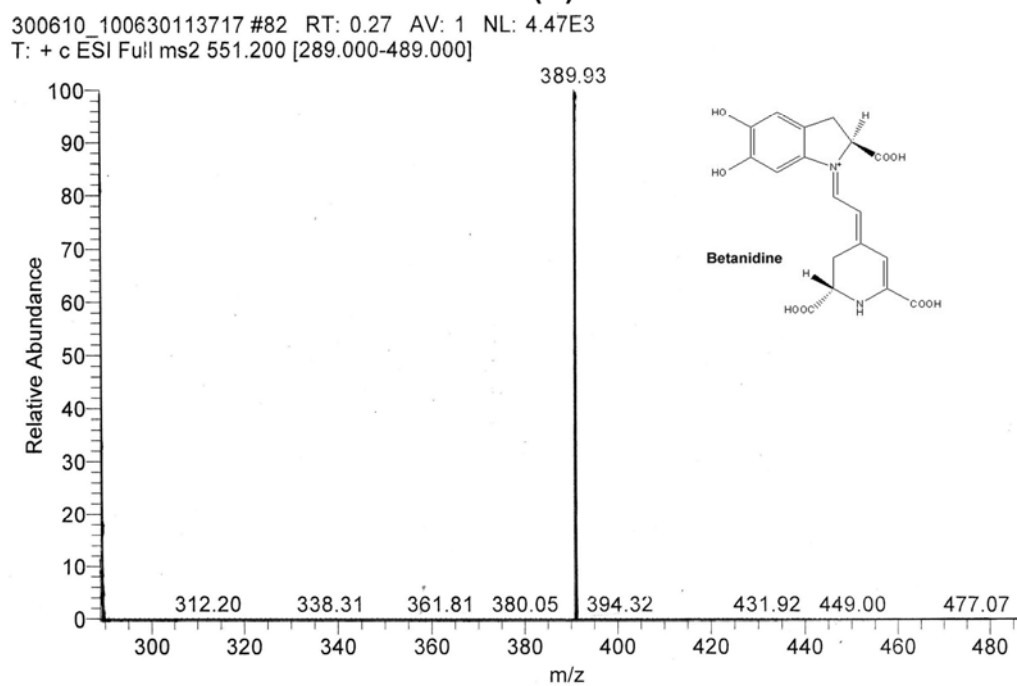
Figure 4.8: Positive ion spray mass spectra corresponding to betanin, parent ion scan of *m/z* 551 (A); daughter ion scan of *m/z* 389 (B).

4.2.5 Quantitative estimation of OFJ

4.2.5.1 Total sugar content

Anthrone reagent method was used to determine total sugar content of OFJ. The fruits of *Opuntia elatior* Mill. contained **11.417 ± 0.0567** (mean ± SD) g/100 ml of total carbohydrates equivalent to glucose.
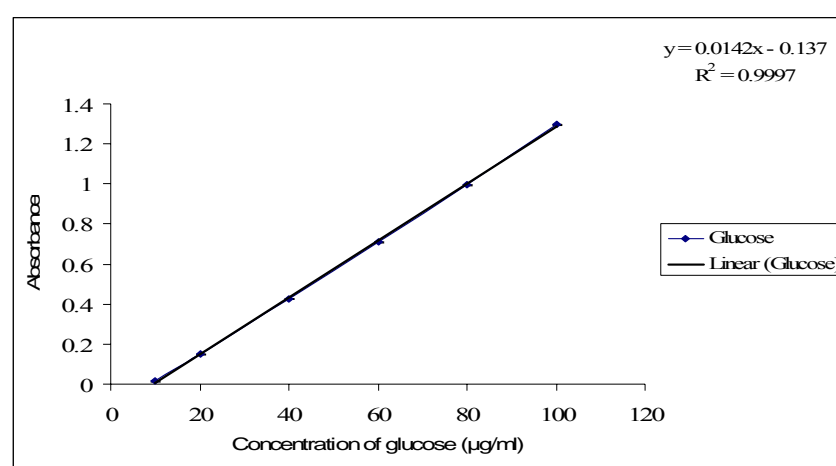
Figure 4.9: Calibration curve for total sugar content by anthrone reagent method.

4.2.5.2 Total phenolic content

Folin Ciocalteu method was used to determine the total phenolic content of OFJ. The fruits of *Opuntia elatior* Mill. contained **49.823 ± 21.294** (mean ± SD) **mg/100 ml** of total phenolics expressed as Gallic acid equivalents.
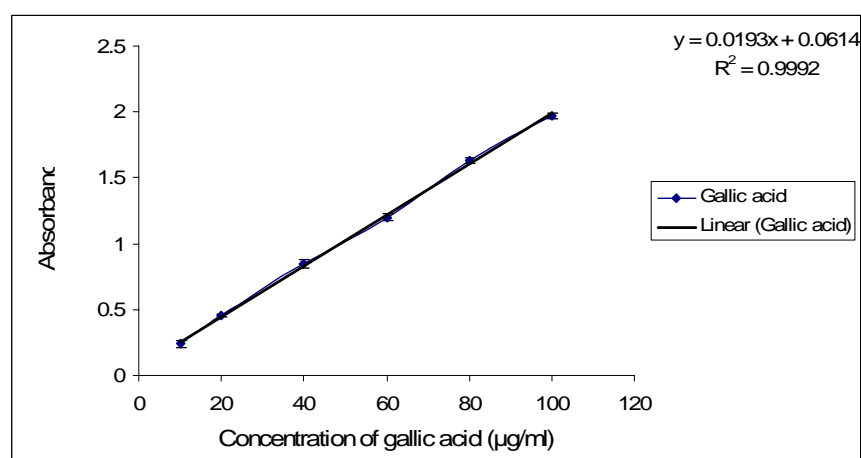
Figure 4.10: Calibration curve for gallic acid by Folin Ciocalteu method.

4.2.5.3 Titratable acidity

The titratable acidity of fruits juice of *Opuntia elatior* Mill. was found **0.942 ± 0.019** (mean ± SD) g/ 100 ml equivalent to citric acid.
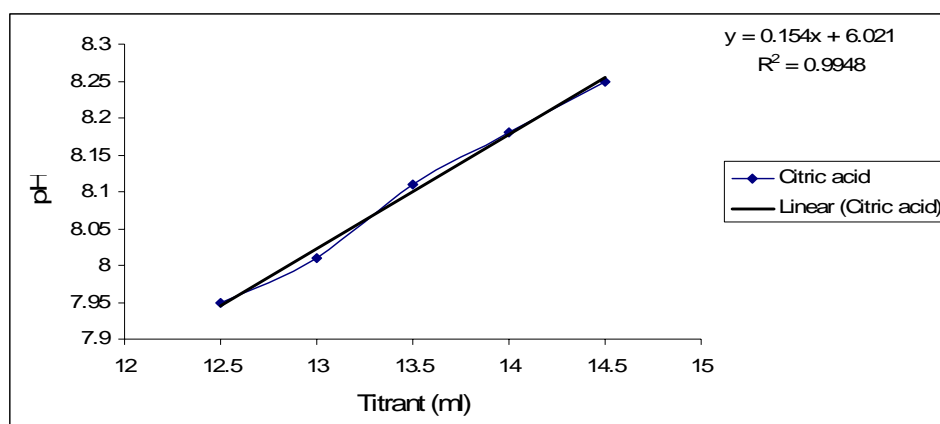
Figure 4.11: Calibration curve for titratable acidity equivalent to citric acid.

4.2.5.4 Total betalains content

This preliminary analysis suggests that the external color of prickly pear fruits depends on the relative concentration of betacyanins (red pigments with maximum absorbance at around 535 nm). The average amount obtained in fruits of *Opuntia elatior* Mill. was **47.101 ± 2.149 mg/100 ml (mean ± SD)** of betacyanin equivalent to betanin. These results are in range of reported literatures of other *Opuntia* species.

4.2.5.5 Elemental analysis

Elemental detection was carried out using atomic absorption spectroscopy (AAS) and summarized in table 4.5. Fruits of *Opuntia elatior* Mill. contained higher amount of potassium followed by calcium and others are in range of reported literature of other *Opuntia* species.

Table 4.5: Elemental analysis of OFJ by AAS.

Elements	mg/100g
Potassium (K)	131.80
Calcium (Ca)	50.24
Sodium (Na)	3.40
Zinc (Zn)	0.51
Iron (Fe)	0.32
Copper (Cu)	0.16

4.3 Pharmacological studies of fruit juice

4.3.1 Acute toxicity study

The OFJ (20 ml/kg) showed no significant change in the various autonomic and behavioral responses of rat compared to the control animals. No mortality was recorded till 48 h in the animals treated with OFJ up to 20 ml/kg oral dose and therefore considered to be safe.

Fruit juice (OFJ) was investigated for haematinic, analgesic, and anti-asthmatic activity using different models.

4.3.2 Haematinic action

4.3.2.1 Mercuric chloride –induced anaemia

4.3.2.1.1 Effect on Body weight

The mean body weight (g) of the albino rats in different treatment groups were recorded at 7 day interval upto 60 days and presented in table 4.6 and figure 4.12. Statistically, highly significant decrease in body weight ($p < 0.001$) was found in $HgCl_2$ treated groups on day 30 with respect to the values of the negative control (Group A) group on same day. On the day 49, significant increase in body weight (g) was obtained 191.2 ± 8.77 ($p < 0.05$) and 201.7 ± 4.82 ($p < 0.001$) in group D10 and D15 treated groups, respectively. However, on the day 60, highly significant enhancement in body weight ($p < 0.001$) was obtained in group C, D10 and D15 treated groups with respect to the values of the group B at the day 30. There is no significant change in group B and D5 treated groups after 30 days. In group E treated group, there was also increasing in body weight after 30 days.

Table 4.6: Measurement of body weight (g) of rats in HgCl$_2$ –induced anaemia.

Days	Groups						
	A	B	C	D5	D10	D15	E
0	214 ± 3.18	216.7 ± 1.14	222.2 ± 1.53	212.7 ± 2.9	218.8 ± 6.52	221.7 ± 5.95	183.8 ± 8.86
7	216 ± 1.94	212 ± 2.56	218 ± 1.86	209 ± 3.05	216.8 ± 5.21	216.3 ± 5.78	183.8 ± 9.07
15	216.3 ± 2.18	203.3 ± 3.32	211.2 ± 2.50	202.7 ± 2.84	202.2 ± 4.75	203.2 ± 4.91	184.5 ± 9.11
21	217.5 ± 1.78	195.2 ± 3.86	200.8 ± 2.28	195.7 ± 3.87	184.3 ± 6.24	184.7 ± 6.97	185.5 ± 9.01
30	223 ± 1.54	168.5 ± 3.86 +++	169.8 ± 2.82 +++	159.2 ± 2.72 +++	161.5 ± 7.11 +++	164.2 ± 6.65 +++	187.3 ± 9.25
42	222.5 ± 1.20	166.7 ± 2.95	179 ± 2.35	158 ± 1.78	172.3 ± 8.13	178.5 ± 5.29	195.2 ± 7.03
49	224.8 ± 0.90	169.5 ± 3.09	198 ± 2.47	165.7 ± 2.56	191.2 ± 8.77 *	201.7 ± 4.82 ***	202.5 ± 5.85
60	227.3 ± 2.06	172.5 ± 3.68	223.2 ± 2.97 ***	176.7 ± 2.90	212.2 ± 6.15 ***	227.7 ± 5.95 ***	208.7 ± 5.59

Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference Vs negative control (group A) at 30 days and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 60 Vs positive control (group B) at 30 days.
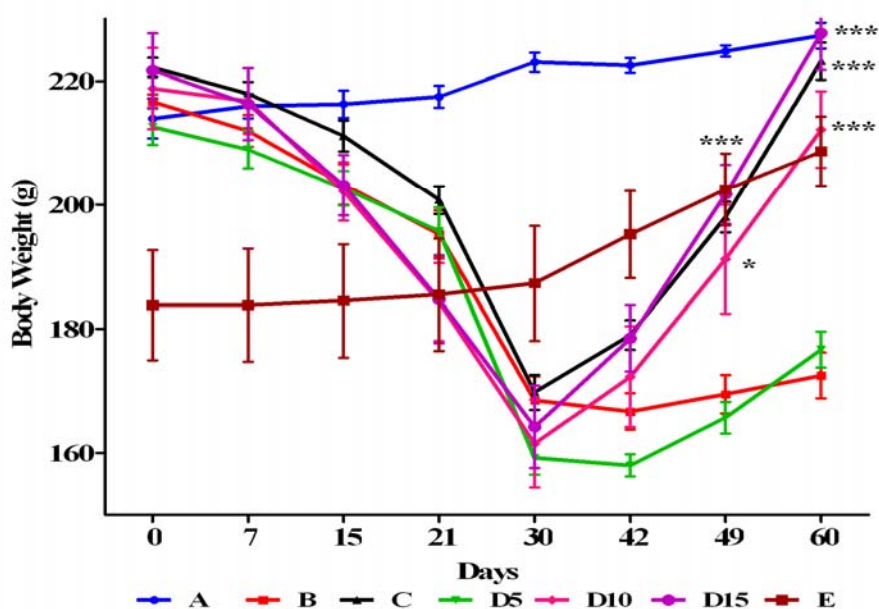
Figure 4.12: Effect of OFJ on body weight (g) in HgCl$_2$ –induced anaemia.
Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 60 Vs positive control (group B) at 30 days.

4.3.2.1.2 Effect on Haemoglobin

Pre- and post – treated mean Hb content (gm %) in all groups were estimated at 7 – day interval up to 60 days in rats (Table 4.7 and Figure 4.13). HgCl$_2$ treated positive control rat demonstrated significant ($p < 0.001$) decrease in mean Hb content compared negative control on day 30, indicated anaemia. The percentage of reduction in Hb concentration was found 25.59 ± 1.274 %, 23 ± 0.84 %, 22.55 ± 2.45 %, 20.87 ± 2.06 % and 22.43 ± 1.57 % in group B, C, D5, D10 and D15 respectively at day 30. We observed significant increase in Hb concentration in group D5 (5 ml/kg, OFJ), group D10 (10 ml/kg, OFJ) and group D15 (15 ml/kg, OFJ) on day 60 ($p < 0.05$, $p < 0.001$, $p < 0.001$, respectively) with respect to positive control on day 30 and it same as standard (group C, $p < 0.001$). Group D5, D10 and D15 treated rats showed significant ($p < 0.001$) percentage recovery compared to positive control and it as same as found in standard group (figure 4.14). The mean Hb level in group D15 was recovered significantly ($p < 0.001$) at day 42, 49 and 60 compared to positive control. We did not found significant change in Hb content in group E (only 15 ml/kg, OFJ) treated animals.

Table 4.7: Estimation of haemoglobin content (gm %) of rats in HgCl$_2$ – induced anaemia.

Days	Groups						
	A	**B**	**C**	**D5**	**D10**	**D15**	**E**
0	15.78 ± 0.24	15.47 ± 0.10	15.53 ± 0.23	15.3 ± 0.32	15.4 ± 0.31	15.82 ± 0.13	15.95 ± 0.11
7	15.82 ± 0.25	14.47 ± 0.14	14.35 ± 0.15	14.25 ± 0.19	14.63 ± 0.24	14.65 ± 0.23	15.85 ± 0.20
15	15.87 ± 0.27	13.53 ± 0.19	13.43 ± 0.18	13.47 ± 0.14	13.53 ± 0.16	13.5 ± 0.19	15.73 ± 0.32
21	16.02 ± 0.36	12.75 ± 0.25	12.75 ± 0.22	12.88 ± 0.2	13 ± 0.22	12.93 ± 0.23	15.98 ± 0.32
30	15.8 ± 0.31	11.73 ± 0.15 +++	12.15 ± 0.19 +++	12.2 ± 0.24 +++	12.47 ± 0.16 +++	12.23 ± 0.22 +++	15.82 ± 0.29
42	15.75 ± 0.28	11.48 ± 0.17	12.87 ± 0.2	12.55 ± 0.21	13.87 ± 0.19	14.13 ± 0.17 ***	16.2 ± 0.38
49	15.7 ± 0.3	11.62 ± 0.16	13.87 ± 0.23 **	13.03 ± 0.19	14.47 ± 0.22 ***	14.73 ± 0.16 ***	16.68 ± 0.42
60	15.78 ± 0.34	11.97 ± 0.19	15.22 ± 0.28 ***	13.77 ± 0.13 *	15.6 ± 0.25 ***	16.2 ± 0.14 ***	16.98 ± 0.32

Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference Vs negative control (group A) at 30 days and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 60 Vs positive control (group B) at 30 days.
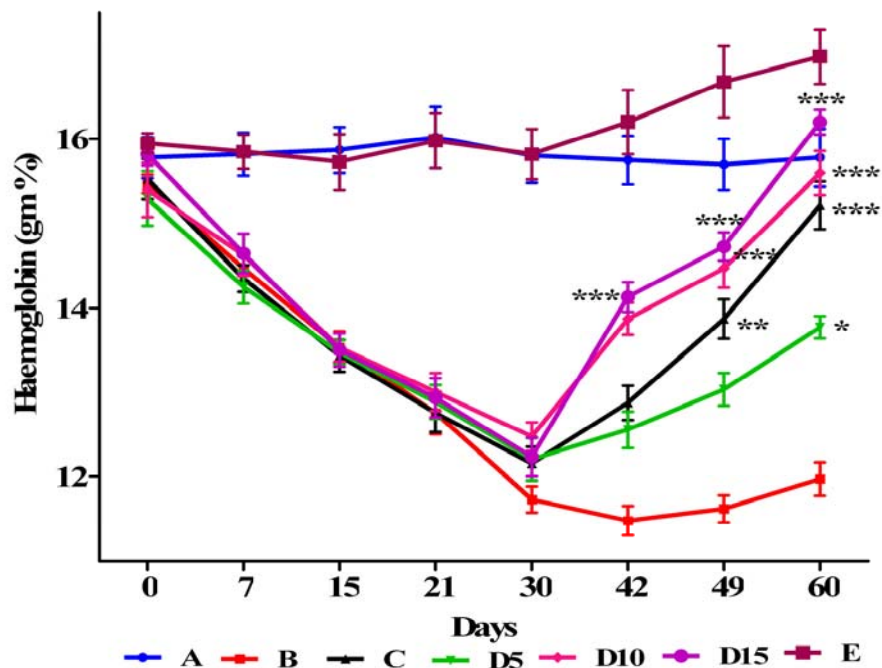
Figure 4.13: Effect of OFJ on Hb content (gm %) in HgCl₂ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 60 Vs positive control (group B) at 30 days.
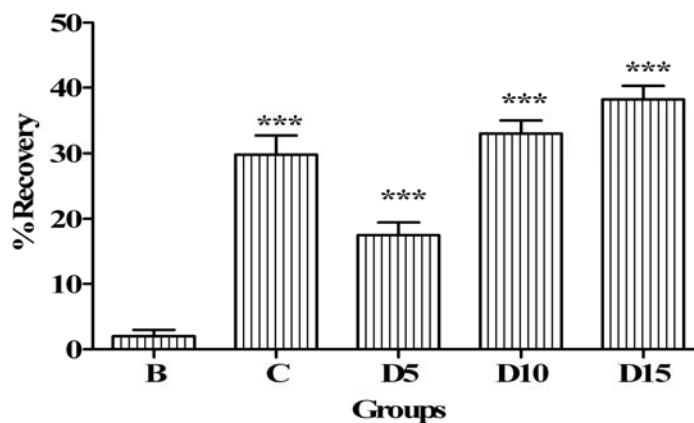
Figure 4.14: The percentage recovery in Hb content of rats in HgCl2 – induced anaemia at day 60. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference Vs positive control (group B) at day 60.

4.3.2.1.3 Effect on Haematological parameters

Pre- and post – treated haematological parameters in all groups were estimated at day 30 and 60 respectively and have been presented in figure 4.15A to C. $HgCl_2$ treated positive control rats at day 30 demonstrated a significant decrease in RBC count ($p < 0.001$), PCV ($p < 0.001$), MCV ($p < 0.001$), MCH ($p < 0.001$) and MCHC ($p < 0.01$), while significant increase was observed in level of RDW ($p < 0.001$) when compared to non treated negative control. Treatment with OFJ (5, 10, 15 ml/kg) for 30 day showed significant increase in RBC ($p < 0.001$) compared to positive control at day 30 and it equivalent to standard. We observed significant increase in PCV ($p < 0.001$) and MCH ($p < 0.001$) in group C and D15, MCV ($p < 0.001$) in group D15 and MCHC ($p < 0.01$) in group C, D10 and D15 treated animals at day 60. It was observed that RDW significantly ($p < 0.001$) reduced in group C, D10 and D15 at day 60 with respect to the values of group B at day 30. There was no significant difference in haematological parameters of group E treated animals.

4.3.2.1.4 Effect on Total and Differential White Blood Cell count

The mean total and differential WBC counts were estimated on day 30 and 60 in $HgCl_2$ –induced anaemia and have been presented in figure 4.16. Mean total WBC count (10^9/L) was significantly ($p < 0.001$) reduced in group B, C, D5, D10 and D15 at the day 30 with respect to the negative control group values on same day. A significantly increase ($p < 0.01$, $p < 0.001$) in neutrophil count (%) and decrease ($p < 0.01$, $p < 0.001$) in lymphocytes (%) in comparison to negative control group values at day 30. There was no significant difference in eosinophil, monocytes and basophil count in pre- and post – treatment. A statistically significant increase ($p < 0.001$) mean total WBC count in group C, D10 and D15 and lymphocytes in group D10 and D15 while significantly ($p < 0.001$) normalized neutrophil counts at the day 60 in comparison with positive control group values at the day 30. There was no change in total and differential WBC count in group E treated animals.

Figure 4.15A: Effect of OFJ on total RBC (A) and PCV (B) in HgCl$_2$ – induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference Vs negative control (group A) at 30 days and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 60 Vs positive control (group B) at 30 days.

137

(C)

(D)
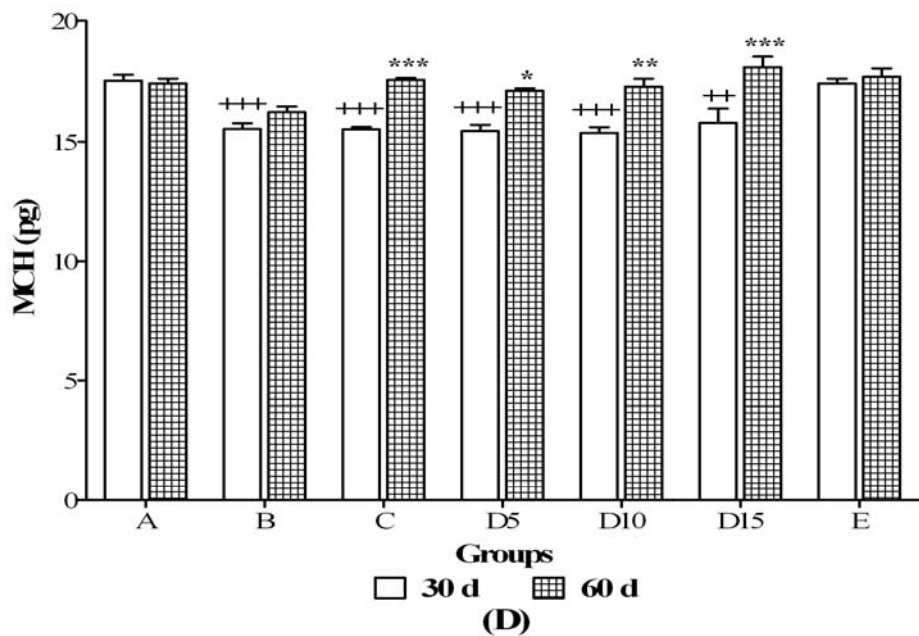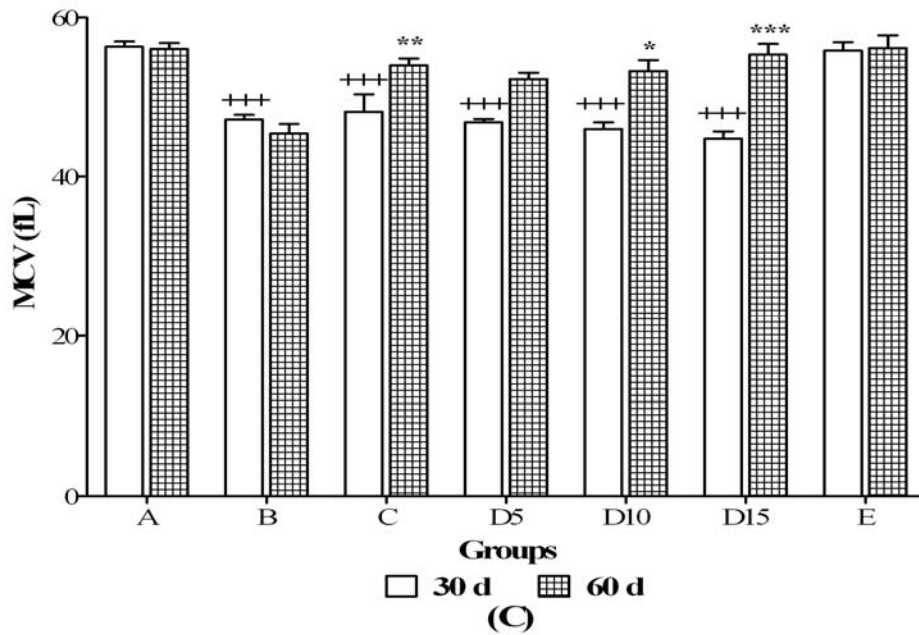
Figure 4.15B: Effect of OFJ on MCV (C) and MCH (D) in HgCl$_2$ – induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference Vs negative control (group A) at 30 days and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 60 Vs positive control (group B) at 30 days.

(E)

(F)
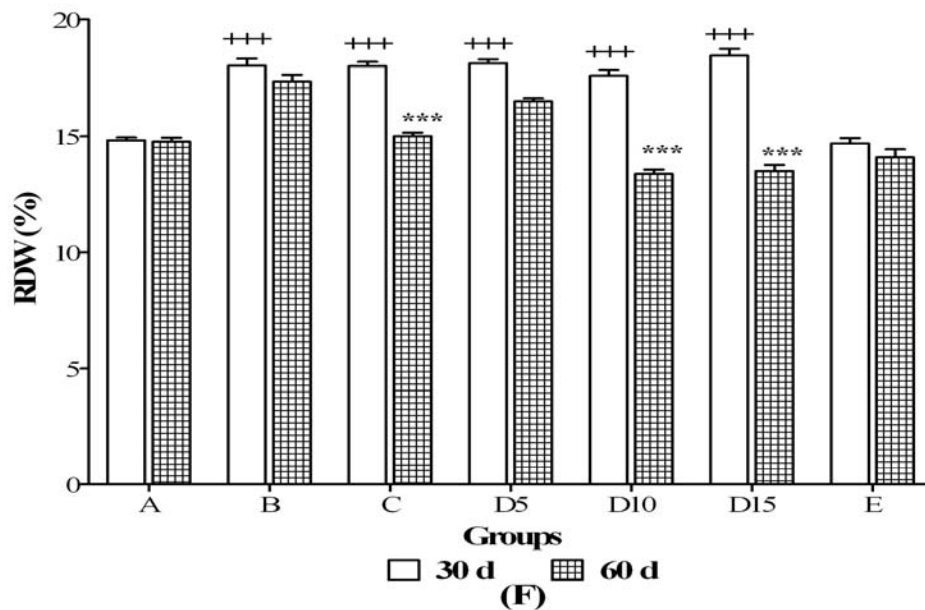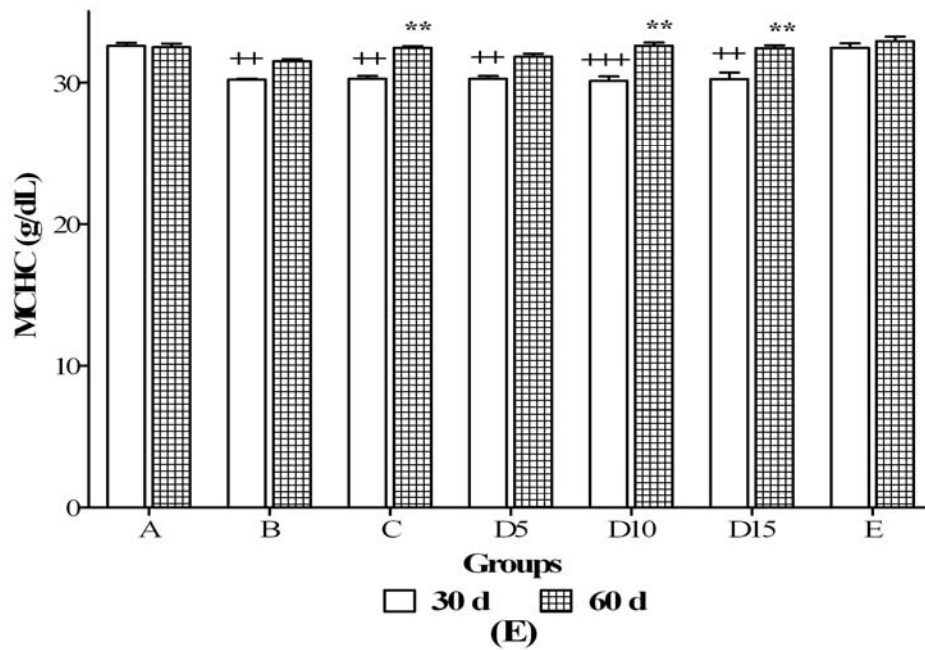
Figure 4.15C: Effect of OFJ on MCHC (E) and RDW (F) in HgCl$_2$ – induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference Vs negative control (group A) at 30 days and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 60 Vs positive control (group B) at 30 days.
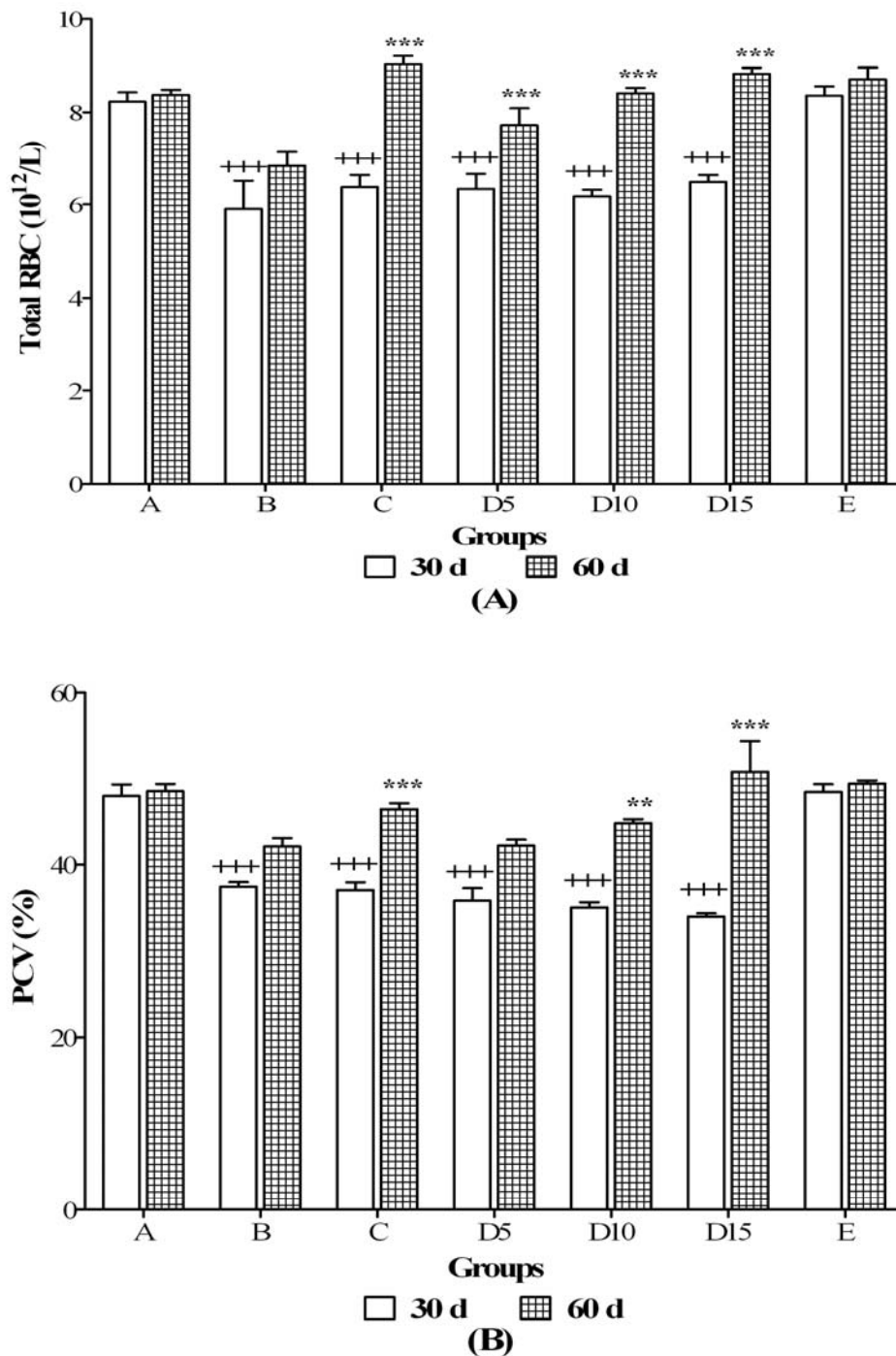
139
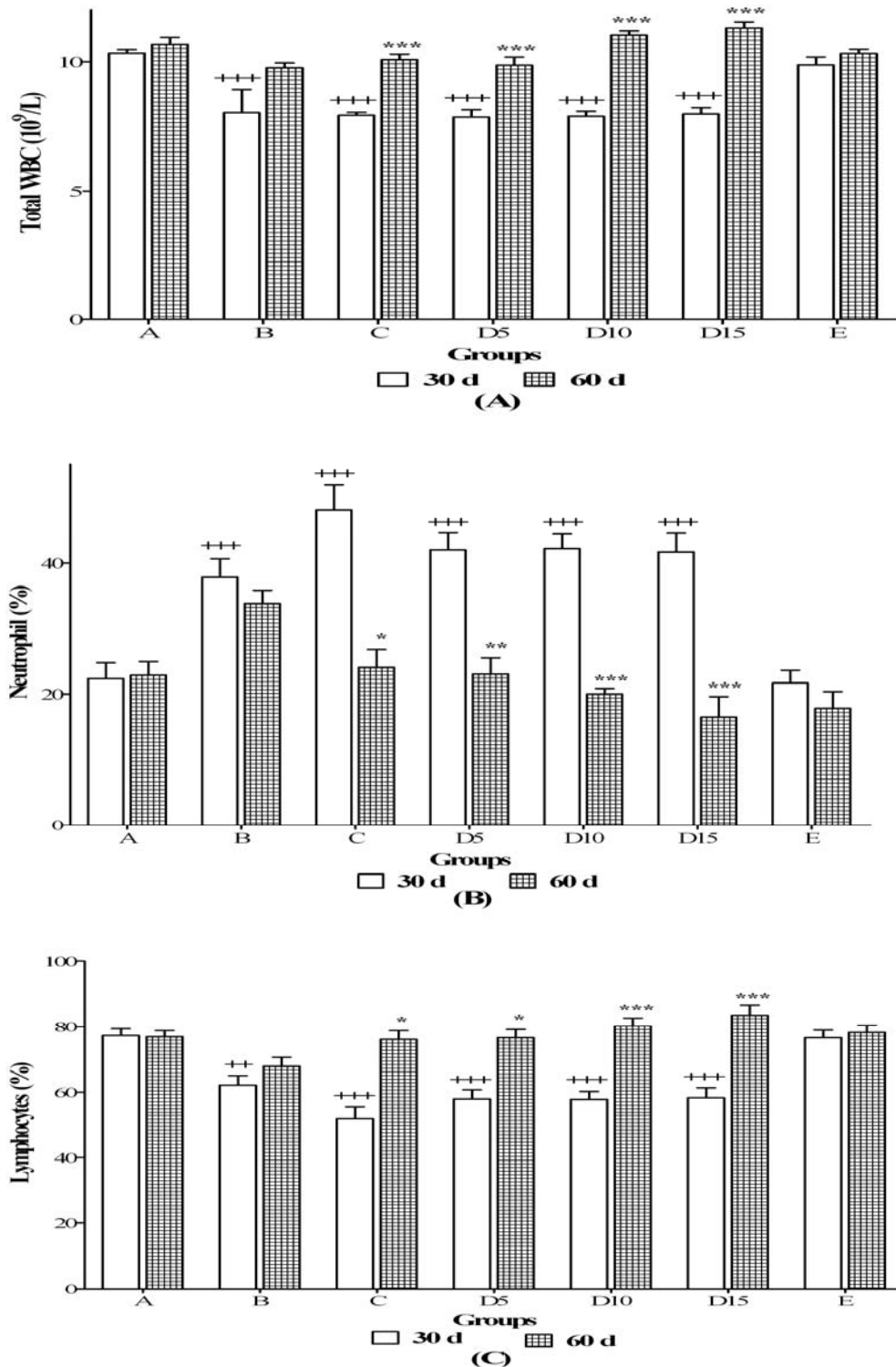
Figure 4.16: Effect of OFJ on total WBC (A), Neutrophils (B) and Lymphocytes (C) in HgCl₂ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference Vs negative control (group A) at 30 days and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 60 Vs positive control (group B) at 30 days.

4.3.2.1.5 Effect on Platelets

The mean platelet (10^9/L), PDW (%) and MPV (%) values were estimated at the day 30 and 60 in all groups and have been presented in figure 4.17. A statistically significant ($p < 0.001$) reduced mean platelet count at the day 30 in comparison to the negative control group values at same day. The mean PDW values was significantly increase 11.15 ± 0.17 ($p < 0.01$) and 11.28 ± 0.31 ($p < 0.001$) in group D10 and D15 at the day 30 in comparison to group A at the same day. Effect of treatment at day 60, mean platelet was significantly ($p < 0.001$) improved in group C, D10 and D15 while PDW and MPV values normalize in comparison to the positive control group values at the day 30.

4.3.2.1.6 Effect on Blood sugar, Cholesterol and triglyceride content

The mean blood sugar, cholesterol and triglyceride level were estimated on the day 30 and 60 and have been presented in figure 4.18. A statistically significant ($p < 0.01$) decrease in blood sugar level in $HgCl_2$ treated groups and no major change found in cholesterol and triglyceride level at the day 30 in comparison to negative control group values at same day. The mean blood sugar (mg/dL) was found 90.02 ± 2.13 ($p < 0.01$), 97.28 ± 2.27 and 102.5 ± 10.22 ($p < 0.001$) in group D5, D10 and D15 at the day 60 respectively in comparison with positive control group values at the day 30.

Figure 4.17: Effect of OFJ on mean platelet count (A), PDW (B) and MPV (C) in HgCl$_2$ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ p < 0.001, ++ p < 0.01, + p < 0.05 for change difference Vs negative control (group A) at 30 days and *** p < 0.001, ** p < 0.01, * p < 0.05 for change difference at day 60 Vs positive control (group B) at 30 days.

142
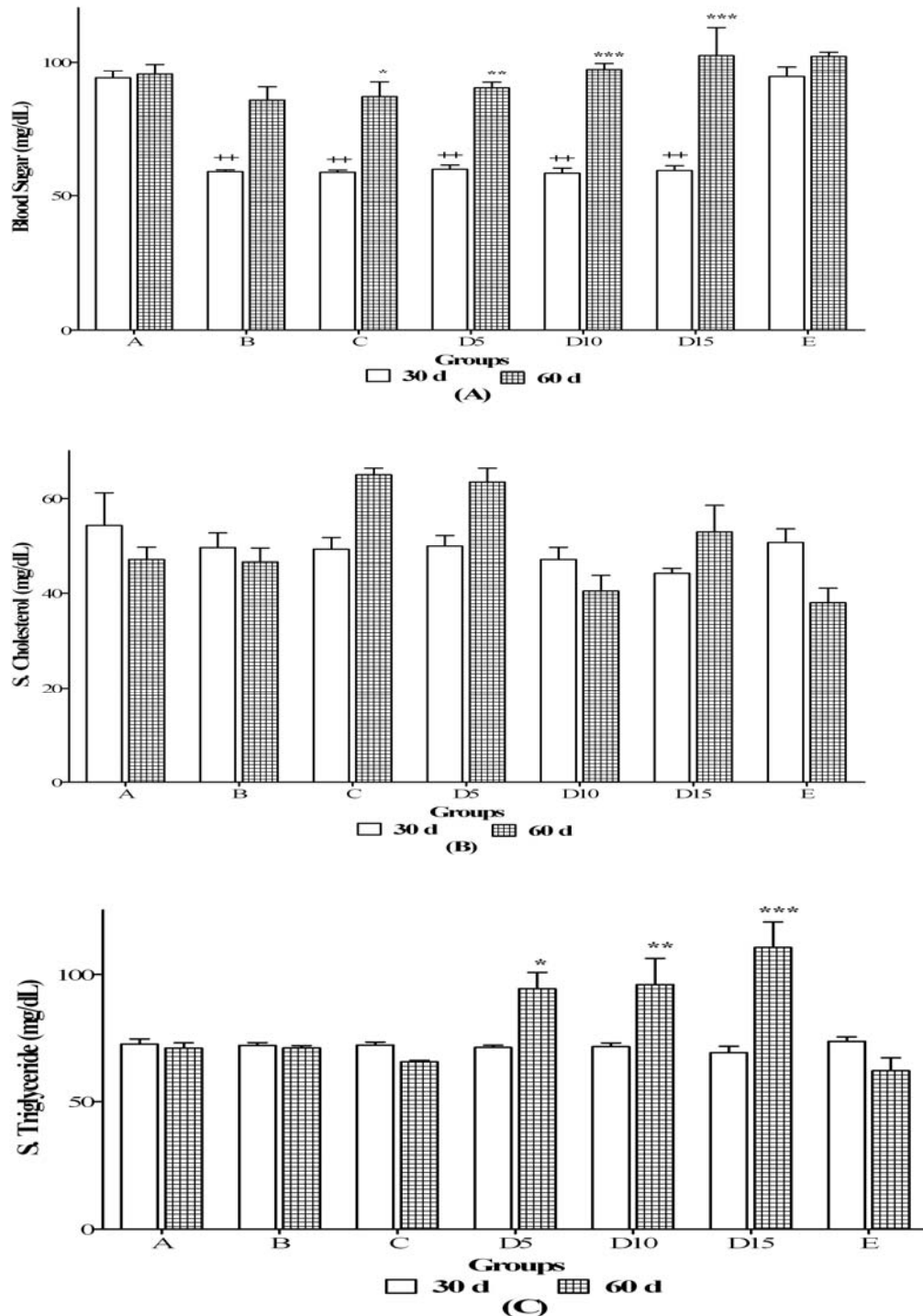
Figure 4.18: Effect of OFJ on blood sugar (A), S. Cholesterol (B) and S. Triglyceride (C) in HgCl₂ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference Vs negative control (group A) at 30 days and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 60 Vs positive control (group B) at 30 days.

4.3.2.1.7 Effect on Kidney functions

Pre- and post – treated kidney function parameters were estimated at day 30 and 60 respectively in $HgCl_2$ –induced anaemia and have been presented in figure 4.19. The mean blood urea level was significantly increase in group D5 ($p < 0.01$) and in group B, C, D10 and D15 ($p < 0.001$) while mean creatinine (mg/dL) level was not change in significant manner at the day 30 with respect to the values of negative control group on same day. In group D10 and D15 treated animals, the mean blood urea (mg/dL) was found 52.77 ± 1.04 ($p < 0.05$) and 50.72 ± 0.64 ($p < 0.01$) while mean creatinine level was also reduced in OFJ treated animals at the day 60 in comparison to positive control group values at the day 30.

4.3.2.1.8 Effect on Liver functions

The liver function parameters were estimated in $HgCl_2$ –induced anaemia in rats at the day 30 and 60 and have been presented in figure 4.20. The mean bilirubin level was significantly increase in group B, D5 ($p < 0.01$) and C, D10, D15 ($p < 0.001$) at the day 30 in comparison to the values of group A at the same day. A statistically significant ($p < 0.001$) reduction in mean total protein was observed while there was not significant change in alkaline phosphatase at the day 30 in comparison to the values of group A at same day. The mean Bilirubin (mg/dL) and alkaline phosphatase (IU/L) were found 0.513 ± 0.009 and 351.3 ± 42.93 ($p < 0.001$), respectively in group D15 while a significant increase ($p < 0.001$) in mean total proteins (g/dL) in group C, D5, D10 and D15 at the day 60 in comparison to the values of group B at the day 30. In group E treated rat, there was not significant change in liver function parameters.
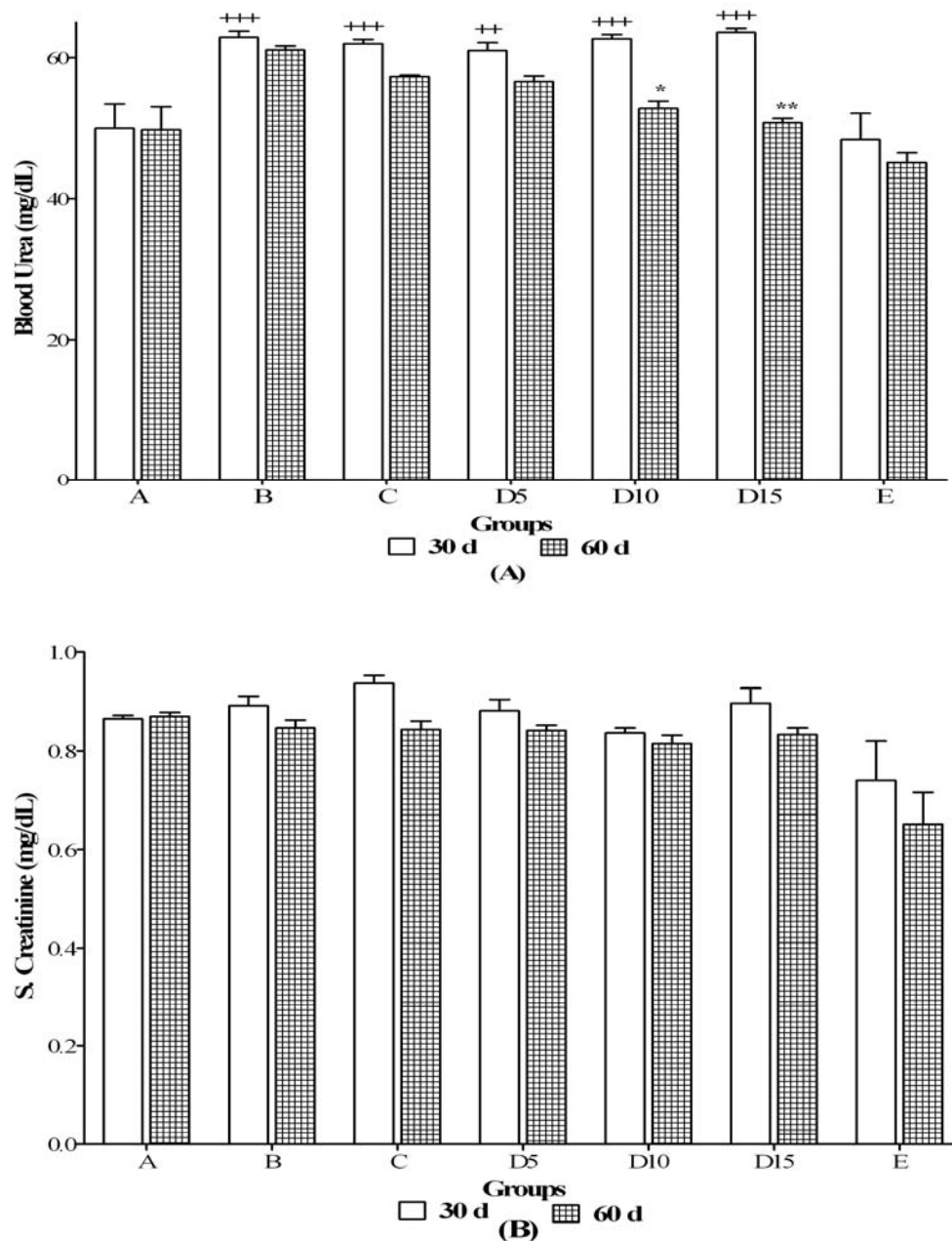
Figure 4.19: Effect of OFJ on blood urea (A) and creatinine (B) level in HgCl₂ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference Vs negative control (group A) at 30 days and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 60 Vs positive control (group B) at 30 days.
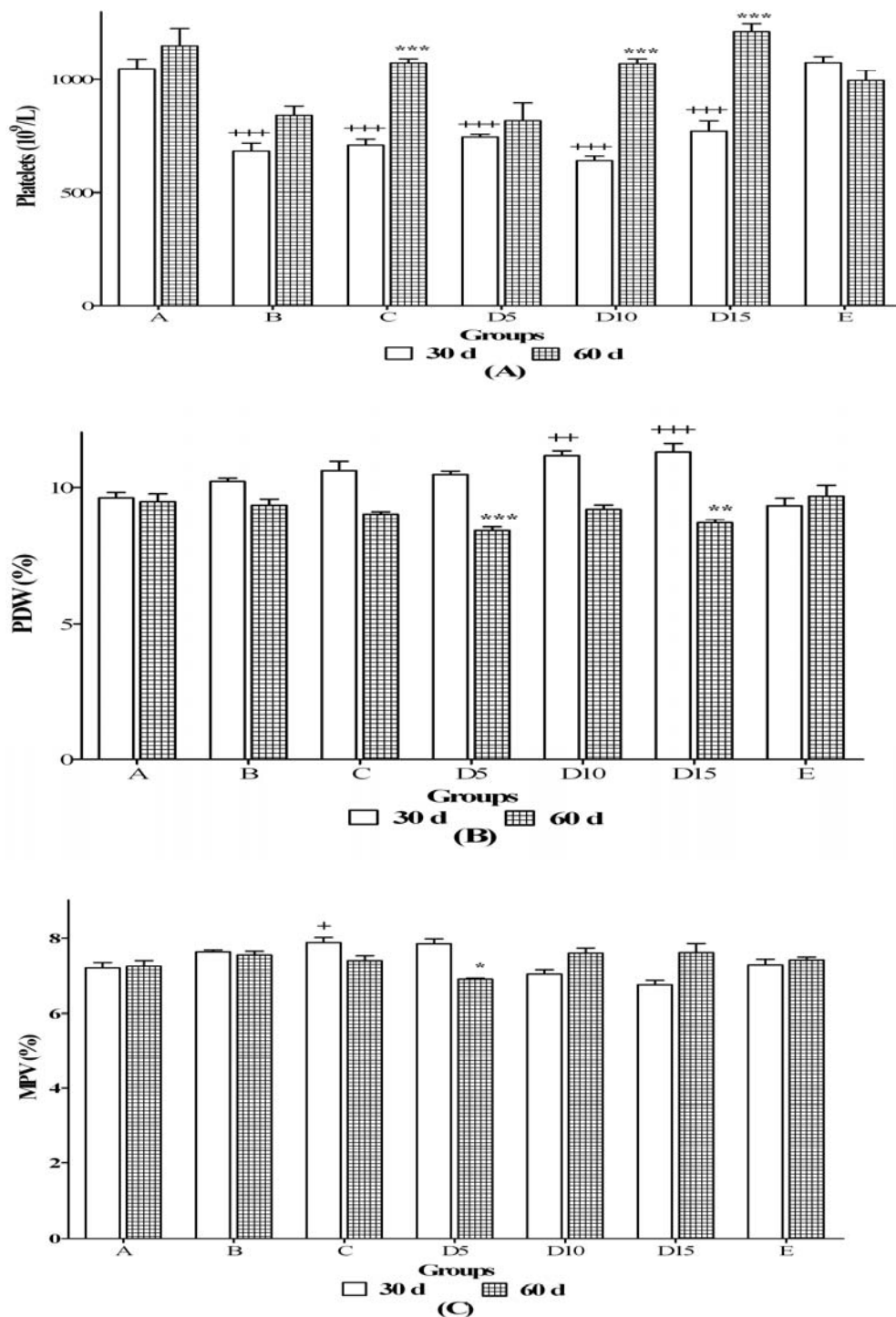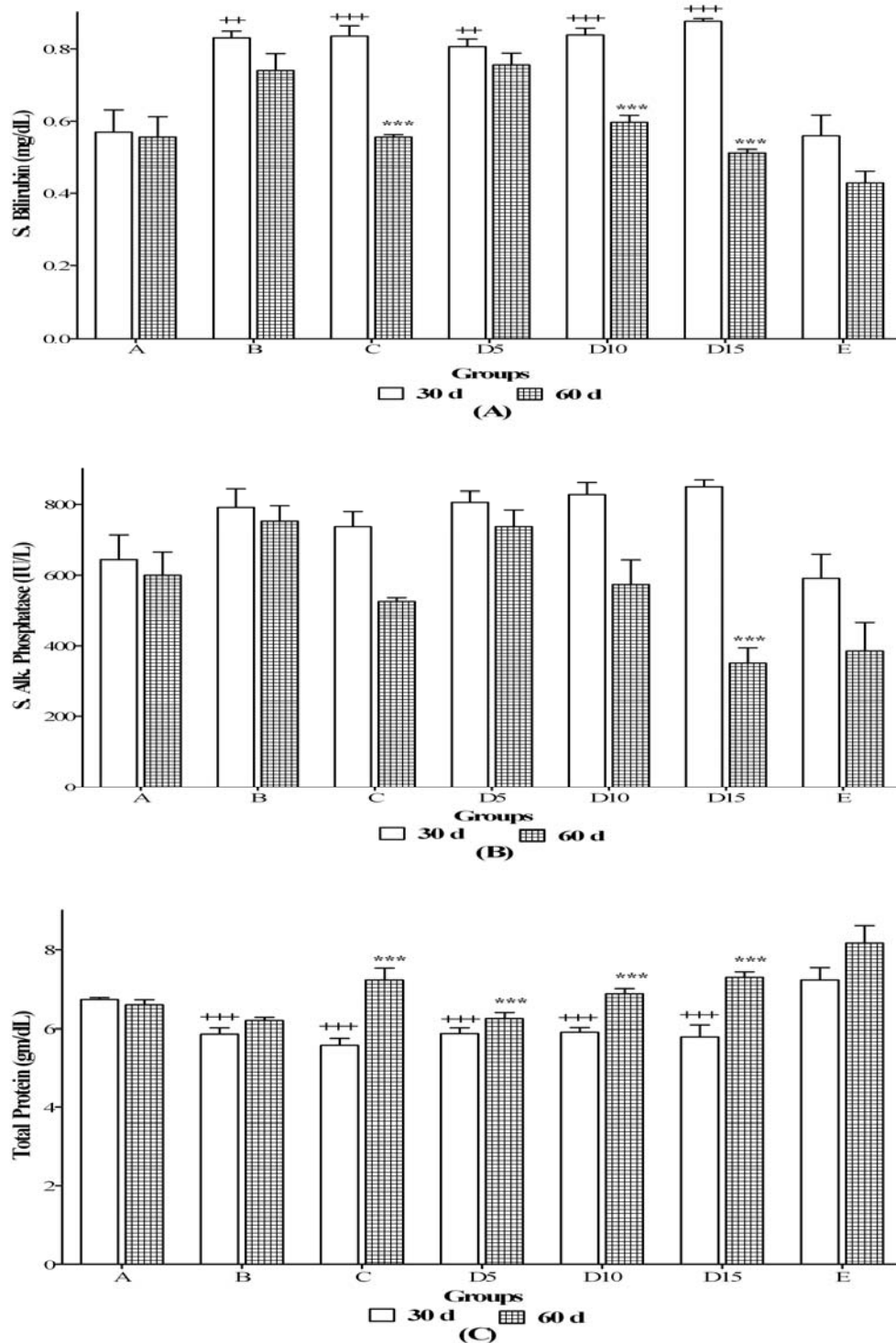
Figure 4.20: Effect of OFJ on S. Bilirubin (A), S. Alkaline phosphatase (B), Total Protein (C) level in HgCl$_2$ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference Vs negative control (group A) at 30 days and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 60 Vs positive control (group B) at 30 days.

146

4.3.2.1.9 Histopathology study

Histopathological section of kidney, liver and spleen were presented in figure 4.21, 4.22 and 4.23, respectively. Kidney section of group A (negative control) rats revealed the normal distinct glomeruli and tubules. In group B rats the kidney showed shrinkage, fibrosis and acute glomerular nephritis. In group C rat fibrosis and acute nephritis were less compared to group B. In fruit juice treated rats better histology was evident, the glomerular and tubular structures were distinct and more improvement compared to group B and C groups kidney sections. The liver was badly damaged in group B rats, showed distortion of hepatocytes, portal tract dilation, and acute inflammatory infiltration. In standard and fruit juice treated rats, quite normal histology was seen. Normal cytoarchitecture of spleen was observed in control group, where as cell depletion, acute inflammatory infiltration, fibrosis and necrosis were observed in the cytoarchitecture of spleen in group B rats. In standard and fruit juice treated rat's spleen mild fatty changes and cell depletion was observed.
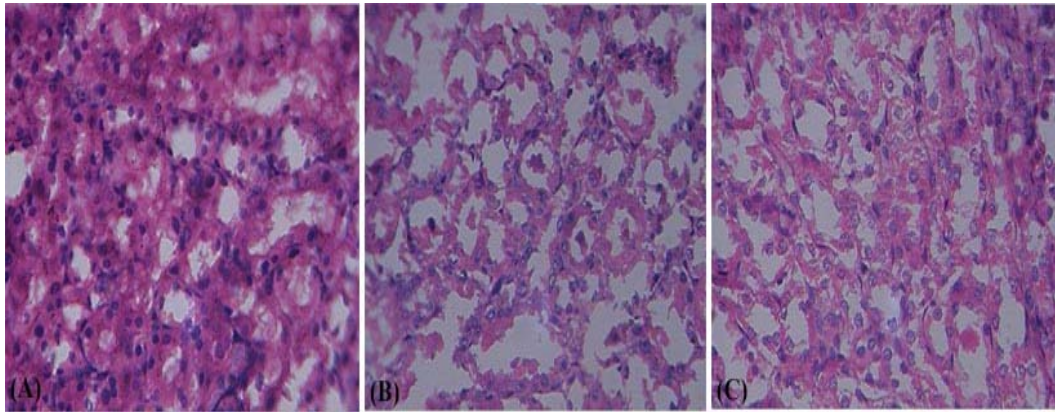
Figure 4.21: Hematoxyline – Eosin sections of kidney (450 x) of negative control (A), positive control (B) and OFJ (C) treated rats in HgCl$_2$ – induced anaemia model.
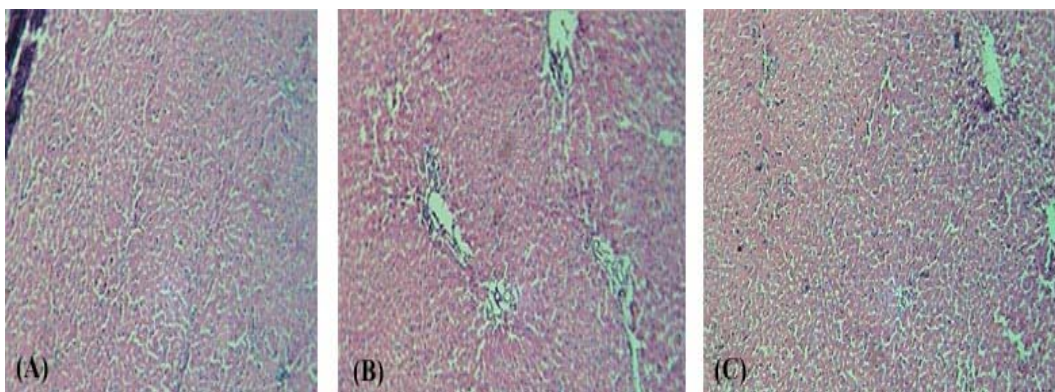
Figure 4.22: Hematoxyline – Eosin sections of liver (450 x) of negative control (A), positive control (B) and OFJ (C) treated rats in HgCl$_2$ – induced anaemia model.
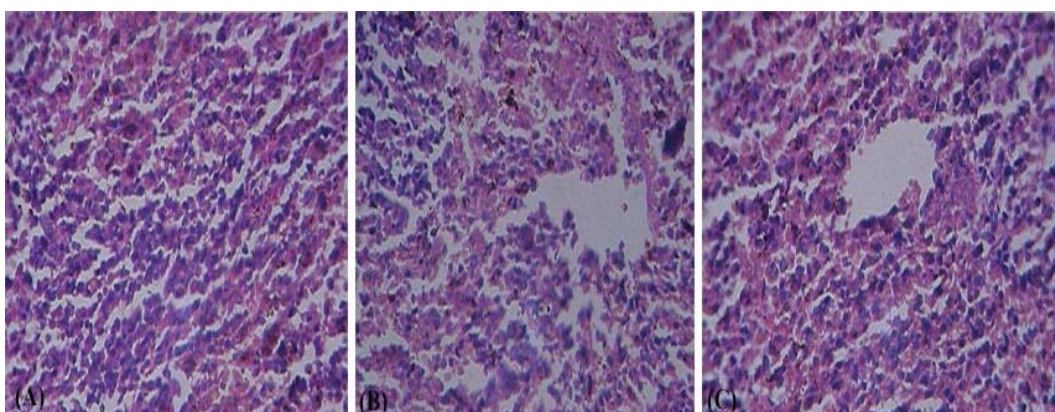
Figure 4.23: Hematoxyline – Eosin sections of spleen (450 x) of negative control (A), positive control (B) and OFJ (C) treated rats in HgCl$_2$ – induced anaemia model.

4.3.2.2 Phenylhydrazine –induced anaemia

4.3.2.2.1 Effect on Body weight

The mean body weight (g) of the albino rats in all groups were recorded on the day 0, 3, 7, 10, 15 and have been presented in figure 4.24. There was not found significant difference in mean body weight of different treatment groups.
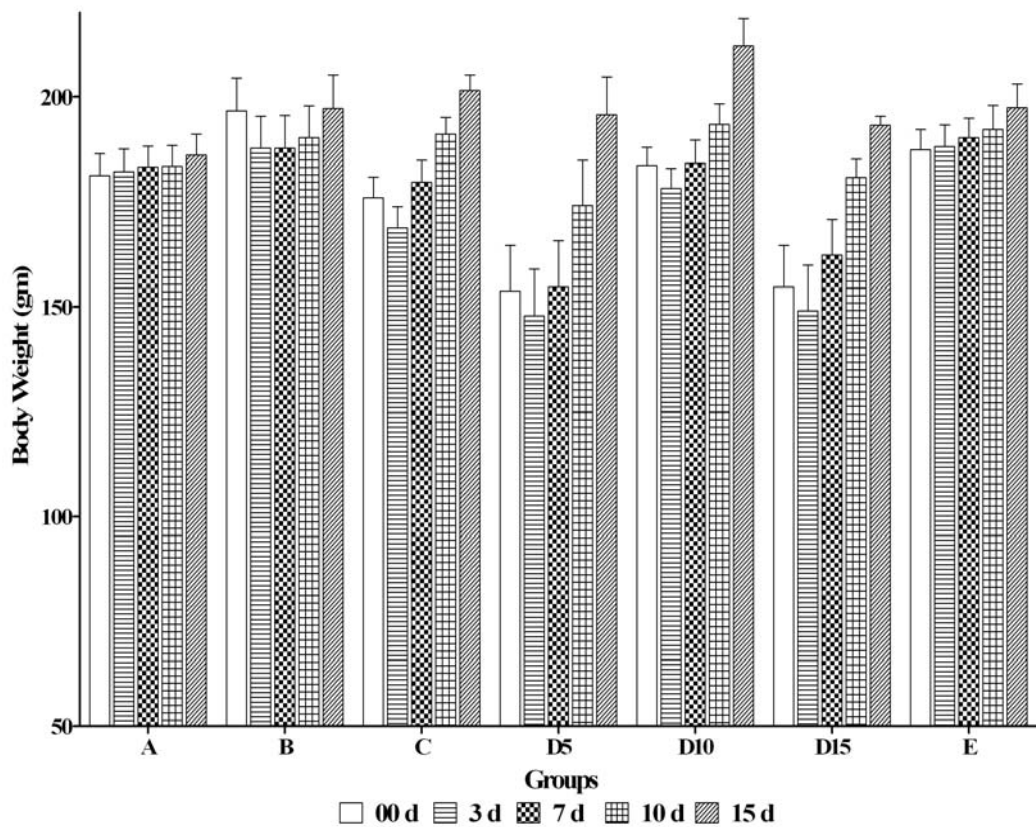
Figure 4.24: Effect of OFJ on body weight in PHZ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test.

4.3.2.2.2 Effect on Haemoglobin content

The mean Hb content of rats at day 3 was highly significant ($p < 0.001$) decrease compare to negative control (group A) after intraperitoneal administration of PHZ (Table 4.8 and Figure 25). PHZ treated positive control (group B) rats demonstrated significant decrease ($p < 0.001$) in Hb content compared to non-treated negative group (group A) at day 3. The percentage reduction in Hb was found 27.82 ± 2.18, 29 ± 2.94, 26.35 ± 2.84, 23.7 ± 4.88 and 25.57 ± 2.86 in group B, C, D5, D10 and D15 on day 3 after PHZ administration. The percentage recovery in Hb content at the day 7, 10 and 15 were presented in figure 26. In group D10 and D15, the Hb content (gm %) was significantly increase 12.75 ± 0.4 ($p < 0.05$) and 12.70 ± 0.17 ($p < 0.05$) at day 7 respectively which was better improvement compared to group C and statistically compare with group B values at the day 3.

Table 4.8: Estimation of haemoglobin content (gm %) of rats in PHZ – induced anaemia.

| Groups | Day | | | | |
	0	3	7	10	15
A	15.32 ± 0.37	15.38 ± 0.42	15.78 ± 0.24	15.3 ± 0.21	15.12 ± 0.36
B	15.23 ± 0.49	11.02 ± 0.11 +++	11.27 ± 0.061	11.58 ± 0.07	12.53 ± 0.30
C	15.63 ± 0.22	10.68 ± 0.25 +++	11.97 ± 0.21	12.98 ± 0.20 **	14.02 ± 0.29 ***
D5	15.8 ± 0.20	11.23 ± 0.23 +++	11.73 ± 0.13	12.57 ± 0.14	14.98 ± 0.19 ***
D10	15.72 ± 0.27	11.6 ± 0.42 +++	12.75 ± 0.40 *	13.85 ± 0.33 ***	15.63 ± 0.34 ***
D15	15.88 ± 0.27	11.35 ± 0.19 +++	12.7 ± 0.17 *	14.02 ± 0.31 ***	15.68 ± 0.37 ***
E	15.48 ± 0.23	15.63 ± 0.18	15.93 ± 0.20	16.13 ± 0.21	16.83 ± 0.42

Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.
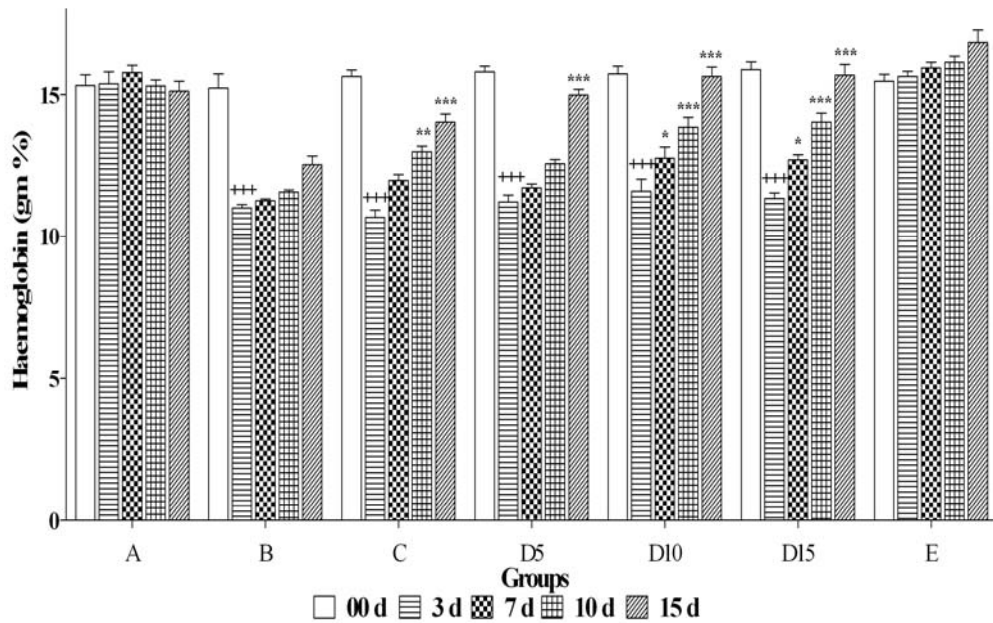
Figure 4.25: Effect of OFJ on Hb content in PHZ –induced anaemia.
Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by
Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for
change difference at day 3 Vs negative control (group A) at day 0 and *** $p <$
0.001, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs
positive control (group B) at day 3.
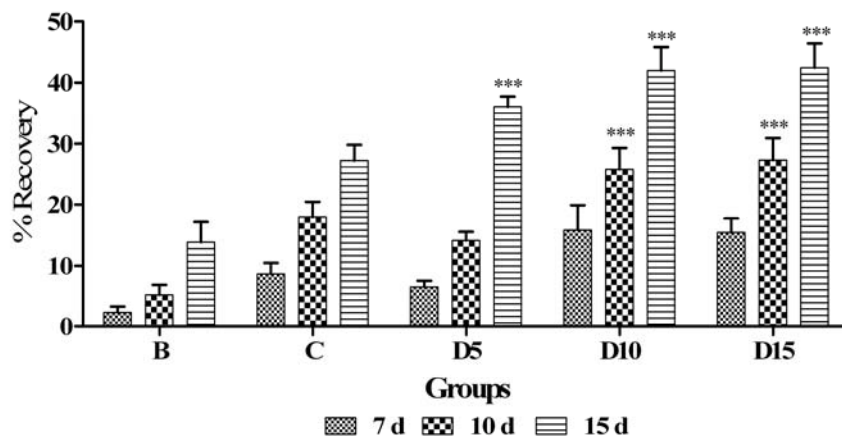
**Figure 4.26: The percentage recovery in Hb content of rats at day 7, 10
and 15 in PHZ –induced anaemia.** Values are Mean ± SEM (n = 6),
analyzed by one way ANOVA followed by Turkey's multiple comparison test,
*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference Vs positive
control (group B) at day 7, 10 and 15.

4.3.2.2.3 Effect on Haematological parameters

The changes in the haematological parameters of the rats during the study are presented in figure 4.27 to 4.32. PHZ treated positive control rat demonstrated significant decrease in mean total RBC count ($p < 0.001$), PCV ($p < 0.001$), MCV and increase in MCH ($p < 0.001$ in group C & D15), MCHC and RDW ($p <0.001$) on the day 3 with respect to the values of negative control (group A) on the day 0. Treatment of anaemic rats with standard and OFJ reversed the effect of PHZ on the day 7. Statistically significant increase in mean total RBC count was found 5.582 ± 0.073 ($p < 0.05$) and 6.65 ± 0.103 ($p <0.001$) at the day 10 and 15 respectively in group D5 while in group D10 and D15 significantly ($p <0.001$) increase on same day with respect to the values of group B at the day 3. The mean PCV (%) level was highly significantly ($p <0.001$) improved in OFJ (5, 10, 15 ml/kg) treated group on the day 10. In group E rats, there was no significant change in the haematological parameters during experimental period.

4.3.2.2.4 Effect on Total and Differential WBC count

Pre- and post – treated mean total and differential WBC counts in PHZ – induced anaemia were presented in figure 4.33 to 4.35. Statistically, highly significant ($p < 0.001$) increase in total WBC count and neutrophils while decrease lymphocytes in PHZ treated rats at the day 3 with the comparison to the values of group A at the day 0. The mean total leukocyte counts were observed 21.67 ± 1.98 ($p < 0.01$), 19.06 ± 0.75 and 19.47 ± 0.78 ($p < 0.001$) in group C, D10 and D15, respectively at the day 10. The mean neutrophils were highly significantly ($p < 0.001$) reduced in group D15 at the day 7 with the comparison to the values of group B at the day 3. The mean lymphocytes were increase more in group D15 rats better than other treatment groups.

Figure 4.27: Effect of OFJ on total Red Blood Cell (RBC) count in PHZ – induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.
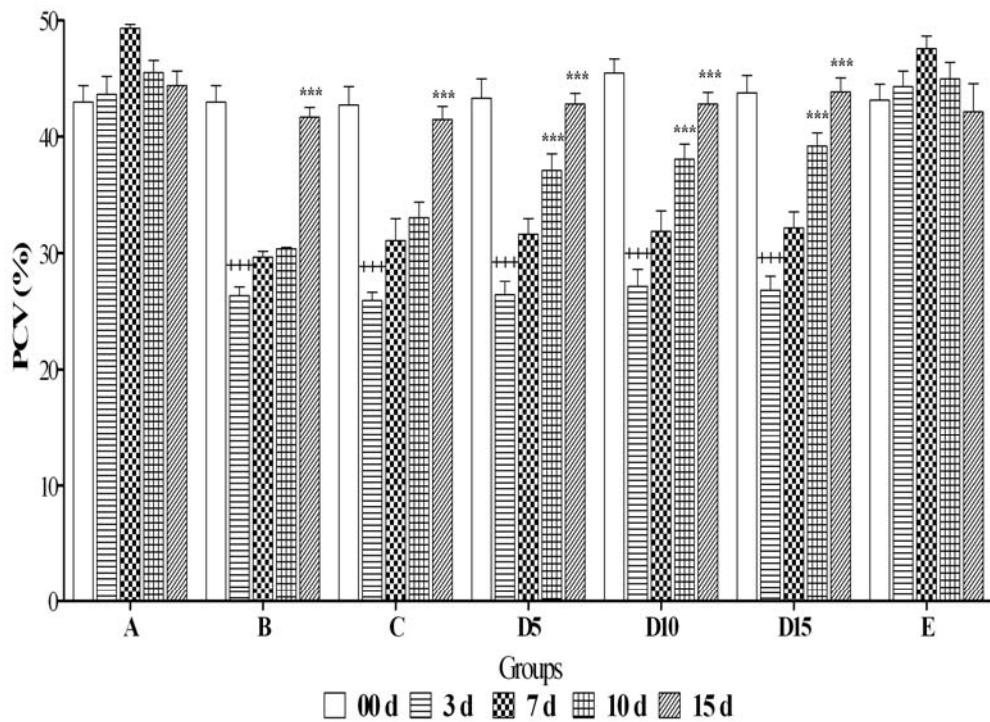
Figure 4.28: Effect of OFJ on Packed Cell Volume (PCV) in PHZ – induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.
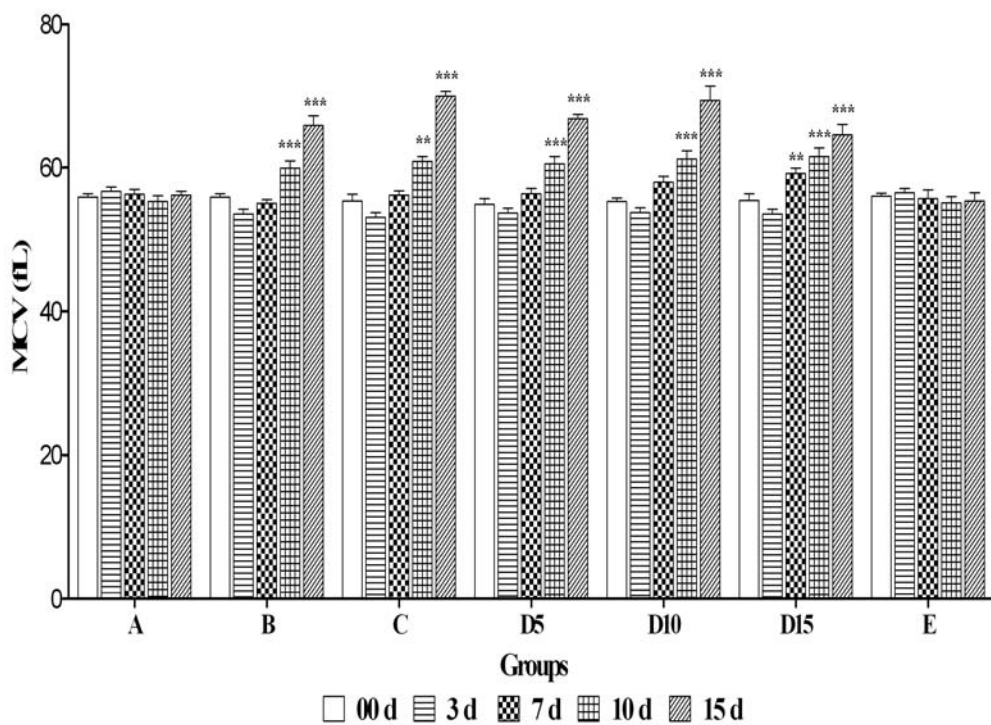
Figure 4.29: Effect of OFJ on Mean Cell Volume (MCV) in PHZ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.
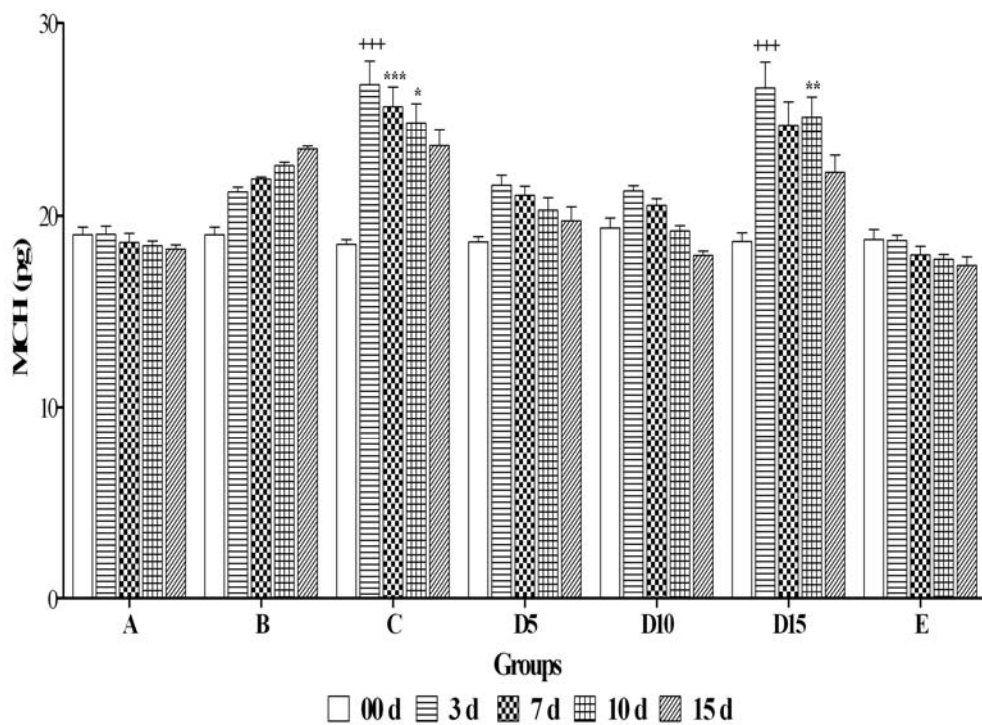
Figure 4.30: Effect of OFJ on Mean Cell Haemoglobin (MCH) in PHZ – induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.
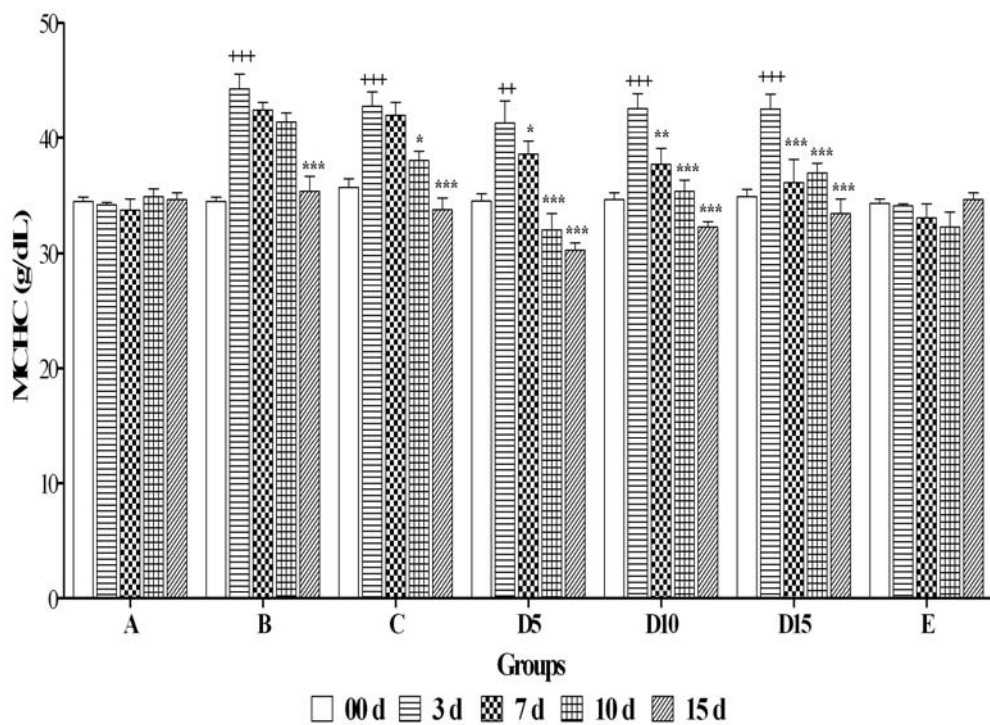
Figure 4.31: Effect of OFJ on Mean Cell Haemoglobin Concentration (MCHC) in PHZ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.
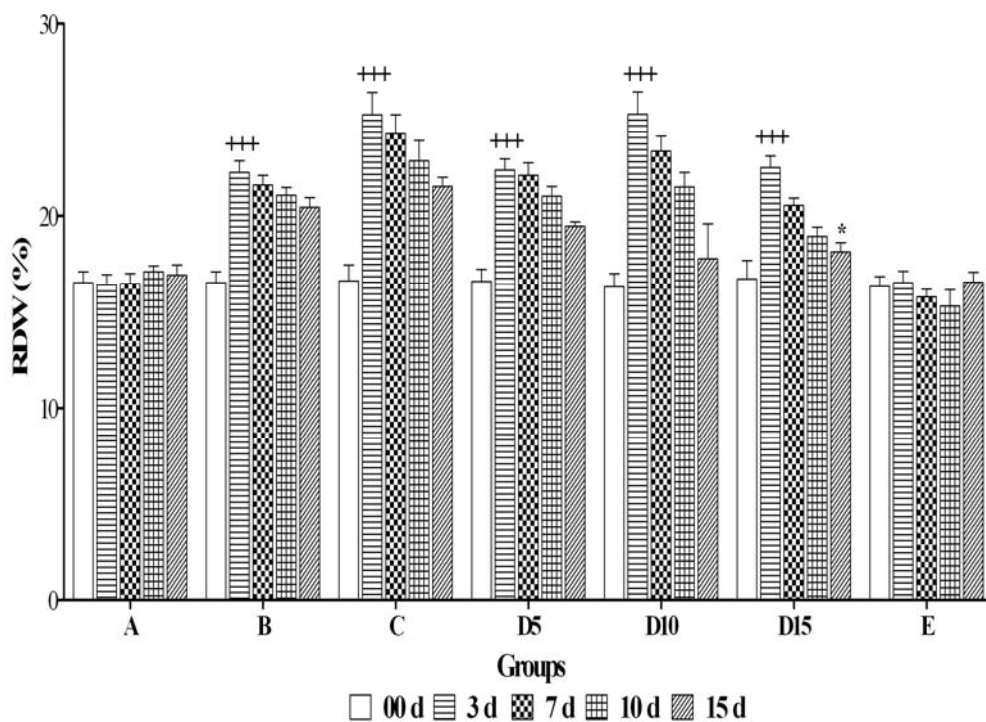
Figure 4.32: Effect of OFJ on Red blood cell Distribution Width (RDW) in PHZ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.
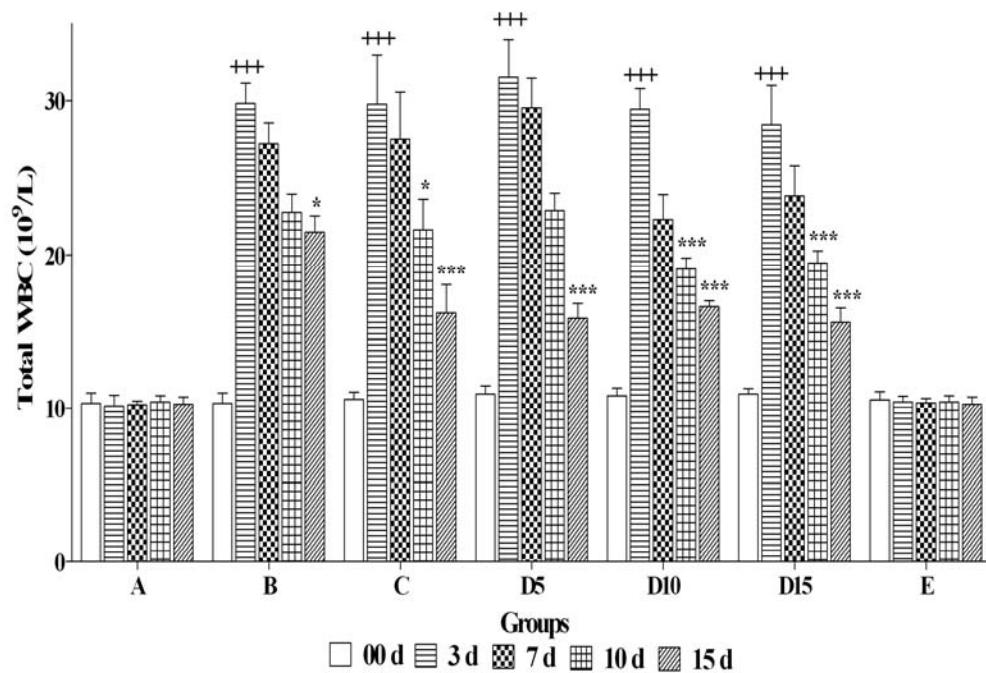
Figure 4.33: Effect of OFJ on Total White Blood Cell (WBC) counts in PHZ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.
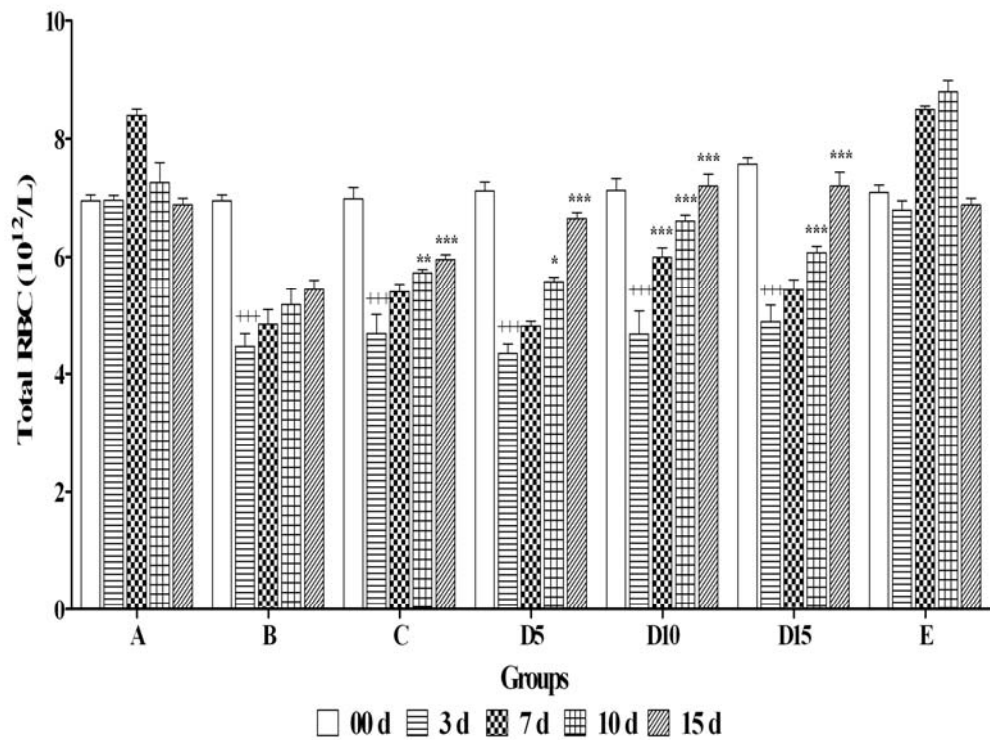
159
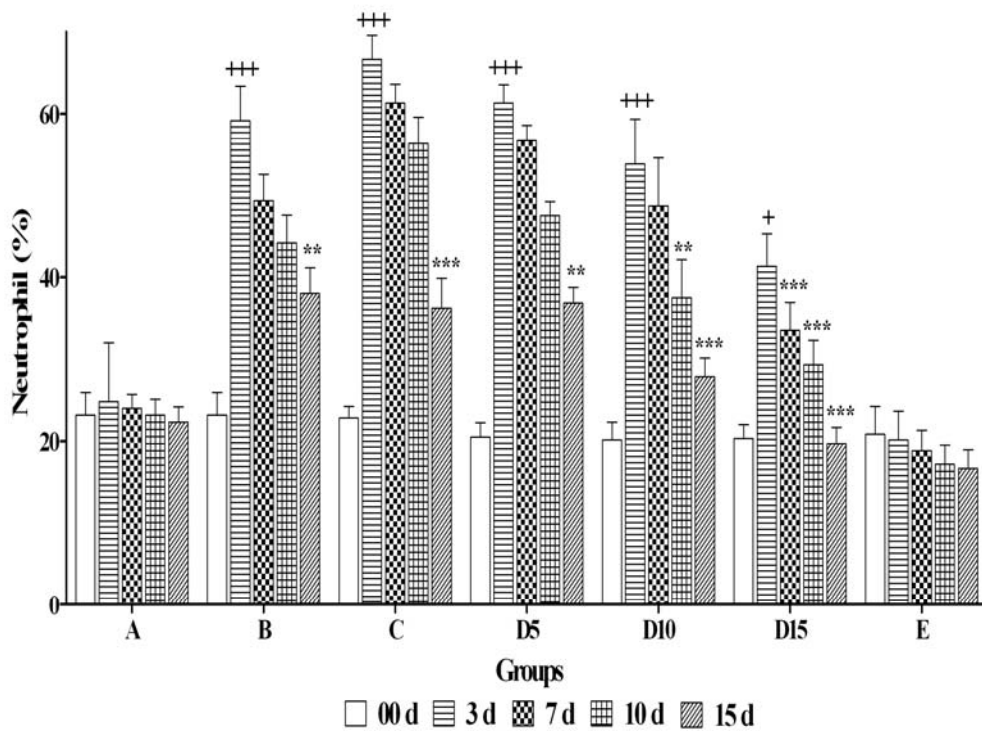
Figure 4.34: Effect of OFJ on Neutrophil count in PHZ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.
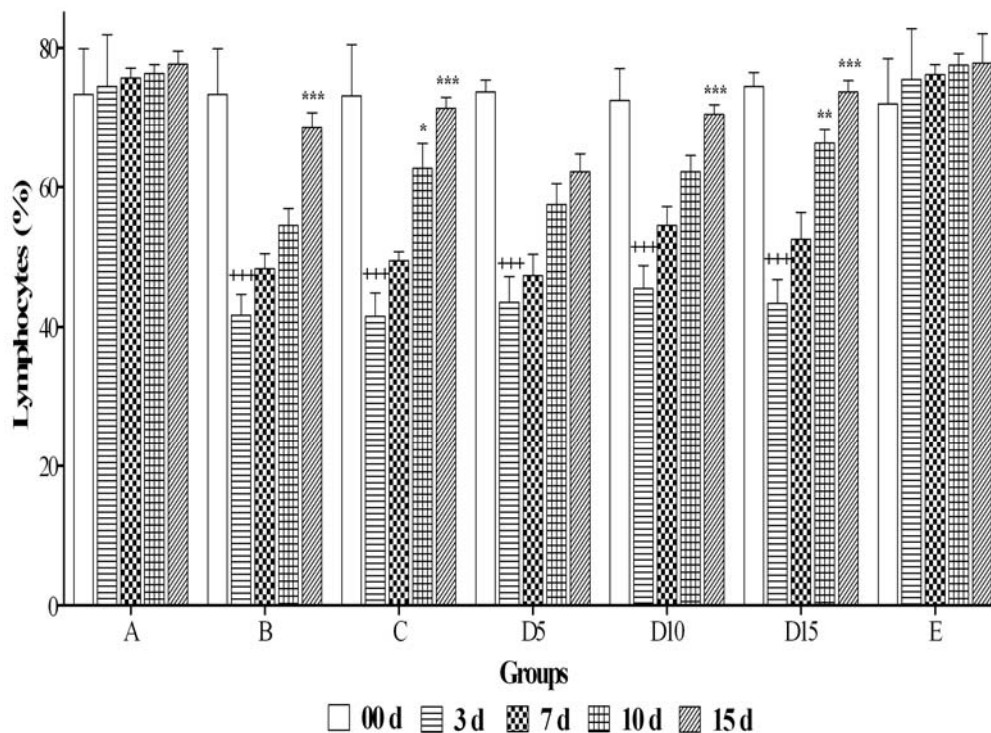
Figure 4.35: Effect of OFJ on Lymphocytes in PHZ –induced anaemia.
Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.

4.3.2.2.5 Effect on Platelets

Statistically, highly significant ($p < 0.001$) increase in mean platelet count and decrease in mean PDW in PHZ treated rat at the day 3 with respect to the values of group A at the day 0 while mean MPV was not significantly affected (Figure 4.36 to 4.38). The mean platelets were observed 1063 ± 20.36 ($p < 0.01$) and 961.5 ± 20.38 ($p < 0.001$) at the day 7 and 10, respectively, in group D10 while 988.2 ± 31.01 ($p < 0.001$) at the day 10 in group D15 with the comparison to the values of group B at the day 3.

Figure 4.36: Effect of OFJ on Platelet count in PHZ –induced anaemia.
Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by
Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for
change difference at day 3 Vs negative control (group A) at day 0 and *** $p <$
0.001, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs
positive control (group B) at day 3.

Figure 4.37: Effect of OFJ on Platelet Distribution Width (PDW) in PHZ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.

Figure 4.38: Effect of OFJ on Mean Platelet Volume (MPV) in PHZ – induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.

164

4.3.2.2.6 Effect on Reticulocytes count

In PHZ –induced anaemia, reticulocytosis was found in rats at the day 3, 7, 10 and 15 (Figure 4.39). The mean reticulocytes were 1.237 ± 0.06 ($p < 0.05$), 1.44 ± 0.073, ($p < 0.001$), 1.267 ± 0.23 ($p < 0.01$), 1.283 ± 0.162 ($p < 0.01$) and 1.317 ± 0.166 ($p < 0.001$) in group B, C, D5, D10 and D15 at the day 3 respectively with comparison to the values of group A at the day 0. At the day 7, in group C, D10 and D15, mean reticulocytes were significantly increase 2.103 ± 0.249 ($p < 0.05$), 2.395 ± 0.156 ($p < 0.001$) and 2.453 ± 0.253 ($p < 0.001$), respectively with respect to the values of group B at the day 3. These indicate fruit juice increase the erythropoietic activity in PHZ –induced anaemia.

Figure 4.39: Effect of OFJ on reticulocyte counts in PHZ –induced anaemia. Values are Mean $\pm$ SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, $+++ p < 0.001$, $++ p < 0.01$, $+ p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and $*** p < 0.001$, $** p < 0.01$, $* p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.

4.3.2.2.7 Effect on Ferritin level

The mean ferritin level was estimated at the day 0, 3, 7, 10 and 15 in rat in PHZ –induced anaemia model and have been presented in figure 4.40. Increase of serum ferritin level was found to be statistically highly significant ($p < 0.001$) on day 7, 10 and 15 in group B with respect to the values of same group on day 3. There was increased in serum ferritin in all groups except group A and E. These indicate in group B rats loss of iron is more compared to other groups on the day 7, 10 and 15. In standard and fruit juice treated rat iron may be available or used for the haemoglobin synthesis. Hyperferritinemia might be produce due to iron overload, acute inflammatory conditions and liver diseases.
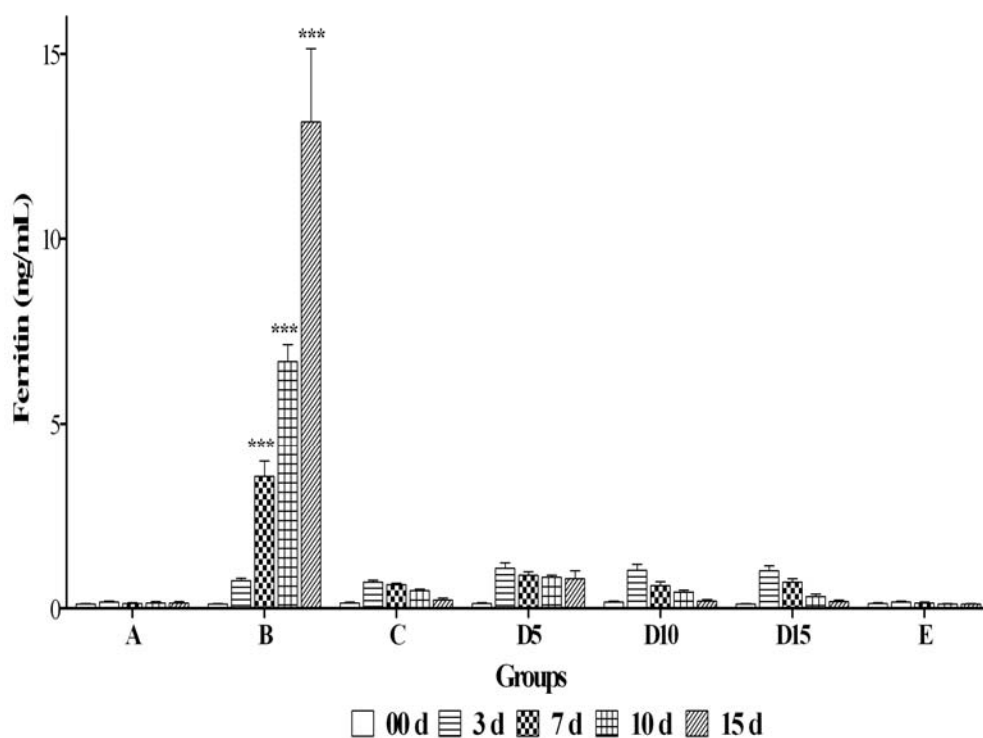
Figure 4.40: Effect of OFJ on Ferritin level in PHZ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.

4.3.2.2.8 Effect on Blood sugar, Cholesterol and Triglycerides content

The mean blood sugar, cholesterol and triglyceride levels in serum were estimated on the day 0, 3, 7, 10 and 15 in PHZ –induced anaemia and have been presented in figure 4.41 to 4.43. The mean blood sugar was significantly increase, 87.83 ± 2.68 ($p < 0.01$), 92.5 ± 2.83 ($p < 0.001$) and 95.67 ± 4.74 ($p < 0.01$) in group D5, D10 and D15, respectively, at the day 15 with respect to the values of group B at the day 3. There was no major change in cholesterol and triglyceride concentration during experimental period. There was increase in blood sugar level might be due to presence of good amount of carbohydrate in fruit juice and served as a good source of energy.

4.3.2.2.9 Effect on Kidney functions

The kidney function parameters were estimated from PHZ –induced anaemic rats on the day 0, 3, 7, 10, 15 and has been presented in figure 4.44 and 4.45. There was elevation in blood urea and creatinine concentration in rats at the day 3. The mean blood urea and creatinine concentration was found 41.47 ± 1.42 ($p < 0.01$) and 0.533 ± 0.033 ($p < 0.01$) in group D10 and 41.73 ± 2.24 ($p < 0.01$) and 0.483 ± 0.087 ($p < 0.001$) in group D15 at the day 10 with comparison to group B values at the day 3.

4.3.2.2.10 Effect on Liver functions

The liver function parameters were estimated on the day 0, 3, 7, 10, and 15 in PHZ –induced anaemia in rats and have been presented in figure 4.46 to 4.48. Statistically, significant increase in mean bilirubin concentration ($p < 0.001$) and decrease in alkaline phosphatase ($p < 0.05$ in group C, D10 and D15) and total proteins ($p < 0.001$) in PHZ –treated rats on the day 3 with respect to the values of group A on the day 0. The fruit juice of *Opuntia elatior* Mill. reversed the effect of PHZ on liver function parameters on the day 7.
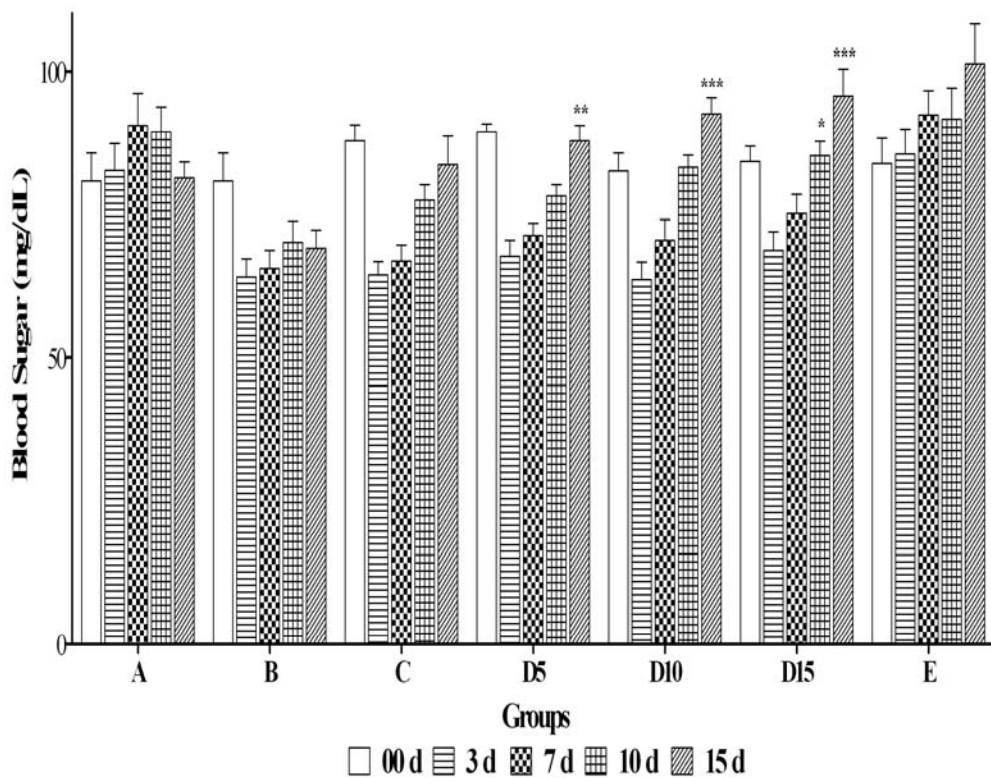
Figure 4.41: Effect of OFJ on Blood Sugar in PHZ –induced anaemia.
Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by
Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for
change difference at day 3 Vs negative control (group A) at day 0 and *** $p <$
0.001, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs
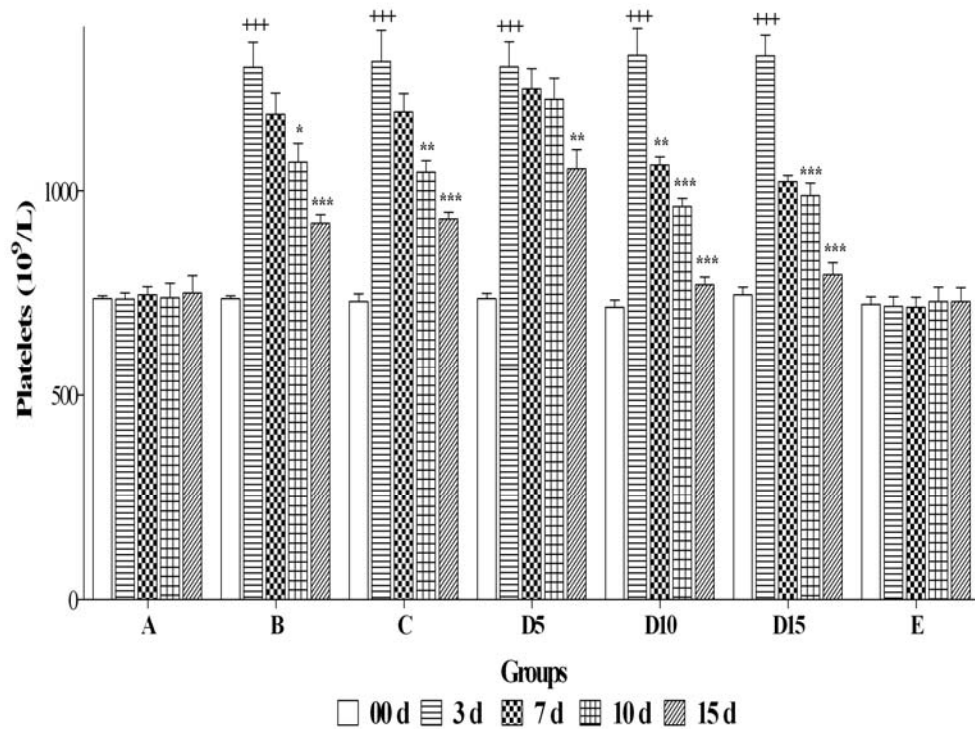positive control (group B) at day 3.
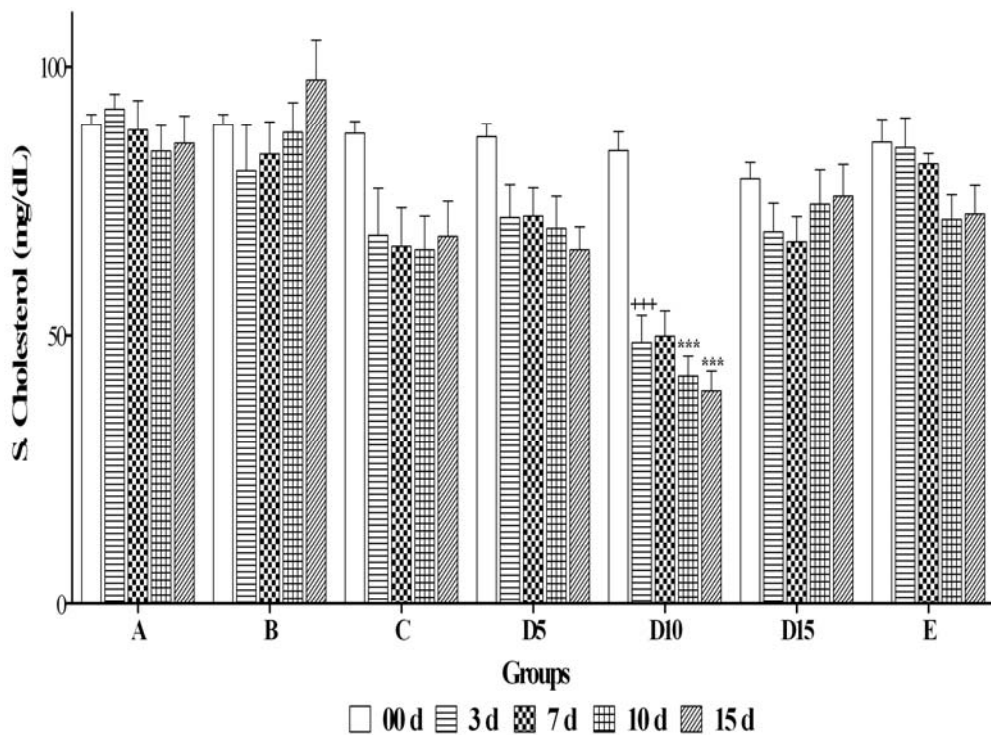
Figure 4.42: Effect of OFJ on S. Cholesterol in PHZ –induced anaemia.
Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by
Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for
change difference at day 3 Vs negative control (group A) at day 0 and *** $p <$
0.001, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs
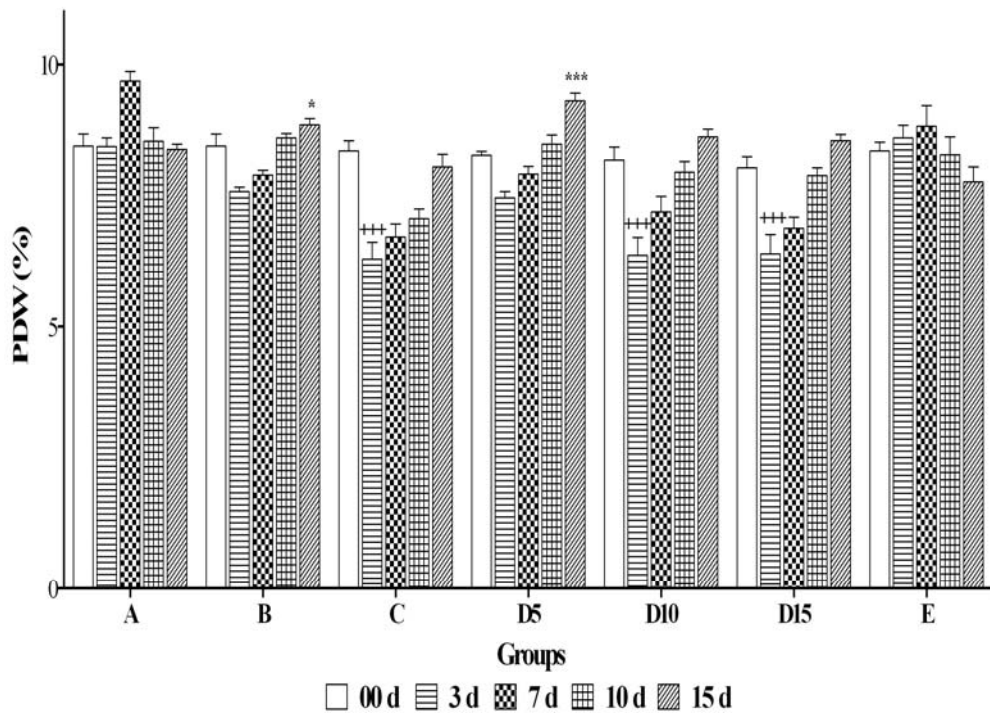positive control (group B) at day 3.
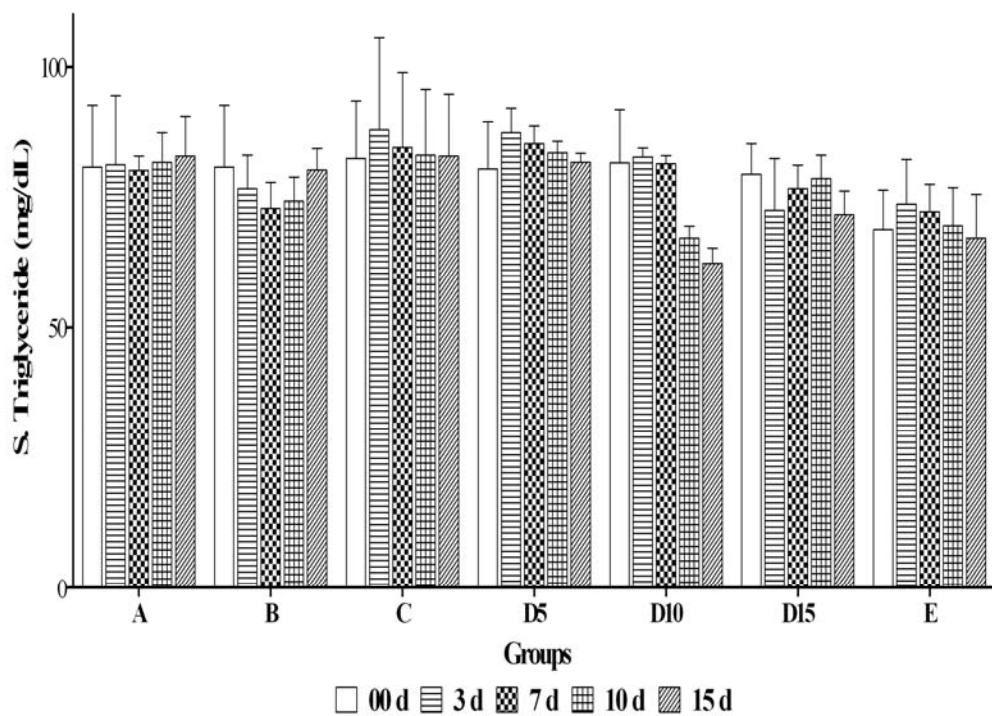
Figure 4.43: Effect of OFJ on S. Triglyceride in PHZ –induced anaemia.
Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.
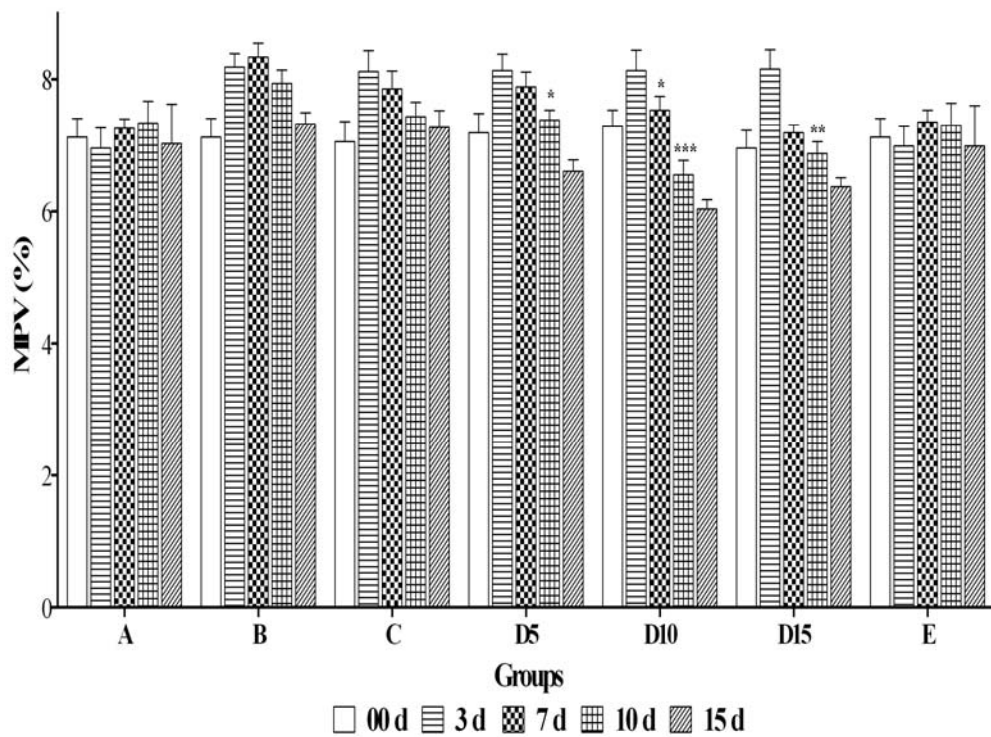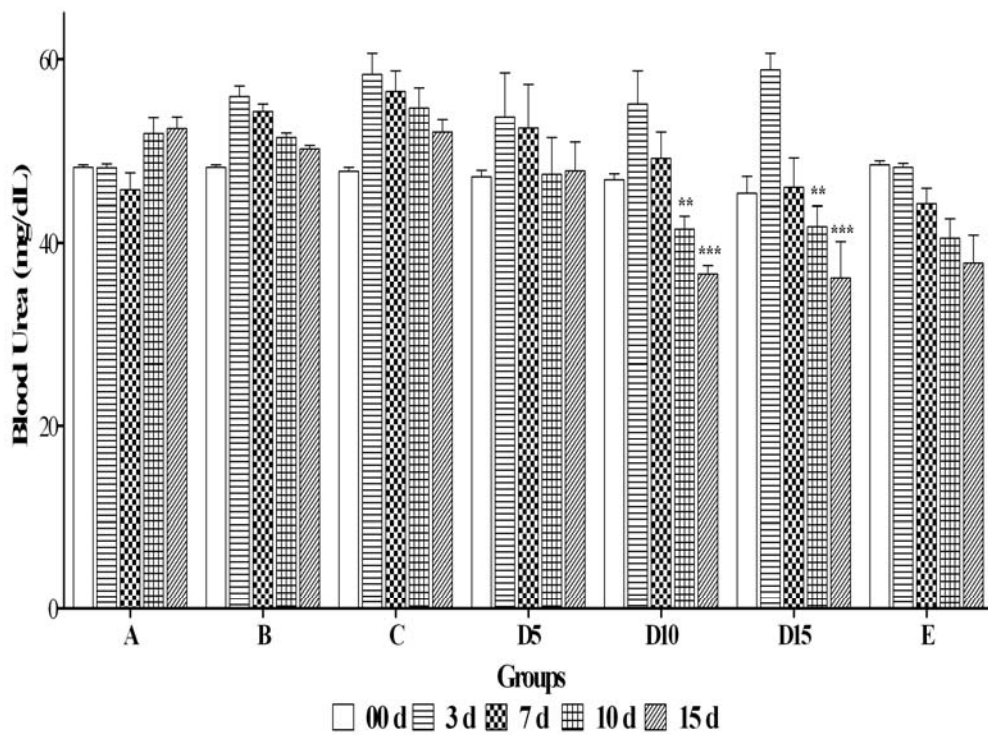
Figure 4.44: Effect of OFJ on Blood Urea in PHZ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.
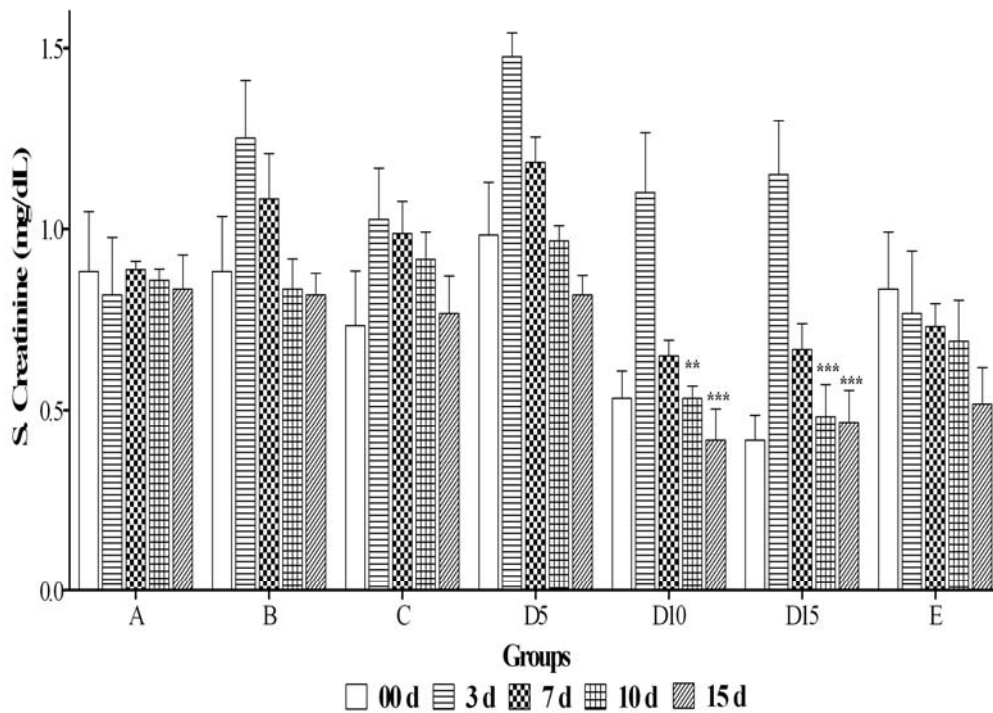
Figure 4.45: Effect of OFJ on S. Creatinine in PHZ –induced anaemia.
Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by
Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for
change difference at day 3 Vs negative control (group A) at day 0 and *** $p <$
0.001, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs
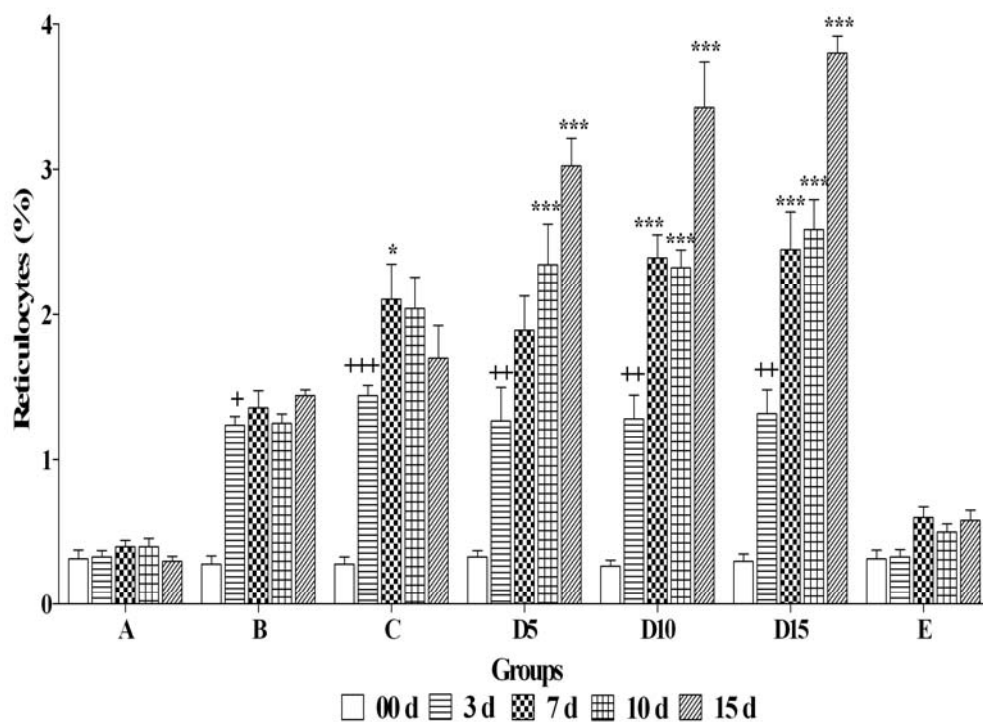positive control (group B) at day 3.
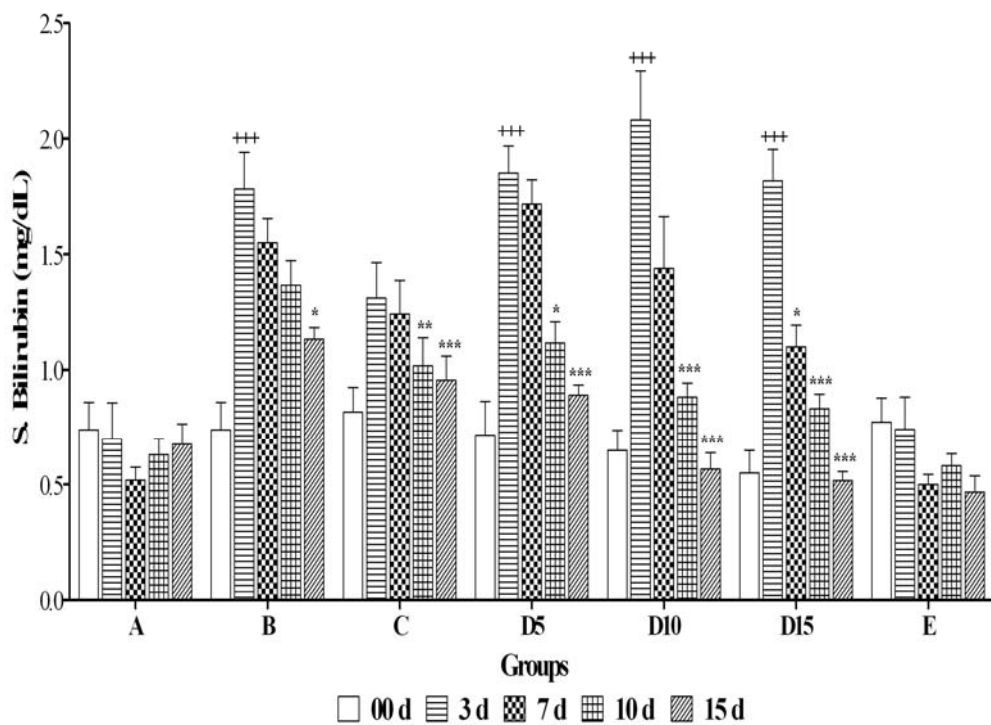
Figure 4.46: Effect of OFJ on S. Bilirubin in PHZ –induced anaemia.
Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by
Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for
change difference at day 3 Vs negative control (group A) at day 0 and *** $p <$
0.001, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs
positive control (group B) at day 3.
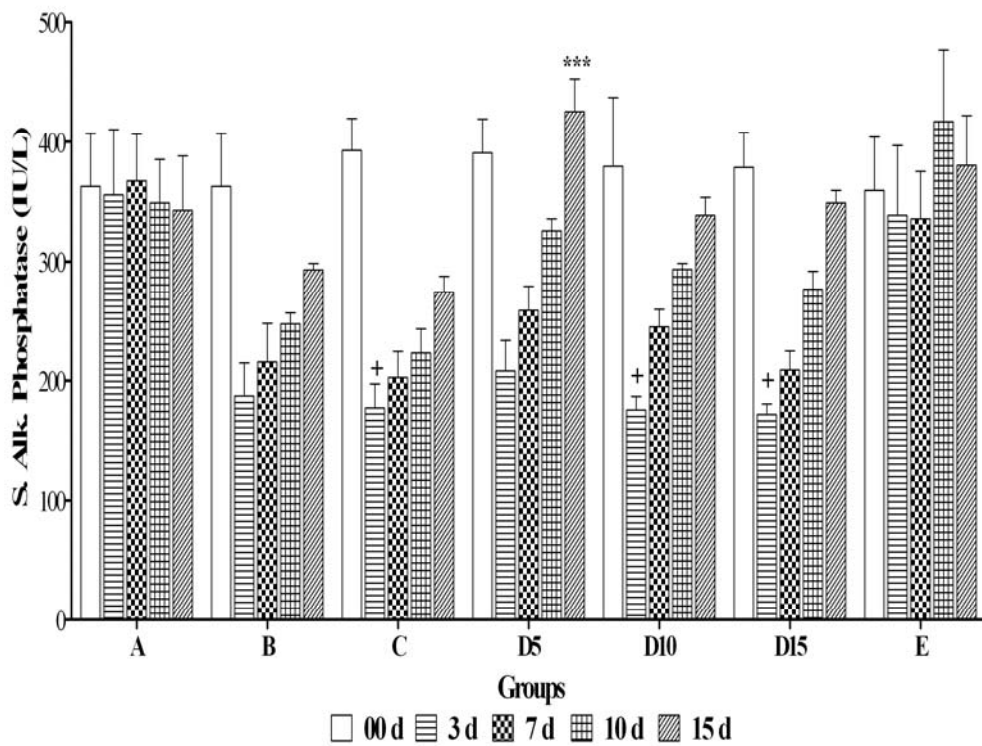
Figure 4.47: Effect of OFJ on S. Alkaline phosphatase in PHZ –induced anaemia. Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test, +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.05$ for change difference at day 3 Vs negative control (group A) at day 0 and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference at day 7, 10 and 15 Vs positive control (group B) at day 3.

174
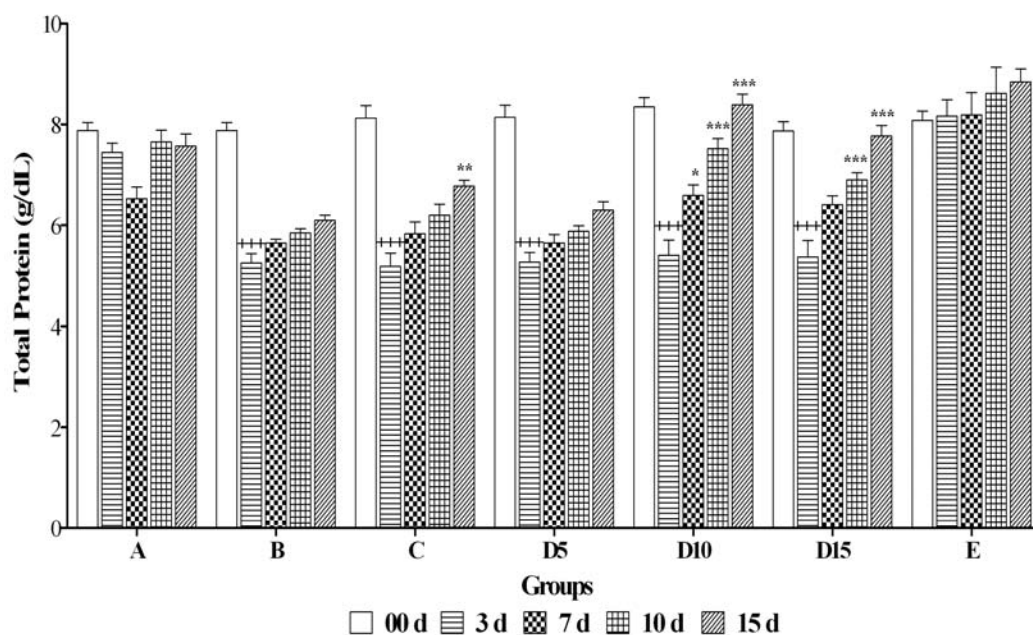
Figure 4.48: Effect of OFJ on Total Protein in PHZ –induced anaemia.
Values are Mean ± SEM (n = 6), analyzed by one way ANOVA followed by
Turkey's multiple comparison test, +++ p < 0.001, ++ p < 0.01, + p < 0.05 for
change difference at day 3 Vs negative control (group A) at day 0 and *** p <
0.001, ** p < 0.01, * p < 0.05 for change difference at day 7, 10 and 15 Vs
positive control (group B) at day 3.

4.3.2.2.11 Histopathology study

Histopathological section of kidney, liver and spleen were presented in figure
4.49, 4.50 and 4.51, respectively. Kidney section of group A (negative control)
rats revealed the normal distinct glomeruli and tubules. In group B rats the
kidney showed shrinkage and acute glomerular nephritis. In fruit juice treated
rats better histology was evident, the glomerular and tubular structures were
distinct and more improvement compared to group B kidney sections. The
liver of group B rats showed distortion of hepatocytes, portal tract dilation,
and acute inflammatory infiltration. In standard and fruit juice treated rats,
quite normal histology was seen. Normal cytoarchitecture of spleen was
observed in control group, where as cell depletion, acute inflammatory
infiltration, fibrosis and necrosis were observed in the cytoarchitecture of
spleen in group B rats. In standard and fruit juice treated rat's spleen mild fatty
changes and cell depletion was observed compared to group B rats.
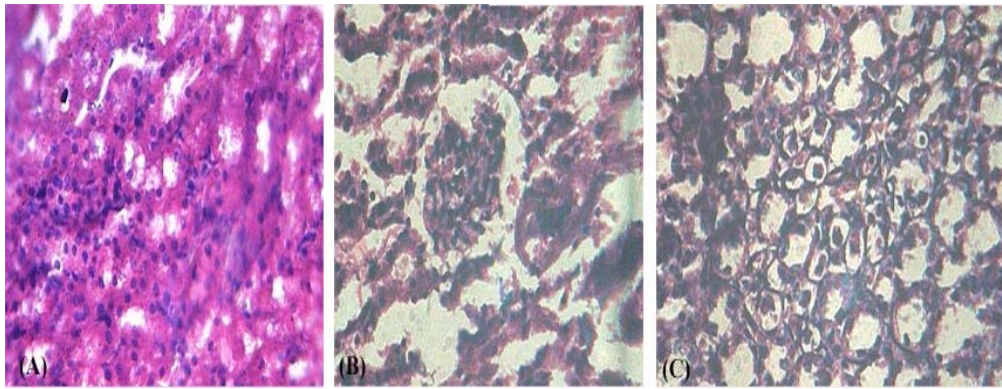
Figure 4.49: Hematoxyline – Eosin sections of kidney (450 x) of negative control (A), positive control (B) and OFJ (C) treated rats in PHZ – induced anaemia model.
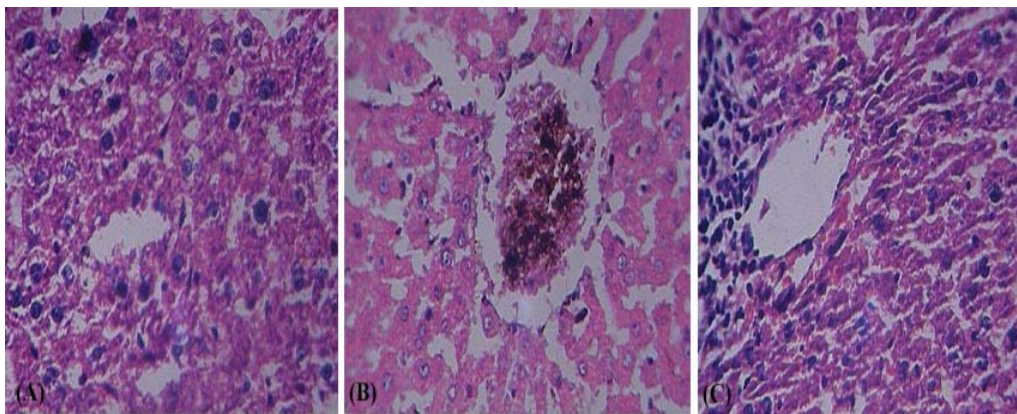
Figure 4.50: Hematoxyline – Eosin sections of liver (450 x) of negative control (A), positive control (B) and OFJ (C) treated rats in PHZ – induced anaemia model.
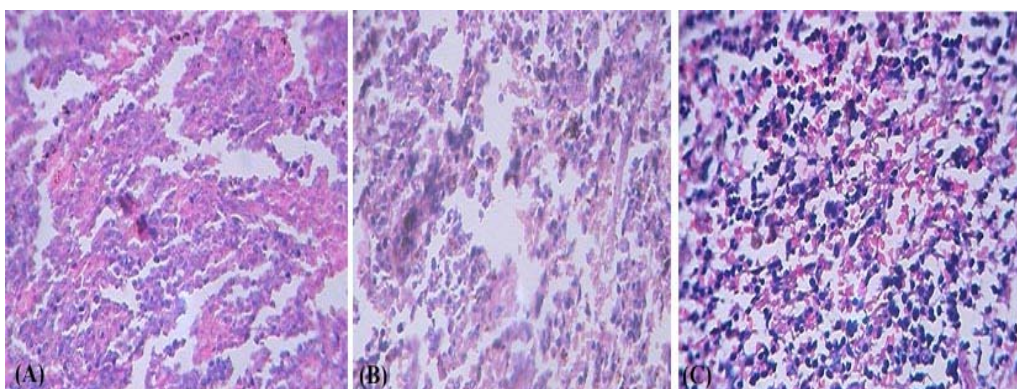
Figure 4.51: Hematoxyline – Eosin sections of spleen (450 x) of negative control (A), positive control (B) and OFJ (C) treated rats in PHZ – induced anaemia model.

4.3.3 Antinociceptive tests

4.3.3.1 Effect on the acetic acid –induced writhing

The results of the acetic acid –induced writhing in rats are given in table 4.9 and figure 4.52. The OFJ (5, 10 and 15 ml/kg, p.o.) inhibited the writhing responses of rat caused by the intraperitoneal administration of acetic acid. The mean numbers of writhes was significantly ($p < 0.001$) reduced in OFJ treated groups after 10 min when compared with control group. The percentage inhibition of the nociceptive responses was presented in table 4.10 and figure 4.53. The maximal inhibition was found 89.67 ± 1.79 % at the dose 15 ml/kg after 20 min. The analgesic effect of the OFJ in this model was dose dependant with the ED50 being 0.919 ml/kg which was equivalent to 1 ml/kg. At the dose of 15 ml/kg fruit juice exerted better analgesic action than diclofenac sodium after 20 min in acetic acid –induced writhing in rat.

Table 4.9: Analgesic effect of OFJ on acetic acid –induced writhing in rat.

Groups	Dose	Number of writhing movements			
		5 min	10 min	15 min	20 min
Control	-	21.5 ± 0.76	33.33 ± 0.84	53.17 ± 1.30	70.5 ± 0.76
Diclofenac sodium	10 mg/kg	3.33 ± 0.42 ***	6.83 ± 0.30 ***	10.67 ± 0.33 ***	15.5 ± 0.34 ***
OFJ	5 ml/kg	9 ± 1.39 **	15 ± 3.04 ***	20.5 ± 4.18 ***	25.5 ± 5.65 ***
OFJ	10 ml/kg	6.16 ± 1.19 ***	15.67 ± 1.83 ***	20.83 ± 2.12 ***	23.83 ± 2.63 ***
OFJ	15 ml/kg	2.33 ± 0.84 ***	3.66 ± 0.84 ***	4.33 ± 1.08 ***	7.33 ± 1.33 ***

Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference Vs vehicle control group.

Table 4.10: Percentage inhibition of OFJ and diclofenac sodium on acetic acid –induced writhing in rat.

Groups	Dose	% Inhibition (Mean ± SEM)			
		5 min	10 min	15 min	20 min
Diclofenac sodium	10 mg/kg	84.33 ± 2.05	79.48 ± 0.86	79.93 ± 0.42	78 ± 0.57
Fruit juice	5 ml/kg	58.05 ± 6.45	55.19 ± 8.78	61.66 ± 7.6	64.01 ± 7.75
Fruit juice	10 ml/kg	70.69 ± 6.29	52.22 ± 6.83	60.32 ± 4.85	66.22 ± 3.66
Fruit juice	15 ml/kg	88.82 ± 4.19	88.83 ± 2.64	91.59 ± 2.32	89.67 ± 1.79

Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test.
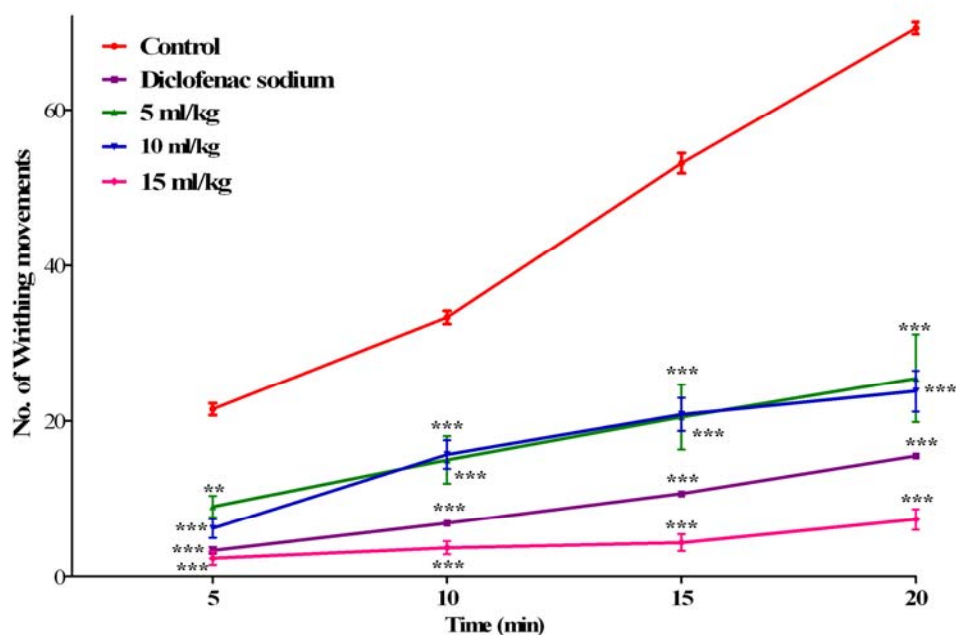
Figure 4.52: Influence of OFJ on acetic acid –induced writhing in rat. Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference Vs vehicle control group.

178
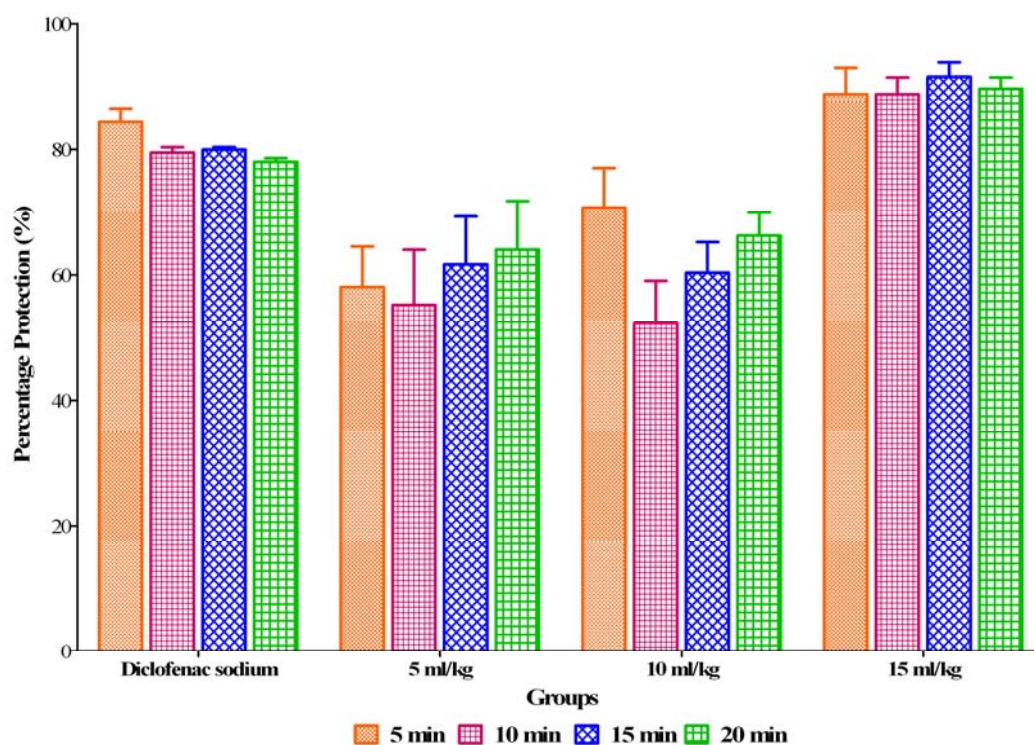
Figure 4.53: Percentage protection OFJ and diclofenac sodium on acetic acid –induced writhing in rat. Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test.

4.3.3.2 Effect on tail immersion test

The results of latency period (hour) and percentage analgesic action of OFJ in tail immersion test were presented in table 4.11 and 4.12, respectively. After a latency period of 0.5 h, the OFJ (15 ml/kg) demonstrated significant reduction ($p < 0.001$) of painful sensation due to tail immersion in warm water compared to control group. The maximum inhibitory effect of fruit juice was 45.41 ± 0.89%, 1 h post-dosing at 15 ml/kg. The maximum antinociceptive action of the OFJ (15 ml/kg) was found 65.56 ± 1.52 % at 3 h as effective as that of tramadol (10 mg/kg) 62.32 ± 6.29 %. The OFJ significantly ($p < 0.001$) increased the reaction time of rat in a dose – dependent manner with the ED_{50} being 2.77 ml/kg after a latency period 3 hour.

179

Table 4.11: Effect of OFJ and tramadol on pain induced by tail immersion test.

Latency period (h)	Control	Tramadol (10 mg/kg)	5 ml/kg	10 ml/kg	15 ml/kg
0	1.582 ± 0.015	1.87 ± 0.010	1.67 ± 0.005	1.58 ± 0.017	1.74 ± 0.010
0.5	1.567 ± 0.048	2.31 ± 0.077 ***	1.873 ± 0.018	1.852 ± 0.113	2.12 ± 0.011 ***
1	1.59 ± 0.093	2.527 ± 0.142 ***	2.1 ± 0.006 ***	2.12 ± 0.055 ***	2.53 ± 0.015 ***
2	1.593 ± 0.039	2.797 ± 0.064 ***	2.34 ± 0.028 ***	2.32 ± 0.018 ***	2.58 ± 0.009 ***
3	1.587 ± 0.096	3.035 ± 0.116 ***	2.57 ± 0.029 ***	2.46 ± 0.003 ***	2.88 ± 0.014 ***
4	1.57 ± 0.077	3.407 ± 0.090 ***	2.858 ± 0.010 ***	2.803 ± 0.127 ***	3.22 ± 0.010 ***
5	1.547 ± 0.085	3.63 ± 0.008 ***	2.88 ± 0.007 ***	2.94 ± 0.049 ***	3.37 ± 0.005 ***
6	1.588 ± 0.167	3.72 ± 0.004 ***	2.94 ± 0.007 ***	3 ± 0.046 ***	3.44 ± 0.01 ***

Values are in minutes Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, *** $p < 0.001$ for change difference Vs vehicle control group.

Table 4.12: Percentage analgesic action (Mean ± SEM) of OFJ on pain induced by tail immersion test.

Time (h)	Control	Tramadol (10 mg/kg)	5 ml/kg	10 ml/kg	15 ml/kg
0.5	0.97 ± 2.72	23.49 ± 3.81	12.18 ± 1.14	17.37 ± 7.71	21.87 ± 1.17
1	0.85 ± 6.93	35.13 ± 7.68	25.76 ± 0.73	34.35 ± 4.41	45.41 ± 0.89
2	0.79 ± 2.75	49.57 ± 3.48	40.11 ± 1.56	46.95 ± 2.32	48.29 ± 0.69
3	0.36 ± 6.18	62.32 ± 6.29	53.9 ± 1.89	55.79 ± 1.69	65.56 ± 1.52
4	0.56 ± 5.49	82.2 ± 4.94	71.17 ± 0.83	77.62 ± 8.60	85.1 ± 1.46
5	2.15 ± 5.53	94.14 ± 0.89	72.47 ± 0.82	86.22 ± 4.01	93.71 ± 1.24
6	0.56 ± 10.73	98.97 ± 1.25	76.06 ± 0.59	90.01 ± 3.78	97.74 ± 1.54

4.3.4 Anti-asthmatic action

4.3.4.1 Effect on Bronchospasm induced by Acetylcholine and Histamine in guinea pigs

Pretreatment with OFJ (5, 10 and 15 ml/kg, p.o.) demonstrated significant increase ($p < 0.001$) and dose dependently delayed the onset of convulsion in guinea pigs due to acute bronchospasm induced by 0.25% histamine and 0.5% acetylcholine aerosol (Table 4.11). The percentage increase in preconvulsion time of OFJ (15 ml/kg) treated animal was comparable to both ketotifen (1 mg/kg) and atropine (2 mg/kg) (Table 4.12).

Table 4.13: Effect of OFJ on preconvulsion time of guinea pig after histamine and acetylcholine aerosol exposure.

Groups	Treatment (p.o.)	Preconvulsion Time (sec)	
		Histamine	Acetylcholine
A	Distilled water (1ml/kg)	95.83 ± 5.06	95.83 ± 5.06
B	Ketotifen (1 mg/kg)	317 ± 21. 62 ***	-
C	Atropine sulfate (2 mg/kg)	-	230.8 ± 14.73 ***
D	OFJ (5 ml/kg)	156.5 ± 8.17 *	169.8 ± 6.90 ***
E	OFJ (10 ml/kg)	219 ± 12.59 ***	216.2 ± 12.38 ***
F	OFJ (15 ml/kg)	290.2 ± 15.1***	223.3 ± 8.34 ***

Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference Vs vehicle control (group A).

Table 4.14: Effect of OFJ on Histamine and Acetylcholine induced bronchospasm in guinea pigs.

Groups	Treatment (p.o.)	% Increase in Preconvulsion Time	
		Histamine	Acetylcholine
B	Ketotifen (1 mg/kg)	69.44 ± 1.64	-
C	Atropine sulfate (2 mg/kg)	-	58. 32 ± 0.89
D	OFJ (5 ml/kg)	37.5 ± 5.75	42.99 ± 4.14
E	OFJ (10 ml/kg)	55.09 ± 4.39	54.62 ± 4.19
F	OFJ (15 ml/kg)	66.82 ± 1.40	57.07 ± 1.68

Values are Mean ± SEM (n = 6).

4.3.4.2 Anticholinergic action on isolated rat ileum

Rat ileum suspended in tyrode solution with 1 g of tension stabilized for 15 min. Acetylcholine (1.83×10^{-7} M to 1.46×10^{-6} M) produced a concentration – dependant contraction of tissue, reaching its maximum within 30 s of tissue contact time. The OFJ at a dose of 50 and 100 μl/ml significantly ($p < 0.001$) inhibited acetylcholine induced contraction response and caused 35% and 43% reductions to the response of 1.47 μM acetylcholine, respectively. OFJ at concentration 10 μl/ml mild significantly ($p < 0.05$) inhibited the response of 1.1 μM acetylcholine (Table 4.13 and Figure 4.54).

Table 4.15: Effect of OFJ on acetylcholine –induced percentage maximum contraction of isolated rat ileum.

Log[Ach] (M)	Percentage maximum contraction (Mean ± SEM)			
	Acetylcholine	Fruit juice of *O. elatior* Mill.		
		10 μl/ml	50 μl/ml	100 μl/ml
-6.73	50.67 ± 5.81	38.71 ± 2.76	27.96 ± 2.59	26.34 ± 2.11 [a]
-6.43	62.88 ± 5.27	48.39 ± 2.49	36.56 ± 2.59 [a]	33.87 ± 1.81 [a]
-6.13	79.65 ± 3.92	66.13 ± 3.50	52.42 ± 2.23 [a]	46.24 ± 3.29 [a]
-5.95	98.19 ± 0.82	79.57 ± 4.53 *	59.95 ± 2.51 [a]	55.91 ± 1.36 [a]
-5.83	100 ± 0	93.55 ± 4.92	65.86 ± 3.07 [a]	57.53 ± 1.93 [a]

Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, [a] $p < 0.001$, *$p < 0.05$ for change difference Vs acetylcholine response.

182
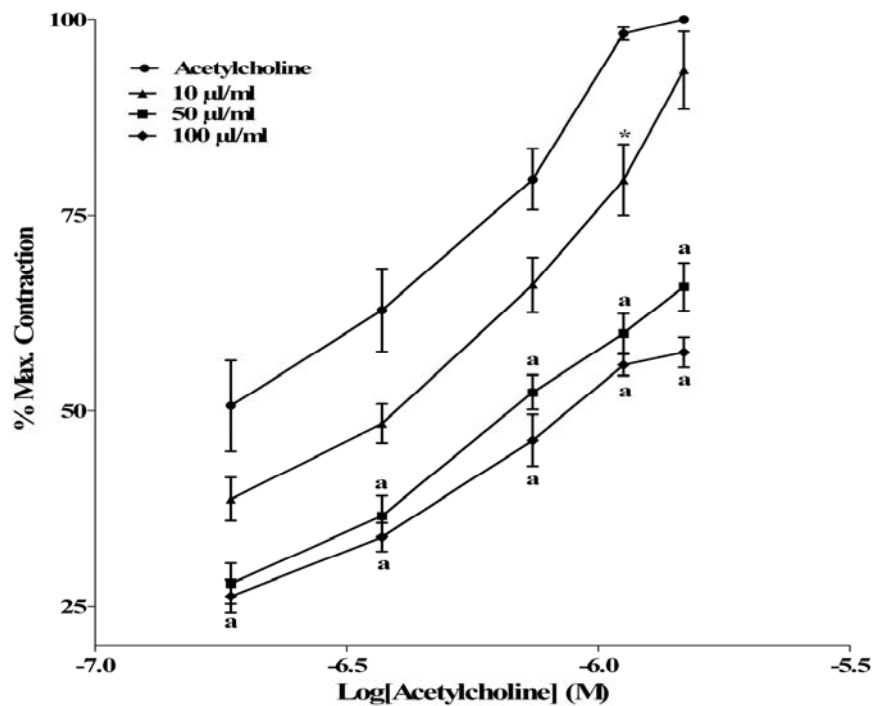
Figure 4.54: Effect of OFJ on tension development in isolated rat ileum.
Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by
Turkey's multiple comparison test, [a] $p < 0.001$, *$p < 0.05$ for change difference
Vs acetylcholine response.

4.3.4.3 Antihistaminic action of isolated Guinea pig ileum

Using the isolated guinea pig ileum as a model, the OFJ (10, 50 and 100 μl/ml)
on histamine –induced contractions were studied. As shown in table 4.14 and
figure 4.55, histamine (1.085×10^{-7} M to 1.085×10^{-6} M) produced dose –
dependant contractions of guinea pig ileum. Pretreatment with OFJ inhibited
the contractions of histamine. OFJ at concentration 50 μl/ml and 100 μl/ml had
significant ($p < 0.01$ and $p < 0.001$) inhibitory effect on the histamine
concentration – response curve, reducing the maximum induced contraction,
and caused 16% and 55% reductions to the response of 1.085×10^{-6} M
histamine.

183

Table 4.16: Effect of OFJ on histamine –induced percentage maximum contraction of isolated guinea pig ileum.

Log[Histamine] (M)	Percentage maximum contraction (Mean ± SEM)			
	Histamine	OFJ		
		10 µl/ml	50 µl/ml	100 µl/ml
-6.964	20.68 ± 0.72	19.57 ± 0.88	18.25 ± 0.79	2.027 ± 0.33***
-6.663	38.81 ± 1.14	35.71 ± 1.91	23.57 ± 1.52*	19.57 ± 1.51***
-6.362	57.33 ± 4.1	52.91 ± 0.97	39.81 ± 5.17**	28.04 ± 1.33***
-6.186	72.26 ± 3.54	67.98 ± 1.71	55.96 ± 5.42**	34.65 ± 1.38***
-6.061	96.91 ± 1.2	90.74 ± 3.41	80.23 ± 3.74**	41.26 ± 1.69***
-5.964	100 ± 0	94.97 ± 4.51	83.96 ± 4.12*	45.23 ± 1.86***

Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, *** $p < 0.001$, **$p < 0.01$, *$p < 0.05$ for change Vs histamine contractions.
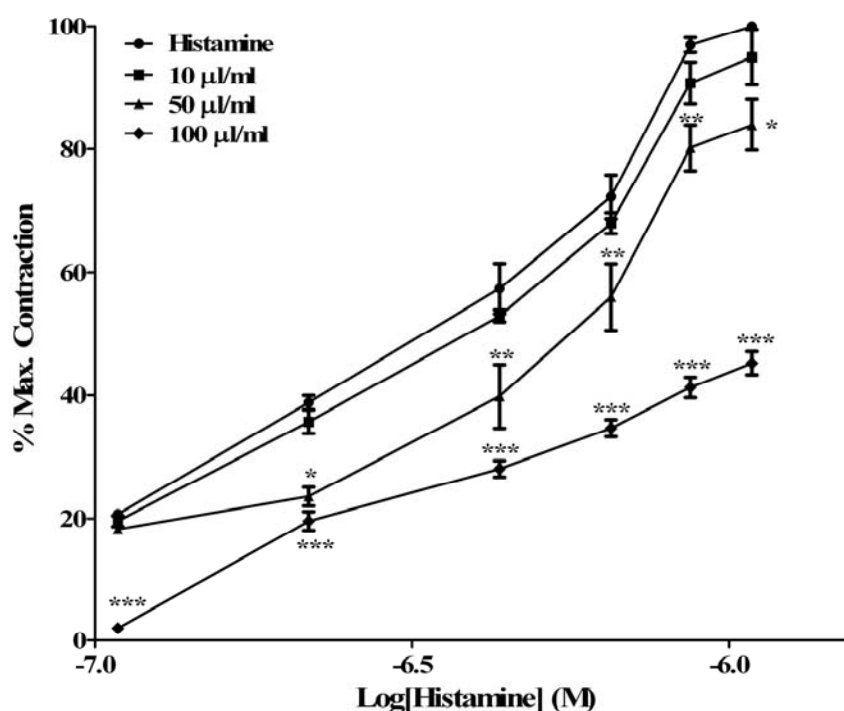
Figure 4.55: Effect of OFJ on tension development in isolated guinea ileum. Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, *** $p < 0.001$, **$p < 0.01$, *$p < 0.05$ for change Vs histamine contractions.

4.3.4.4 Effect on mast cell degranulation

The OFJ (10 – 200 μl/ml) were studied for the effect on sensitized rat peritoneal mast cell degranulation induced by immunological (egg albumin) and non-immunological (compound 48/80) stimuli and compared with that of reference compound (sodium cromoglycate and ketotifen, 10 μg/ml).

Egg albumin (1 ml, 10% w/v) incubated mast cell demonstrated 93.17% of degranulation. Sodium cromoglycate and ketotifen as a reference standard produced an inhibition of 75.33% and 80.33%, respectively. OFJ produced significant ($p < 0.001$) concentration – dependant inhibition of mast cell degranulation (Table 4.15 & Figure 4.56).

Compound (48/80) (10 μg/ml) incubated mast cell demonstrated 92.83% of degranulation. Sodium cromoglycate and ketotifen as a reference standard produced an inhibition of 79.33% and 84.33%, respectively. OFJ produced significant ($p < 0.001$) concentration – dependant inhibition of mast cell degranulation with respect to positive control group (Table 4.16 & Figure 4.57).

Further, we observed better inhibition with OFJ (200 μl/ml) than sodium cromoglycate in egg albumin induced mast cell degranulation. The IC_{50} of fruit juice is being 12.24 and 18 μl/ml for egg albumin and compound 48/80 induced mast cell degranulation, respectively. Figure 4.58 represented the photographs of intact and degranulated mast cells.

Table 4.17: Effect of OFJ on egg albumin induced rat mast cell degranulation.

Groups	Dose	% Mast cell degranulated	% Inhibition of degranulation
Negative Control	-	12.17 ± 2.34	87.83 ± 2.34
Positive Control	-	93.17 ± 0.87	6.833 ± 0.87
Na cromoglycate	10 µg/ml	25.67 ± 1.40 [$]	75.33 ± 1.43 [$]
Ketotifen	10 µg/ml	19.67 ± 1.52 [$]	80.33 ± 1.52 [$]
OFJ	10 µl/ml	51.83 ± 1.79 [$]	48.17 ± 1.77 [$]
	20 µl/ml	43.67 ± 1.83 [$]	56.33 ± 1.83 [$]
	40 µl/ml	39.17 ± 0.65 [$]	60.83 ± 0.65 [$]
	60 µl/ml	37.83 ± 1.27 [$]	62.17 ± 1.27 [$]
	80 µl/ml	29.83 ± 1.97 [$]	70.17 ± 1.97 [$]
	100 µl/ml	26.83 ± 2.25 [$]	73.17 ± 2.25 [$]
	200 µl/ml	21.33 ± 1.02 [$]	78.67 ± 1.02 [$]

Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, [$] $p < 0.001$ for change difference Vs positive control group.
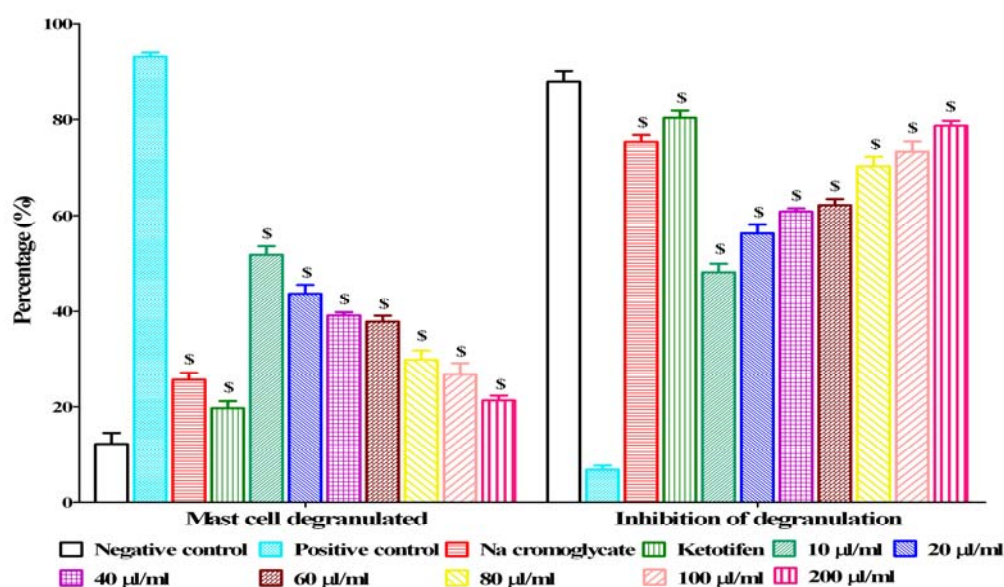
Figure 4.56: Effect of OFJ on egg albumin induced rat mast cell degranulation. Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, [$] $p < 0.001$ for change difference Vs positive control group.

Table 4.18: Effect of OFJ on compound 48/80 induced rat mast cell degranulation.

Groups	Dose	% Mast cell degranulated	% Inhibition of degranulation
Negative Control		11.17 ± 1.6	88.83 ± 1.6
Positive Control		92.83 ± 1.04	7.167 ± 1.04
Na cromoglycate	10 µg/ml	20.67 ± 1.74 [$]	79.33 ± 1.74 [$]
Ketotifen	10 µg/ml	17.33 ± 1.90 [$]	84.33 ± 1.82 [$]
OFJ	10 µl/ml	58.67 ± 1.70 [$]	41.33 ± 1.70 [$]
	20 µl/ml	47.83 ± 2.37 [$]	52.17 ± 2.37 [$]
	40 µl/ml	42.33 ± 1.43 [$]	57.67 ± 1.43 [$]
	60 µl/ml	37.17 ± 1.55 [$]	62.83 ± 1.55 [$]
	80 µl/ml	33.5 ± 1.78 [$]	66.5 ± 1.78 [$]
	100 µl/ml	28.17 ± 2.62 [$]	71.83 ± 2.62 [$]
	200 µl/ml	27.17 ± 1.51 [$]	72.83 ± 1.51 [$]

Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, [$] $p < 0.001$ for change difference Vs positive control group.
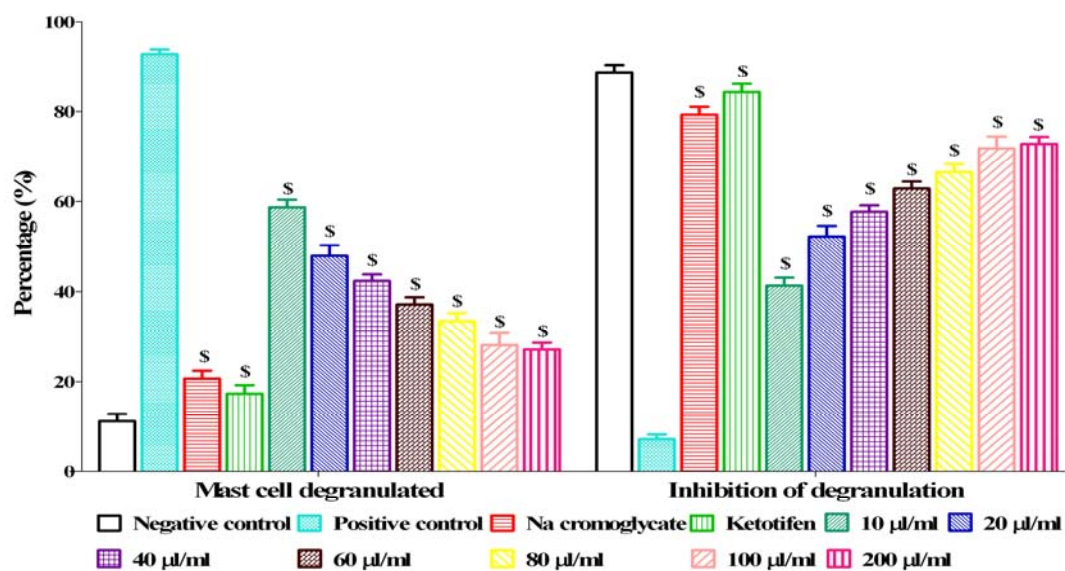
Figure 4.57: Effect of OFJ on compound 48/80 induced rat mast cell degranulation. Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, [$] $p < 0.001$ for change difference Vs positive control group.

187
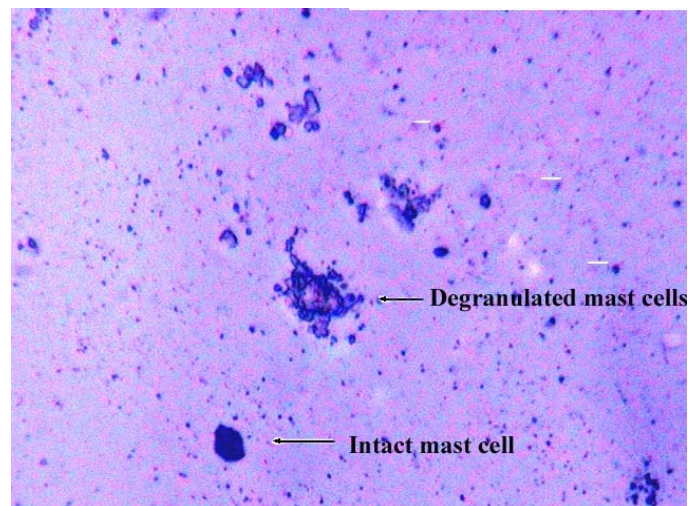
Figure 4.58: Intact and degranulated mast cells.

4.3.4.5 Carrageenan-induced rat paw edema

Paw size (cm) of rat was measured at one hour interval for each group in carrageenan –induced edema and are presented in table 4.17. Percentages of inflammation, calculated for each group are presented in table 4.18 and figure 4.59. Percentages of inhibition at 3 hour are reported in table 4.19 and over 5 hours are presented in figure 4.60.

In control animals, the sub plantar injection of carrageenan produced a local edema that increased progressively to reach maximal intensity at 5 h after the injection of the phlogistic agent (90.68 ± 4.21 %). OFJ at oral dose of 10 ml/kg and 15 ml/kg showed a significant suppression of carrageenan –induced rat paw edema after 2 h when compared with the control group ($p < 0.01$ and $p < 0.001$, respectively). Diclofenac sodium also showed a clear inhibition of the inflammation induced by carrageenan after 2 h when compared with the control group ($p < 0.001$). Pretreatment by OFJ significantly reduced ($p < 0.001$) the carrageenan –induced edema in a dose dependent manner, 3 h after carrageenan injection, to reach a maximal inhibition at this time with the dose 15 ml/kg (54.69 ± 5.98 %) with the ED_{50} being 9.282.

Table 4.19: Effect of OFJ on carrageenan induced rat paw edema over 5 hour.

Group	Dose	Time (hour) and Paw size (cm) (Mean ± SEM)					
		0 h	1 h	2 h	3 h	4 h	5 h
Control	-	2.8 ± 0.036	3.383 ± 0.060	3.95 ± 0.056	4.517 ± 0.087	5.117 ± 0.094	5.333 ± 0.080
Diclofenac sodium	10 mg/kg	2.6 ± 0.025	2.95 ± 0.034	3.233 ± 0.042***	3.267 ± 0.055***	3.45 ± 0.084***	3.35 ± 0.061***
OFJ	5 ml/kg	2.617 ± 0.030	3.083 ± 0.060	3.55 ± 0.088	3.95 ± 0.152**	4.15 ± 0.108***	4.167 ± 0.076***
OFJ	10 ml/kg	2.667 ± 0.049	3.083 ± 0.060	3.4 ± 0.1**	3.483 ± 0.098***	3.633 ± 0.120***	3.617 ± 0.172***
OFJ	15 ml/kg	2.567 ± 0.021	2.967 ± 0.066	3.25 ± 0.088***	3.333 ± 0.084***	3.45 ± 0.117***	3.45 ± 0.099***

Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference Vs vehicle control group.

Table 4.20: Percentage inflammation produced by carrageenan in rat paw.

Time (hour)	Groups				
	Control	Diclofenac sodium (10 mg/kg)	OFJ (5 ml/kg)	OFJ (10 ml/kg)	OFJ (15 ml/kg)
1	20.89 ± 2.23	13.49 ± 1.37	17.84 ± 1.89	15.65 ± 1.20	15.67 ± 3.12
2	41.08 ± 1.18	24.35 ± 0.68 *	35.58 ± 2.0	27.41 ± 1.98	26.59 ± 3.00
3	61.34 ± 2.67	25.63 ± 1.56 ***	50.92 ± 5.44	30.58 ± 2.19 ***	29.87 ± 3.08 ***
4	82.87 ± 3.87	32.68 ± 2.90 ***	58.64 ± 4.11 ***	36.09 ± 2.33 ***	34.38 ± 4.208 ***
5	90.68 ± 4.21	28.86 ± 2.17 ***	59.21 ± 1.95 ***	35.36 ± 4.41 ***	34.41 ± 3.62 ***

Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference Vs vehicle control group.

Table 4.21: Effect of OFJ on carrageenan – induced rat paw edema at 3 hour.

Group	Dose	Inhibition (%) (Mean ± SEM)	ED_{50}
Control (Saline)	-	-	
Diclofenac sodium	10 mg/kg	60.99 ± 2.48	9.282 ± 0.9117 ml/kg
Fruit Juice	05 ml/kg	21.35 ± 9.90	
Fruit Juice	10 ml/kg	51.69 ± 5.25	
Fruit Juice	15 ml/kg	54.69 ± 5.98	
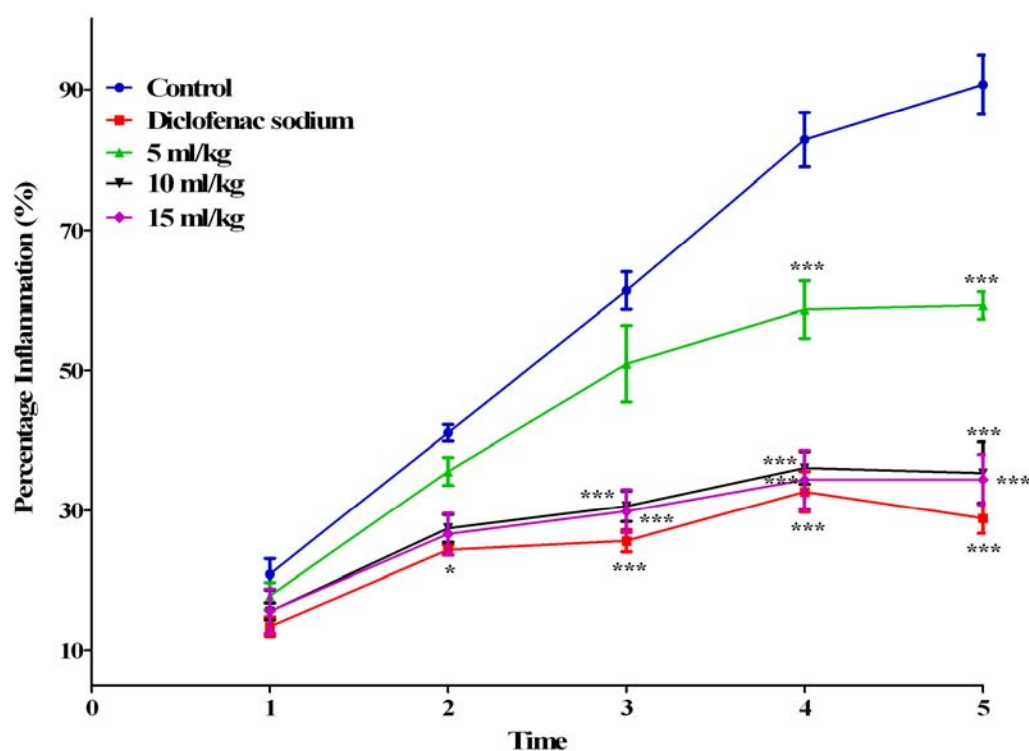
Figure 4.59: Influence of OFJ on percentage inflammation in carrageenan –induced rat paw edema. Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for change difference Vs vehicle control group.

190
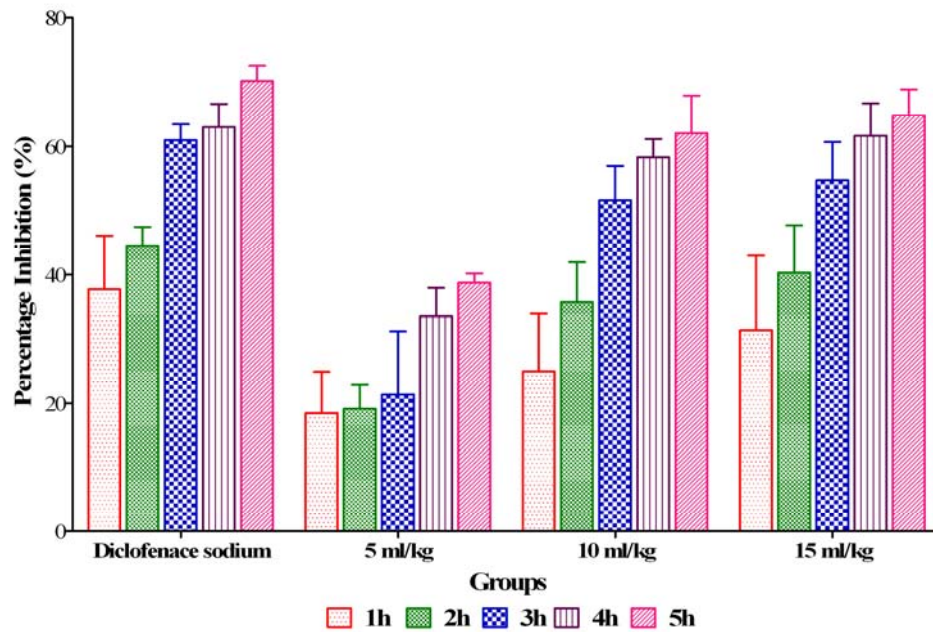
Figure 4.60: Percentage inhibition produced by OFJ and diclofenac sodium on carrageenan –induced rat paw edema over 5 hour. Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test.

4.3.4.6 Neutrophil adhesion test

The percentage of neutrophil adhesion were estimated on day 14 and presented in table 4.20 and figure 4.61. Pretreatment with OFJ at dose (10 and 15 ml/kg, p.o.) induced a significant ($p < 0.001$) decrease in the *in vitro* neutrophil adhesion to nylon fibers with respect to that of control group, which correlated the decrease in percentage of neutrophils. However, fruit juice at dose of 5 ml/kg did not show any significant change in neutrophil adhesion when compared with respective control group.

Table 4.22: Effect of OFJ on neutrophil adhesion.

Groups	Dose (ml/kg)	Neutrophil Index		Neutrophil adhesion (%)
		Untreated Blood	**Fiber Treated Blood**	
Control	-	334333.3 ± 51590	202416.7 ± 31617	39.55 ± 3.12
OFJ	5	633250 ± 36560	403933.3 ± 26453	36.26 ± 1.48
	10	432816.7 ± 58965	323966.7 ± 45790	25.38 ± 1.68 ***
	15	440900 ± 99970	342966.7 ± 77568	22.04 ± 1.08 ***

Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, *** $p < 0.001$ for change difference Vs vehicle control group.
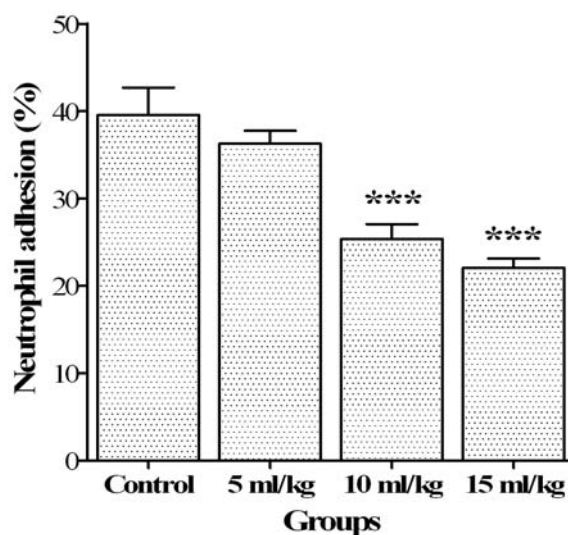
Figure 4.61: Effect of OFJ on neutrophil adhesion test. Values are Mean ± SEM (n = 6); analyzed by one way ANOVA followed by Turkey's multiple comparison test, *** $p < 0.001$ for change difference Vs vehicle control group.

192

4.4 Antimicrobial action of fruit peel extracts

In vitro antimicrobial potential of the different concentrations of peel extracts of *Opuntia elatior* Mill. fruits against gram positive bacteria, gram negative bacteria and fungi those are known to cause infections which are presented in figure 4.62, 4.63 and 4.64, respectively. All the peel extracts demonstrated antimicrobial actions in a dose dependant manner against both test bacteria and fungi. Petroleum ether extract (100 µg/ml) demonstrated maximum zone of inhibition in gram positive bacteria. Benzene and petroleum ether extracts (100 µg/ml) demonstrated maximum antibacterial action against *P. aurugenosa*, *E. coli* and *S. abony*, respectively. Methanol extract (100 µg/ml) showed maximum antifungal action against all the test fungi.

Table 4.23: *In vitro* **antimicrobial activity order of fruit peel extracts of** *Opuntia elatior* **Mill.**

Micro organisms	Antimicrobial activity order of peel extracts
B. pumilus	Petroleum ether > Methanol > Benzene > Chloroform > Dis. water
M. luteus	Petroleum ether > Methanol > Chloroform > Dis. water > Benzene
P. aurugenosa	Benzene > Dis. water > Methanol > Petroleum ether > Chloroform
E. coli	Benzene > Methanol > Petroleum ether > Chloroform > Dis. water
S. abony	Petroleum ether > Methanol > Chloroform > Dis. water > Benzene
C. albicans	Methanol > Petroleum ether > Benzene > Dis. water > Chloroform
A. niger	Methanol > Benzene > Petroleum ether > Dis. water > Chloroform
S. cereviasea	Methanol > Petroleum ether > Benzene > Chloroform > Dis. water
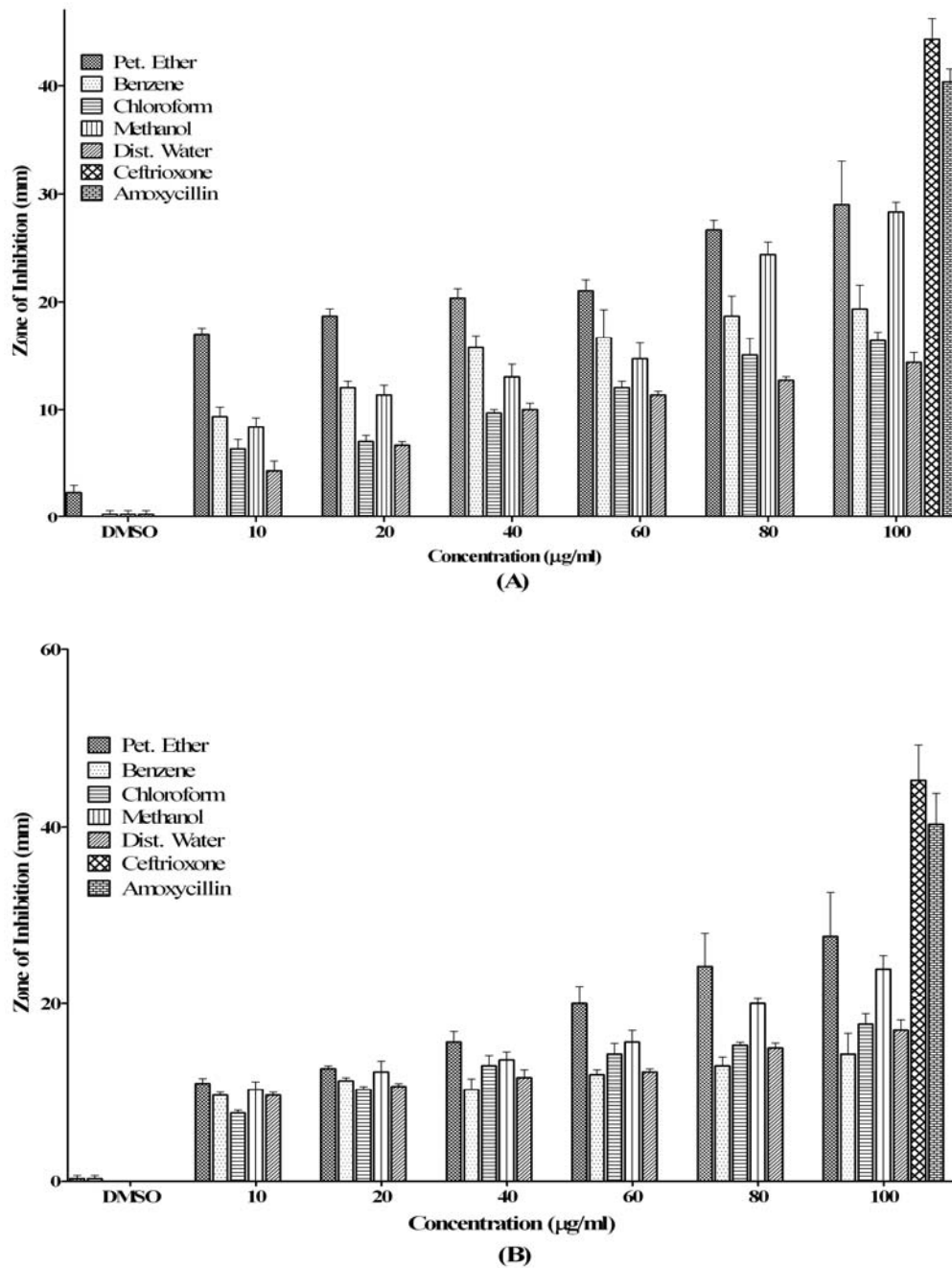
Figure 4.62: Effect of peel extracts on gram positive bacteria *B. pumilus* (A) and *M. luteus* (B). The graph lists values of the observed zone of inhibition (in mm diameter) excluding the diameter of well (8 mm) for peel extracts. Results are Mean ± SEM (n =3). Concentration of standard compound was 100 µg/ml. Dimethyl sulfoxide (DMSO) was used as a blank.
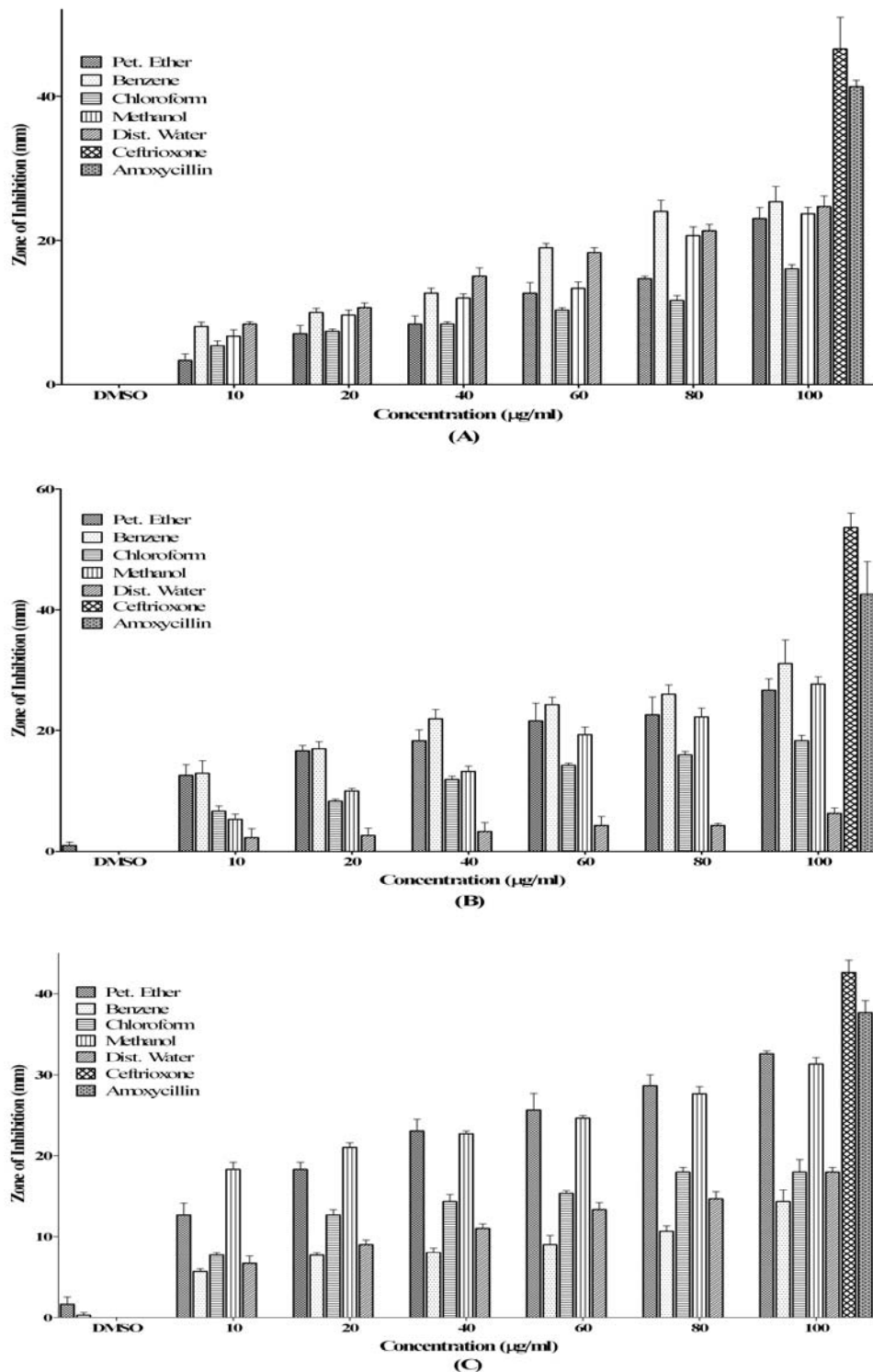
Figure 4.63: Effect of peel extracts on gram negative bacteria *P. aeruginosa* (A), *E. coli* (B) and *S. abony* (C). The graph lists values of the observed zone of inhibition (in mm diameter) excluding the diameter of well (8 mm) for peel extracts. Results are Mean ± SEM (n =3). Concentration of standard compound was 100 µg/ml. Dimethyl sulfoxide (DMSO) was used as a blank.

195
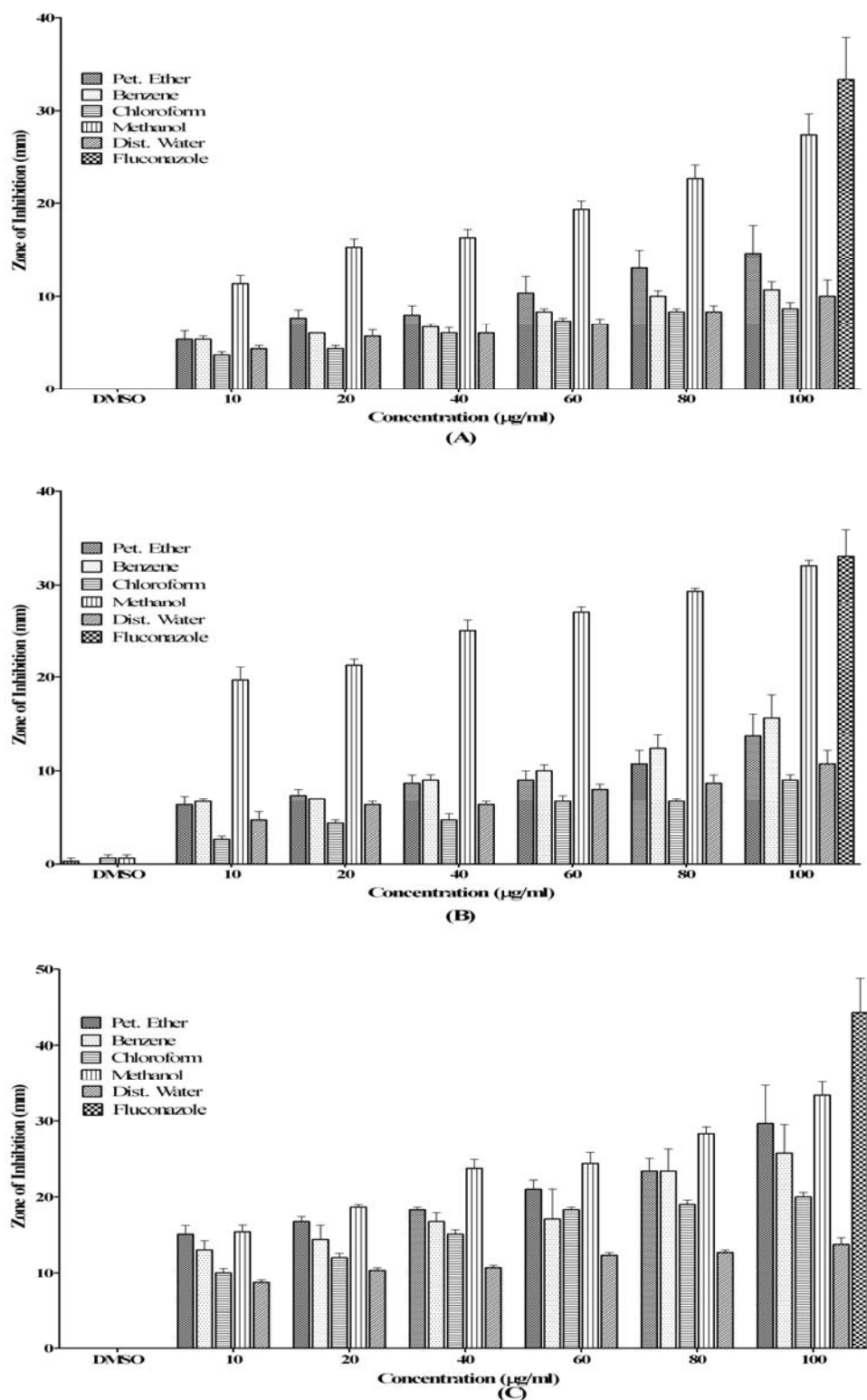
Figure 4.64: Effect of peel extracts on fungi *C. albican* **(A),** *A. niger* **(B) and** *S. cereviasea* **(C).** The graph lists values of the observed zone of inhibition (in mm diameter) excluding the diameter of well (8 mm) for peel extracts. Results are Mean ± SEM (n =3). Concentration of standard compound was 100 µg/ml. Dimethyl sulfoxide (DMSO) was used as a blank.

196

5. Discussion

5. Discussion

In the present study, pharmacognostical, phytochemical and pharmacological studies have been carried out to establish the authenticity of the plant based on morphological, physicochemical and phytochemical data and haematinic, analgesic and anti-asthmatic activities in the support of traditional and folkloric use of fruits of *Opuntia elatior* Mill.

The macroscopic studies carried out to authenticate the plant *Opuntia elatior* Mill. revealed that the characteristics of various parts were identical to those reported earlier by Kirtikar & Basu (1999) and The Wealth of India (2001). Phylloclade is an aerial modification of stem, broadly ovate to oblong in shape and greenish in color. Flowers are arising from areoles and contain perianth pink color in centre with yellow at the edge. Fruits are berry pyriform, reddish purple when ripe and greenish when unripe. It is indehiscent, depressed at the apex and many seeded pulpy fruit.

The phytochemical analysis was carried out for standardization of fruit juice contained carbohydrates, flavonoids, phenolics and betalains. Piga (2004) and Moßhammer et al. (2006) reported the presence of color pigment betalains and sugar content in high amount and low acidity of fruit which make it very sweet and delicious. The fruits were preliminary evaluated by estimation of proximate analysis. The average weight of fruit was 24.568 ± 7.134 g/unit and among percentage of peel and seed was very low compared to the edible portion. A comparatively low ash value indicates presence of less inorganic matter. The fruits are very good source of watery content as high amount of moisture content and low amount of solid content. The fruit juice can resist microbial growth as low pH and acidic in nature. The density of fruit juice was comparatively near to the water as high moisture content. Viscosity of juice was 53.62±0.402 centipoise at 100 rpm.

Results of extraction of fruits peel indicate maximum extractive values attained with polar solvents like methanol and water whereas with non polar solvents like petroleum ether, benzene and chloroform were comparatively less. Methanol and aqueous extract of peel was found to be 23.37% and 18.16% respectively.

Qualitative chemical tests of peel extract showed presence of carbohydrate, fats & fixed oil, flavonoids, phenolics, steroids & terpenoids, waxes, saponins and betalains whereas fruit juice showed presence of carbohydrates, flavonoids, phenolics and color pigment betalains.

A comparison of R_f values and spot color obtained in TLC of carbohydrates, it can be concluded that the fruit juice contained reducing hexose monosaccharides like galactose and glucose (Egon, 2007). TLC of betalain indicates presence of betacyanins type of color pigments as Rf values was low (0.081) in BAW and high (0.916) in 1% Aq. HCl (Harborne, 2007).

Harborne (2007) reported visible spectrum of betacyanin in methanol-HCl give maximum absorbance in the range of 532 – 554 nm. The spectrophotometric analysis suggests that the external color of prickly pear fruits depends on the relative concentration of betacyanins (red pigments with maximum absorbance at around 535 nm) and betaxanthins (yellow pigments with maximum absorbance at around 480 nm) (Wybraniec et al., 2001; Fernandez-Lopez & Almela, 2001; Stintzing et al., 2003, 2005). The absence of a peak at 484 nm would indicate that in fruits of *Opuntia elatior* Mill., betaxanthins are to be found in a very low level and spectrophotometrically is very difficult to distinguish them from betacyanins, which are present in a much higher concentration at 535 nm in methanol and water extracts of fruit.

Since betacyanins and betaxanthins possess similar spectroscopic and chromatographic properties, HPLC is an invaluable means of separating and analyzing them. Tentative identification of these betalains can be deduced from their chromatographic behavior, and corroborative data may be provided by an analysis of their absorption spectra. HPLC is an excellent means in the

198

analysis of betalains. The most common support is C_{18}-derivatized silica providing adequate efficiency and retention of betacyanins as well as their sufficient resolution on conventional stationary phases. Because betacyanins exist in aqueous solution in different ionized forms at varying pH values, the use of typical acidic eluents with or without buffers is a useful factor governing their separation (Schliemann et al., 1996, 2000, 2001; Wybraniec et al., 2001, 2006). Fernandez-Lopez et al. (2002) analyzed presence of betalains from the fruits of *Opuntia stricta, Opuntia undulata* and *Opuntia ficus-indica* and found HPLC patterns of betalains with retention time at 16.8 min (λ_{max} 484 nm), 19.6 min, and 22.8 min (λ_{max} 537 nm) assigned to indicaxanthin, betanin and isobetanin, respectively. From the respective retention times in comparison to literature, the spectral properties provided by the photodiode array detector and data reported by other researchers peak 1 with retention time 21.76 min at 484 nm was identified as indicaxanthin which present in very low amount and peak 2 with retention time 22.76 min at 535 nm as betanin, present in high amount in fruits of *Opuntia elatior* Mill.

MS provides molecular weight and structural information of the chromatographic bands so that fully-resolved peaks are not required, thus shortening chromatographic runs and reducing sample preparation while ensuring high sensitivity and selectivity. This technique is commonly used in investigations on betalain pigments (Schliemann et al., 1996, 2000, 2001; Wybraniec et al., 2001). Ferndndez-Lopez et al. (2002) screened the presence of betalain pigments in fruits of *Opuntia stricta, Opuntia undulata* and *Opuntia ficus-indica,* also Yahia & Castellanos-Santiago (2008) identified betalains from the fruits of 10 Mexican prickly pear cultivars by HPLC and ESI-MS. The presence of betanin was confirmed by its identical spectral properties (maximum absorbance at 535 nm), and the presence of their protonated molecular ions [M+H]$^+$ with *m/z*551, while the prominent secondary ion at *m/z*390 due to the presence of protonated aglycones [betanidine + H]$^+$. From the respective molecular mass in comparison to literature and data reported by other researchers, it was identified as betanin.

Total sugars range from 12 – 17 ºBrix and are mainly of the reducing type with glucose being the predominant sugar and fructose being the second sugar thus the fruit pulp is very sweet. Directly absorbable high glucose concentrations in cactus fruits represented as instantly available energy source for brain and nerve cells while being sweeter may enhance the fruit's flavor. Some authors have also reported the occurrence of galactose and maltose. The total sugar content (11.41 g/100 ml) equivalent to glucose obtained in fruits of *Opuntia elatior* Mill. was low with respect to other *Opuntia* species (Feugang et al., 2006; Piga, 2004; Stintzing et al., 2001).

In fruits belonging to species *Opuntia ficus-indica* and *Opuntia dillenii* contained 117 ± 10 and 45.2 ± 7.4 mg/100 g of total phenolics respectively (Diaz Media et al., 2007). Su Feng Chang et al. (2008) reported total phenolics (91.5 ± 1.5) and flavonoids (29.2 ± 1.5) along with gallic acid (4 ± 0.6), catechin (22.7 ± 0.7) and epicatechin (10.9 ± 0.2) as mg/100 g fresh sample of *Opuntia dillenii* Haw fruits. The total phenolic content (49.82 mg/100 ml) equivalent to gallic acid obtained in fruits of *Opuntia elatior* Mill. was in range of other species.

Citric acid is the major organic acid in cactus pear followed by malic acid, quinic, shikimic and also oxalic acids were found while isocitric, fumaric, glycolic, and succinic acids were only found in traces (Moßhammer et al., 2006; Stintzing et al., 2001). The titratable acidity (0.94%) equivalent to citric acid serves as a low acid food in fruits of *Opuntia elatior* Mill.

The most convenient way to quantify betalains is spectrophotometric method. First, Nelsson (1970) established a method to quantify pigments in beetroot. The total content of betacyanin was determined using the formula reported by Chethana, (2007); Cai et al., (2005); Ferndndez-Lopez et al., (2002). The total betacyanin content (47.10 mg/100 ml) equivalent to betanin obtained from fruits of *Opuntia elatior* Mill. was higher compare to *Opuntia ficus-indica* and *Opuntia undulata* Griff. while lower compare to *Opuntia*

stricta Haw (Fernandez-Lopez & Almela, 2001; Ferndndez-Lopez et al., 2002).

The mineral composition is characterized by high amounts of potassium followed by calcium while other minerals are in the normal range of fruits with respect to reported literature of other *Opuntia* species (Feugang et al., 2006; Piga, 2004; Stintzing et al., 2001).

This study aimed to evaluate the haematinic effect of *Opuntia elatior* Mill. fruit on $HgCl_2$ and PHZ –induced anaemia. Before explaining the possible protective role of fruit, it seems essential to describe the mechanism of Hg induced anaemia. Hg ions bind with –SH groups in the bio-membranes, and damage them via lipid peroxidation (Clarkson, 1972). Hg also binds with lysosomal membranes and renders them labile (Lauwery & Buchet, 1972). It inhibits protein synthesis (Nakada et al., 1980), alters the tertiary structure of RNA and DNA (Gruenwedel & Davidson, 1966) and affects their synthesis. Hg disturbs the structure and function of inner mitochondrial (Humes & Weinberg, 1983). All these effects can be held responsible for anaemia due to inorganic Hg –induced cellular demage (EHC-118, 1991).

Iron deficiency is the commonest cause of hypochromic microcytic anaemia. In iron deficiency, the amount of iron lost from the body exceeds the amount absorbed. The physiological demand for iron exceeds iron uptake. First there is depletion of the iron store of the body followed by reduction in the plasma level of iron and development of hypochromic microcytic anaemia. Hypochromic microcytic anaemia can be interpreted based on reduction of haemoglobin content, total RBC count, PCV, MCV, MCH, MCHC and increase in RDW values is the indicator of hypochromic microcytosis (Godkar & Godkar, 2004) and that's why we precise the estimation of these haematological parameters. The results obtained after 30 days indicated that $HgCl_2$ –induced hypochromic microcytic or haemolytic anaemia due to iron loss. The results after treatment indicated that the fruit juice of *Opuntia elatior* Mill. increased significantly the haemoglobin, total RBC count, RBC indices (MCV, MCH, MCHC), PCV and decreased RDW.

Loss of body weight is a common clinical feature of anaemia. It was observed that there was a remarkable increase in body weight in animals treated with OFJ at dose 10 ml/kg (212.2 g) and 15 ml/kg (227.7 g) after continuous treatment for 30 days which was better than standard treated group. Only OFJ (15 ml/kg) treated rat demonstrated slight increase in body weight (208.7 g) after 30 days but comparatively less than disease treated groups. The reversal of body weight by fruit juice could be considered as a significant effect. It indicates reversal of the toxicant induced tissue degenerative changes. Body weight change is the sum of the effects occuring in different parts of the body and reversal of the toxicant induced decrease is an index of good tissue or cytoprotective activity of the test drugs.

Haemoglobin estimation is considered as the marker for evaluating the correction of anaemia. At the dose of 10 ml/kg and 15 ml/kg of fruit juice showed good percentage of recovering in haemoglobin, 32.99 % and 38.18 %, respectively, which was higher than standard treated group (29.8 %) indicating correction of anaemia. The haematinic action of fruit juice was dose dependant manner. It was observed that there was slight increase in haemoglobin content but not significant in group E higher dose treated rat.

The mean total and differential WBC count give the information regarding defense system of the body. However, fruit juice of *Opuntia elatior* Mill. improved the total and differential WBC count in $HgCl_2$ –induced anaemia. The results were dose dependant and reversed the effect of $HgCl_2$ in rats. Thrombocytopenia (decrease in platelet count) is often associated with prolonged bleeding and poor clot retraction. It also occurs in aplastic, haemolytic, megaloblastic anaemia, hyperplenism, acute leukemia and in immune thrombocytopenia (Godkar & Godkar, 2004). The fruit juice of *Opuntia elatior* Mill. improved the platelet functions and the results was dose dependant in $HgCl_2$ –induced anaemia model.

The increment of blood sugar indicated that the fruit juice was a source of energy due to presence of high amount of carbohydrates. The fruit juice has not major effect on serum cholesterol and triglyceride levels.

Urea is the major nitrogen containing metabolic product of protein catabolism in humans. In leukemia and haemolytic anaemia, release of leukocyte protein contributes to high plasma urea. In gastrointestinal disease, plasma proteins and haemoglobin can be released into the gut and digested. This may contribute to high plasma urea. Creatinine formed as the end product of creatine metabolism is a waste product. The plasma blood urea and creatinine increases in renal diseases (Godkar & Godkar, 2004). Fruit juice of *Opuntia elatior* Mill. showed tendency towards reversal of these toxicant induced changes. The changes observed after $HgCl_2$ administration can be mainly attributed to the toxicant induced kidney damage. Reversal of most of these changes by fruit juice administration indicates that they do have some element of cytoprotective activity. The kidney is badly damaged by $HgCl_2$ exposure (Rathore and Vaghese, 1994). Fitzhuge et al. (1950) studied Hg-acetate (25 ppm) –induced changes in kidney of rats and reported a dose dependant change in its structure and function. Among human beings, inorganic Hg salt ingestion result in anuria and uraemia from acute tubular necrosis (Kazantzis et al., 1962)

Liver is often the primary target for the toxic effects of xenobiotics. It is known that the detoxification of the toxic materials which enter the body occurs mainly in the liver. Therefore, liver can be used as an index for the toxicity of xenobiotics. Total bilirubin may rise in irritation of liver; this reflects liver cell damage or bile duct damage within the liver itself. Proteins are synthesized in liver; low level indicates that the synthetic function of liver has been markedly diminished. Alkaline phosphatase is the marker enzyme produced within the cells of the liver, as the cells are damaged, leaks into the blood stream leading to a rise in the serum levels. It is an enzyme, which is associated with the biliary tract, and it elevated; biliary tract damage and

inflammation should be considered (Godkar & Godkar, 2004). From the bilirubin, alkaline phosphatase and total protein content observations, it seems that fruit juice of *Opuntia elatior* Mill. improves the liver function significantly. The liver showed $HgCl_2$ –induced pathological changes (Rathore and Vaghese, 1994). Ashe et al. (1953) had reported severe hepatic effects in rabbits exposed to metallic Hg vapors. Accidental, fatal Hg vapor inhalation exposures in a young child caused hepatocellular damage and biochemical alterations (Jafee et al., 1983).

Spleen is the store house of dead RBC and breakdown of haemoglobin also occurs in the spleen. Hemolytic anaemia leads to accelerated breakdown of haemoglobin causing larger iron deposition in spleen (Chatterjee, 1994). This is likely to be the cause of fibrosis and necrosis observed in the spleen in $HgCl_2$ treated groups. This disturbance in the cytoarchitecture was significantly reversed by test drug administration. In this respect fruit juice was comparatively better because in addition to attenuating the fibrosis, it restored cellularity to moderate level thus inhibiting the toxicant induced cell depletion.

Numerous *in vitro* studies have demonstrated the beneficial effect of phenolics and betalains as antioxidant action. These are generally attributed to the ability of antioxidant to neutralize reactive oxygen species such as singlet oxygen, hydrogen peroxide (H_2O_2), or suppression of the xanthine/xanthineoxidase system, all of which may induce oxidative injury i.e. lipid peroxidation (Budinsky et al., 2007) and as we know inorganic Hg induced lipid peroxidation, inhibition of protein synthesis and cellular damage which results in anamia.

The fruit juice of *Opuntia elatior* Mill. reversed anaemia induced by $HgCl_2$ in dose dependant manner. The antioxidant phenolics and betanin constituents and mineral compositions appear most likely as the active ingredients responsible for haematinic effect of *Opuntia elatior* Mill. fruits. This results support at least partially the traditional use of fruits in the treatment of anaemia.

Phenylhydrazine produces both aryl and hydroxyl radicals when incubated with rat liver microsomes (Gannett et al., 1997) and oxidised by hydrogen peroxide at pH 7.4 and 37°C (Rehse and Shahrouri, 1998). The radicals induced oxidative stress on the red cell membrane resulting in haemolysis by lipid peroxidation (Cighetti et al., 1999). Sub-chronic intoxication of rats with PHZ (10 mg/kg/day for 8 days) resulted in a marked haemolytic anaemia characterised by decreased RBC, Hb and PCV (Unami et al., 1996). Similar results were obtained in our study when experimental rats were administered PHZ in order to induce anaemia. In addition, Ferrali et al. (1997) observed increased reticulocytosis, methaemoglobinemia and haemocatheresis in PHZ intoxicated rats. The main function of the RBC is the transportation of oxygen in to the tissues of the body. At such, any pathological or physiological condition that affects the RBC alters its function and this may be detrimental to the body. In this study PHZ altered the function of RBC by haemolysis characterised by decreased levels of RBC, Hb and PCV. PHZ increases reactive oxygen species (ROS) and lipid peroxidation, and decreases glutathione (GSH); these effects are reversed by N-acetyl cysteine, a known ROS scavenger (Amer et al., 2004). Thus, PHZ-induced haemolytic injury seems to be derived from oxidative alterations to red blood cell proteins rather than to membrane lipids (McMillan et al., 2005).

Anaemia is a disease characterized by a reduction in the concentration of haemoglobin, circulating red blood cell and pack cell volume per unit of the peripheral blood below the normal for the age and sex of the patient (Aguwa, 1996; Oma, 1991). Blood parasites, bacterial infections, viral infections, drugs/chemical agents and metabolic diseases may result in destruction of red blood cells leading to haemolytic anaemia (Ramzi et al., 1994). Administration of PHZ to rats also resulted in an increase in the MCV and

MCH values which are indicators of macrocytosis thus describing the anaemia as macrocytic. This condition is also common in Vitamin B_{12} and folate deficiencies probably as a result of iron deficiency (loss of iron). Macrocytic anaemia has also been reported in rats infected with *Trypanosoma Brucei brucei* (Erah et al., 2003) and this has been linked to iron deficiency anaemia (Mwangi et al., 1995).

In this study, PHZ altered the haematological parameters by haemolysis characterized by decrease in haemoglobin concentration, total RBC counts and PCV on day 3. However, the haematological parameters were restored to normal range after treatment with fruit juice of *Opuntia elatior* Mill. The middle administrated dose of 10 ml/kg reduced the recovery time of the blood parameters from 15 days in the anaemic control to 10 days. Also the recovery was progressive such that after 15 days of continuous treatment, the haemoglobin concentration was higher in group D10, D15 and E treated rats than in the negative control group. It was also observed that the recovery of the treated groups was dose dependant with the highest dose of 15 ml/kg effecting the highest change. Giving the highest dose 15 ml/kg fruit juice to normal rats did not significant alter the haematological parameters.

Leucocytosis with neutrophilia and lymphopenia is at its maximum on day 3, hypersegmented neutrophils were observed rarely, phagocytosing blood lymphocytes are frequent on day 3. The fruit juice normalized the total and differential WBC counts after PHZ administration in rats. The results indicate that fruits of *Opuntia elatior* Mill. improve the host defense mechanism of the body. PHZ treatment induces hypercellularity with erythroid hyperplasia (Criswell et al., 2000). The fruit juice also improved the platelet functions in PHZ treated rats.

This anaemia which resulted from the early lysis of the red blood cells was naturally reversed 7 days later by the regeneration of these blood cells due to the increase of the reticulocytes. Our results indicate that the fruit juice 10 ml/kg and 15 ml/kg dose increased significantly the number of reticulocytes, mainly 7 days after PHZ administration. Moreover, the fruit juice of *Opuntia*

elatior Mill. potentiates the increase of the number of reticulocytes. The fruit juice could stimulate erythropoisis process.

The measurement of serum ferritin level provided a reliable estimate of iron store. The increase of serum ferritin level in group B treated rats indicates that it may be due to liver damage by PHZ. The fruit juice restored the liver functions and did not make significant change in serum ferritin level. Based on the biochemical and histopathological results, *Opuntia elatior* Mill. fruit juice also reserved the toxic effect of PHZ on liver, kidney and spleen. There is not significant change in cholesterol and triglyceride level in fruit juice treated rats.

The speedy and progressive recovery of anaemic rats responding to treatement of *Opuntia elatior* Mill. fruits may be due to increased erythropoiesis. The improvement in the haematological indices exhibited by fruit juice might be connected with the minerals, phenolics and betacyanin content of the fruits of *Opuntia elatior* Mill. The phenolics and betacyanin have remarkable anti oxidant activity. These constituents might have direct influence on the protection of haemolysis by reactive oxygen species generated by PHZ. These results support the folkloric use of *Opuntia elatior* Mill. fruits in the treatment of anaemia.

The potential antinociceptive as central analgesic by using tail immersion test and peripheral analgesic by using acetic acid induced writhing test of the fruits of *Opuntia elatior* Mill. was investigated. The antinociceptive tests used in the present work were chosen in order to test different nociceptive stimuli, namely cutaneous thermic (tail immersion) and chemical visceral (writhing) stimuli. The results indicate that oral administration of the fruit juice of *Opuntia elatior* Mill. exhibit central and peripheral analgesic properties, since it exerted a significant and dose-dependent protective effect on chemical (acetic acid injection) and thermic (heat) painful stimuli. Such an efficacy on these two stimuli is characteristic of central analgesics like morphine and tramadol while peripheral analgesics like diclofenac sodium, aspirin are known to be inactive on thermic painful stimuli.

The results demonstrate that the fruits of *Opuntia elatior* Mill. attenuated the nociceptive responses to chemical stimuli in the acetic acid-induced abdominal constriction. The mean number of abdominal contractions was reduced from 25 to 7 at the respective doses of 5 and 15 ml/kg. Diclofenac sodium, the peripheral analgesic drug also produced similar antinociceptive action. It has been postulated that acetic acid acts indirectly by inducing the release of endogenous mediators which stimulate the nociceptive neurons sensitive to non-steroidal anti-inflammatory drugs and opiods (Collier et al., 1968; Dai et al., 2002). This test is generally used for the screening of central and peripheral analgesic effects (Koster et al., 1959; Vogel and Vogel, 1997). The centrally acting protective effect of the extract was also corroborated in our study by the tail immersion test results.

The analgesic efficacy and potency of acutely administered tramadol is comparable to that of codeine, pentazocine, or dextropropoxyphene (Hennies et al., 1988), while its analgesic and antinociceptive potency is only 5- to 10-fold lower than that of morphine (Lehmann et al., 1990). It is believed that tramadol works by μ-opioid receptors (Raffa et al., 1992) despite its relatively low binding affinity (Hennies et al., 1988). Thus, it is speculated that nonopioid mechanisms are involved in tramadol analgesia. In accordance with the recognized implication of noradrenaline and serotonin in pain modulation, tramadol has been shown to inhibit the re-uptake of noradrenaline and serotonin, thereby increasing the concentration of these two neurotransmitters in selected brain areas, thus raising the pain threshold (Driessen and Reimann, 1992; Raffa et al., 1992).

Lyophilized aqueous extract (100–400 mg/kg, i.p.) of the fruits of *Opuntia dillenii* (Ker-Gawl) Haw was evaluated for analgesic activity using writhing and hot plate test in mice and rat, respectively and also anti-inflammatory activity using carrageenan-induced paw edema in rats, the results exhibited dose dependent action (Loro et al., 1999). Taking this into consideration, it seems that the fruit juice of *Opuntia elatior* Mill. contains morphine and tramadol like components and other peripherally acting

principles. According to this study, we can concluded that the fruits of *Opuntia elatior* Mill. is endowed with central and peripheral analgesic properties might be due to presence of phenolics and betanin content. In future experiments, studies with purified fractions of fruit will be conducted for further research for deriving mechanism involved in analgesic action.

Since bronchodilators, mediator release inhibitors and anti-inflammatory drugs are the different classes of drugs used conventionally in the treatment of bronchial asthma; various animal models and experimental protocols were used in the present study to evaluate anti-asthmatic activity of fruit of *Opuntia elatior* Mill.

Bronchial asthma is characterized by increased airway reactivity to spasmogens (Cockcroft, 1983). An initial event in asthma appears to be the release of inflammatory mediators (e.g. Histamine, Tryptase, Leukotrienes and prostaglandins).Some of these mediators directly cause acute bronchoconstriction,
airway hyperresponsiveness and bronchial airway inflammation. Spasmolytic drugs like beta adrenergic agonists, xanthine derivatives and anticholinergics relax the airway smooth muscles and are used as quick relief medications in acute asthmatic attacks. Beta adrenergic agonists promote bronchodilation by direct stimulation of beta adrenergic receptors in the airway smooth muscle, that lead to relaxation of bronchial smooth muscle by rapid decrease in airway resistance in vivo. Specific β2 agonists like salbutamol, salmeterol etc. are used since long for symptomatic relief in asthma.

In present study, significant increase in preconvulsion time was observed due to pretreatment with fruit juice of *Opuntia elatior* Mill., when the guinea pigs were exposed to either acetylcholine or histamine aerosol. This

bronchodilating effect of fruit juice at high dose was comparable to ketotifen and atropine sulfate. Spasmolytic effect of *Opuntia elatior* Mill. fruit was also evaluated by observing the effect of fruit juice (10, 50 and 100 μl/ml) on acetylcholine and histamine induced ileum contractions to seek for scientific evidence for beneficial use of fruits in spasm produced by any means. The results showed antagonistic effects of the fruit juice against the contraction induced by the standard spasmogens. The results of this study indicated a right ward shift in the log dose- response curve of acetylcholine and histamine in the presence of the fruit juice of *O. elatior* Mill. The maximum effects of acetylcholine and histamine induced contractions were inhibited in the presence of the fruit juice. The non-parallel rightward shift in acetylcholine and histamine log dose- response curves obtained in the presence of the fruit juice, with lowered maximum contraction effect to acetylcholine and histamine would indicate a non-competitive or an irreversible antagonistic effect of *Opuntia elatior* Mill. fruits at muscarinic and histamine H_1 receptors (Linden et al., 1993). In this case the antagonist binds irreversibly to receptor site or to another site that inhibits response to the agonist.

Control of tension in gastrointestinal smooth muscle is dependent on the intracellular Ca^{2+} concentration. In general, there are two types of excitation–contraction coupling based on the type of mechanism responsible for changes in Ca^{2+} concentration. Electromechanical coupling requires changes in membrane potential, which in turn activate the voltage-dependent Ca^{2+} channel to trigger an influx of Ca^{2+} (Sadraei et al., 2003). Acetylcholine and histamine caused contraction via specific receptors and can produce changes in tension (Elorriaga et al., 1996). Both acetylcholine and histamine have functional roles in natural contraction of gastrointestinal tract. Acetylcholine is a neurotransmitter at post-ganglionic parasympatic neurons that innervate the gut. The response to acetylcholine is mediated by activation of two types (M_2 and M_3) of muscarinic receptors (Goyal, 1988; Levey, 1993). Activation of these receptors results in an increase in intracellular Ca^{2+}, an effect mediated by inositol triphosphate acting on internal calcium stores (Caulfield, 1993; Eglen et al., 1996; Elorriaga et al., 1996). Serotonin (5-HT) is also an important substance in the gastrointestinal tract and is present in

both enterochromaffin cells of the mucosa and neurons of the mesenteric plexus; it affects both secretion and motor activity (Briejer et al., 1995; Kunze and Furness, 1999). The histamine contraction is mediated by the release of acetylcholine from the cholinergic neurone as well as activation of serotonergic receptors on the smooth muscles of ileum (Sander-Bush and Mayer, 1996). This experiment showed that *Opuntia elatior* Mill. fruits possess a significant inhibitory effect on rat and guinea pig ileum contraction via antihistaminic and antimuscarinic action.

In addition to bronchodilating activity, a significant number of therapeutic approaches for bronchial asthma have been designed based on the antagonism of specific mediators released from mast cells. Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens. Degranulated cells liberate mediators of inflammation such as histamine, leukotrienes, platelet activating factors and chemotactic factors for eosinophils, neutrophils etc. from mast cells. The unique mediator profile of mast cells, elicited upon activation through their high-affinity receptors for IgE, include pre-formed granule-associated inflammatory mediators (histamine, neutral proteases, pre-formed cytokines, and proteoglycans) that are released by exocytosis. Finally, activated mast cells synthesize and secrete a host of proinflammatory, chemoattractive, and immunomodulatory cytokines over a period of several hours (Okayama et al., 1995; Toru et al., 1998; Kobayashi et al., 1998). The bioactivities of these mediators include brochoconstriction (cys-LTs, histamine, PGD_2), vasodilation and tissue edema (histamine, cys-LTs), leukocyte infiltration (cys-LTs, PGD_2, tryptases, cytokines and chemokines), collagen matrix turnover and stromal cell growth (tryptases, cytokines), and hyperplasia of bronchial smooth muscle (tryptases, cys-LTs). These properties of mast cells, and their normal residence in bronchi, would seem to position them for a potentially relevant role in the pathophysiology of asthma (Boyce, 2003). Degranulation of mast cells has been taken as the criteria of positive anaphylaxis. Ketotifen fumarate, a well-known mast cell stabilizer, reduces synthesis of prostaglandins E_2, thromboxane A_2, leukotriene C_4 and B_4. It also inhibits release of histamine, serotonin and other inflammatory mediators from

mast cells. Simultaneously it blocks H_1 receptors. Cromolyn sodium, which is developed from the structural modification of Khellin is the mast cell stabilizer used in the treatment of mild to moderate asthma by raising cAMP levels due to inhibition of the enzyme phosphodiesterase (Saraf et al., 2000).

In the present study, the fruit juice of *Opuntia elatior* Mill. was found to inhibit the degranulation of mast cells induced by an immunological and a non-immunological stimulus. It is known that the physiological stimulus for the release of histamine from mast cells is provided by a combination of antigen with specific antibody fixed on the cell surface. This combination is believed to transiently increase the permeability of membrane to calcium ions showing an absolute requirement for calcium ions for the secretory process to occur (Razin et al., 1995). Anaphylactic and compound 48/80 induced secretion from mast cells share a common requirement as far as the presence of calcium ions is concerned. However, compound 48/80 can utilize intracellular calcium stores to initiate the release process, even in the absence of calcium in the extracellular medium (Burka, 1984). On the other hand, anaphylactic release requires the presence of calcium in the extracellular medium which moves onto the cell via calcium gates in the membranes (West, 1983; Saraf et al., 2000). A significant protection of rat peritoneal mast cells from disruption by antigen and compound 48/80 by fruit juice of *Opuntia elatior* Mill. points towards its ability to interfere the release and /or synthesis of mediators of inflammation, indicating its mast cell stabilizing activity. Hence it may be assumed that the cytoprotective effect induced by fruit juice of *Opuntia elatior* Mill. on mast cell surface could be due to its ability to alter the influx of calcium ions.

Further, airway inflammation has been demonstrated in all forms of asthma. Even in mild asthma, there is an inflammatory response involving infiltration, particularly with activated eosinophils and lymphocytes, with neutrophils and mast cells. The degree of bronchial hyperresponsiveness and airway obstruction is closely linked to the extent of inflammation (Bousquet et al., 2000). Anti-inflammatory drugs suppress the inflammatory response by inhibiting infiltration and activation of inflammatory cells as well as their

synthesis, or release of mediators and the effects of inflammatory mediators. Carrageenan rat paw edema is a suitable test for evaluating anti-inflammatory drugs which has been frequently used to assess the anti-edematous effect of natural products (Basu and Nag Chaudhuri, 1991). Carrageenan –induced inflammation is useful in detecting orally active anti-inflammatory agents. Oedema formation due to carrageenan in the rat paw is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The edema produced at the peak (3 h) is thought to be due to the release of kinin-like substances, especially bradykinin. The second phase is sensitive to most clinically effective anti-inflammatory drugs (Van Arman et al., 1965; Vinegar et al., 1969; DiRosa et al., 1971; Crunkhon and Maecock, 1971). It is well established fact that non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory activity by inhibition of prostaglandin biosynthesis (Van, 1971). The anti-edematogenic mechanism of action of *Opuntia elatior* Mill. fruit may also be related to prostaglandin synthesis inhibition. Inflammation pain results from the release of hyperalgesic mediators – prostaglandins and catecholamines – which are supposed to act by regulating the sensitivity of pain receptors (Ferreira, 1972; Ferreira and Nakamura, 1979).

The neutrophil, an end cell unable to divide and with limited capacity for protein synthesis is, nevertheless, capable of a wide range of responses, in particular chemotaxis, phagocytosis, exocytosis and both intracellular and extracellular killing (Dale and Foreman, 1984). Neutrophils are present in much larger numbers than any other inflammatory cell in the circulation and in tissue stores, particularly the lung. Neutrophils are one of the first inflammatory cells to be recruited into the airways after either allergen exposure or injury (Susan and Qutayba, 2007). In acute inflammation, activated neutrophils are the major effector cells of this inflammatory response, releasing interleukins, tumour necrosis factor α, leukotriene B_4, platelet activating factor (PAF), proteases, and products of the respiratory burst reaction (Hayllar and Bjarnason, 1991; Roos and Dolman, 1990; McColl and Showell, 1994). Mucosal recruitment of neutrophils involves sequential adhesion and transmigration across endothelial, lamina propria and epithelial

compartments (Madar, 1994). Subsequent adhesion to apical epithelial membranes results in activated neutrophils persisting in crypt abscesses with local release of chemotactic and chemoactivating substances (Nathan, 1987; Opal et al., 1994). In addition to producing a number of functionally diverse substances, polymorphonuclear cells also express receptors for a number of mediators including IL-8, IL-9 and the high-affinity IgE receptor (Soussi-Gounni et al., 2001; 2001a). These receptors have been implicated in different inflammatory reactions, including allergic asthma. Neutrophil recruitment from the circulation into the interstitium during inflammation is an extension of a physiological process across an adapted, permissive cell layer. Such transmigration involves the tethering, rolling, tight adhesion, and diapedesis of marginalised flowing cells (Adams and Shaw, 1994). Neutrophil adhesion to the vascular endothelium as described in the 'traffic signal' paradigm, results from the sequential recruitment of selectins, β_2-integrins, tissue bound factors (IL-8, PAF) and products of the immunoglobulin gene superfamily(Springer, 1994). Mucosal inflammation is associated with an increase in the expression of vascular and leucocyte adhesion molecules (Balsitis et al., 1994; Dippold et al., 1993). A number of cellular adhesion molecules are involved in the adhesion of neutrophils to the site of tissue inflammation. Neutrophils must adhere to the endothelium and subsequently migrate through the vessels before entering the tissue. Neutrophil rolling and arrest on endothelium is mediated through successive interactions of selectins and β_2-integrins (Susan and Qutayba, 2007). Neutrophil adhesion to endothelium is enhanced by activation of adenosine A_1 receptors. Binding to the adenosine A_2 receptor results in inhibition of the respiratory burst reaction and decreased binding to fibrinogen (Prescott et al., 1990; Bouma et al., 1994; Dianzani et al., 1994; Cronstein et al., 1992; Meenan et al., 1996). In present study, fruit juice of *Opuntia elatior* Mill. reduced significantly percentage of neutrophil adhesion. This may help in decreasing the release of various cytokines and might be binding to A_1 and/or A_2 receptor on endothelium and results in producing anti-inflammatory action.

In conclusion our data suggests that fruits of *Opuntia elatior* Mill. has potential anti-asthmatic activity that may be due to its bronchodilator, mast cell stabilization, anti-inflammatory and reduction of neutrophil adhesion property. The fruit juice was found reddish purple in color due to presence of betanin in higher concentration. So betanin might be responsible for anti-asthmatic action due to its anti-oxidant and/or other property.

The peel of various fruits serves the protective action against microbial invasion. So we aimed to evaluate the antimicrobial activity of fruit peel of *Opuntia elatior* Mill. The peel extracts of fruit exhibited antimicrobial actions in a dose dependant manner against both test bacteria and fungi. Antimicrobial activity of the peel extracts is directly concerning with the components that they contain. Petroleum ether, benzene and methanol extracts showed maximum inhibitory action against gram positive bacteria, gram negative bacteria and fungi, respectively. The *in vitro* antimicrobial activity order of peel extracts summarize in table 4.21. Among constituents of plants, polyphenols have received a great deal of attention, in recent years, due to their diverse biological functions. The antimicrobial activity of the polyphenols, tannins and flavonoids, is well documented. The tannin-rich peels are byproducts of food industry, and are only used in animal feeds in many developed countries. In fact, the antimicrobial activity of peels has been demonstrated against pathogenic bacteria by Ahmad and Beg (2001), Al-Zoreky (2009), Machado et al. (2003), Shan et al. (2007). In future experiments, studies with purified fractions of peel extracts will be conducted for identification of antimicrobial leads.

Taken together, these phytochemical results indicate that the flavonoids betacyanin equivalent to betanin is the active principle in the fruits of *Opuntia elatior* Mill. The fruits are exhibiting haematinic effect against anaemia induced by mercuric chloride and phenylhydrazine while antinociceptive effect against thermal and chemical stimuli. Fruits also appear anti-asthmatic property. Furthermore, peel extracts of fruit appear antimicrobial property against bacteria and fungi. These findings on haematinic, analgesic and anti-asthmatic effects of fruit juice, further add value to the nutritional characteristics of the fruits of *Opuntia elatior* Mill.

6. Summary

6. Summary

Pharmacognostic analysis of fruits of *Opuntia elatior* Mill. confirms the authenticity of the plant as per previous reports. Phylloclade is an aerial modification of stem, broadly ovate to oblong in shape and greenish in color. Flowers are arising from areoles and contain perianth pink color in centre with yellow at the edge. Fruits are berry pyriform, reddish purple when ripe and greenish when unripe. It is indehiscent, depressed at the apex and many seeded pulpy fruit.

The phytochemical analysis was carried out for standardization of fruit juice contained carbohydrates, flavonoids, phenolics and betalains. The fruits were preliminary evaluated by estimation of proximate analysis. The average weight of fruit was 24.568 ± 7.134 g/unit and among percentage of peel and seed was very low compared to the edible portion. A comparatively low ash value indicates presence of less inorganic matter. The fruits are very good source of watery content as high amount of moisture content and low amount of solid content. The fruit juice can resist microbial growth as low pH and acidic in nature. The density of fruit juice was comparatively near to the water as high moisture content. Viscosity of juice was 53.62 ± 0.402 centipoise at 100 rpm.

Results of extraction of fruits peel indicate maximum extractive values attained with polar solvents like methanol and water whereas with non polar solvents like petroleum ether, benzene and chloroform were comparatively less. A comparison of R_f values and spot color obtained in TLC of carbohydrates, it can be concluded that the fruit juice contained reducing hexose monosaccharides like galactose and glucose. TLC of betalain indicates presence of betacyanins type of color pigments.

Visible spectrum of betacyanin in methanol and water extracts of fruit demonstrated maximum absorbance at 535 nm indicate presence of betacyanin. HPLC chromatogram exhibited peak 1 with retention time 21.76 min at 484 nm was identified as indicaxanthin which present in very low amount and peak 2 with retention time 22.76 min at 535 nm as betanin, present in high amount in fruits of *Opuntia elatior* Mill. LC – MS suggested the presence of betanin confirmed by its identical spectral properties and the

presence of their protonated molecular ions $[M+H]^+$ with m/z551, while the prominent secondary ion at m/z390 due to the presence of protonated aglycones [betanidine + H]$^+$.

The total sugar content (11.41 g/100 ml) equivalent to glucose, total phenolic content (49.82 mg/100 ml) equivalent to gallic acid and titratable acidity (0.94%) equivalent to citric acid obtained in fruits of *Opuntia elatior* Mill. The total betacyanin content (47.10 mg/100 ml) equivalent to betanin obtained from fruits of *Opuntia elatior* Mill. was higher compare to *Opuntia ficus-indica* and *Opuntia undulata* Griff. while lower compare to *Opuntia stricta* Haw. The mineral composition is characterized by high amounts of potassium followed by calcium while other minerals are in the normal range of fruits.

The fruit juice of *Opuntia elatior* Mill. reversed anaemia induced by $HgCl_2$ and phenylhydrazine in dose dependant manner. It was observed that there was a remarkable increase in body weight in animals treated with fruit juice at dose 10 ml/kg (212.2 g) and 15 ml/kg (227.7 g) after continuous treatment for 30 days. At the dose of 10 ml/kg and 15 ml/kg of fruit juice showed good percentage of recovering in haemoglobin, 32.99 % and 38.18 %, respectively, which was higher than standard treated group (29.8 %) indicating correction of anaemia. fruit juice of *Opuntia elatior* Mill. improved the total and differential WBC with platelet functions and the results was dose dependant. The increment of blood sugar indicated that the fruit juice was a source of energy due to presence of high amount of carbohydrates. The fruit juice has not major effect on serum cholesterol and triglyceride levels. The kidney, liver and spleen functions were restored by fruit juice of *Opuntia elatior* Mill. The antioxidant phenolics and betanin constituents and mineral compositions appear most likely as the active ingredients responsible for haematinic effect of *Opuntia elatior* Mill. fruits. This results support at least partially the traditional use of fruits in the treatment of anaemia.

Phenylhydrazine produces both aryl and hydroxyl radicals when incubated with rat liver microsomes and oxidised by hydrogen peroxide. PHZ-induced haemolytic injury seems to be derived from oxidative alterations to red blood cell proteins rather than to membrane lipids. In this study, PHZ altered the haematological parameters by haemolysis characterized by

decrease in haemoglobin concentration, total RBC counts and PCV on day 3. However, the haematological parameters were restored to normal range after treatment with fruit juice of *Opuntia elatior* Mill. The speedy and progressive recovery of anaemic rats responding to treatement of *Opuntia elatior* Mill. fruits may be due to increased erythropoiesis. The improvement in the haematological indices exhibited by fruit juice might be connected with the minerals, phenolics and betacyanin content of the fruits of *Opuntia elatior* Mill. The phenolics and betacyanin have remarkable anti oxidant activity. These constituents might have direct influence on the protection of haemolysis by reactive oxygen species generated by PHZ.

The potential antinociceptive as central analgesic by using tail immersion test and peripheral analgesic by using acetic acid induced writhing test of the fruits of *Opuntia elatior* Mill. was investigated. According to antinociceptive tests, we can concluded that the fruits of *Opuntia elatior* Mill. is endowed with central and peripheral analgesic properties might be due to presence of phenolics and betanin content.

Bronchial asthma is characterized by increased airway reactivity to spasmogens, mediator releases and inflammation. Various animal models and experimental protocols were used in the present study to evaluate anti-asthmatic activity of fruit of *Opuntia elatior* Mill. In present study, significant increase in preconvulsion time was observed due to pretreatment with fruit juice of *Opuntia elatior* Mill., when the guinea pigs were exposed to either acetylcholine or histamine aerosol. The non-parallel rightward shift in acetylcholine and histamine log dose- response curves obtained in the presence of the fruit juice, with lowered maximum contraction effect to acetylcholine and histamine would indicate a non-competitive or an irreversible antagonistic effect of *Opuntia elatior* Mill. fruits at muscarinic and histamine H_1 receptors.

Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens. In the present study, the fruit juice of *Opuntia elatior* Mill. was found to inhibit the degranulation of mast cells induced by an immunological (egg albubim) and a non-immunological (compound 48/80) stimulus. A significant protection of rat peritoneal mast cells from disruption by antigen and compound 48/80 by fruit juice of *Opuntia*

218

elatior Mill. points towards its ability to interfere the release and /or synthesis of mediators of inflammation, indicating its mast cell stabilizing activity. Hence it may be assumed that the cytoprotective effect induced by fruit juice of *Opuntia elatior* Mill. on mast cell surface could be due to its ability to alter the influx of calcium ions.

Fruit juice of *Opuntia elatior* Mill. was found effective against carrageenan –induced inflammation and also reduced the neutrophil adhesion which suggested the anti-inflammatory action.

The peel of various fruits serves the protective action against microbial invasion. The peel extracts of *Opuntia elatior* Mill. fruits exhibited antimicrobial actions in a dose dependant manner against both test bacteria and fungi. Antimicrobial activity of the peel extracts is directly concerning with the components that they contain. Petroleum ether, benzene and methanol extracts showed maximum inhibitory action against gram positive bacteria, gram negative bacteria and fungi, respectively.

In conclusion, our pharmacognostical data authenticate the plant and phytochemical results indicate that the flavonoids betacyanin equivalent to betanin is the active principle in the fruits of *Opuntia elatior* Mill. The fruits are exhibiting haematinic effect against anaemia induced by mercuric chloride and phenylhydrazine while antinociceptive effect against thermal and chemical stimuli. Fruits also appear anti-asthmatic property due to its bronchodilator, mast cell stabilization, anti-inflammatory and reduction of neutrophil adhesion property. Furthermore, peel extracts of fruit appear antimicrobial property against bacteria and fungi. These findings on haematinic, analgesic and anti-asthmatic effects of fruit juice, further add value to the nutritional characteristics of the fruits of *Opuntia elatior* Mill.

7. Reference

7. Reference

Adams, D. H., Shaw, S., 1994. Leucocyte-endothelial interactions and regulation of leucocyte migration. Lancet 343, 831-836.

Agbor, G.A., Oben, J.E., and Ngogang, J.Y., 2005. Haematinic activity of *Hibiscus cannabinus.* African Journal of Biotechnology 4(8), 833-837.

Aggarwal, A.N., Chaudhry, K., Chhabra, S.K., D'Souza, G.A., Gupta, D., Jindal, S.K., Katiyar, S.K., Kumar, R., Shah, B., and Vijayan, V.K., 2006. Prevalence and Risk Factors for Bronchial Asthma in Indian Adults: A Multicentre Study. Indian Journal of Chest Disease and Allied Science 48, 13-22.

Aguwa, C. N., 1996. Therapeutic Basis of Clinical Pharmacy in the tropics. 2nd ed. Uptimal Pub. Enugu, Nigeria. pp. 379.

Ahmad, A., Davies, J., Randall, S., Skinner, G.R.B., 1996. Antiviral properties of extract of *Opuntia streptacantha.* Antiviral Research 30, 75-85.

Ahmad, I. and Beg, A.Z., 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology 74, 113–133.

Al-Zoreky, N. S., 2009. Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. International Journal of Food Microbiology 134, 244–248.

Amer, J., Goldfarb, A., Fibach, E., 2004. Flow cytometric analysis of the oxidative status of normal and thalassemic red blood cells. Cytometry 60A, 73–80.

Anderson, E.E., 2001. The cactus family, Timber press, Portland, OR, 15 – 72, ISBN 0881924989.

Angelique, L. and Ralf, J. 2008. Alternative drug discovery approaches for orphan GPCRs. Drug Discovery Today 13, 52–58.

Anonymous, 1989. The Ayurvedic Pharmacopoeia of India. Part I, Vol. III, published by Govt. of India, Ministry of Health & Family Welfare, Dept. of Indian system of Medicine & Homoeopathy, New Dehli, 190-191.

Anonymous, 1996. The Indian Pharmacopoeia. 2nd edition, Vol. II. Controller of Publication, New Delhi (1996) 631- 636.

Anonymous, 2003. The Ayurvedic Formulary of India Vol. I and II. Controller of Publications, Ministry of Health & Family Welfare, Government of India.

AOAC, 1995. In; AOAC Official Methods of Analysis, Patricia Cunniff (Ed.), 16th edition, Vol. II, Virginia, USA, page no. 10.

Ashe, W., Largent, E., Dutra, F., 1953. Behaviour of mercuric in the animal organism following inhalation. Archive of Indian Hygiene and Occupational Medicine 17, 19-48.

Aster, J. C., 2007. Red blood cell and bleeding disorders. In: In: Robbins and Cotran Pathologic Basis of Disease, Kumar, V., Abbas, A. K. and Fausto, N. (Ed), 7th edition, published by Elsevier, a division of Reed Elsevier India Private Limited, Noida, U. P., India, 619-627.

Aydin, S., Demir, T., Ozturk, Y., Baser, K.H.C., 1999. Analgesic activity of *Nepeta italica* L. Phytotherapy Research 13, 20–23.

Balsitis, M., Morrell, K., Mahida, Y., Hawkey, C. J., 1994. Cell adhesion molecule expression by colonic mucosa in ulcerative colitis. Eur J Gastroenterol Hepatol 6, 351-358.

Barbera G., Carimi F. and Inglese P., 1992. Past and present role of the Indian-fig prickly-pear *(Opuntia ficus-indica* (L.) Miller, Cactaceae) in agriculture in Sicily. Economic Botany 46, 10-20.

Barnard, H., Dreef, E.J., and van Krieken, J.H.J.M., 1990. The ruptured spleen. A histological, morphometrical and immunohistochemical study. Histology and Histopathology 5, 299-304.

Barry, K. A., 2005. The role of eosinophils in the pathogenesis of asthma. Trends in Molecular Medicine 11(4), 148-152.

Barry, K. A., Phipps, S., and Robinson, D. S., 2004. A role for eosinophils in airway remodeling in asthma. Trends in Immunology 25(9), 477-482.

Basu, A., Nag Chaudhuri, A.K., 1991. Preliminary studies on the antiinflammatory and analgesic activities of *Calotropis procera* root extract. Journal of Ethnopharmacology 31(3), 319–324.

Batista, A.M., Mustafa, A.F., McAllister, T., Wang, Y., Soita, H., McKinnon, J.J., 2003. Effects of variety on chemical composition, in situ nutrient disappearance and in vitro gas production of spineless cacti. Journal of Science and Food Agricultural 83, 440–445.

Beaven, G.H., & White, J.C., 1954. Oxidation of phenylhydrazine in the presence of oxyhemoglobin and the origin of Heinz bodies in erythrocytes. Nature 173, 389-391.

Ben Salem, H., Abdouli, H., Nefzaoui, A., El-Mastouri, Ben Salem, L., 2005. Nutritive value, behaviour, and growth of Barbarine lambs fed on oldman saltbush (*Atriplex nummularia* L.) and supplemented or not with barley grains or spineless cactus (*Opuntia ficus-indica* f. inermis) pads. Small Ruminant Research 59, 229-237.

Benjamin, N., Kushwah, A., Sharma, R. K. & Katiyar, A. K., 2006. Histopathological changes in liver, kidney & muscles of pesticides exposed malnourished and diabetic rats. Indian Journal of Experimental Biology 44, 228-332.

Benson, L., 1982. The cacti of the United States and Canada, Stanford University Press, Stanford, California, 1044, ASIN B000PWOAY0.

Ben-Thlija, A., 2002. Nutritional value of several *Opuntia* species. Master Thesis, Oregon State University, Corvallis/USA, 1987; cit. in: Nefzaoui, A., Ben Salem, H., Forage, fodder, and animal nutrition, in: Nobel, P. S. (Ed.), Cacti. Biology and Uses, University of California Press, Berkeley, Los Angeles, London, 199-210.

Berger, J., 2007. Phenylhydrazine haematotoxicity. Journal of Applied Biomedicine 5, 125-130.

Betty, M., Lillo, L.E., Saenz, C., Urzua, C.C. Zarate, O., 2006. Chemical characterization of the mucilage from fruits of *Opuntia ficus-indica*. Carbohydrate Polymers 63, 263–267.

Beutler, E., 2001. Hemolytic anemia due to chemical and physical agents. In Beutler E, Coller BS, Lichtman MA, Kipps TJ, Seligsohn U (eds): Williams Hematology, 6th edition, New York, pp. 629–632.

Bilyk, A., 1979. Extractive Fractionation of Betalaines. Journal of Food Science 44, 1249-1251.

Bilyk, A., 1981. Thin Layer Chromatographic Separation of Beet pigments. Journal of Food Science 46, 298-299.

Bouma, M. G., Stad, R. K., van den Wildenberg, F. A. J. M., Buurman, W. A., 1994. Differential regulatory effects of adenosine on cytokine release by activated human monocytes. J Immunology 153, 4159-4168.

Bousquet, J., Jeffery, P. K., Busse, W. W., 2000. Asthma: From bronchoconstriction to airways inflammation and remodeling. American Journal of Respiratory and Critical Care Medicine 161, 1749-1745.

Boyce, J. A., 2003. The role of mast cells in asthma. Prostaglandins, Leukotrienes and Essential Fatty Acids 69, 195–205.

Bradding, P., Walls, A. F. and Holgate, S. T., 2006. The role of the mast cell in the pathophysiology of asthma. Journal of Allergy and Clinical Immunology 117, 1277-84.

Briejer, M.R., Akkermans, L.M., Schuurkes, J.A., 1995. Gastrointestinal prokinetic bezamide: the pharmacology underlying stimulation of motility. Pharmacological Review 47, 631–657.

Bruckner, H., & Westhauser, T., 2003. Chromatographic determination of L- and D amino acids in plants. Amino Acids 24, 43–55.

Brunner, F.P., de Rougemont, D., Robbiani, M., Seiler, H., Thiel, G., 1985. Renal mercury content in HgCl2-induced acute renal failure in furosemide/saline-protected and nonprotected rats. Nephron 41(1), 94-9.

Bruno de Benoist, Erin McLean, Ines Egli, Mary Cogswell, editor, 2008. Worldwide prevalence of anaemia 1993 – 2005: WHO global database on anaemia. WHO, Geneva, ISBN 9789241596657.

Budinsky, A., Wolfram, R. Oguogho, A., Efthimiou, Y., Stamatopoulos, Y., Sinzinger, H., 2007. Regular ingestion of *Opuntia robusta* lowers oxidation injury. Prostaglandins, Leukotrienes and Essential Fatty Acids 65(1), 45-50.

Burka, J. F., 1984. Effect of compound 48/80 and formyl-methyl-leucyl-phenylalanine on isolated guinea pig airways, Int. Archs. Allergy Appl. Immunol. 73, 309-311.

Bwititi, P., Musabayane, C.T., Nhachi, C.F.B., 2000. Effects of *Opuntia megacantha* on blood glucose and kidney function in streptozotocin diabetic rats. Journal of Ethnopharmacology 69, 247–252.

Cai, Y. Z., Sun, M, & Corke, H., 2005. Characterization and application of betalain pitments from plants of the Amaranthaceae. Trends in Food Science & Technology 16, 370-376.

Cai, Y., & Corke, H. 1999. Amaranthus betacyanin pigments applied in model food systems. Journal of Food Science 64, 869–873.

Cantwell, M., 1995. Post-harvest management of fruits and vegetables stems. In: Agro-ecology, Cultivation and Uses of Cactus Pear. Eds: Barbera G, Inglese P, Pimienta-Barrios E, FAO-Plant Production and Protection Paper, Rome 132, 120-136.

Cantwell, M., Rodriguez-Felix, A., Robles-Contreras, F., 1992. Postharvest physiology of prickly pear cactus stems. Scientia Horticulturae 50, 1-9.

Cassano, A., Conidi, C., Timpone, R., D'Avella, M., Drioli, E., 2007. A membrane- based process for the clarification and the concentration of cactus pear juice, Journal of Food Engineering 80, 914-921.

Caulfield, M.P., 1993. Muscarinic receptor characterization, coupling and function. Pharmacological Therapeutics 58, 319–379.

Chakrabarti, S., Sonaye, B., Naik, A.A., Nadkarni, P.P., 1995. Erythrocyte membrane protein damage by oxidation products of phenylhydrazine. Biochemical and Molecular Biology International 35, 255–263.

Chalker-Scott, L., 1999. Environmental significance of anthocyanins in plant stress responses. Photochemistry and Photobiology 70, 1-9.

Charak Samhita, 1949. Shri Gulab Kunverba Ayurvedic Society, Jamnagar, Ayurvedic Mudranalaya, Jamnagar, Vol. IV**,** 1952-2032

Chatterjee, C. C., 1994. Human physiology. Vol. I, 10th Ed. Medical Allied Agency, Kolkata, India.

Chethana, S., Nayak, C. A., Raghavarao, K.S.M.S., 2007. Aqueous two phase extraction for purification and concentration of betalains. Journal of Food Engineering 81, 679–687.

Chowdhury, A.R., Vachhrajani, K.D., Makhija, S., and Kashyap, S.K., 1986. Histomorphometric and biochemical changes in the testicular tissues of rats treated with mercuric chloride. Biomedica Biochima Acta 45(7), 949-56.

Cicero A.F.G., Derosa, G. & Gaddi, A., 2004. What do herbalists suggest to diabetic patients in order to improve glycemic control? Evaluation of scientific evidence and potential risks. Acta Diabetologica 41, 91-98.

Cighetti, G., Debiasis, S., Paroni, R., Allevi, 1999. Free and total malondialdehyde assessment in biological matrices by GC/MS. What is needed for an accurate detection? Anal Chem. 266(2), 222-229.

Clarkson, T. W., 1972. Recent advances in toxicology of mercury with emphasis on the alkyl mercurials. Critical Review in Toxicology, 203.

Claro, L. M., Leobart, M. S. S., Comar, S. R., do Nascimento, A. J., 2006. Effect of vitamins C and E on oxidative processes in human erythrocytes. Cell Biochemical Funct. 24, 531–535.

Cockcroft, D. W., 1983. Mechanism of perennial allergic asthma. Lancet 2, 253-256.

Collier, H. D. J., Dinnin, L.C., Jonhson, C.A., Scheinder, C., 1968. The abdominal response and its suppression by analgesic drugs in the mouse. British Journal of Pharmacology 32, 295–310.

Criswell, K. A., Sulkanen, A. P., Hochbaum, A. F., Bleavins, M. R., 2000. Effects of phenylhydrazine or phlebotomy on peripheral blood, bone marrow and erythropoietin in Wistar rats. Journal of Applied Toxicology 20, 25–34.

Cronstein, B. N., Levin, R. I., Philips, M., Hirschhorn, R., Abramson, S. B., Weissman, G., 1992. Neutrophil adherence to endothelium is enhanced via adenosine A_1 receptors and inhibited via adenosine A_2 receptors. Journal of Immunology 148, 2201-2206.

Crunkhon, P., Meacock, S.E.R., 1971. Mediators of the inflammation induced in the rat paw by carrageenan. British Journal of Pharmacology 42, 392–402.

Cruse, R.R., 1973. Desert plant chemurgy: a current review. Economic Botany 27, 210-230.

Curtin, L.S.M., 1949. By the Prophet of the Earth. Santa Fe, New Mexico: San Vicente Foundation.

Dai, Y., Ye, W.C., Wang, H., Matsuga, H., Kubo, M., But, P.P.H., 2002. Antipyritic and antinociceptive effects of *Chenopodium album* L. in mice. Journal of Ethnopharmacology 81, 245–250.

Dale, M.M., Foreman, J.C., 1984. Textbook of Immunopharmacology. Blackwell, London, pp. 52–54.

Datta, A.C., 2003. Botany. 6th edition, Oxford University Press, New Delhi, India, 566-67.

Datta, K., Soni, J. L., Datta, I. C., 1990. An avian model for the study of acute hemolytic anemia in the domestic fowl (*Gallus domesticus*). Biomedica Biochimica Acta 49, 607–611.

Datta, S.C., 1988. "A handbook of systematic botany" , fourth edition, New Age Publishers, New Delhi, page no. 229-231.

Dawson, Y., 2007. Pharmacology. 3rd edition, published by Elsevier's Health Sciences Rights Department, Philadelphia, USA, 55-62.

deJong, K., Geldwerth, D., Kuypers, F. A., 1997. Oxidative damage does not alter membrane phospholipids asymmetry in human erythrocytes. Biochemistry 36, 6768 – 6776.

Delgado-Vargas, F., Jimenez, R., Paredes-Lopes, O., 2000. Natural Pigments: Carotenoids, Anthocyanins, and Betalains – Characteristics, Biosynthesis, Processing, and Stability. Critical Review in Food Science and Nutrition 40(3), 173-289.

Diallo, A., Gbeassor, M., Vovor, A., Eklu-Gadegbeku, K., Aklikokou, K., Agbonon, A., Abena, A. et al., 2008. Effect of *Tectona grandis* on phenylhydrazine-induced anaemia in rats. Fitoterapia 79, 332-336.

Dianzani, C., Brunelleschi, S., Viano, I., Fantozzi, R., 1994. Adenosine modulation of primed human neutrophils. European Journal of Pharmacology 263, 223-226.

Diaz Media, E.M., Rodriguez, E.M., and Diaz Romero, C., 2007. Chemical characterization of *Opuntia dillenii* and *Opuntia ficus-indica* fruits, Food Chemistry 103(1), 38-45.

Dieter, M.P., Luster, M.I., Boorman, G.A., Jameson, C.W., Dean, J.H., Cox, J.W., 1983. Immunological and biochemical responses in mice treated with mercuric chloride. Toxicology and Applied Pharmacology 68, 218–228.

Dippold, W., Wittig, B., Schwaeble, W., Mayet, W., Meyer zum Buschenfelde, 1993. Expression of intercellular adhesion molecule 1 (ICAM-1, CD54) in colonic epithelial cells. Gut 34, 1593-1597.

DiRosa, M., Giroud, J.P., Willoughby, D.A., 1971. Studies of the acute inflammatory response induced in rats in different sites by carragenan and turpentine. Journal of Pathology 104, 15–29.

Domínguez López A., 1995. Use of the fruits and stems of the prickly pear cactus (*Opuntia* spp.) into human food. Food Science and Technology International 1, 65–74.

Downie, S.R. and Palmer, J.D., 1994. A chloroplast DNA phylogeny of the Caryophyllales based on structural and inverted repeat restriction site variation. Systematic Botany 19, 236-252.

Driessen, B., Reimann, W., 1992. Interaction of the central analgesic, tramadol, with the uptake and release of 5-hydroxytryptamine in the rat brain in vitro. British Journal of Pharmacology 105(1), 147–51.

Duke, J.A., and Vasquez, R., 1994. Amazonian Ethnobotanical Dictionary. Boca Raton, Florida: CRC Press.

Eglen, R.M., Hedge, S.S., Watson, N., 1996. Muscarinic receptor subtypes and smooth muscle function. Pharmacological Review 48, 531–565.

Egon, S., 2007. Thin layer chromatography – A laboratory hand book, 2nd edition, Springer – Verlag Berlin, Heidelberg, 807 – 810.

EHC-118, 1991. Environmental Health Criteria: Inorganic Mercury. World Health Organizaion (WHO), Geneva publication.

Elorriaga, M., Anselmi, E., Hernandez, J.M., Docon, P., Ivorra, D., 1996. The source of Ca^{2+} for muscarinic receptor-induced contraction in rat ileum. Journal of Pharmacy and Pharmacology 48, 817–819.

Ennouri, M., Evelyne, B., Laurence, M. & Hamadi, A., 2005. Fatty acid composition and rheological behaviour of prickly pear seed oils. Food Chemistry 93, 431–437.

Ennouri, M., Fetoui, H., Bourret, E., Zeghal, N., F., Attia, H., 2006. Evaluation of some biological parameters of *Opuntia ficus-indica* 1. Influence of a seed oil supplemented diet on rats. Bioresource Technology 97, 1382–1386.

Ennouri, M., Fetoui, H., Bourret, E., Zeghal, N., Guermazi, F., Attia, H., 2006a. Evaluation of some biological parameters of *Opuntia ficus-indica*. 2. Influence of seed supplemented diet on rats. Bioresource Technology 97, 2136–2140.

Ennouri, M., Fetoui, H., Hammami, M., Bourret, E., Attia, H., Zeghal, N., 2007. Effects of diet supplementation with cactus pear seeds and oil on serum and liver lipid parameters in rats. Food Chemistry 101, 248–253.

Erah, O. P., Asonye, C. C., Okhamafe, O. A., 2003. Response of *Trypanosoma Brucei brucei* induced anaemia to a commercial herbal preparation. African Journal of Biotechnology 2(9), 307-311.

Eun Ha Lee, Hyoung Ja Kim, Yun Seon Song, Changbae Jin, Kyung Tae Lee, Jungsook Cho, Young Sup Lee, 2003. Constituents of the stems and fruits of *Opuntia ficus-indica* var. *saboten*. Archives of Pharmaceutical Research 26(12), 1018-1023.

Evans, W. C., 1996. Trease and Evans Pharmacognosy, 14th edition, Harcourt Brace and Company Asia, PTE Ltd., 290.

Evans, W.C., 2005. Trease and Evans Pharmacognosy. 15th edition, Elsevier, A division of Reed Elsevier India Private Limited, New Delhi, India, 20-22.

Falade, O.S., Otemuyiwa, I.O., Oladipo, A., Oyedapo, O.O., Akinpelu, B.A., & Adewusi, S.R.A., 2005. The chemical composition and membrane stability activity of some herbs used in local therapy for anemia. Journal of Ethanopharmacology, 102, 15-22.

Fernandez, M.L., Lin, E.C., Trejo, A. & McNamara, D.J., 1992. Prickly pear (*Opuntia* sp.) pectin reverses low density lipoprotein receptor suppression induced by a hypercholesterolemic diet in guinea pigs. Journal of Nutrition 122, 2330-2340.

Fernandez-Lopez, J. A., & Almela, L., 2001. Application of high-performance liquid chromatography to the characterization of the betalain pigments in prickly pear fruits, Journal of Chromatography A 913, 415–420.

Ferndndez-Lopez, J.A., Castellar, R., Obon, J.M., Almela, L., 2002. Screening and Mass-Spectral Confirmation of Betalains in Cactus Pears. Chromatographia 56, 591- 595.

Ferrali, M., Signorini, C., Sugherini, Pompella, A., Lodovic, M., Caciotti, B., Ciccoli, L., and Comporti, M., 1997. Release of free redox-active iron in the liver and DNA oxidative damage following phenylhadrazine intoxication. Biochem. Pharmacol. 53, 1743-1751.

Ferreira, S.H., 1972. Prostaglandins, aspirin-like drugs and analgesia. Nature New Biology 240 (102), 200–203.

Ferreira, S.H., Nakamura, M., 1979. I-Prostaglandin hiperalgesia, a cAMP:Ca^{2+}dependent process. Prostaglandins 18(2), 179–190.

Feugang, J.M., Konarski, P., Zou, D., Stintzing, F.C., Zou, C., 2006. Nutritional and medicinal use of cactus pear (*Opuntia* spp.) cladodes and fruits. Frontiers in Bioscience 11, 2574-2589.

Filipović, M., and Cekić, S., 2001. The role of eosinophils in asthma. Medicine and Biology 8(1), 6-10.

Fitzhugh, O.C., Nelson, A.R., Lang, E.P., 1950. Chronic oral toxicities of mercuriphenyl and mercuric salts. Archives in Indian Hygiene and Occupational Medicine 2, 433-442.

Flanagan, J.P. & Lessler, M.A., 1970. Controlled phenylhydrazine-induced reticulocytosis in the rat. The Ohio Journal of Science 70 (5), 300-304.

Foster, P. S., Hogan, S. P., Yang, M., Mattes, J., Young, I. G., Matthaei, K. I., Kumar, R. K., Mahalingam, S. and Webb, D. C., 2002. Interleukin-5 and eosinophils as therapeutic targets for asthma. TRENDS in Molecular Medicine 8(4), 162-167.

Frati A., 1992. Medical implication of prickly pear cactus. In: Proc. 3rd Annual Texas prickly pear council. Eds: Felkar P, Moss LR, 24–25 July, Kingsville, Texas, 29-34.

Frati, A.C., Altamirano-Bustamante, E., Rodriguez-Barcenas, N., Ariza-Andraca, R. and Lopez-Ledesma, R. 1989. Hypoglycemic action of *Opuntia streptacantha* Lemaire: study using raw extracts. Archivos de investigación médica 20(4), 321-325.

Frati, A.C., Del Valle-Martines, L.M., Ariza-Andraca, C.R., Islas-Andrade, S. and Chavez-Negrete, A., 1989a. Hypoglycemic action of different doses of nopal (*Opuntia streptacantha* Lemaire) in patients with type II diabetes mellitus. Archivos de investigación médica 20(2), 197-201.

Frati, A.C., Gordillo, B.E., Altamirano, P. & Ariza, C.R., 1988. Hypo-glycemic effect of *Opuntia streptaeantha* Lemaire in NIDDM. Diabetes Care 11, 63-66.

Frati, A.C., Jimenez, E. & Ariza, C.R., 1990a. Hypoglycemic effect of *Opuntia ficus-indica* in non insulin-dependent diabetes mellitus patients. Phytotherapy Research 4, 195-197.

Frati, A.C., Licona-Quesada, R., Araiza-Andraca, C.R., Lopez-Ledesma, R. and Chavez-Negrete, A., 1990. Activity of *Opuntia streptacantha* in

healthy individuals with induced hyperglycemia. Archivos de investigación médica 21(2), 99-102.

Frati, A.C., Xilotl Diaz, N., Altamirano, P., Ariza, R., and Lopez-Ledesma, R. 1991. The effect of two sequential doses of *Opuntia streptacantha* upon glycemia. Archivos de investigación médica 22(3-4), 333 – 336.

Fukino, H., Hirai, M., Hsueh, Y.M., and Yamane, Y., 1984. Effect of zinc pretreatment on mercuric chloride-induced lipid peroxidation in the rat kidney. Toxicology and Applied Pharmacology 73, 395-401.

Galanter, J. M. and Lazarus, S., 2008. Integrative inflammation pharmacology: Asthma. In: Principles of pharmacology The pathophysiologic basis of drug therapy, Golan, D.E., Tashjian, A.H., Armstrong, E.J. and Armstrong, A.W. (Ed.), 2nd edition, published by Wolters Kluwer Health and Lippincott Williams & Wilkins, New Delhi, India, 825-833.

Galati, E.M., Monforte, M.T., Tripodo, M.M., d'Aquino, A., Mondello, M.R., 2001. Antiulcer activity of *Opuntia ficus-indica* (L.) Mill. (Cactaceae): ultrastructural study. Journal of Ethnopharmacology 76, 1-9.

Galati, E.M., Pergolizzi, S., Miceli, N., Monforte, M.T., Tripodo, M.M., 2002a. Study on the increment of the production of gastric mucus in rats treated with *Opuntia ficus-indica* (L.) Mill. cladodes. Journal of Ethnopharmacology 83, 229-233.

Galati, E.M., Tripodo, M.M., Trovato, A. d'Aquino, A. & Monforte, M.T., 2003. Biological activity of *Opuntia ficus-indica* cladodes II: Effect on experimental hypercholesterolemia in rats. Pharmaceutical Biology 41(3), 175-179.

Galati, E.M., Tripodo, M.M., Trovato, A., Miceli, N., Monforte, M.T., 2002. Biological effect of *Opuntia ficus-indica* (L.) Mill. (Cactaceae) waste matter. Journal of Ethnopharmacology 79, 17-21.

Galt, A.H., and Galt, J.W., 1978. Peasant use of some wild plants on the island of Pantelleria, Sicily. Economic Botany 32, 20-26.

Ganguly, A.K., Govindachari, T.R., Mohamed, P.A., 1965. Structure of opuntiol, a constituent of *Opuntia elatior*. Phytochemistry 21, 93–99.

Gannett, P. M., Lawson, T. S., Kolar, C., Toth, B., 1997. Aryl radical formation during the metabolism of arylhydrazines by microsomes. Chemical Research Toxicology 10(12), 1372-1377.

Gaud, R.S. and Gupta, G.D., 2006, "Practical Physical Pharmacy", First Edition, CBS Publishers & Distributors, New Delhi, Page No. 29 – 36.

Ghule, B.V., Murugananthan, G., Nakhat, P.D., & Yeole, P.G., 2006. Immunostimulant effects of *Capparis zeylanica* Linn. leaves. Journal of Ethnopharmacology 108, 311-315.

Gibbs, K. P., and Cripps, D., 2007. Asthma. In: Clinical pharmacy and therapeutics, Walker, R. and Whittlesea, C., (Ed.), 4th edition, Churchill Livingstone Elsevier, Elsevier's Health Sciences Rights Department, Philadelphia, USA, 367-372.

Girardi, G., and Elias, M.M., 1995. Mercuric chloride effects on rat renal redox enzyme activities: SOD protection. Free Radical Biology and Medicine 18, 61-66.

Godkar P. B. & Godkar D. P., 2004. Textbook of medical laboratory technology, 2nd edition, published by Bhalani Publishing House, Mumbai, India.

Gokhale, A. B., & Saraf, M. N., 2002. Recent developments in asthma therapy. Indian Drugs, 39(3), 121-132.

Golab, J., Olszewska, D., Mroz, P., Kozar, K., Kaminski, R., Jalili, A., Jakobisiak, M., 2002. Erythropoietin restores the antitumor effectiveness of photodynamic therapy in mice with chemotherapy-induced anemia. Clinical Cancer Research 8, 1265–1270.

Goldberg, B., Stern, A., 1977. The mechanism of oxidative hemolysis produced by phenylhydrazine. Molecular Pharmacology 13, 832–839.

Gonzfilez, A.T., Ortiz, G.G., Puebla-Pdrez, A.M., Huizar-Contreras, M.D., Mazariegos, M.R.M., Arreguin, S.M., Calva, E., 1996. A purified extract from prickly pear cactus *(Opuntia fuliginosa)* controls experimentally induced diabetes in rats. Journal of Ethnopharmacology 55, 27-33.

Goyal, R.K., 1988. Identification, localisation and classification of muscarinic receptor subtypes in the gut. Life Science 43, 2209–2220.

Goycoolea, F.M. & Cardenas, A., 2003. Pectins from *Opuntia* spp.: A Short Review, Journal of the Professional Association for Cactus Development, 17-29.

Greenburg, A. G., 1996. Pathophysiology of Anemia. American Journal of Medicine 101 (suppl 2A), 7S-11S.

Grenand, P., Moretti, C. and Jacquemin, H., 1987. Pharmacopees Traditionnelles en Guyane: Creoles, Palikur, Wayapi. Memoires ORSTROM 108. Paris: Editions del'ORSTROM.

Gruenwedel, D. W. and Davidson, N., 1966. Complexing and denaturation of DNA by methyl mercuric hydroxide. Journal of Molecular Biology 21, 129-141.

Gstraunthaler, G., Pfaller, W., and Kotanko, P., 1983. Glutathione depletion and *in vitro* lipid peroxidation in mercury or maleate induced acute renal failure. Biochemical Pharmacology 32, 2969-2972.

Guevara, J.C., Yahia, E.M., Brito de la Fuente, E., 2001. Modified atmosphere packaging of prickly pear cactus stems (*Opuntia* spp.). Lebensmittel – Wissenschaft und- Technologie 34, 445–451.

Gupta, R. et al. 2005. Nature's medicines: traditional knowledge and intellectual property management. Case studies from the NIH, USA. Current Drug Discovery Technology 2, 203–219.

Gupta, R.S., Sharma, R., Sharma, A., Chaudhudery, R., Bhatnager, A.K., Dobha, M.P., Joshi, Y.C., Sharma, M.C., 2002. Antispermatogenic effect and chemical investigation of *Opuntia dillenii*. Pharmaceutical Biology 40, 411–415.

Gurbachan S. & Felker, P., 1998. Cactus: new world foods. Indian Horticulture 43, 29–31.

Gurbachan singh, 2003. General Review of Opuntias in India. Journal of the Professional Association for Cactus Development, 30-46.

Gutierrez, L.L.P., Mazzotti, N.G., Araújo, A.S.R., Klipel, R.B., Fernandes, T.R.G., Llesuy, S.F. and Belló-Klein, A., 2006. Peripheral markers of oxidative stress in chronic mercuric chloride intoxication. Brazilian Journal of Medical and Biological Research 39, 767-772.

Habibi, Y., Heyraud, A., Mahrouz, M., Vignon, M. R., 2004. Structural features of pectic polysaccharides from the skin of *Opuntia ficus-indica* prickly pear fruits. Carbohydrate Research 339, 1119-1127.

Habibi, Y., Mahrouz, M. Marais, M. F., Vignon, M. R., 2004a. An arabinogalactan from the skin of *Opuntia ficus-indica* prickly pear fruits. Carbohydrate Research 339, 1201-1205.

Habibi, Y., Mahrouz, M., Vignon, M.R., 2005. Isolation and structural characterization of protopectin from the skin of *Opuntia ficus-indica* prickly pear fruits. Carbohydrate Polymers 60, 205-213.

Hall, G. W., 2007. Understanding haemolytic anaemia. Paediatrics and Child Health 17(8), 333-339.

Han, Y. N., Choo, Y., Lee, Y.-C., Moon, Y.-I., Kim, S.-D., Choi, J.-W., 2001. Monoamine oxidase B inhibitors from the fruits of *Opuntia ficus-indica* var. *saboten*. Archives of Pharmaceutical Research 24, 51–54.

Harborne, J. B., 2007. "Phytochemical Methods – A guide to modern techniques of plant analysis"; 3rd edition, Springer International edition, 66-74.

Hassanien, M.F.R. and Morsel, J.T., 2003. Agro waste products from prickly pear fruit processing as a source of oil. Fruit processing 13, 242-248

Hayllar, J., and Bjarnason, I., 1991. Sulphasalazine in ulcerative colitis. Gut 32, 462-463.

Hennies, H. H., Friderichs, E., Schneider, J., 1988. Receptor binding, analgesic and antitussive potency of tramadol and other selected opiods. Arzneim-Forsch/Drug Research 38(7), 877– 80.

Hess, S.M. & Milonig, R.C. 1972. Assay for anti-inflammatory drugs. In: Lepow, I. H., Ward, P.A. (Eds.), Inflammation, Mechanisms and Control. Academic Press, New York, pp. 1-12.

Hill, H. A. O. and Thornalley, P. J., 1982. Free radical production during phenylhydrazine-induced hemolysis. Canadian Journal of Chemistry 60, 1528–1531.

Hodges, V. M., Rainey, S., Lappin, T. R., Maxwell, A. P., 2007. Pathophysiology of anemia and erythrocytosis. Critical Reviews in Oncology/Hematology 64, 139–158.

Hong-Fang Ji, et al., 2009. Natural products and drug discovery. EMBO Reports 10(3), 194–200.

Hughes, B., 2009. 2008 FDA drug approvals. Natural Review in Drug Discovery 8, 93–96.

Hughes, W. L., 1957. A physicochomical rationale for the biological activity of mercury and its compounds. Annual New York Academic Science 65, 454-460.

Humes, H. D. and Weinberg, J. M., 1983. Cellular energetics in acute renal failure. In: Brenner B.M., Lazarus J.M. (eds.) Acute renal failure. W.B. Saunders, Philadelphia, 47-98.

Hussain, A. N. and Kumar, V., 2007. The Lung. In: Robbins and Cotran Pathologic Basis of Disease, Kumar, V., Abbas, A. K. and Fausto, N. (Ed), 7th edition, published by Elsevier, a division of Reed Elsevier India Private Limited, Noida, U. P., India, 711-728.

Ibanez-Camacho, R. & Roman-Ramos, R., 1979. Hypoglycemic effect of *Opuntia* cactus. Archivos de investigación médica 10, 223–230.

Ibanez-Camacho, R., Meckes-Lozoya, M. & Mellado-Campos, V., 1983. The hypoglycemic effect of *Opuntia streptacantha* studied in different animal experimental models. Journal of Ethnopharmacology 7, 175-181.

Ismail, T.S., Gapalakrisan, S., Begum, V.H., Elango, V., 1997. Anti-inflammatory activity of *Salacia oblonga* Wall and *Azima tetracantha* Lam. Journal of Ethnopharmacology 56, 145–152.

Jadhav, S.H., Sarkar, S.N., Ram, G.C., Tripathi, H.C., 2007. Immunosuppressive effect of subchronic exposure to a mixture of eight heavy metals, found as groundwater contaminants in different areas of India, through drinking water in male rats. Archives in Environmental Contamination and Toxicology 53, 450–458.

Jaffe, K. M., Shurtleff, D. B. and Robertson, W. O., 1983. Survival after caute mercury vapour poisoning-role of intensive supportive care. American Journal of Disease in Children 137, 749-751.

Jain, A. S., Surana, S. J., Gokhale, S. B., Tatiya, A. U. and Bothara, R. C., 2007. Antimicrobial Properties of *Eranthemum roseum* (Vahl) R.Br. Iranian Journal of Pharmaceutical Research 6(2), 131-133.

Jain, S. K., and Tarafder, C.R., 1970. Medicinal plant lore of the Santals. Economic Botany 24, 241-278.

Jianqin, J., Wencai, Y., Zhen, C., Fengchang, L., Zhida, M., 2002. Two new phenolic carboxylic acid esters from *Opuntia vulgaris*. Journal of Chinese Pharmaceutical Science 11, 1–3.

Jianqin, J., Yanfang Li, Zhen Chen, Zhida Min, Fengchang Lou, 2006. Two novel C29-5β-sterols from the stems of *Opuntia dillenii*. Steroids 71, 1073-1077.

Joel Corrales-Garcıa, Cecilia, B., Peña-Valdivia, Yolanda Razo-Martınez, Margarita Sánchez-Hernández, 2004. Acidity changes and pH-buffering capacity of nopalitos (*Opuntia* spp.), Postharvest Biology and Technology 32, 169–174.

Jones, P., Raeini-Sarjaz, M., Ntanios, F., Vanstone, C., Feng, J. & Parsons, W., 2000. Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. Journal of Lipid Research 41, 697–705.

Joshi, K., Chavan, P., Warude, and Patwardhan, B., 2004. Molecular markers in herbal drug technology. Current Science, 87, 415.

Jung-Hoon Kima, Shin-Mi Park, Hyun-Joo Ha, Chang-Jong Moon, Tae-Kyun Shin, Jung-Mi Kim, Nam-Ho Lee, Hyoung-Chun Kim, Kyung-Jin Jang, Myung-Bok Wie, 2006. *Opuntia ficus-indica* attenuates neuronal injury in in vitro and in vivo models of cerebral ischemia. Journal of Ethnopharmacology 104, 257–262.

Jungsook Cho, Hyang Dok-Go, Kwang Heun Lee, Hyoung Ja Kim, Eun Ha Lee, Jiyong Lee, Yun Seon Songb, Yong-Ha Leeb, Changbae Jinb, Yong Sup Leeb, 2003. Neuroprotective effects of antioxidative flavonoids, quercetin, (+)-dihydroquercetin and quercetin 3-methyl ether, isolated from *Opuntia ficus-indica* var. *saboten.* Brain Research 965, 130–136.

Kanemoto, J.T., Kasugai, T., Yamatodani, A., Ushio, H., Mochizuki, T., Tohya, K., Kimura, M., Nishimura, M., Kitamura, Y., 1993. Supernormal histamine release and normal cytotoxic activity of beige (Chediak-Higashi syndrome) rat mast cells with giant granules. Int Arch Allergy Immunol 100, 99–106.

Karbownik, M., Reiter, R.J., Garcia, J.J. and Dun-Xian T., 2000. Melatonin reduced phenylhydrazine-induced oxidative damage to cellular membranes: evidence for the involvement of iron. The International Journal of Biochemistry & Cell Biology 32, 1045-1054.

Karimi, M., Mirzaei, M., Dehghani, A., 2004. Prevalence of Anemia, Iron Deficiency and Iron Deficiency Anemia in 6-60 Month Old Children in Yazd's Rural Area. International Pediatrics, 19(3), 180-184.

Kazantzis, G., Schiller, K., Asscher, A., 1962. Albuminuria and the nephrotic syndrome following exposure to mercury and its compounds. Qatar Journal of Medicine 3, 403-419.

Kirtikar, K.R. and Basu, B.D., 1999. Indian Medicinal Plants, 2nd edition, International Book Distributors, Dehradun, India. II, 1173-1178.

Kobayashi, H., Okayama, Y., Ishizuka, T. et al., 1998. Production of IL-13 by human lung mast cells in response to Fce receptor cross-linkage, Clin. Exp. Allerg. 28, 1219–1227.

Kokate, C. K., 1996. Practical Pharmacognosy, 1st edition, Vallabh Prakashan, India, 110-114.

Kong, et al., 2009. Where is the hope for drug discovery? Let history tell the future. Drug Discovery Today 3–4, 115–119.

Kossori, R.L., Villaume, C., Boustani, E., Sauvaire, Y., Mejean, L. 1998. Composition of pulp, skin and seeds of prickly pear fruit (*Opuntia ficus-indica* sp.). Plant Foods for Human Nutrition 52, 263 – 270.

Koster, R., Anderson, N., Debber, E. J., 1959. Acetic acid for analgesic screening. Federation Proceedings 18, 412-416.

Kujala, T. S., Loponen, J. M., Klika, K. D., Pihlaja, K., 2000. Phenolics and betacyanins in red beetroot (*Beta Vulgaris*) root: Distribution and effect of cold storage on the content of total phenolics and three individual compounds. Journal of Agriculture and Food Chemistry 48, 5338-5342.

Kumar, V. et al., 2008. Pharmaceutical issues in the development of a polypill for the treatment of cardiovascular diseases. Drug Discovery Today: Therapeutic Strategy 5, 63–71.

Kunze, W.A., Furness, J.B., 1999. The enteric nervous system and regulation of intestinal motility. Annual Review of Physiology 61, 117–142.

Kuti, J.O., 2004. Antioxidant compounds from four *Opuntia* cactus pear fruit varieties. Food Chemistry 85, 527–533.

Lauwerys, R. and Buchet, J. P., 1972. Study on the mechanism of lysosomal labilization by inorganic mercury *in vitro*. European Journal of Biochemistry 26, 535-542.

Lecavalier, P.R., Chu, I., Villeneuve, D., Valli, V.E., 1994. Combined effects of mercury and hexachlorobenzene in the rat. Journal of Environmental Science and Health B 29, 951–961.

Lee, Y.C., Hwang, K.H., Han, D.H., Kim, S.D., 1999. Compositions of *Opuntia ficus-indica*. Korean Journal of Food Science and Technology 29, 847–853.

Lee, Y.C., Pyo, Y.H., Ahn, C.K. & Kim, S.H., 2005. Food functionality of *Opuntia ficus-indica* var. cultivated in Jeju Island. Journal of Food Science and Nutrition 10, 103-110.

Lehmann, K. A., Kratzenberg, U., Schroeder-Bark, B., Horrichs-Haermeyer, G., 1990. Post-operative patient-controlled analgesia with tramadol: analgesic efficacy and minimum effective concentrations. Clin J Pain 6(3), 212– 220.

Levey, A.I., 1993. Immunological localization of M1–M5 muscarinic acetylcholine receptors in peripheral tissue and brain. Life Science 52, 441–448.

Lewis, R. A., Austen, K. F., Drazen, J. M. et al., 1980. Slow-reacting substance of anaphylaxis: identification of leukotrienes c-1 and d from human and rat sources, Proc. Natl. Acad. Sci. USA 77, 3710–3714.

Lim, Y.K., Enner, A., Ali, A.B., Wang, Y., I-Hong Hsu, S., Chong, S.M., Baumman, H., et al., 2000. Haptoglobin reduces renal oxidative DNA and tissue damage during phenylhydrazine-induced hemolysis. Kidney International 58, 1033–1044.

Linden, A., Lofdahl, C.G., Ullman, A. and Skoogh, B.E., 1993. Non-adrenergic noncholinergic responses stabilize smooth muscle tone with and without parasympathetic activation in guinea-pig isolated airways. Eur. Respir. J. 6, 425-433.

Loro, J. F., del Rio, I., Perez-Santana, L., 1999. Preliminary studies of analgesic and anti-inflammatory properties of *Opuntia dillenii* aqueous extract. Journal of Ethanopharmacology 67, 213-218.

Luangaram, S., Kukongviriyapan, U., Pakdeechote, P., Kukongviriyapan, V., and Pannangpetch, P., 2007. Protective effects of quercetin against phenylhydrazine-induced vascular dysfunction and oxidative stress in rats. Food and Chemical Toxicology 45, 448-455.

Lund, B.O., Miller, D.M., and Woods, J.S., 1991. Mercury-induced H_2O_2 production and lipid peroxidation *in vitro* in rat kidney mitochondria. Biochemical Pharmacology 42 Suppl. S181-S187.

Mabry, T.J., 1977. The order Centrospermae. Annals of the Missouri Botanical Garden 64, 210-220.

Machado, T., Pinto, A., Pinto, M., Leal, I., Silva, M., Amaral, A., Kuster, R., Netto dosSantos, K., 2003. In vitro activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant *Staphylococcus aureus*. International Journal of Antimicrobial Agents 21, 279–284.

Madara, J. L., 1994. Migration of neutrophils through epithelial monolayers. Trends in Cell Biology 4, 4-7.

Malainine, M.E., Dufresne, A., Dupeyre, D., Vignon, M.R., Mahrouz, M., 2003. First evidence of weddelite crystallites in *Opuntia ficus-indica* parenchyma. Zeitschrift fuer Naturforschung, C: Biosciences 58c, 812 – 815.

Malhotra, P., Savita Kumari, Kumar, R., Varma, S., 2004. Prevalence of anemia in adult rural population of north India. Journal of Association of Physicians of India, 52, 18-20.

Manis, J. & Schachter, D., 1966. Active transport of iron by intestine: effect of erythropoiesis stimulated by phenylhydrazine. Nature 209, 1356.

Masoli, M., Fabian, D., Holt, S., Beasley, R., 2003. Global burden of asthma. Global Initiative for Asthma (GINA), 1-20. Through http://www.ginasthma.com

McColl, S. R., Showell, H. J., 1994. Neutrophil derived inflammatory mediators. In: Hellewell PG, Williams TJ, eds. Immunophannacology of neutrophils. London: Academic Press, 95-114.

McConn, M.M., & Nakata, P.A., 2004. Oxalate reduces calcium availability in the pads of the prickly pear cactus through formation of calcium oxalate crystals. Journal of Agriculture and Food Chemistry 52, 1371-1374.

McLean, E., de Benoist, B., Egli, I., and Cogswell, M. (Ed.), 2008. Worldwide prevalence of anaemia 1993–2005 : WHO global database on anaemia. Published by World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland, 1-14.

McMillan, D. C., Powell, C. L., Bowman, Z. S., Morrow, J. D., Jollow, D. J., 2005. Lipids versus proteins as major targets of prooxidant, direct-acting hemolytic agents. Toxicology Science 88, 274–283.

Meckes-Lozyoa, M. and Roman-Ramos, R. 1986. *Opuntia streptacantha*: a coadjutor in the treatment of diabetes mellitus. American Journal of Chinese Medicine 14 (3-4), 116 – 118.

Meenan, J., Mevissen, M., Monajemi, H., Radema, S. A., Soule, H. R., Moyle, M., Tytgat, G. N. J., van Deventer, S. J. H., 1996. Mechanisms underlying neutrophil adhesion to apical epithelial membranes. Gut 38, 201-205.

Mehra, M. and Kanwar K.C., 1979. Absorption, distribution and excretion of Hg203 in mice. Bulletin Environmental Contamination and Toxicology 21, 733-738.

Mencia-Huerta, J. M., Razin, E., Ringlet, W. E. et al., 1983. Immunologic and ionophore-induced generation of leukotriene B4 from mouse bone marrow-derived mast cells, J. Immunol. 130, 1885–1890.

Mercadante, S., Gebbia, V., Marrazzo, A. and Filosto, S., 2000. Anaemia in cancer: pathophysiology and treatment. Cancer Treatment Reviews 26, 303–311.

Misra, H.P. and Fridovich, I., 1976. The oxidation of phenylhydrazine: superoxide and mechanism. Biochemistry 15, 681–687.

Moerman, D.E., 1998. Native American Ethnobotany. Portland, Oregon: Timber Press.

Montalemberk, M., Girot, R., 1996. "Les Oligoelements," Lefer Ricour G, Ghisoifi J, Putet G, Goulet O (eds). Traits de Nutrition Pédiatrique ed. Paris Maloine. pp.193-207.

Moore, M. 1989. Medicinal Plants of the Desertand Canyon West. Santa Fe: Museum of New Mexico Press.

Moßhammer, M.R., Stintzing, F.C. and Carle, R., 2006. Cactus Pear Fruits (*Opuntia* spp.): A Review of Processing Technologies and Current Uses. Journal of the Professional Association for Cactus Development, 1 – 25.

Moßhammer, M.R., Stintzing, F.C., and Carle, R., 2005. Colour studies on fruit juice blends from *Opuntia* and *Hylocereus* cacti and betalain-containing model solutions derived there from. Food Research International 38, 975 – 981.

Moßhammer, M.R., Stintzing, F.C., and Carle, R., 2006a. Evaluation of different methods for the production of juice concentrates and fruit

powders from cactus pear. Innovative Food Science and Emerging Technologies 7, 275 – 287.

Mtambo, M.M.A., Mushi, E.J., Kinabo, L.D.B., Maeda-Machangud, A., Mwamengele, G.L.M., Yongolo d, M.G.S., Temu, R.P.C., 1999. Evaluation of the efficacy of the crude extracts of *Capsicum frutescens*, *Citrus limon* and *Opuntia vulgaris* against Newcastle disease in domestic fowl in Tanzania. Journal of Ethnopharmacology 68, 55–61.

Muller, G., Majdoub, H., Roudesli, S., Le Cerf, D., & Grisel, M., 2001. Prickly pear nopals pectin from *Opuntia ficus-indica* physico-chemical study in dilute and semi dilute solutions. Carbohydrate Polymers 46, 69-79.

Munoz de Chavez, M., Chavez, A., Valles, V., Roldan, J.A., 1995. The nopal: a plant of manifold qualities. World Review of Nutrition and Dietetics 77, 109–134.

Muriel, C., Jean-Yves, L. H., 1998. Prevalence of and Risk Factors of Anaemia in young children in Southern Cameroon. American Journal of Tropical Medicine and Hygiene 58(5), 606-611.

Murthy, K. J. R. and Sastry, J.G., 2005 Economic burden of asthma. In: National Commission on Macroeconomics and Health (NCMH) Background Papers—Burden of Disease in India, published by Ministry of Health & Family Welfare, Govt. of India, New Delhi, India, pp 251-263.

Mwangi, S. M., McOdimba, F., Logan, H. L., 1995. The effect of Trypanosoma brucei brucei infection on rabbit plasma iron and zinc concentrations. Acta Tropican 59, 283-291.

Naeshiro, I., Yoshioka, M., Chatani, F., Sato, S., 1998. Changes in the plasma erythropoietin level in rats following fasting, ageing, and anaemia. Comparative Haematology International 8, 87–93.

Nakada, S., Nomoto, A. and Imura, N., 1980. Effect of methyl mercury and inorganic mercury and inorganic mercury on protein synthesis in mammalian cells. Ecotoxicology Enviornmental Safari 4, 184-190.

Nakanishi, A., Kinuta, K., Abe, T., Araki, K., Yoshda, Y., Liang, S., Li, S. A., Takei, K., Kinuta, M., 2003. Formation of meso, N-

diphenylprotoporphyrin IX by an aerobic reaction of phenylhydrazine with oxyhemoglobins. Acta Medicine Okayama 57, 249–256.

Nassar, A. G.; 2008. Chemical Composition and Functional Properties of Prickly Pear (*Opuntia ficus-indica*) Seeds Flour and Protein Concentrate, World Journal of Dairy & Food Sciences 3 (1), 11-16.

Nassberger, L., Johansson, A.C., Bjorck, S., Sjoholm, A.G., 1991. Antibodies to neutrophil granulocyte myeloperoxidase and elastase: autoimmune responses in glomerulonephritis due to hydralazine treatment. Journal of International Medicine 229, 261–265.

Nathan, C. F., 1987. Neutrophil activation on biological surfaces: massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes. J Clin Invest 80, 1550-1560.

Ndhlala, A.R., Kasiyamhura, A., Mupura, C., Chitindingu, K., Benhura, M.A., Muchuweti, M., 2007. Phenolic composition of *Flacourtia indica*, *Opuntia megacantha* and *Sclerocarya birrea*. Food Chemistry 103, 83-87.

Nelson, C., Erikson, K., Pinero, D. J., Beard, J. L., 1997. *In Vivo* dopamine metabolism is altered in iron deficient anaemic rats. Journal of Nutrition 127(12), 2282-2288.

Nelsson, T. 1970. Studies into the pigments in beetroot. Lantbrukshögskolans Annaler 36, 179–219.

Nerd, A., Dumoutier, M., Mizrahi, Y., 1997. Properties and post-harvest behavior of the vegetable cactus Nopalea cochenillifera. Postharvest Biology and Technology 10, 135-143.

Newman, D.J. and Cragg, G.M., 2007. Natural products as sources of new drugs over the last 25 years. Journal of Natural Product 70, 461–477.

Nicolas, G., Chauvet, C., Viatte, L., Danan, J.L., Bigard, X., Devaux, I., Beaumont, C., Kahn, A., Vaulont, S., 2002. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. Journal of Clinical Invest 110, 1037–1044.

Nobel, P.S., Cavelier, J., Andrade, J.L., 1992. Mucilage in cacti: Its apoplastic capacitance associated solutes, and influence on tissue water relations. Journal of Experimental Botany 43, 641-648.

Nordal, A., Krogh, A., Ogner, G., 1965. Further observations on the occurrence of phorbic acid in plants. Acta Chemica Scandinavica 19, 1705-1708.

O'Sullivan, S., Dahlen, B., Dahlen, S. E., Kumlin, M., 1996. Increased urinary excretion of the prostaglandin D_2 metabolite 9 alpha, 11 beta-prostaglandin F2 after aspirin challenge supports mast cell activation in aspirin-induced airway obstruction, Journal of Allergy and Clinical Immunology 98, 421–432.

Ochoa, J.M., 2003. Cactus pear (*Opuntia* spp.) verities main characteristics at Republica Argentina. Cactusnet 8, 5-9.

Okayama, Y., Petit-Frere, C., Kassel, O. et al., 1995. IgE-dependent expression of mRNA for IL-4 and IL-5 in human lung mast cells, Journal of Immunology 155, 1796–1808.

Oladiji, A.T., Jacob, T.O., Yakubu, M.T., 2007. Anti-anaemic potentials of aqueous extract of *Sorghum bicolor* (L.) moench stem bark in rats. Journal of Ethanopharmacology, 111, 651-656.

Olajide, O.A., Makinde, J.M. & Awe, S.O., 1999. Effects of the aqueous extract of *Bridelia ferruginea* stem bark on carrageenan-induced oedema and granuloma tissue formation in rats and mice. Journal of Ethnopharmacology 66, 113-117.

Oma, N. U., 1991. Iron Deficiency anaemia. Clinical Pharmacy and Herbal Medicine 7(116), 12-15.

Omolo, O.J., Chhabra, S.C., Nyagah, G., 1997. Determination of iron content in different parts of herbs used traditionally for anaemia treatment in East Africa. Journal of Ethnopharmacology, 58, 97-102.

Opal, S. M., Palardy, J. E., Marra, M. N., Fisher, C. J., McKelligon, B. M., Scott, R. W., 1994. Relative concentrations of endotoxin binding proteins in body fluids during infection. Lancet 344, 429-431.

Pandit, S., Biswas, T.K., Debnath, P.K., Saha, AV., Chowdhury, U., Shaw, B.P., Sen, S., Mukherjeem, B., 1999. Chemical and pharmacological evaluation of different ayurvedic preparations of iron. Journal of Ethnopharmacology 65, 149–156.

Park, E.H. & Chun, M.J., 2001. Wound healing activity of *Opuntia ficus-indica*. Fitoterapia 72, 165-167.

242

Park, E.H., Kahnga, J.H., Leeb, S.H., Shinb, K.H., 2001. An anti-inflammatory principle from cactus. Fitoterapia 72, 288-290.

Parmar, C. & Kaushal, M.K., 1982. Wild Fruits. Kalyani Publishers, New Delhi, India, 54-57.

Parodi, S., De Flora, S., Cavanna, M., Pino, A., Robbiano, L., Bennicelli, C., Brambilla, G., 1981. DNA-damaging activity in vivo and bacterial mutagenicity of sixteen hydrazine derivatives as related quantitatively to their carcinogenicity. Cancer Res. 41, 1469–1482.

Itano, H.A., Hirota, K., Hosokawa, K., 1975. Mechanism of induction of haemolytic anaemia by phenylhydrazine. Nature 256, 665–667.

Patil, G.G., Mali, P.Y., & Bhadane, V.V., 2008. Folk remedies used against respiratory disorders in Jalgoan district, Maharashtra. Natural Product Radiance 7(4), 354 – 358.

Patwardhan, B. and Hooper, M., 1992. Ayurveda and future drug development. International Journal of Alternative and Complementary Medicine, 10(12), 9-11.

Patwardhan, B. and Mashelkar, R. A., 2009. Traditional medicine-inspired approaches to drug discovery: can Ayurveda show the way forward? Drug Discovery Today 14, 15/16, 804-811.

Patwardhan, B., 2005. Traditional Medicine: Modern Approach For Affordable Global Health. CIPIH Studies, WHO, Geneva. Through http://www.who.int/intellectualproperty/studies/traditional_medicine/en/index.html.

Patwardhan, B., Joshi, K. and Ghodke, Y., 2006. Genetic basis to concept of Prakriti. Current Science, 90(7), 896.

Patwardhan, B., Vaidya, D. B. and Chorghade, M., 2004. Ayurveda and natural products drug discovery. Current Science, 86(6), 789-799.

Pazdernik, T. L. and Kerecsen, L., 2009. Rapid review: Pharmacology. 2nd edition, published by Elsevier, a division of Reed Elsevier India Private Limited, Noida, U. P., India, 137-146.

Piga, A., 2004. Cactus Pear: A fruit of Nutraceutical and Functional Importance. Journal of the Professional Association for Cactus Development, 9 – 22.

Pimienta-Barrios, E., 1993. Vegetable cactus (Opuntia), in: Williams, J. T. (Ed.), Pulses and Vegetables, Chapman & Hall, London, 177 –191.

Pinkava, D.J., 2002. On the evolution of the continental North American Opuntioideae (Cactaceae). In: D. R. Hunt and N. P. Taylor, eds. 2002 Studies in the Opuntioideae (Cactaceae). Sherborne. Pp. 59-98.

Plummer, D. T., 2006. An introduction to practical biochemistry, 3rd edition, Tata – McGraw-Hill publishing company limited, New Delhi, India, 168-180.

Pokhrel, P. K. and Lau-Cam, C.A., 2000. *In vitro* and *in vivo* effects of taurine and structurally related sulfur-containing compounds against phenylhydrazine-induced oxidative damage to erythrocytes. Taurine 4: Taurine and excitable tissues. Advance in Experimental Medical Biology 483, 503–522.

Prescott, S. M., Zimmerman, G. A., McIntyre, T. M., 1990. Platelet activating factor. J Biol Chem 265(17), 381-384.

Puxeddu, I., Ribatti, D., Crivellato, E. and Levi-Schaffer, F., 2005. Mast cells and eosinophils: A novel link between inflammation and angiogenesis in allergic diseases. Journal of Allergy and Clinical Immunology 116, 531-536.

Qiu, Y., Chen, Y., Pei, Y., Matsuda, H., Yoshikawa, M., 2002. Constituents with radical scavenging effect from *Opuntia dillenii*, Structures of new α-pyrones and flavonol-glycoside. Chemical and Pharmaceutical Bulletin 50, 1507–1510.

Qiu, Y., Chen, Y., Pei, Y., Matsuda, H., Yoshikawa, M., 2003. New constituents from the fresh stems of *Opuntia dillenii*. Journal of Chinese Pharmaceutical Science 12, 1–5.

Raffa, R. B., Friderichs, E., Reimann, W., Shank, R. P., Codd, E. E., Vaught, J. L., 1992. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an datypicalT opioid analgesic. J Pharmacol. Exp. Ther. 260(1), 275–85.

Raghavendra, H. L., Yogesh, H. S., Gopalakrishna, B., Chandrashekar, V. M., Sathishkumar, B. P., and Vadlapudi Kumar, 2009. An overview of herbal medicine. International Journal of Pharmaceutical Sciences 1(1), 1 - 20.

Ramadan, F. M. & Morsel, J. T., 2003. Oil cactus pear (*Opuntia ficus-indica* L.). Food Chemistry, 82, 339–345.

Ramadan, M.F., & Morsel, J.T., 2003a. Recovered lipids from prickly pear [*Opuntia ficus-indica* (L.) Mill] peel: a good source of polyunsaturated fatty acids, natural antioxidant vitamins and sterols. Food Chemistry 83, 447-456.

Ramzi, S. C., Vinay, K., Stanley, L. R., 1994. Pathologic Basis of Disease, 5th edn. Pub. W.B. Saounders Company. pp. 586-590.

Rang, H.P. and Dale, M.M., 2003. Pharmacology, 6th edition, Churchil Livingstone Elsevier, Phaladelphia, USA, 338-350.

Rathore, H.S. & Siddiqui, S., 2000. Prevention of mercuric chloride-induced haematological effects in mice with a Homoeopathic drug. Indian Drugs 38(8), 383- 385.

Rathore, H.S. and Vaghese, J., 1994. Effect of mercuric chloride on the survival, food-intake, body weight, histological and haematological changes in mice and their prevention with Liv-52. Indian Journal of Occupational Health 37(2), 42-54.

Razin, E., Pechi, I., Rivera, J., 1995. Signal transduction in activation of mast cells and basophils. Immunol. Today 16, 370-372.

Redondo, P. A., Alvarez, A. I., Diez, C., Fernandezrojo, F., Prieto, J. G., 1995. Physiological response to experimentally induced anemia in rats − a comparative study. Laboratory Animal Science 45, 578–583.

Rehse, A., Shahrouri, T., 1998. New NO donors with antithrombotic and vasodilating activities, Part 24. Hydrazine derivatives. Archives in Pharmacy 331(10), 308-312.

Retamal, N., Duran, J. M., Fernandez, J., 1987. Seasonal variations of chemical composition in prickly pear (*Opuntia ficus-indica* (L.) Miller). Journal of Science of Food and Agriculture 38, 303–311.

Richardson, M., 1987. Flavonols and C-glycosylflavonoids of the Caryophyllales. Biochemical Systematics and Ecology 6, 283 –286.

Riordan, O. D. K., Sharp, P., Sykes, R. M., Epstein, O., Debnam, E.S., 1995. Response to PHZ-induced haemolytic anaemia. European Clinical Invest. 25, 722.

Ritter, M. J., Lewis, L. D., Mant, T. G. K. and Ferro, A., 2008. A textbook of clinical pharmacological and therapeutics, 5th edition, Hodder Arnold, an imprint of Hodden Education, part of Hachette Livre UK, London, 231-236.

Robinson, D. S., 2004. The role of the mast cell in asthma: Induction of airway hyperresponsiveness by interaction with smooth muscle? J Allergy Clin Immunol 114, 58-65.

Robinson, H. 1974. Scanning electron microscope studies of the spines and glochids of the Opuntioideae (Cactaceae). American Journal of Botany 61, 278-283.

Rodriguez-Felix, A., & Cantwell, M., 1988. Developmental changes in composition and quality of prickly pear cactus cladodes (nopalitos). Plant Foods for Human Nutrition 38, 83–93.

Rodriguez-Felix, A., 2002. Postharvest physiology and technology of cactus pear fruits and cactus leaves. Acta Horticulturae 581, 191–199.

Rokushima, M., Omi K., Imura, K., Araki, A., Furukawa, N., Itoh, F., Miyazaki, M., et al., 2007. Toxicogenomics of drug-induced hemolytic anemia by analyzing gene expression profiles in the spleen. Toxicological Sciences 100 (1), 290-302.

Roman-Ramos, R., Flores-Saenz, J.L., Alarcon-Aguilar, F.J., 1995. Anti-hyperglycemic effect of some edible plants. Journal of Ethnopharmacology 48, 25- 32.

Roman-Ramos, R., Flores-Saenz, J.L., Partida-Hernandez, G., Lara-Lemuz, A. and Alarcon-Aguilar, F. 1991. Experimental study of the hypoglycemic effect of some antidiabetic plants. Archivos de investigación médica 22(1), 87 – 93.

Roos, D., and Dolman, K., 1990. Neutrophil involvement in inflammatory tissue damage. Netherlands J Med 36, 89-94.

Sadraei, H., Asghari, G., Hekmatti, A. A., 2003. Antispasmodic effect of three fractions of hydroalcoholic extract of *Pycnocycla spinosa*. Journal of Ethnopharmacology 86, 187-190.

Salt, T.A., Tocker, J.E., Adler, J.H., 1987. Dominance of D5-sterols in eight species of the Cactaceae. Phytochemistry 26, 731–737.

Sander-Bush, E. and Mayer, S. E., 1996. 5-Hydroytryptamine (serotonine) receptor agonists and antagonists. In: Hardman JG, Limbird LE, Gilman AG, editors. Goodman & Gilman's the pharmacological basis of therapeutics. McGraw Hill.

Saraf, M. N., Gokhale, A. B., Dikshit, V. J., Damre, K. R., Kulkarni, K. R., 2000. Influence of ethanolic extract of *Tephrosia purpurea* Linn. on mast cells and erythrocytes membranes integrity. Indian Journal of Experimental Biology 38, 837-840.

Sarkar, P.K., Prajapati, P.K., Choudhary, A.K., Shukla, V.J. and Ravishankar, B., 2007. Haematinic evaluation of Lauha Bhasma and Mandura Bhasma on HgCl$_2$- induced anemia in rats. Indian Journal of Pharmaceutical Sciences 69(6), 791-795.

Sauder, P.H.F., Livardjani, F., and Jaeger, A., 1988. Acute mercuric chloride intoxication, effects on hemodialysis and plasma exchange on mercury kinetics. Clinical Toxicology 26, 189-197.

Sawynock, J., 1987. GABAergic mechanisms of analgesia: an update. Pharmacology Biochemistry and Behaviour 26, 463–474.

Schepetkin, I.A., Xie, G., Kirpotina, L.N., Klein, R.A., Jutila, M.A., Quinn, M.T., 2008. Macrophage immunomodulatory activity of polysaccharides isolated from *Opuntia polyacantha*. International Immunopharmacology 8 (10), 1455−1466.

Schliemann, W. et al. 1996. Betacyanin from plants and cell cultures of *Phytolacca americana*. Phytochemistry 40(4), 1039-1046.

Schliemann, W. et al. 2000. Betalains from Christmas cactus. Phytochemistry 54, 419-426.

Schliemann, W. et al. 2001. Betalains of *Celosia argentea*. Phytochemistry 58, 159-165.

Schmid, E.F. and Smith, D.A., 2004. Is pharmaceutical R&D is just a game of chance or can strategy make a difference? Drug Discovery Today 9, 18–26.

Sepulveda E., Saenz C., Aliaga E., Aceituno C., 2007. Extraction and characterization of mucilage in *Opuntia* spp. Journal of Arid Environments 68, 534-545.

Seth, S. D. and Seth, V., 2009. Textbook of Pharmacology, 3rd edition, published by Elsevier, a division of Reed Elsevier India Private Limited, Noida, U.P., India. pp VII. 8.

Shan, B., Cai, Y.-Z., Brooks, J., Corke, H., 2007. The in vitro antibacterial activity of dietary species and medicinal herb extracts. International Journal of Food Microbiology 117, 112–119.

Sharma, M. L., Soni, J.L., Atta, K., 1991. Pathomorphological changes in calf erythrocytes during phenylhydrazine-induced acute hemolytic anemia. Archives in Experimental Veterinary Medicine 5, 45–78.

Shaw, B.P., Dash, S., and Panigrahi, A.K., 1991. Effect of methyl mercuric chloride treatment on haematological characteristics and erythrocyte morphology of Swiss mice. Environmental Pollution 73(1), 43-52.

Sheth, U.K., Dadkar, N.K., Kamat, N.G., 1972. Selected topics in experimental pharmacology. Kothari book depot Bombay, India, 5-63.

Shetlar, M.D. & Hill, H.A., 1985. Reactions of hemoglobin with phenylhydrazine: a review of selected aspects. Environmental Health Perspectives 64, 265-281.

Silja, V.P., Verma, S.K. & Mohanan, K.V., 2008. Ethanomedicinal plant knowledge of the *Mullu kuruma* tribe of Wayanad district, Kerala. Indian Journal of Traditional Knowledge, 7(4), 604-612.

Singh, J., Singh, A. K. and Khanuja, S. P. S., 2003. Medicinal plants: India's opportunities. Pharmaceutical Bio World, 1, 59-66.

Smith, C.E., 1967. Plant remains. The Prehistory of the Tehuacan Valley. Environment and substistence. University of Texas Press, Austin, TX. pp 259.

Soussi-Gounni, A., Koussih, L., Nutku, E., Lamkhioued, B., Nicolaides, N. C., Levitt, R. C. et al., 2001. Functional expression of IL-9 receptor by human neutrophils from asthmatic donors: role in IL-8 release. Journal of Immunology 166, 2768-2774.

Soussi-Gounni, A., Lamkhioued, B., Koussih, L., Ra, C., Renzi, P. M., Hamid, Q., 2001a. Human neutrophils express the high-affinity receptor for immunoglobulin E (Fc epsilon RI): role in asthma. FASEB J. 15, 940-949.

Springer, T. A., 1994. Traffic signals for lymphocyte recirulation and leukocyte emigration: the multi-step paradigm. Cell 76, 301-314.

Sreekanth, D., Arunasree, M.K.,. Roy, K.R., Reddy, T.C., Reddy, G.V., Reddanna, P., 2007. Betanin a betacyanin pigment purified from fruits of *Opuntia ficus-indica* induces apoptosis in human chronic myeloid leukemia Cell line-K562. Phytomedicine 14, 739-746.

Stevens, R. L. and Austen, K. F., 1989. Recent advances in the cellular and molecular biology of mast cells, Immunol. Today 10, 381–386.

Stintzing F.C., Herbach, K.M., Mosshammer, M.R., Carle, R., Yi, W.G., Sellappan, S., Akoh, C.C., Bunch, R. & Felker, P., 2005. Color, betalain pattern, and antioxidant properties of cactus pear (*Opuntia* spp.) clones. Journal of Agriculture and Food Chemistry 53, 442-451.

Stintzing, F. C. & Carle, R., 2007. Betalains – emerging prospects for food scientists. Trends in Food Science & Technology 18, 514-525.

Stintzing, F. C., Conrad, J., Klaiber, I., Beifuss, U., Carle, R., 2004. Structural investigations on betacyanin pigments by LC NMR and 2D NMR spectroscopy, Phytochemistry 65, 415-422.

Stintzing, F. C., Moßhammer, M. R., & Carle, R., 2005a. Development of a process for the production of a betalain-based colouring foodstuff from cactus pear. Innovative Food Science and Emerging Technologies 6, 221– 231.

Stintzing, F. C., Moßhammer, M. R., Carle, R., 2006. Evaluation of different methods for the production of juice concentrates and fruit powders from cactus pear. Innovative Food Science and Emerging Technologies 7, 275-287.

Stintzing, F. C., Schieber, A., & Carle, R., 2003. Evaluation of colour properties and chemical quality parameters of cactus juices. European Food Research and Technology 216, 303–311.

Stintzing, F.C. & Carle, R., 2004. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. Trends in Food Science and Technology 15, 19-38.

Stintzing, F.C. & Carle, R., 2005. Cactus stems (*Opuntia* spp.): A review on their chemistry, technology, and uses. Molecular Nutrition and Food Research 49, 175-194.

Stintzing, F.C., Schieber A., and Carle, R., 2001. Phytochemical and nutritional significance of cactus pear. European Food Research and Technology 212, 396-407.

Strack, D., Vogt, T., Schliemann, W., 2003. Recent advances in betalain research. Phytochemistry 62, 247-269.

Stuppy, W., 2002. Seed characters and the classification of the Opuntioideae, Succulent Plant Research 6, 25 – 58.

Su Feng Chang, Chiu-Lan Hsieh, and Grow-Chin Yen, 2008. The protective effect of *Opuntia dillenii* Haw fruit against low-density lipoprotein peroxidation and its active compounds. Food Chemistry 106, 569-575.

Susan, C. F. and Qutayba, H., 2007. Images in allergy and immunology: neutrophils in asthma. Journal of Allergy and Clinical Immunology 119, 1282-1286.

Sutton, B.G., Ting, I.P., Sutton, R., 1981. Carbohydrate metabolism of cactus in a desert environment. Plant Physiology 68, 784–787.

Telang, S.A., 1973. 2-Hydroxymethyl-4-methoxy-a-pyrone from *Opuntia polyacantha*. Phytochemistry 12, 2059.

Teles, F.F.F., Price, R.L., Whiting, F.M., Reid, B.L., 1994. Circadian variation of non-volatile organic acids in the prickly pear (*Opuntia ficus-indica* L.). Revista Ceres 41, 614–622.

Teles, F.F.F., Stull, J., Brown, W. & Whitting, F., 1984. Amino and organic acids of prickly pear cactus (*Opuntia ficus-indica* L.). Journal of Science of Food and Agriculture 35, 421–425.

Teles, F.F.F., Stull, J.W., Brown, W.H., Whiting, F.M., 1983. Amino and organic acids of the prickly pear cactus (*Opuntia ficus-indica* L.). Journal of Science of Food and Agriculture 35, 421– 25.

Teles, F.F.F., Whiting, F.M., Price, R.L., Borges, V.E.L., 1997. Protein and amino acids of nopal (*Opuntia ficus-indica* L.). Revista Ceres 44, 205–214.

Teles, F.F.F., Whiting, F.M., Price, R.L., Brown, W.H., Reid, B.L., Wegner, T.N., Renuncio, E., 1994a. Prickly pear (*Opuntia ficus-indica*) cactus as a source of vitamin A. Revista Ceres 41, 396–406.

Teramura, A. H., 1983. Effects of ultraviolet-B radiation on the growth and yield of crop plants. Physiologia Plantarum 58, 415–427.

Tesoriere L., Butera, D., Allegra, M., Fazzari, M. & Livrea, M.A., 2005. Distribution of betalain pigments in red blood cells after consumption of cactus pear fruits and increased resistance of the cells to ex vivo induced oxidative hemolysis in humans. Journal of Agriculture and Food Chemistry 53, 1266-1270.

Tesoriere, L., Butera D., Di Gaudio, F., Bongiorno, A., Allegra, M., Pintaudi, A.M., Kohen, R. & Livrea, M.A., 2002. Antioxidant activities of Sicilian prickly pear (*Opuntia ficus-indica*) fruit extracts and reducing properties of its betalains: Betanin and indicaxanthin. Journal of Agriculture and Food Chemistry 50, 6895-6901.

Tesoriere, L., Butera, D. D'Arpa, D., Di Gaudio, F., Allegra, M., Gentile, C. & Livrea, M.A., 2003. Increased resistance to oxidation of betalain-enriched human low density lipoproteins. Free Radical Research 37, 689–696.

Tesoriere, L., Fazzari, M., Allegra, M. & Livrea, M.A., 2005a. Biothiols, taurine, and lipid-soluble antioxidants in the edible pulp of Sicilian cactus pear (*Opuntia ficus-indica*) fruits and changes of bioactive juice components upon industrial processing. Journal of Agriculture and Food Chemistry 53, 7851-7855.

Tesoriere, L., Gentile C., Allegra, M., Livrea, M.A., & D'Alessio, P., 2004. Antioxidant betalains from catus pear (*Opuntia ficus-indica*) inhibit endothelial ICAM-1 expression. Annals of the New York Academy of Science 1028, 481–486.

The Wealth of India, 2001. A Dictionary of Indian Raw Material & Industrial products, raw material, National Institute of Science Communication, CSIR, New Delhi, India, VIII, 100-104.

Thirupati, K., Kumar, S.S., Raju, V.S., Ravikumar, B., Krishna, D.R, Krishna Mohan, G., 2008. A review of medicinal plants of the genus Cordia: their chemistry and pharmacological uses. Journal of Natural Products 8(1), 1-10.

Thomas, L. H. and Warner, J. A., 1996. The Eosinophil and its Role in Asthma. General Pharmacology 27(40), 593-597.

Torphy, T. J. and Douglas, W. P. H., 2008. Drugs used in Asthma. In. Craig, R. C. and Stitzel, R. E., editor. Modern Pharmacology With Clinical Applications, 5th edition, Lippincott, pp. 458-461.

Toru, H., Pawankar, R., Ra, C., Yata, J., Nakahata, T., 1998. Human mast cells produce IL-13 by high-affinity IgE receptor crosslinking: enhanced IL-13 production by IL-4-primed human mast cells, J. Allerg. Clin. Immunol. 102, 491–502.

Trejo-González, A., Gabriel-Ortiz, G., Puebla-Pérez, A.M., Huízar-Contreras, M.D., Munguía-Mazariegos, M. R., Mejía-Arreguín, S. & Calva, E., 1996. A purified extract from prickly pear cactus (*Opuntia fuliginosa*) controls experimentally induced diabetes in rats. Journal of Ethnopharmacology 55, 27-33.

Trombetta, D., Puglia, C., Perri, D., Licata, A., Pergolizzi, S., Lauriano, E.R., De Pasquale, A., Saija, A., Bonina, F.P., 2006. Effect of polysaccharides from *Opuntia ficus-indica* (L.) cladodes on the healing of dermal wounds in the rat. Phytomedicine 13, 352–358.

Unami, A., Nishina, N., Terai, T., Sato, S., Tamura, T., Noda, K., Mine, Y., 1996. Effect of cisplatin on erythropoietin production in rats. Journal of Toxicology Science 21(3), 157-65.

Undem, B. J., 2006. Pharmacotherapy of asthma. In: Laurence L. Brunton, John S Lazo, Keith L. Parker, (Editor). Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th ed. McGraw-Hill, Medical Publishing Division, New Delhi, India.

Vaidya, A.D.B. and Devasagayam, T.P.A., 2007. Current status of herbal drugs in India: an overview. Journal of Clinical Biochemistry and Nutrition 41, 1–11.

Vaidya, R.A. et al., 2003. Ayurvedic pharmaco-epidemiology: a new discipline proposed. Journal of Association of Physicians India 51, 528.

Van Arman, C.G., Begany, A.J., Miller, L.M., Pless, H.H., 1965. Some details of the inflammations caused by yeast and carrageenan. Journal of Pharmacology and Experimental Therapeutics 150, 328–334.

Vane, J.R., 1971. Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. Nature 231, 232–235.

Varghese, J., Shrilakshmi, K. and Rathore, H.S., 1997. Prevention of mercuric chloride induced cerebellar damage in mice with a multiherbal hepatic drug results and possibilities. Indian Journal of Occupational Health 40(1), 1-6.

Verity, M. A. and Reith, A., 1967. Effect of mercurial compounds on structure-linked latency of lysosomal Hydrolases. Biochemical Journal 105, 685-690.

Vignon, M. R., Habibi, Y., & Mahrouz, M., 2005. D-Xylans from seed endosperm of *Opuntia ficus-indica* prickly pear fruits. Comptes Rendus Chimie 8, 1123–1128.

Vignon, M. R., Habibi, Y., & Mahrouz, M., 2005a. Arabinan-rich polysaccharides isolated and characterized from the endosperm of the seed of *Opuntia ficus-indica* prickly pear fruits. Carbohydrate Polymers 60, 319–329.

Viloria-Matos, A., Moreno-Alvarez, M. J., Hidalgo-Baez. D., 2001. Isolation and Identification of Betacyanin from fruits of *Opuntia boldinghii* Br. et R. by HPTLC. Ciência e Tecnologia de Alimentos 3(3), 140 – 143.

Vinegar, R., Schreiber, W., Hugo, R., 1969. Biphasic development of carrageenan oedema in rats. Journal of Pharmacology and Experimental Therapeutics 166, 96–103.

Vogel, H.G., Vogel, W.H., 1997. Drug discovery and evaluation. Pharmacology assay. Ed. Springer. Berlin, pp. 370–376.

Wagner, A. J., Arnout, R. A., and Demetri, G. D., 2008. Pharmacology of Hematopoiesis and Immunomodulation. In: In: Principles of pharmacology The pathophysiologic basis of drug therapy, Golan, D.E., Tashjian, A.H., Armstrong, E.J. and Armstrong, A.W. (Ed.), 2nd edition, published by Wolters Kluwer Health and Lippincott Williams & Wilkins, New Delhi, India, 781-790.

Walsh, A., 1955. Application of atomic absorption spectra to chemical analysis. Spectrochim Acta 7, 108–117.

Watkins, W.J. and Renau, T.E., 2003. Burger's Medicinal Chemistry and Drug discover, 6th edition. A Wiley-Interscience Publication, New York, Vol. 5, 881-911.

Wehren,W., 2002. Eignung und Futterwert, besonders Amino saurezusammensetzung von unkonventionellen Proteinquellen in semi-ariden Gebieten Tunesiens. Dissertation, Universitat Bonn, Cacti. Biology and Uses, University of California Press, Berkeley, Los Angeles,

London, pp. 1976; cit. in Nefzaoui, A., Ben Salem, H., Forage, fodder, and animal nutrition, in: Nobel, P. S. (Ed.), 199–210.

Wei Liu, Yu-Jie Fu, Yuan-Gang Zu, Mei-Hong Tong, Nan Wu, Xiao-Lei Liu, Su Zhang, 2009. Supercritical carbon dioxide extraction of seed oil from *Opuntia dillenii* Haw. and its antioxidant activity. Food Chemistry 114, 334-339.

Wenzel, S. E., 2003. The role of leukotrienes in asthma. Prostaglandins, Leukotrienes and Essential Fatty Acids 69, 145–155.

West, G. B., 1983. Histamine releasers and rat mast cells. Int. Archs. Allergy Appl. Immunol. 72, 284-286.

Wilkinson, P.C., 1978. Neutrophil adhesion test. In: Vane, J.K., Ferreria, S.H. (Eds.), Handbook of Experimental Pharmacology, I, first ed. Springer Verlag, Berlin, p. 109.

Williams, D. A. and Lemake, T. L., 2002. Foye's Principles of Medicinal Chemistry, 5th edition, Lippincott Williams and Wilkins, New York, London, 892-901.

Winter, C.A. Risley, E.A., Nuss, C.W. 1962. Carraaneenan-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine 111, 544-547.

Wolfram, R., Budinsky, A., Efthimiou, Y., Stomatopoulos, J., Oguogho, A., Sinzinger, H., 2003. Daily prickly pear consumption improves platelet function. Prostaglandins, Leukotrienes and Essential Fatty Acids 69, 61–66.

Woodcock, J. and Woosley, R., 2008. The FDA critical path initiative and its influence on new drug development. Annual Review in Medicine 59, 1–12.

Woodruff, P. G., and Fahy, J. V., 2002. A Role for Neutrophils in Asthma? American Journal of Medicine 112, 498 –500.

World Health Organization (WHO), 2002. Quality controls methods for medicinal plant materials. Geneva, AITBS publisher and distributors, Delhi, 28-30.

Wybraniec, S. et al. 2001. Betacyanin from vine cactus *Hylocereus polyrhizus*. Phytochemistry 58, 1209-1212.

Wybraniec, S., 2006. Effect of tetraalkylammonium salts on retention of betacyanins and decarboxylated betacyanins in ion-pair reversed-phase high-performance liquid chromatography. Journal of Chromatography A 1127, 70-75.

Wybraniec, S., 2008. Chromatographic investigation on acyl migration in betacyanins and their decarboxylated derivatives. Journal of Chromatography B 861, 40-47.

Wybraniec, S., Nowak-Wydra, B., & Mizrahi, Y.; 2006. ^{1}H and ^{13}C NMR spectroscopic structural elucidation of new decarboxylated betacyanins. Tetrahedron Letters 47, 1725-1728.

Wybraniec, S., Nowak-Wydra, B., Mitka, K., Kowalski, P., Mizrahi, Y., 2007. Minor betalains in fruits of *Hylocereus* species. Phytochemistry 68, 251-259.

Xie, L. D., Gu, L., Yan, Z. Y., Yao, W. J., Sun, D. G., Wen, Z. Y.,2003. The microrheological changes in the course of erythrocyte senescence after phenylhydrazine injection. Clinical Hemorheol Microcirc. 28, 5–11.

Yahia, E. M., & Castellanos-Santiago, E., 2008. Identification and Quantification of Betalains from the Fruits of 10 Mexican Prickly Pear Cultivars by High-Performance Liquid Chromatography and Electrospray Ionization Mass Spectrometry. Journal of Agriculture and Food Chemistry 56, 5758–5764.

Yeshoda, K. M., 1942. Phenylhyrazine anaemia in rats. Current Science 11, 360–363.

Yi-Zhong Cai, Mei Sun, and Harold Corke, 2005. Characterization and application of betalain pigments from plants of the Amaranthaceae. Trends in Food Science & Technology 16, 370-376.

Zimmermann, G. R. et al., 2007. Multi-target therapeutics: when the whole is greater than the sum of the parts. Drug Discovery Today 12, 34–42.

Zimmermann, L., Antebi, H., Morvan, B. C., Alcindor, G., 1997. In vitro peroxidation of plasma and erythrocyte lipids during WR 1339 induced hyperlipidemia in Wistar rats. Annal in Pharmacy France 55 (6), 246-253.

Zou, D.M., Brewer, M., Garcia, F., Feugang, J.M., Wang, J., Zang, R., Liu, H. & Zou, C.P., 2005. Cactus Pear - a Natural Product in Cancer Chemoprevention. Nutrition Journal 4, 25.

Zourgui, L., El Golli, E., Bouaziz, C., Bacha, H., Hassen, W., 2008. Cactus (*Opuntia ficus-indica*) cladodes prevent oxidative damage induced by the mycotoxin zearalenone in Balb/C mice. Food and Chemical Toxicology 46, 1817-1824.

Zyuz'kow, G.N., Abramova, E.V., Dygai, A.M. and Gol'dberg, E.D., 2004. Mechanisms of regulation of erythropoiesis during hemolytic anemia. Bulletin of Experimental Biology and Medicine 10, 334-337.

8. Annexure

8. Annexure

1. Plant Authentification Certificate

निस्कैयर
NISCAIR

राष्ट्रीय विज्ञान संचार एवं सूचना स्रोत संस्थान
NATIONAL INSTITUTE OF SCIENCE COMMUNICATION
AND INFORMATION RESOURCES
(वैज्ञानिक एवं औद्योगिक अनुसंधान परिषद्)
(Council of Scientific and Industrial Research)
14, सत्संग विहार मार्ग, नई दिल्ली 110 067
14, SATSANG VIHAR MARG, NEW DELHI 110 067 &
डा. के. एस. कृष्णन् मार्ग, नई दिल्ली – 110 012
Dr. K. S. KRISHNAN MARG (Near Pusa Gate), NEW DELHI 110 012

Ref. NISCAIR/RHMD/Consult/-2008-09/1068/99   3 October 2008

Dr. H.B. Singh
Scientist F & Head
Raw Materials Herbarium & Museum
Phone: 25841143
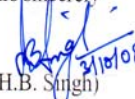E-mail: hbs@niscair.res.in; hbsbhati@yahoo.com

Dear Mr. Chauhan

Kindly refer to your letter No. rbpmpc/08-09/327 dated 27 September 2008, regarding
identification of one crude drug sample. The sample has been identified as given below:

Sl No.	Sample received as	Part	Sample identified as
1	Hathiothor (Opuntia sp.)	Whole plant	*Opuntia elatior* Mill. Syn. *O. nigricans* Haw. ; *O. burgeriana*; *Cactus tuna* var. *elatior*; *C. elatior*

With regards

Yours sincerely

(Dr. H.B. Singh)

Mr. Sanjay P. Chauhan
Lecturer
Smt. R.B. Patel Mahila Pharmacy College
Kailashnagar, Bhawanagar road
ATKOT-360 040 (District Rajkot)
GUJARAT

दूरभाष/Phone: सत्संग विहार मार्ग/Satsang Vihar Marg: 91-11-26560141, 26560143, 26560165   फैक्स/Fax: 91-11-26862228
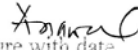
2. IAEC Certificate

IAEC/RBPMPC/09-10/01

CERTIFICATE

This is certify that the project title "**Phytochemical and Pharmacological Study (Screening) of Opuntia species**" has been approved by the IAEC.

18/07/2009
Signature with date
(Dr. N. P. Jivani)
Chairman of IAEC

Signature with date
(Dr. Y. K. Agrawal)
CPCSEA nominee

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

3. Receipt for Provisional Patent Filed for Pharmaceutical Preparation for Asthma

भारत सरकार / GOVERNMENT OF INDIA

Intellectual Property Office

पेटेन्ट शाखा, बौद्धिक संपदा भवन, ॲन्टॉप हिल डाकघर के बाजू में,

एस. एम. रोड, ॲन्टॉप हिल, मुंबई - 400 037.

The Patent Division Bouddhik Sampada Bhavan, Beside Antop Hill Post Office,

S. M. Road, Antop Hill, Mumbai - 400 037, India

Phone : 24137701 Fax : 24130387 E-mail : mumbai-patent@nic.in, Website : http://www.ipindia.nic.in

INTELLECTUAL PROPERTY INDIA Patents/Design/ Trade Marks/ Geographical Indications.

रसीद / Receipt

C.B.R. NO : 3213   Date/Time : 04/03/2010 16:09:43

To

CHAUHAN SANJAYKUMAR PRAKASHBHAI   Agent Number:

RAJPUT STREET, AT & POST: VALLBHIPUR TAL: VALLBHIPUR DIST: BHAVANAGAR 364310

Serial Number	Reference Number/Application Type	Application Number	Title/Remarks	Amount Paid	Amount Computed	Fee Payment
1	ORDINARY APPLICATION Pages:-9 , Claims:-0	579/MUM/2010	PHARMACEUTICAL PREPARATION FOR ASTHMA	1000	1000	Full
Total Amount				1000	1000	

Received a sum of Rs. 1000 (Rupees One Thousand only) through

Payment Mode	Bank Name	Cheque/Draft Number	Cheque/Draft Date	Amount in Rs
Cheque	BANK OF INDIA	015453	26/02/2010	1000

For Controller of Patents & Designs

4. Receipt for Provisional Patent Filed for Pharmaceutical Preparation for Haematological Disorder

भारत सरकार / GOVERNMENT OF INDIA
Intellectual Property Office
पेटेन्ट शाखा, बौद्धिक संपदा भवन, ॲन्टॉप हिल डाकघर के बाजू में,
एस. एम. रोड, ॲन्टॉप हिल, मुंबई - 400 037.
The Patent Division Bouddhik Sampada Bhavan, Beside Antop Hill Post Office,
S. M. Road, Antop Hill, Mumbai - 400 037, India
Phone : 24137701 Fax : 24130387 E-mail : mumbai-patent@nic.in. Website : http://www.ipindia.nic.in

INTELLECTUAL
PROPERTY INDIA
Patents/Design/
Trade Marks/
Geographical
Indications.

रसीद / Receipt

C.B.R. NO : 3214

Date/Time : 04/03/2010 16:14:03

To

Agent Number:

CHAUHAN SANJAYKUMAR PRAKASHBHAI
RAJPUT STREET, AT & POST: VALLBHIPUR TAL: VALLBHIPUR DIST: BHAVANAGAR 364310

Serial Number	Reference Number/Application Type	Application Number	Title/Remarks	Amount Paid	Amount Computed	Fee Payment
1	ORDINARY APPLICATION Pages:-9 , Claims:-0	580/MUM/2010	PHARMACEUTICAL PREPARATION FOR HAEMATOLOGICAL DISORDERS	1000	1000	Full
Total Amount				1000	1000	

Received a sum of Rs. 1000 (Rupees One Thousand only) through

Payment Mode	Bank Name	Cheque/Draft Number	Cheque/Draft Date	Amount in Rs
Cheque	BANK OF INDIA	015454	26/02/2010	1000

For Controller of Patents & Designs