EVALUATION OF ANTI-INFLAMMATORY AND HEPATOPROTECTIVE POTENCY OF A SELECTED MEDICINAL PLANT

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CERTIFICATE

This is to certify that the Ph.D. thesis entitled "**Evaluation of anti-inflammatory and hepatoprotective potency of a selected medicinal plant**" embodies the original results of bonafide experimental work carried out by **Mr. Yogeshbhai Baravalia** under my guidance and supervision at the Department of Biosciences, Saurashtra University, Rajkot.

It is further certified that he has put seven terms for research work and that this work has not been submitted to any other University/Institution for the award of Ph.D. degree. His thesis is recommended for acceptance for the award of the Ph.D. degree by the Saurashtra University.

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Place: Rajkot
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Chapter 1

INTRODUCTION

CHAPTER 1: INTRODUCTION

1.1. INFLAMMATION

Inflammation is an important physiological reaction which occurs in response to a wide variety of injurious agents (e.g. bacterial infection, physical trauma, chemicals or any other phenomenon) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair (Nathan, 2002). Inflammatory processes are required for immune surveillance, optimal repair, and regeneration after injury (Vodovotz et al., 2008). The inflammatory process protects our body from diseases by releasing cells and mediators that combat foreign substances and prevent infection (Frank and Fries, 1991; El-Gamal et al., 2010). However, sustained, excessive or inappropriate inflammation is the cause of numerous diseases including rheumatoid arthritis, psoriasis and inflammatory bowel disease (Franklin et al., 2008). Inflammation is a major component of the damage caused by autoimmune diseases, and is a fundamental contributor of various infectious and non-infectious diseases such as cancer, diabetes, cardiovascular disease, rheumatoid arthritis, Alzheimer's and arteriosclerosis. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever (Lucas et al., 2006; Kassuya et al., 2009).

Inflammation is a complex pathophysiological process mediated by a variety of signalling molecules produced by leucocytes, macrophages and mast cells undergoing various cellular responses such as phagocytic uptake, and the production of inflammatory mediators such as nitric oxide (NO), prostaglandin E2 (PGE2) and tumour necrosis factor (TNF)-α (Kinne et al., 2000; Yu et al., 2010), that bring about edema formation as a result of extravasation of fluid and proteins and accumulation of leucocytes at the inflammatory site (White, 1999). In addition, it is broadly accepted that cytokines, produced by either immune or central nervous system cells, might directly sensitize the peripheral nociceptors (Obreja et al., 2002).

Inflammation is an important cellular response triggered by various mechanical, chemical or immunological stress factors and it is regulated by a delicate balance

between local factors that finally determine the outcome of the disease process: progression or resolution. The inflammatory response is a complex and highly regulated sequence of events that start with an initial production of pro-inflammatory mediators that recruit professional inflammatory cells to the site of injury to clear the offending trigger (Huwiler and Pfeilschifter, 2009). This is followed by an anti-inflammatory phase, in which resident tissue cells may acquire the potential for protecting themselves from further activation and injury.

More recently, inflammation was described as "the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality" or "the reaction to injury of the living microcirculation and related tissues" (Spector and Willoughby, 1963). Although, in ancient times inflammation was recognised as being part of the healing process, up to the end of the 19^{th} century, inflammation was viewed as being an undesirable response that was harmful to the host.

Based on visual observation, the ancients characterised inflammation by five cardinal signs, namely redness (rubor), swelling (tumour), heat (calor; only applicable to the body extremities), pain (dolor) and loss of function (functio laesa). The first four of these signs named by Celsus in ancient Rome (30-38 B.C.) and the last by Galen (A.D. 130-200) (Hurley, 1972).

The classical description of inflammation accounts for the visual changes seen. The sensation of heat is caused by the increased movement of blood through dilated vessels into the environmentally cooled extremities. Redness is due to the additional number of erythrocytes passing through the area. Swelling (edema) is the result of increased passage of fluid from dilated and permeable blood vessels into the surrounding tissues, infiltration of cells into the damaged area, and in prolonged inflammatory responses deposition of connective tissue. Pain is due to the direct effects of mediators, either from initial damage or that resulting of sensory nerves due to oedema. Loss of function refers to either simple loss of mobility in a joint, due to the oedema and pain, or to the replacement of functional cells with scar tissue.

Inflammatory process has two phases: acute and chronic. Acute and chronic inflammations are known to be complicated processes induced by several different classes of chemical mediators, e.g. prostaglandins, leukotrienes and platelet-activating factor, etc. Anti inflammatory agents exert their effect through a spectrum of different modes of action (Samuelsson et al., 1978).

Acute inflammatory response is characterized by an increase in vascular permeability and cellular infiltration leading to oedema formation as a result of extravasation of fluid and proteins and accumulation of leukocytes at the inflammatory site for short time (Posadas et al., 2004).

Chronic inflammation is the reaction arising when the acute response is insufficient to eliminate the pro-inflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and infiltration of neutrophils with exudation of fluid. It occurs by means of development of proliferative cells which can either spread or form granuloma. Chronic inflammation may also occur due to the persistence of infection or antigen, recurring tissue injury, or a failure of endogenous anti-inflammatory mechanisms.

Chronic (or acute) inflammation is a multiple process mediated by activating inflammatory or immune cells (Lundberg, 2000), among which macrophages play a central role in managing many different immunopathological phenomena including the overproduction of proinflammatory cytokines and inflammatory mediators, generated by activated iNOS and COX-2 (Walsh, 2003). Under inflammatory conditions, immune cells are also stimulated by adhesion molecule activation signals in order to enhance the migration capacity to inflamed tissue and finally to form heterotypic cell clustering between the immune cells, endothelial cells and inflamed cells (Tao et al., 2009).

Macrophages in the inflammatory reaction initially requires an interaction between surface receptors such as Toll-like receptors (TLR) and stimuli (Takeda and Akira, 2001), and subsequent up-regulation of intracellular signalling events mediated by enzymes such as phosphoinositide 3-kinases (PI3K) and mitogen activated protein kinases (MAPKs) as well as transcription factors (e.g., nuclear factor [NF]-κB and

activator protein [AP]-1) (Sekine et al., 2006). Overall, these events lead macrophages to express pro-inflammatory genes such as inducible NO synthase (iNOS) and cyclooxygenase (COX)-2 (Burmester et al., 1997; Bresnihan, 1999). Because large amounts of macrophage-derived inflammatory mediators can cause collateral or severe damage such as septic shock, rheumatoid arthritis and arteriosclerosis (Michaelsson et al., 1995; Stuhlmuller et al., 2000), the effective blockade of these inflammatory responses is an important therapeutic target.

Inflammatory diseases are a major cause of morbidity of the work force throughout the world. These have been called the "King of Human Miseries" (Chatterjee and Pal, 1984). Pain is an objectionable sensory and emotional incident associated with actual or potential tissue inflammation. Pyrexia or fever is caused as a secondary impact of inflammation (Khan et al., 2007). Inflammation, pain and fever are all associated with enhanced production of prostaglandins (Rang et al., 2003). Thus, most anti-inflammatory agents are expected to possess analgesic and antipyretic activity (Tripathi, 2001; Dewanjee et al., 2009).

1.1.1. Analgesia

Pain is an unpleasant subjective experience that is the net effect of a complex interaction of the ascending and descending nervous systems involving biochemical, physiological, psychological, and neocortical processes (Chisholm-Burns et al., 2008). Pain can affect all areas of a person's life including sleep, thought, emotion, and daily activities. There are several ways to classify pain, but the first distinction usually made is that between acute and chronic pain. Pain is a subjective sensation which cannot be measured objectively, and its intensity is not always a direct reflection of the nociceptive inputs provoking it. Nociceptive inputs which are easily ignored by an individual in one situation may be unbearable in another (Buschmann, 2002).

The management and treatment of pain is probably one of the most common and yet difficult aspects of medicinal practice. Analgesic therapy is domain by two major classes of analgesic drugs; viz. opioids and non steroidal anti-inflammatory drugs (NSAIDs). Both classes of analgesic drugs produce serious side effects, such as

gastrointestinal disturbance, renal damage (with NSAIDs drugs), etc. (Dahl and Reader, 2000; Bures et al., 2002).

1.1.2. Inflammatory diseases

Inflammation is a physiological response of a body to stimuli, including infections and tissue injury. However, excessive or persistent inflammation causes a variety of pathological conditions (Palladino et al., 2003; Kang et al., 2008). As the primary interface between the body and the external environment, the skin provides the first line of defense against traumatic injury and invasion by microbial pathogens. In addition to its properties as a physical barrier, the skin has many active defence mechanisms (Kupper and Fuhlbrigge, 2004) and regulation of these mechanisms is crucial, as inappropriate or misdirected immune activity is implicated in the pathogenesis of a large variety of inflammatory skin disorders. While some of these conditions are easily remedied, treatments for chronic inflammatory diseases such as psoriasis and atopic dermatitis are not 100% successful (Chi et al., 2003). High levels of inflammatory cytokines and reactive oxygen species are proposed to contribute to the pathophysiological mechanisms associated with various inflammatory skin disorders (Trouba et al., 2002).

Many degenerative diseases such as rheumatoid arthritis, shoulder tendonitis, gouty arthritis, polymyalgia rheumatica, heart disease, asthma, and inflammatory bowel disease are often associated with inflammatory processes (Polya, 2003; Iwalewa et al., 2007). Furthermore, oxidative and inflammatory processes are among the pathological features associated with the central nervous system in Alzheimer's disease (AD) (Howes and Houghton, 2003).

Rheumatoid arthritis (RA) and osteoarthritis (OA) are frequent and important diseases with complex pathophysiology. There is convincing evidence that cytokines (e.g., IL-1 and TNF), prostaglandins (PG), and nitric oxide (NO) play critical roles in the development and perpetuation of inflammation and cartilage and meniscus damage in rheumatoid arthritis and osteoarthritis.

Obese individuals have high circulating levels of a range of inflammatory markers produced by adipose tissue, including TNF-α, interleukin-1 (IL-1), and IL-6 (Bullo-Bonet et al., 1999; Yudkin et al., 2000). These factors, whose levels can be reduced by weight loss, are likely to contribute to vascular damage in obese individuals.

Since its discovery in the early 1990s, COX-2 has emerged as a major factor in inflammatory reactions in peripheral tissues (Hinz and Brune, 2002). By extension, COX-2 expression in brain has been associated with pro-inflammatory activities, which are thought to be instrumental in the neurodegenerative processes occurring in acute and chronic diseases.

Many malignancies arise in the areas of infection and inflammation (Ebert et al., 2002; Martinez-Maza and Breen, 2002). There is a growing body of evidences that chronic inflammation is strongly associated with incidence of cancer. For example, colon cancer can arise from inflammatory bowel disease such as chronic ulcerative colitis and Crohn's disease persistent more than 10 years.

1.1.3. Standard drugs for inflammation and side effects

Many steroids, specifically glucocorticoids and Mineralocorticoids reduce inflammation or swelling by binding to corticoid receptors. These drugs are often referred to as corticosteroids. Long-term corticosteroids use has several severe side effects eg. hyperglycemia, insulin resistance, diabetes mellitus, osteoporosis, anxiety effects etc. (Donihi et al., 2006).

There are over 50 different NSAIDs available (Chiroli et al., 2003) and they can be divided into different groups based on their chemical structure, pharmacokinetics and selectivity towards Cox-1 or Cox-2 (FitzGerald and Patrono, 2001; Bancos et al., 2009). NSAIDs can be classified (Paul, 2004) broadly into two types based on their chemical structure. Most NSAIDs are carboxylic acids; but a few, most noticeably phenylbutazones, are enolic acids. Carboxylic acid containing drugs include salicylate derivatives (eg. aspirin), carbocyclic and hetrocyclic acid derivatives (eg. indomethacin), fenamic acid derivatives (eg. Ibuprofen, ketoprofen, fenbufen, flurbiprofen, suprofen and naproxen) and phenyl acetic acid derivatives (eg.

diclofenac, aceclofenac, etc.). Enolic acid containing drugs include oxicam derivatives (eg. piroxicam, tenoxicam and meloxicam) and pyrazoles (eg. phenylbutazone and oxyphenbutazone). Non acidic group compounds include nabumenton (Derle, 2006).

Most of the NSAIDs have three major types of action (Vane, 1998):

1) Anti-inflammatory action for treating several conditions including rheumatoid arthritis, osteoarthritis, musculoskeletal disorders and pericarditis.
2) Analgesia for treating pain of mild to moderate intensity. Their maximum therapeutic efficiency is much lower than that of the opioids, but they do not cause dependence.
3) Antipyretic action, which mediates by the release of endogenous pyrogen from monocytes and macrophages in the presence of infection or inflammation.

Non-steroidal anti-inflammatory drugs (NSAIDs) typically relieve inflammation and associated pain by inhibiting cyclooxygenase enzymes involved in the production of prostaglandins. These enzymes exist in two isoforms (COX-1 and COX-2) coded by distinct genes on different chromosomes (Polya, 2003). NSAIDs can cause liver damage (Purcell et al., 1991), renal failure (Fored et al., 2001), aseptic meningitis (Nguyen and Juurlink, 2004) and can interfere with bone fracture healing (Wheeler and Batt, 2005). NSAID use is associated with a high risk of upper gastrointestinal symptoms and lesions such as oesophagitis, gastritis, peptic ulcers, and their severe complications including bleeding and perforation (Cryer and Kimmey, 1998) and results mostly from inhibition of Cox-1 in the gastric mucosa.

Diclofenac reduces inflammation, swelling and arthritic pain by inhibiting prostaglandins synthesis and/or production (Todd and Sorkin, 1988; Skoutakis et al., 1988). The drug also affects polymorphonuclear leukocytes function *in vitro*, thereby reducing chemotaxis, superoxide toxic radical formation, oxygen-derived free radical generation, and neutral protease production (Mahgoub, 2002). Diclofenac has also been reported to suppress inflammation induced by various phlogistic agents in experimental animal models (Al-Tuwaijri and Mustafa, 1992). However, it may cause side effects including gastrointestinal disorders when administered by oral route and cutaneous lesions by intramuscular injection (Lopes et al., 2006; Suwalsky et al.,

2009). There are several published reports of cases of diclofenac-associated hepatotoxicity (Purcell et al., 1991; Aydin et al., 2003).

Indomethacin is used in the treatment of disorders such as rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis. Indomethacin produce its therapeutic and toxic effect by inhibiting prostaglandin synthesis in various tissues (Lione and Scialli, 1995). However long time use of indomethacin causes gastrointestinal complications, including intestinal perforation (Cassady et al., 1989); bronchopulmonary dysplasia and respiratory distress syndrome (Eronen et al., 1994).

Aspirin is the most widely used drug in the world today, because of its ability to act as anti-inflammatory medicine (Serhan et al., 2004; Schwab et al., 2007). However, patients with a history of peptic ulcer or other gastrointestinal disorders, are prone to gastroduodenal lesions on prolonged use of aspirin. The toxicity of aspirin is both dose- and disease-dependent.

Ibuprofen is also a commonly and successful used NSAIDs. However, long term use of ibuprofen, sulindac, phenylbutazone, and piroxicam has been associated with hepatotoxicity (Manoukian and Carson, 1996).

The coxibs like rofecoxib, lumiracoxib, celecoxib and etoricoxib were reported to be associated with reduced gastrointestinal toxicity from the upper gastrointestinal tract when compared to non-selective NSAIDs; however there are also reports that coxibs are associated with serious cardiovascular events (Pilotto et al., 2009) and hepatotoxicity (Alegria et al., 2002).

Based on all these findings, the US Food and Drug Administration (FDA) in 2005 mandated that all NSAIDs should include a warning to highlight the potential increase in the risk of serious cardiovascular events, along with the warning about potential severe life-threatening gastrointestinal events. The same has been delivered by the European Medicines Agency (EMEA) as well as by a large number of national drug agencies all over the world. Thus, a careful evaluation of the risk profiles for adverse events before prescribing non-selective NSAIDs and coxibs is strongly recommended (Layton et al., 2008).

1.1.4. Inflammatory mediators

The inflammatory response is a complex and highly regulated sequence of events that start with an initial production of pro-inflammatory mediators that recruit professional inflammatory cells to the site of injury to clear the offending trigger. Macrophages play major roles in the immune and inflammatory responses involved in host defence. Activated macrophages secrete a number of different inflammatory mediators, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), reactive oxygen species (ROS), prostaglandin E2 (PGE2), nitric oxide (NO), etc (Kaplanski et al., 2003; Bosca et al., 2005).

1.1.4.1. Cyclooxygenase (COX)

COX is the key enzyme that catalyses the first two steps in the biosynthesis of the prostaglandins (PGs). The COX pathway leads to the generation of prostaglandins and thromboxanes, which mediate the pain and edema associated with inflammation. There are two isoforms of COX: COX-1 and COX-2. COX-1 is detectable, but COX-2 is not detectable in most normal tissues, however, COX-2 can be induced by many factors such as pro-inflammatory cytokines, phlogistic factors, etc. Studies indicated that COX-2 plays an important role in inflammation (Oshima et al., 1996; Shu et al., 2006). Thus, those agents that could suppress the activity or protein expression of COX-2 are likely to be valuable medicine for anti-inflammation and pain ease. Thus, decreasing of synthesis and activity of COX-2 can result in anti-inflammatory action both in localized and systemic conditions (Salvemini et al., 1993).

1.1.4.2. Prostaglandins

Prostaglandins (PGs) are generated by a variety of cell types, including activated macrophages (Harris et al., 2002). The rate-limiting enzyme in PG synthesis is cyclooxygenase (COX). Prostaglandins are the end products of the metabolism of arachidonic acid by cyclooxygenases (COX) and prostaglandin synthases (PGS), and comprise a series of classical pro-inflammatory mediators like PGD_2, PGE_2, $PGF_2\alpha$, and PGI_2.

1.1.4.3. Arachidonic acid

The lipoxygenase pathway utilizes arachidonic acid by 5-lipoxygenase to produce the lipoxygenase products e.g. leucotrienes (LTs) which are also involved in inflammatory reactions as pro-inflammatory mediators. Leukotrienes, i.e. LTC_4 and LTD_4 cause edema together with increased microvascular permeability.

1.1.4.4. Thromboxane

Thromboxane A_2 (TXA_2) is an arachidonic acid metabolite produced during the catalysis of arachidonic acid by the sequential action of COX and thromboxane synthase (TXS), and is well established as a potent vasoconstrictor. This metabolite participates in various physiological and pathological processes ranging from synaptic transmission to inflammation (Turini and DuBois, 2002). Platelets represent the best known cell type to produce TXA_2 in response to various stimuli. However, many other cells and tissues are also able to synthesize TXA_2 (Nakahata, 2008).

1.1.4.5. Leukotrienes

Leukotrienes (LT) are end products of the metabolism of arachidonic acid by 5-lipoxygenase. Leukotrienes have physiological roles in innate immune responses and pathological roles in a variety of inflammatory and allergic diseases, such as rheumatoid arthritis, inflammatory bowel disease, psoriasis, allergic rhinitis, but most prominently in bronchial asthma (Werz and Steinhilber, 2005).

1.1.4.6. Polyunsaturated fatty acids (PUFA)

Linoleic acid (LA) and α-linolenic acid (ALA) belong to the n−6 (ω−6) and n−3 (ω−3) series of polyunsaturated fatty acids (PUFA). LA and ALA are precursors for the synthesis of higher unsaturated species: arachidonic acid deriving from LA, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) deriving from ALA. One possible mechanistic explanation for these anti-inflammatory and anti-tumorigenic effects may be that an increased consumption of EPA and DHA results in an increased incorporation of these fatty acids into phospholipids at the expense of

arachidonic acid. Consequently, they also replace arachidonic acid as a substrate for COX and LO that results in a reduced formation of PGE2, TXA2, LTB4 and LTE4 (Huwiler and Pfeilschifter, 2009).

1.1.4.7. Histamine

Histamine (HA) is a biogenic amine that affects a variety of functions in the human body. It has been known to play a role in inflammation, gastric acid secretion, and neurotransmission (Passani et al., 2007; Huang and Thurmond, 2008). Multiple receptors exist for histamine in mammalian tissues and these have been classified into 4 distinct receptor types (H_1R, H_2R, H_3R, and H_4R), all of which are G-protein coupled receptors (GPCRs) (Schneider et al., 2002). Histamine appears to play a complex role in pain modulation. Histamine released from mast cells is an established mediator of acute allergic reactions and chronic inflammation. Histamine and other mediators of inflammation increase vascular permeability at various times after injury. Chemical-induced vascular permeability (such as seen with acetic acid) causes an immediate sustained reaction that is prolonged over 24 h (Okoli et al., 2007).

1.1.4.8. Nitric oxide (NO)

It is widely known that nitric oxide (NO), synthesized from L-arginine by nitric oxide synthase (NOS), is involved in diverse physiological processes. An excess in NO production is largely thought of as causing a variety of inflammatory diseases, such as sepsis, psoriasis, arthritis, multiple sclerosis, and systemic lupus erythromatosus (Clancy et al., 1998).

1.2. INTRODUCTION TO LIVER

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles (Shanmugasundaram and Venkataraman, 2006). Liver functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Additionally, it also handles the metabolism and excretion of drugs and other xenobiotics from the body thereby

providing protection against foreign substances by detoxifying and eliminating them (Saleem et al., 2010).

Liver cells possess the antioxidant defence system consisting of antioxidants such as GSH, ascorbic acid, and vitamin E and antioxidant enzymes such as SOD, catalase, and GPx to protect own cells against oxidative stress, which causes destruction of cell components and cell death (Kaplowitz and Tsukamoto, 1996).

The liver is a major target organ for toxicity of xenobiotics and drugs, because most of the orally ingested chemicals and drugs first go to liver where they are metabolized into toxic intermediates. A large number of xenobiotics are reported to be potentially hepatotoxic (Ajith et al., 2007). Hepatocytes, which make up the majority of the liver structure, are very active in the metabolism of exogenous chemicals, and this is one of the major reasons why the liver is a target for toxic substances (Timbrell, 2001). During the detoxification of xenobiotics, reactive oxygen species (ROS) are generated which cause oxidative stress (Kohen and Nyska, 2002) which leads to the hepatic damage.

1.2.1. Liver diseases

Liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. About 20,000 deaths occur every year due to liver disorders. Some of the commonly known disorders are viral hepatitis, alcohol liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, metabolic liver disease, drug induced liver injury, gallstones, etc. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,50,000 new cases each year (Gupta and Misra, 2006). According to WHO estimates, globally 170 million people are chronically infected with hepatitis C alone and every year 3–4 millions are newly added into the list. Also, there are more than 2 billion infected by hepatitis B virus (HBV) and over 5 million are getting infected with acute HBV annually (Negi et al., 2008).

Depending on the duration of the disease the liver diseases are classified as acute or chronic. If the disease does not exceed six months it is considered as acute liver disorder while diseases of longer duration are classified as chronic.

Acute viral hepatitis and drug reactions account for the majority of cases of acute liver disease. Hepatitis A and B are the commonest causes of viral hepatitis in Europe and hepatitis E is common in India. Hepatitis C is not usually recognised as an acute infection because it rarely causes jaundice at this stage.

Chronic liver damage is a worldwide common pathology characterized by inflammation and fibrosis that can lead to chronic hepatitis, cirrhosis and cancer (Tessitore and Bollito, 2006; Kohle et al., 2008). Chronic hepatitis or long term intoxification can severely injure hepatic cells. Initially, the damaged cells are denatured, but subsequently transformed to hypertrophic fibrosis and necrosis, and eventually may progress to hepatoma.

Hepatic fibrosis is usually initiated by hepatocyte damage. Biologic factors such as hepatitis virus, bile duct obstruction, cholesterol overload, schistosomiasis, etc; or chemical factors such as CCl_4 administration, alcohol intake, etc. were known to contribute to liver fibrosis. Hepatic fibrosis is major features of a wide range of chronic liver injuries including metabolic, viral, cholestatic and genetic disease. The failure of bile salt excretion in cholestasis leads to retention of hydrophobic bile salts within the hepatocytes and causes apoptosis and/or necrosis (Miyoshi et al., 1999).

Oxidative stress has been implicated in the pathogenesis of various liver diseases including alcoholic liver disease, nonalcoholic fatty liver disease, and chronic hepatitis C (Seki et al., 2005; Kitase et al., 2005). In many patients, hepatitis such as non-alcoholic fatty liver disease becomes chronic and eventually progresses to more serious liver pathologies, such as fibrosis, cirrhosis, or even carcinogenesis, causing devastating economic losses and mortality (Albano et al., 2005).

Drug/chemical-mediated hepatic injury is the common sign of drug toxicity (Lee, 2003) and accounts for greater than 50% of acute liver failure cases. Hepatic damage is the largest obstacle to the development of drugs and is the major reason for

withdrawal of drugs from the market (Cullen and Miller, 2006). Drug-induced liver disease can be predictable (high incidence and dose-related) or unpredictable (low incidence and may or may not be dose-related). Unpredictable reactions, also referred to as idiosyncratic, can be viewed as either immune-mediated hypersensitivity or nonimmune reactions. Most potent predictable hepatotoxins are recognized in the animal testing or clinical phase of drug development.

1.2.2. Drugs for liver diseases

The liver is quantitatively the most important site of drug metabolism. However, many drugs are known to cause hepatic injury. Conventional and synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects.

Steroids, vaccines, and antiviral drugs, have been used as therapies for liver pathologies, have potential adverse side-effects, especially if administered chronically or sub-chronically. Current medical treatments for these liver diseases are often ineffective, and therefore efforts are being made to seek new effective medications (Seeff et al., 2001). Developing pharmacologically effective agents from natural products has become a new trend by virtue of their little toxicity or few side effects. There are few plant derived drugs in the market which are used for the liver disorders.

1.2.2.1. Silymarin

Silymarin, derived from the seeds of *Silybum marianum* L. (Family: Asteraceae or Compositae), commonly known as milk thistle, has been used for centuries as a natural remedy for liver and biliary tract diseases (Saller et al., 2001). Milk thistle protects and regenerates the liver in most liver diseases such as cirrhosis, jaundice, and hepatitis (Flora et al., 1998). Silymarin offers good protection in various models of experimental liver disease. It acts by antioxidative, antilipid peroxidative (Pascual et al., 1993), antifibrotic (Mourelle et al., 1989), membrane stabilizing, immunomodulatory and liver regenerating mechanisms (Pradhan and Girish, 2006).

Limitations

Silymarin is insoluble in water and typically administered as a sugar coated tablet (Thakur, 2002) or as an encapsulated standardized extract. The absorption by oral route is as low as 2-3 percent of the silybin recovered from rat bile in 24 h. About 20-40 percent of the administered dose of silymarin is excreted in bile as sulphates and glucuronide conjugates in human beings (Saller et al., 2001).

Side Effects

Silymarin has low level of toxicity. Although, silymarin has a good safety record, there are few reports of gastrointestinal disturbances and allergic skin rashes (Negi et al., 2008).

1.2.2.2. Liv-52

Liv-52 was introduced in 1954 as a specially formulated Ayurvedic herbal remedy for the treatment of viral hepatitis and has been widely prescribed for infective hepatitis since then (Mukerjee and Dasgupta, 1970). Experimentally, Liv-52 prevented injurious effects of carbon tetrachloride and other toxic substances on the liver.

Liv.52 is available as tablets and syrup containing the following herbs: *Capparis spinosa*; *Cichorium intybus*; *Solanum nigrum*; *Terminalia arjuna*; *Cassia occidentalis, Achillea millefolium*; *Tamarix galica* and *Phyllanthus amarus*. These herbs are processed and formulated according to the principles of Ayurveda, which are aimed at enhancing efficacy and avoiding toxicity (Charak and vimanasthan, 1981).

1.2.3. Hepatotoxicant

Liver toxicity mainly occurs due to alcohol, viral and induced by drugs. The third factor for the cause of acute liver disease is the use of drugs like paracetamol, pain killers and antibiotics.

1.2.3.1. Non-steroidal anti-inflammatory drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAIDs) are the centrepiece of pharmacotherapy for most rheumatological disorders, and are used in large numbers as analgesics and antipyretics, both as prescription drugs and over the counter purchases. Non-steroidal anti-inflammatory drugs (NSAIDs), which are often used for the relief of non-specific fever (Radwan, 2000), continue to be important for the palliation of pain. They are the most frequently used medications for the treatment of a variety of common chronic and acute inflammatory conditions (Manoukian and Carson, 1996), and continue to be important for the palliation of pain and in decreasing inflammation and fever (Skoutakis et al., 1988; McGettigan and Henry, 2000).

Nearly all of the NSAIDs have been implicated in causing liver injury (Rabinovitz and Van Thiel, 1992). Diclofenac, and particularly sulindac, are reported to be more commonly associated with hepatotoxicity (Bjorkman, 1998). Several NSAIDs have been withdrawn from clinical use because of associated hepatotoxicity (Rabkin et al., 1999). The new more selective COX-2 inhibitors (e.g. celecoxib, rofecoxib, nimesulide) are also associated with hepatotoxicity (Merlani et al., 2001). Hepatotoxicity from NSAIDs can occur at any time after drug administration, but like most adverse drug reactions, most commonly occurs within 6–12 weeks of initiation of therapy (Aithal and Day, 1999).

There are two main clinical patterns of hepatotoxicity due to NSAIDs (Rabinovitz and Van Thiel, 1992; Aithal and Day, 1999). The first is an acute hepatitis with jaundice, fever, nausea, greatly elevated transaminases and sometimes eosinophilia. The alternative pattern is with serological and histological (periportal inflammation with plasma and lymphocyte infiltration and fibrosis extending into the lobule) features of chronic active hepatitis. Some of the NSAIDs which causes liver damage are listed below.

1.2.3.1.1. Diclofenac

Diclofenac sodium has antipyretic, analgesic and anti-inflammatory effects, is an inhibitor of cycloxygenase enzyme. Like other nonsteroidal anti-inflammatory drugs,

clinical use of diclofenac has been associated with a small but significant incidence of hepatotoxicity, ranging from mild, asymptomatic, reversible increases in liver function tests to jaundice and hepatitis, including several reports of fatal hepatitis (Purcell et al., 1991; Banks et al., 1995).

In many cases, the clinical and biochemical features of diclofenac hepatotoxicity suggest the involvement of reactive or toxic metabolites. These products presumably were formed via the hepatic cytochrome P_{450} (CYP)-catalyzed oxidation of diclofenac to reactive benzoquinone imines that are trapped by GSH (glutathione) conjugation. It is, therefore, possible that reactive benzoquinone imines may be formed and contribute to diclofenac mediated hepatic injury (Tang et al., 1999).

1.2.3.1.2. Sulindac

Liver injury from sulindac appears within a few days to six weeks after therapy is initiated. Fever, rash, eosinophilia, and edema are frequently found in association with evidence of liver injury. It is considered as one of the most likely NSAIDs to produce hepatic injury (Garcia Rodriguez et al., 1992).

1.2.3.1.3. Nimesulide

The anti-inflammatory drug, nimesulide is a selective COX-2 inhibitor, with only residual activity against COX-1 (Giuliano et al., 2001). It is almost exclusively metabolized and cleared by the liver (Chatterjee and Sil, 2007). The drug can cause several types of liver damage, ranging from mild abnormal function such as increase in serum amino transferase activity to severe organ injuries such as hepatocellular necrosis or intrahepatic cholestasis (Lucena et al., 2001).

1.2.3.1.4. Bromfenac

This acetic acid derivative was introduced in 1997 as a non-narcotic analgesic of the phenyl acetate class for short-term pain relief, but was removed from the market in 1998 owing to several instances of fulminant hepatic failure (FHF) leading to death or transplant that occurred after prolonged administration (Goldkind and Laine, 2006).

1.2.3.1.5. Indomethacin

Indomethacin has produced hepatocellular necrosis, sometimes accompanied by microvesicular steatosis and striking cholestasis (Fenech et al., 1967); children are more vulnerable and the drug is not recommended in the pediatric age group based on several deaths involving hepatocellular necrosis (Boardman and Hart, 1967).

1.2.3.1.6. Ibuprofen

Ibuprofen was withdrawn from use in the 1960s because of fatal hepatocellular injury.

1.2.3.2. Alcohol

Alcohol administration causes accumulation of reactive oxygen species, which in turn causes lipid peroxidation of cellular membranes and proteins and DNA oxidation resulting in hepatocyte injury (Zhou et al., 2002). Alcohol treatment of rats is known to cause the translocation of fat from the peripheral adipose tissue to liver, kidney and brain for accumulation (Nadro et al., 2006).

1.2.3.3. Carbon tetrachloride (CCl_4)

Many compounds including clinically useful drugs can cause cellular damage through metabolic activation of the compound to highly reactive substances such as free radicals. One such toxicant is carbon tetrachloride (CCl_4); the hepatotoxicity of CCl_4 is attributed to the formation of trichloromethyl and trichloromethyl peroxyl radicals, initiating lipid peroxidation and resulting in fibrosis and cell necrosis (Kadiiska et al., 2000). Long-term administration of CCl_4 causes chronic liver injury (Hernandez-Munoz et al., 1990).

1.2.3.4. Acetaminophen

Acetaminophen (APAP) has been widely used as a medicine for pain and fever relief (Whitcomb, 1994). It is commonly considered as a ‘‘safe drug’’ when taken within the suggested therapeutic dose. However, APAP can be hepatotoxic when an

overdose is administered. Clinically, APAP has been demonstrated to be nephrotoxic and hepatotoxic (Bonkovsky et al. 1994).

1.2.3.5. Galactosamine

Galactosamine (GalN) has been proposed to be hepatotoxic due to its ability to destruct liver cells (Anandan et al., 1999). Its toxicity is of clinical importance because there is a close resemblance between the multifocal necrosis produced by d-GalN and the lesion of viral hepatitis in humans. This amino sugar is known to selectively block the transcription and indirectly hepatic protein synthesis and as a consequence of endotoxin toxicity, it causes fulminant hepatitis within 8 h after administration (Ravikumar et al., 2005).

‘‘Burden of disease” is an all encompassing term that captures not only the frequency (such as the incidence and prevalence) of the disease but also reflects how the disease impacts other aspects of the health of a population. These include negative impact of disease on longevity (such as pre-mature death and years of lost life), morbidity (pain and impaired health related quality of life), and economic consequences of the disease (such as direct health care expenditures in caring for the disease and indirect costs related to lost income from premature death or disability). Therefore, one needs to take all these aspects into account to understand the true magnitude of a disease’s burden. Such understanding is also essential in formulating health care policies to prioritize health interventions and to allocate scarce resources across a range of medical diseases. For example expensive interventions (e.g., screening to detect early disease, treatment) will add cost and therefore may increase the overall disease burden, however these interventions may actually reduce the overall disease burden by prolonging life, and improving quality of life (Kanwal and El-Serag, 2009).

1.3. HERBAL MEDICINE

India is a rich source of medicinal plants and a number of plant derived extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Siddha. Use of herbal medicines can be traced back as far as 2100 B.C. in ancient China (Xia dynasty) and India (Vedic period). The first written reports date back to

600 B.C. with the Charaka Samhita of India and the early notes of the Eastern Zhou dynasty of China that became systematized around 400 B.C. The recipes, once formulated, were usually expanded rather than abandoned during subsequent centuries. Expansion was stimulated by a growing understanding of the natural evolution of frequently encountered diseases and by emerging hypotheses regarding their causes (Schuppan et al., 1999).

The use of medicinal plants in curing diseases is as old as man (Aibinu et al., 2007). The World Health organization (WHO) has long recognized and drawn the attention of many countries to the ever increasing interest of the public in the use of medicinal plants and their products in the treatment of various ailments. These plants which are found in our environment enjoy wide acceptability by the population and serve as cheaper alternatives to orthodox medicine (Akah and Nwabie., 1994). Since ancient times of civilization, people have been relying on plants as either prophylactic or therapeutically arsenal to restore and maintain health, and plants are well known as an important source of many biologically active compounds.

Plant derived natural products such as flavonoids, terpenes and alkaloids (Witherup et al., 1990; Shukla et al., 2010) have received considerable attention due to their diverse pharmacological properties including inflammatory, antipyretic and analgesic activities. Consumption of natural products reduce the risk of developing pathological conditions, including cancer, nervous system disorders, cardiovascular, genetic, and inflammatory diseases (Jurenka, 2009; Newman and Cragg, 2007). Plants contain numerous bioactive molecules that can improve the body's resistance to cellular stress and prevent the cytotoxicity of various agents.

Natural products and their derivatives have traditionally been the most common sources of drugs, and still represent a fairly large percentage of the pharmaceutical market (Kirkpatrick, 2002). It has long been recognised that natural product structures have the characteristics of high chemical diversity, biochemical specificity and other molecular properties that make them favourable as lead structures for drug discovery (Okoye and Osadebe, 2010).

Plants are a rich source of active ingredients for health care products, with many blockbuster drugs being directly or indirectly derived from plants (Newman et al., 2000). Examples of some drugs are shown in table 1. However, many high value plant-derived natural products remain undiscovered or unexplored for their pharmacological activity (Raskin et al., 2002).

India has been identified as one of the top twelve mega bio-diversity center of the world. This is because India has a vast area with wide variation in climate, soil, altitude and latitude (Tiwari, 2008). India is rich in all the three levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In India thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times (Parekh et al., 2005). India with its biggest repository of medicinal plants in the world may maintain an important position in the production of raw materials either directly for crude drugs or as the bioactive compounds in the formulation of pharmaceuticals and cosmetics etc (Tiwari, 2008). Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity (Manna and Abalaka, 2000). Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive (Parekh and Chanda, 2007a).

Due to the known side effects of approved pharmaceuticals, patients often turn to alternative medicine which is considered “natural” and “healthy”. Herbal medicine is thus gaining popularity, but lack of knowledge of the mechanisms and side effects of these preparations as well of safety regulations for their preparation may have serious consequences (Boullata and Nace, 2000).

Table 1: Plant derived drugs, with their clinical uses and sources.

Drug	Action or clinical use	Plant source
Bromelain	Anti-inflammatory; proteolytic agent	*Ananas comosus* (L.) Merrill
Codeine	Analgesic	*Papaver somniferum* L.
Colchicine	Antitumor agent; antigout	*Colchicum autumnale* L.
Danthron	Laxative	*Cassia* spp.
Digitalin	Cardiotonic	*Digitalis purpurea* L.
Digoxin	Cardiotonic	*Digitalis lanata* Ehrh.
Quinine	Antimalarial	*Cinchona ledgeriana* Moens ex. Trimen
Salicin	Analgesic	*Salix alba* L.
Silymarin	Antihepatotoxic	*Silybum marianum* (L.) Gaertn.
Taxol	Antitumor	*Taxus brevifolia* Nutt.

1.3.1. Standardization of herbal medicine

The use of herbal medicines continues to expand rapidly across the world. Many people now take herbal medicines or herbal products for their health care in different national health-care settings. According to WHO, 80% if the rural population in developing countries depend on traditional medicines to meet their primary health care needs (Bannerman et al., 1993). Authentication and standardization are prerequisite steps while considering source materials for herbal formulation in any system of medicine (Ahmad et al., 2009).

In traditional systems of medicine, the drugs are primarily dispensed as water decoction or ethanolic extract. Fresh plant parts, juice or crude powder are a rarity rather than a rule. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination, etc. (Kamboj 2000). It is very important that a system of standardization is established for every plant medicine in the market because the

scope for variation in different batches of medicine is enormous. World Health Organization (WHO) encourages, recommends and promotes traditional /herbal remedies in national health care programmes because these drugs are easily available at low cost, safe and people have faith in them. The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards (Raina, 2003). Some of the standardization test for herbal medicines are listed below (Ritch, 2000).

Macro and microscopic examination: For identification of right variety and search of adulterants.

Foreign organic matter: Remove of matter other than source plant to get the drug in pure form.

Ash values: It is criteria to judge the identity and purity of crude drug – Total ash, sulfated ash, water soluble ash and acid insoluble ash etc.

Moisture content: To check moisture content is helps in prevent degradation of product.

Extractive values: These are indicating the approximate measure of chemical constituents of crude drug.

Crude fiber: To determine excessive woody material criteria for judging purity.

Qualitative chemical evaluation: It covers identification and characterization of crude drug with respect to phytochemicals constituent.

Quantitative chemical evaluation: To estimate amount the major class of constituents.

Toxicological studies: Pesticide residue, potentially toxic elements, and microbial count approach to minimize their effect in final product.

1.3.2. Toxicological aspects of herbal medicine

Phytotherapy has never stopped gaining in popularity. In low and middle income countries, it often represents the main, if not, only therapeutic system to which majority of people are referred to for their primary health care (WHO, 2007; Mukinda and Eagles, 2010). Its widespread use is further substantiated by the affordability, knowledge of medicinal plants and the belief that they are harmless (Springfield et al., 2005), since these treatments are “natural” and commonly used for self-medication

without supervision. Although medicinal plants may cause several biological activities in humans, very little is known regarding the potential toxicity for many of these bioactive substances. The increase in number of users as oppose to the scarcity of scientific evidences on the safety of the medicinal plants have raised concerns regarding toxicity and detrimental effects of these remedies (Saad et al., 2006). Because they are considered natural and are available without a prescription, many users ignore the potential for toxicity (Larrey, 1997). Many herbal remedies have not been submitted to rigorous scientific testing and are largely justified by prescribers via trial-and-error experience. Acute toxicity test gives clues on the range of doses that could be toxic to the animal; it could also be used to estimate the therapeutic index (LD_{50}/ED_{50}) of drugs and xenobiotics (Rang et al., 2001; Maikai et al., 2008).

Non-steroidal anti-inflammatory drugs (NSAIDs) are used in large numbers as analgesics and antipyretics. But they are having many side effects in the body organs. Diclofenac, is one of the non-steroidal anti-inflammatory drugs, and widely used in treatment of several rheumatic diseases, and as an analgesic and anti-inflammatory agent. There are many reports that long term use of diclofenac has toxic effects to the liver as well as other organs (Sallustio and Holbrook, 2001). Natural products/plant derived extracts has great capacity to overcome this problem. Therefore, the prime objective of the present study is to select a medicinal plant which can be used as anti-inflammatory agent as well as in hepatic disorders/damage; and may not have side effects to the other organs of the body.

1.4. SELECTION OF THE PLANT FOR PRESENT STUDY

When selecting a plant for pharmacological activities, four basics methods are usually followed (Suffness and Douros, 1979):

a) Random choice of plant species
b) Choice based on ethnomedical use
c) Follow up of existing literature on the use of the species
d) Chemotaxonomic approaches

Comparison of the four methods showed that the choice based on folklore has given about 25% more positive leads than other methods. Based on the second and third

approach, selection of the plant has been made in the present work. In light of the above context, *Woodfordia fruticosa* Kurz. flowers were selected for the study.

1.4.1. *Woodfordia fruticosa*

Woodfordia fruticosa Kurz. (syn. *Woodfordia floribunda* Salisb.) belongs to the family Lythraceae, is a much branched beautiful shrub, 1-3 m high. It is the plant of tropical and subtropical regions with a long history of medicinal use. English names of the plant are Fire Flame Bush and Shiranjitea. The plant is abundantly present throughout India, ascending up to an altitude of about 1500 m, and also in a majority of the countries of South East and Far East Asia like Malaysia, Indonesia, Sri Lanka, China, Japan and Pakistan as well as Tropical Africa (Kirtikar and Basu, 1935). The original Sanskrit name Agnijwala or Tamra-pushpi appears to be derived from the bright red colour of the flower and the bark. Locally (In Gujarat) it is known as Dhavdi (Shome et al., 1981; Khare, 2004). The bark of the plant, characteristically cinnamon-brown coloured and smooth, peels off in fibres and the young shoots are terete, often clothed with fine white pubescence. The leaves are 1.5-13 x 0.8-4 cm, opposite or sub-opposite, decussate, sometimes in whorls of 3, sessile. Flowers are brilliant red.

Considering the above, the objectives set forth are:

- Review of Literature for;
    - Reported anti-inflammatory activity of some medicinal plants
    - Reported hepatoprotective activity of some medicinal plants
    - *Woodfordia fruticosa* and their reported activities
- Pharmacognostic study of *Woodfordia fruticosa* flowers
- Physicochemical study of *Woodfordia fruticosa* flowers
- Phytochemical study of *Woodfordia fruticosa* flowers
- Anti-inflammatory study of *Woodfordia fruticosa* flowers
- Hepatoprotective study of *Woodfordia fruticosa* flowers
- Toxicity study of *Woodfordia fruticosa* flowers

Chapter 2

REVIEW OF LITERATURE

CHAPTER 2: REVIEW OF LITERATURE

2.1. ANTI-INFLAMMATORY STUDY

Inflammation is the primary response to infection or injury that functions to clear the injurious material or agent and promote tissue repair. It is characterized by the sequential release of mediators including; bioactive amines, eicosanoids, cytokines, chemokines and growth factors that regulate increased vascular permeability and recruitment of blood borne leukocytes. Increased vascular permeability also results in extravasation of plasma proteins that further amplify the inflammatory reaction. Inflammation is essentially a salutary response that normally resolves with the restoration of normal tissue structure and function, however when inflammation persists it can cause tissue damage and loss of function (Rodriguez-Vita and Lawrence, 2010). Many degenerative diseases such as rheumatoid arthritis, shoulder tendonitis, gouty arthritis, polymyalgia rheumatica, heart disease, asthma, cancer, and inflammatory bowel disease are often associated with inflammatory processes (Iwalewa et al., 2007; Fawole et al., 2010).

During inflammatory responses, the activation of phospholipase A2 induces the mobilization of fatty acids, in particular arachidonic acid from the membrane lipid pool (Fiorucci et al., 2001). Arachidonic acid is then oxidized by constitutive cyclooxygenase-1 (COX-1) or inducible cyclooxygenase-2 (COX-2) enzymes, leading to the production of prostaglandins. Prostaglandins are a group of inflammatory mediators, implicated in many pain-related ailments (Rang and Dale, 1987).

COX-1 was constitutionally present in low abundance in most human tissues, acting as a housekeeping enzyme by regulating normal physiological processes such as the maintenance of gastric mucosal integrity, kidney function, and platelet aggregation. Conversely, COX-2 was selectively upregulated after exposure to inflammatory mediators or trauma, causing subsequent inflammatory responses and mediation of pain. Therefore COX-2 selective NSAID is an ideal drug, with analgesic, antipyretic, and anti-inflammatory benefits without hepatic damage or other side effects.

There are number of steroidal and non steroidal anti-inflammatory drugs available in the market; and they are having good potential as anti-inflammatory and antipyretic drugs (eg. diclofenac, aspirin, indomethacin, etc.), but they cause undesired, un pleasant and serious adverse side effects on liver and gastrointestinal track. Therefore, development of new and more powerful anti-inflammatory and hepatoprotective drugs is needed to substitute the chemical therapeutics (Conforti et al., 2009).

There are a number of models used for studying anti-inflammatory potential of medicinal plants. People all over the world are using one of the below mentioned models for evaluating the anti-inflammatory and analgesic potential of medicinal plants.

2.1.1. Models for acute inflammation

1. Agar induced paw edema
2. Arachidonic acid induced paw edema
3. Capsaicin induced ear edema
4. Carrageenan induced paw edema
5. Croton oil induced ear edema
6. Dextran induced paw edema
7. Formaldehyde induced paw edema
8. Egg-albumen induced paw edema
9. Histamine induced paw edema
10. Prostaglandin induced ear edema
11. Serotonin induced paw edema
12. TPA (12-*O*-tetradecanoylphorbol-13-acetate) induced ear edema
13. Xylene induced ear edema
14. Zymosan induced peritonitis

2.1.2. Model for chronic inflammation

1. Cotton pellet induced granuloma
2. Complete Freund's Adjuvant

2.1.3. Models for analgesia

1. Acetic acid induced writhing
2. Formaldehyde induced paw licking
3. Hot plate test
4. Rotarod test
5. Tail flick test

Amongst all these models, carrageenan induced rat paw edema is the most widely used animal model for evaluation of anti-inflammatory property of medicinal plants. The search for new pharmacologically active agents for inflammatory disorders obtained by screening natural sources is in high demand.

2.2. HEPATOPROTECTIVE STUDY

Natural products and their active principles as sources for new drug discovery and treatment of diseases have attracted attention in recent years. Herbs and spices are generally considered safe and proved to be effective against various human ailments. Herbal based therapeutics for liver disorders has been in use in India for a long time and has been popularized in world over by leading pharmaceuticals (Saleem et al., 2010). The currently observed rapid increase in consumption of herbal remedies worldwide has been stimulated by several factors, including the notion that all herbal products are safe and effective (Said et al., 2002). The 21st century has seen a paradigm shift towards therapeutic evaluation of herbal products in liver disease models by carefully synergizing the strength of the traditional systems of medicine with that of the modern concept of evidence based medicinal evaluation, standardization and randomized placebo controlled clinical trials to support clinical efficacy (Thyagarajan et al., 2002).

To evaluate the hepatoprotective potential of medicinal plants in animal models some chemical compounds and drugs are used as a toxicant; few of them are listed below:

2.2.1. Hepatotoxicant

1. Acetaminophen
2. Aflatoxin B_1
3. Azathioprine
4. Carbon tetrachloride (CCl_4)
5. D-galactosamine
6. Diclofenac
7. Diethylnitrosamine
8. Ethanol
9. Isoniazid
10. Lipopolysaccharide
11. Nimesulide
12. Poloxamer (PX)- 407
13. Rifampicin
14. Sodium oxalate
15. tert-Butyl hydroperoxide
16. Thioacetamide

Among the listed chemical compounds and drugs, carbon tetrachloride and acetaminophen are the most widely used hepatotoxicants.

In the present study, review has been done for some medicinal plants, which have been evaluated for their anti-inflammatory (Table 2) and hepatoprotective (Table 3) potency using different animal models. The plant name, part and solvent used for the extraction and different parameters done are given.

There are many medicinal plants in the literature, which are reported either for anti-inflammatory or hepatoprotective activity. But very few plants are there, which are reported for both anti-inflammatory and hepatoprotective property (Lau et al., 2002; Al-Howiriny et al., 2003; Tanna et al., 2009). Therefore, the search for potentially effective anti-inflammatory agent with hepatoprotective property continues.

2.3. *Woodfordia fruticosa* Kurz.

In India, *Woodfordia fruticosa* Kurz. is a much used medicinal plant in Ayurvedic and Unani systems of medicines (Chopra et al., 1956; Watt, 1972; Dymock et al., 1995). Although all parts of this plant possess valuable medicinal properties, there is a heavy demand for the flowers, both in domestic and international markets specialized in the preparation of herbal medicines (Oudhia, 2003).

The flower is pungent, acrid, cooling, toxic, sedative and anthelmintic, and is useful in thirst, dysentery, leprosy, erysipelas, blood diseases, leucorrhoea, menorrhagia and toothache. It is considered as 'Kapha' (mucilage type body secretion) and 'Pitta' (energy-dependent metabolic activity) suppressant in the Ayurvedic concepts of medicine (Sharma, 1956). Many marketed drugs comprise flowers, fruits, leaves and buds mixed with pedicels and thinner twigs of the plant (Chopra et al., 1956; Ahuja, 1965).

The flowers are used in the preparation of Ayurvedic fermented drugs called "Aristhas" (hot extraction followed by month-long slow fermentation) and "Asavas" (cold percolation followed by month-long slow fermentation) (Atal et al., 1982). Aristhas are believed to be general health tonics in nature, having overall health stimulating properties via ameliorating and/or delaying one or other systemic disorders. Of the 18 aristhas mentioned in the Indian Ministry of Health & Family Welfare's monograph (CCRIMH, 1978), 17 have been found to contain *Woodfordia fruticosa*. Tribal people in the Chhattisgarh district of central India uses fresh flowers to stop bleeding in emergency cuts, but they prefer to employ dried flower powder to heal wounds more efficiently. It is also one of the ingredients of a preparation used to increase fertility in women (Burkill, 1966; Dey, 1984).

Flowers used for the ayurvedic preperation "Kutajarista" for Sprue, dysentery, diarrhoea (Shenoy and Yoganarasimhan, 2008), "Lukol" for the leucorrhoea DUB (dysfunctional uterine bleeding) and symptoms of pelvic inflammatory disease. Oil based flower extract has always been recommended for open wounds (Tewari et al., 2001; Das et al., 2007). The dried flowers powder sprinkled over ulcers and wounds to diminish discharge and promote granulation (Khorya and Katrak, 1984). They are also used as tonic in disorders of mucous membranes, hemorrhoids and in derangement of the liver (Chopra et al., 1956; The Wealth of India, 1988; Mhaskar et al., 2000). An Ayurvedic medicine called "Balarishta", a drug of 'Asava' and 'Aristha' group, contains *Woodfordia fruticosa* flowers as one of the major constituents and is used in burning sensation in stomach (Agnimandya), weakness (Daurbalya) and rheumatic diseases (Vataja roga) (Anonymous, 1978). A popular crude drug (Sidowaya or Sidawayah) of Indonesia and Malaysia mainly contains dried flowers of *Woodfordia fruticosa* (Burkill, 1966). It has been used as an

astringent to treat dysentery and sprue and also for the treatment of bowel complaints, rheumatism, dysuria and hematuria in many southeast Asian countries.

Water decoction of the fresh flowers, either alone or in combination with ginger (*Zingiber officinale*) or intrajua (*Wrightia tinctoria*), is used for the treatment of dysentery. Oral use of powdered bark in managing diarrhea is well known. Successful treatment of otorrhoea by dried powdered flowers in tribal areas of Chhatisgarh is reported to be popular (Oudhia, 2003). Management of female-specific disorders like leucorrhoea and dysmenorrhoea with flower-based preparations is very popular among these tribes. However, many of these tribal therapies are not supported by systematic ethnobotanical and ethnopharmacological research. Some of the reported activity of different parts of *Woodfordia fruticosa* are listed in Table 4.

2.3.1. Chemical constituents

The extracts of *Woodfordia fruticosa* flowers showed the presence of carbohydrates, gums, flavonoids, sterols and phenolic compounds/tannins (Khushalani et al., 2006). A series of publications have appeared on the structural characterization of the secondary metabolites of the plant. The compounds identified are predominantly phenolics, particularly hydrolysable tannins and flavonoids. The following chemical constituents are found in different part of the *Woodfordia fruticosa*.

Octacosanol and β-sitosterol (Chauhan et al., 1979a), steroid sapogenin hecogenin and *meso*-inositol from the flowers (Chauhan et al., 1979b), lupeol, betulin, betulinic acid, oleanolic acid and ursolic acid from the leaves (Dan and Dan, 1984) .

The phenolic constituents include gallic acid in leaves and stems (Kalidhar et al., 1981; Kadota et al., 1990), ellagic acid in leaves and flowers (Nair et al., 1976), bergenin (a *C*-glycoside of gallic acid) and the norbergenin in stems (Kalidhar et al., 1981), chrysophanol-8-*O*-β-D-glucopyranoside in flowers (Chauhan et al., 1979a), and the naphthaquinone pigment lawsone in leaves (Saoji et al., 1972).

The flavonoid constituents: six quercetin glycosides; 3-rhamnoside from flowers (Chauhan et al., 1979b), 3-β-L-arabinoside (polystachoside) from flowers and leaves

(Nair et al., 1976), and 3-*O*-α-L-arabinopyranoside, 3-*O*-β-D-xylopyranoside, 3-*O*-(6″-galloyl)-β-D-glucopyranoside from leaves (Kadota et al., 1990). Three myricetin glycosides; 3-*O*-β-D-galactoside in flowers and leaves (Nair et al., 1976), and 3-*O*-α-L-arabinopyranoside, 3-*O*-(6″-galloyl)-β-D-galactopyranoside in leaves (Kadota et al., 1990), as also naringenin 7-glucoside and kaempferol 3-*O*-glucoside in flowers (Chauhan et al., 1979b).

A large number of new and known hydrolysable tannins have been isolated from the flowers. The known tannins reported are: 1,2,3,6-tetra-*O*-galloyl-β-D-glucose, 1,2,4,6-tetra-*O*-galloyl-β-D-glucose, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose, tellimagrandin, gemin D, heterophyllin A and oenothein B (Yoshida et al., 1989a, 1990), woodfordins A-C (Yoshida et al., 1989b, 1990), woodfordin D, oenothein A (Yoshida et al., 1991), as also isoschimawalin A and woodfordins E-I (Yoshida et al., 1992; Kuramochi-Motegi et al., 1992).

Table 2: List of medicinal plants, their family, part, solvent and model used for anti-inflammatory activity

Sr. No.	Plant (Family)	Part used	Solvent used	Model used	References
1	*Acacia modesta* Wall. (Mimoseae)	Leaves	70% Methanol	- Carrageenan - Acetic acid - Hot plate - Formalin	Bukhari et al., 2010
2	*Aconitum heterophyllum* Wall. ex Royle. (Valeraneaceae)	Root	Ethanol	- Cotton pellet	Verma et al., 2010
3	*Albizia lebbeck* Benth. (Mimosaceae)	Bark	Petroleum ether, Chloroform, 95% Ethanol	- Carrageenan - Dextran - Cotton pellet	Babu et al., 2009
4	*Allanblackia monticola* Staner LC. (Guttiferae)	Stem bark	Methylene chloride:Methanol (1:1)	- Carrageenan - Dextran - Histamine - Serotonin - Arachidonic acid - Hot plate	Nguemfo et al., 2007
5	*Aniba canelilla* (H.B.K.) Mez. (Lauraceae)	Bark	Bark wood oil	- Acetic acid - Hot plate - Formalin	De Lima et al., 2009
6	*Annona Squamosa* L. (Annonaceae)	Bark	Petroleum ether	- Carrageenan - Acetic acid - Hot plate	Chavan et al., 2010
7	*Aquilaria sinensis* (Lour.) Gilr. (Thymelaeaceae)	Leaves	50% Ethanol	- Carrageenan - Xylene - CMC-Na induced leukocyte emigration - Acetic acid - Hot plate	Zhou et al., 2008
8	*Arctium minus* (Hill) Bernh. ssp. Minus. (Asteraceae)	Leaves	Water, Ethanol	- Carrageenan - p-benzoquinone	Erdemoglu et al., 2009

Table 2: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Model used	References
9	*Aristolochia indica* L. (Aristolochiaceae) *Argemone mexicana* L. (Papaveraceae) *Alpinia speciosa* Schum. (Zingiberaceae) *Gymnema sylvestre* (Retz.) Schult. (Asclepidaceae)	Leaves Whole plant Leaves, Stem Leaves, Stem	Methanol	- Carrageenan	Vaghasiya et al., 2010
10	*Artemisia copa* Phil. (Compositae)	Aerial parts	Water, 50% Ethanol, Dichloromethane	- Carrageenan - TPA - Acetic acid - Hot plate - Formalin	Mino et al., 2004
11	*Austroplenckia populnea* Reiss. (Celastraceae)	Bark	96 % Ethanol	- Carrageenan - Dextran - Histamine - Acetic acid - Hot plate	Andrade et al., 2007
12	*Baccharis dracunculifolia* DC. (Asteraceae)	Leaves	Ethanol/water (7:3)	- Acetic acid - Formalin - Carrageenan - Histamine	dos Santos et al., 2010
13	*Baccharis illinita* DC. (Asteraceae)	Leaves	96% Ethanol	- TPA - Capsaicin - Arachidonic acid	Boller et al., 2010
14	*Bauhinia purpurea* Linn. (Leguminoseae)	Leaves	Water	- Carrageenan - Acetic acid - Hot plate - Formalin	Zakaria et al., 2007
15	*Bauhinia racemosa* L. (Caesalpiniaceae)	Stem bark	Methanol	- Carrageenan - Dextran - Histamine - Serotonin - Cotton pellet - Acetic acid - Hot plate - Yeast induced hyperpyrexia	Gupta et al., 2005

Table 2: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Model used	References
16	*Bowdichia virgilioides* Kunth. (Leguminosae, Papilonoideae)	Bark, Leaves	Water	- Carrageenan - Acetic - Hot plate - Formalin	Thomazzi et al., 2010
17	*Caesalpinia bonducella* F. (Caesalpiniaceae)	Seed	Seed oil	- Carrageenan - Acetic acid - Hot plate	Shukla et al., 2010
18	*Camellia sinensis* L. (Theaceae)	Leaves	Water (boiling)	- Carrageenan - Arachidonic acid	Roy et al., 2008
19	Celosia argentea L. (Amaranthaceae)	Leaves	95% Ethanol	- Carrageenan - Cotton pellet	Bhujbal et al., 2008
20	*Chloranthus eretus* (Buch-Ham) Verd. (Chloranthaceae)	Leaves	90% Methanol	- Carrageenan - Histamine - Serotinin - Cotton pellet	Tag et al., 2009
21	*Cissampelos pareira* L. (Menispermaceae)	Roots	50% Ethanol	- Acetic acid - Hot plate	Amresh et al., 2007
22	*Citrullus colocynthis* Schrad. (Cucurbitaceae)	Root, Seed, Stem, Leaves, Fruit	Water	- Carrageenan - Acetic acid	Marzouk et al., 2010
23	*Coccinia indica* Wight & Arn. (Cucurbitaceae)	Leaves	Water	- Carrageenan - Tail flick	Niazi et al., 2009
24	*Combretum micranthum* G. Don. (Combretaceae)	Leaves	Water	- Carrageenan - Acetic acid - Cotton pellet	Olajide et al., 2003
25	*Coronopus didymus* (L.) Smith. (Brassicaceae)	Whole plant	Ethanol	- Carrageenan - Histamine - dextran	Busnardo et al., 2010
26	*Dodonaea polyandra* Merr. & L.M.Perry. (Sapindaceae)	Leaves	80% Ethanol	- Croton oil	Simpson et al., 2010
27	*Dregea volubilis* (L.f.) Benth. ex Hook.f. (Asclepiadaceae)	Leave	Methanol Petroleum ether, Chloroform	- Carrageenan	Hossain et al., 2010

Table 2: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Model used	References
28	*Drimys angustifolia* Miers. (Winteraceae)	Leaves, Stem bark	Water	- Carrageenan - Acetic acid - Hot plate - Tail flick	Witaicenis et al., 2007
29	*Ecbolium viride* (Forsk) Merrill. (Acanthaceae)	Root	80 % Ethanol	- Carrageenan - Cotton pellet	Lalitha and Sethuraman, 2010
30	*Geranium pretense* subsp. *Finitimum* (Geraniaceae)	Aerial parts	Methanol	- Carrageenan - PGE2 - p-benzoquinone	Kupeli et al., 2007
31	*Hygrophila spinosa* T. Anders. (Acanthaceae)	Leaves	Petroleum ether, Chloroform, Ethanol, Water	- Carrageenan	Patra et al., 2009
32	*Hypericum rumeliacum* Boiss. subsp. apollinis (Boiss. & Heldr.) Robson & Strid. (Guttiferae)	Aerial parts	Methanol	- Carrageenan	Galati et al., 2008
33	*Lamiophlomis rotata* (Benth.) Kudo. (Labiatae)	Aerial parts	Water	- Carrageenan - Xylene - Acetic acid - Hot plate - Formalin	Li et al., 2010
34	*Lantana trifolia* L. (Verbenaceae)	Leaf	Ethanol	- Carrageenan - Histamine - Serotonin - Acetic acid - Tail flick	Silava et al., 2005
35	*Leonotis nepetaefolia* R. Br. (Lamiaceae)	Leaves, Stem, Flowers	Hexane, Ethyl acetate, Methanol	- TPA	Delgado et al., 2004
36	*Leucas aspera* Spreng. (Labiatae)	Aerial parts	95% Ethanol	- Formalin	Goudgaon et al., 2003
37	*Ligustrum robustum* L. O. Kuntze. (Theaceae)	Leaves	Ethanol	- Acetic acid - Croton oil	Lau et al., 2002
38	*Lindera erythrocarpa* Makino. (Lauraceae)	Fruit	Ethanol	- Croton oil	Wang SY et al., 2008

Table 2: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Model used	References
39	*Mallotus philippinensis* Muell.-Arg. (Euphorbiacea)	Stem wood	Ethanol	- Formaldehyde - Carrageenan	Arfan et al., 2010
40	*Malva parviflora* Linn. (Malvaceae)	Stem	Methanol	- Carrageenan - Histamine - Acetic acid - Formalin	Afolayan et al., 2010
41	*Moringa oleifera* L. (Moringaceae)	Leaves	Water	- Carrageenan - Acetic acid - Hot plate - Formalin	Sulaiman et al., 2008
42	*Mortonia greggii* Gray. (Celastraceae)	Roots, Leaves	Acetone	- Carrageenan - TPA	Arciniegas et al., 2004
43	*Olax viridis* Oliv (Olacaeceae)	Root bark	Hexane	- Egg albumen	Ajali and Okoye, 2009
44	*Onosma* L. species (Boraginaceae)	Roots	70% Chloroform, 70% Ethanol	- Carrageenan - p-benzoquinone induced abdomen constriction	Tosun et al., 2008
45	*Paederia scandens* (Lour.) Merri. (Rubiaceae)	Whole plant	Methanol	- Formalin - Acetic acid - Hot plate - Tail flick	Chu et al., 2008
46	*Parquetina nigrescens* (Afzel) Bullock. (Periploceae)	Leaves	Water	- Carrageenan - Formalin - Hot plate - Cotton pellet	Owoyele et al., 2009
47	*Pedilanthus tithymaloides* L. Poit. (Euphorbiaceae)	Stem, Leave	Tincture	- Carrageenan	Abreu et al., 2006
48	*Petroselinum crispum* (Mill.) (Umbelliferae)	Leaves	Ethanol	- Carrageenan - Cotton pellet	Al-Howiriny et al., 2003
49	*Pimenta racemosa* var. ozua. (Mirtaceae)	Leaves	Water	- Carrageenan - TPA - Formalin - Acetic acid - Hot plate	Garcia et al., 2004
50.	*Pinus brutia* Ten. (Pinaceae)	Bark	-	- Carrageenan	Ince et al., 2009

Table 2: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Model used	References
51	*Pinus densiflora* Siebold & Zucc. (Pinaceae)	Pollen	70% Ethanol	- Carrageenan - Formalin - Arachidonic acid	Choi et al., 2007
52	*Piper sarmentosum* Roxb. (Piperaceae)	Leaves	Water	- Acetic acid - Hot plate - Carrageenan	Zakaria et al., 2010
53	Piper sp. (Piperaceae)	Leaves	Methanol	- Carrageenan - Cotton pellet - Dextran	Vaghasiya et al., 2007
54	*Piptadenia stipulacea* Benth. (Fabaceae)	Aerial parts	Ethyl acetate	- Acetic acid - Hot plate - Formalin - Zymosan A	de Queiroz et al., 2010
55	*Plumeria acuminate* Ait. (Apocynaceae)	Leaves	Methanol	- Carrageenan - Dextran - Histamine - Serotonin - Cotton pellet	Gupta et al., 2006
56	*Polyalthia longifolia* var. pendula. (Annonaceae)	Leaves	Toluene, Chloroform, Acetone, Methanol	- Carrageenan	Tanna et al., 2009
57	*Rhus chirindensis* (Baker F) (Anacardiaceae)	Stem bark	Water	- Egg albumin - Acetic acid - Hot plate	Ojewole, 2007
58	*Russelia equisetiformis* (Schlect&Chan) (Scrophulariacae)	Whole plant	Methanol	- Egg albumin - Agar	Awe et al., 2010
59	*Schima wallichii* Choisy. (Ternstroemiaceae)	Bark	70% Ethanol	- Carrageenan - Dextran - Cotton pellet	Dewanjee et al., 2009
60	*Sclerocarya birrea* (A. Rich.) (Anacardiaceae)	Stem bark	Water, Methanol	- Carrageenan - Histamine - Serotonin - Complete Freund's Adjuvant - Formalin	Fotio et al., 2009
61	*Smilax china* Linn. (Liliaceae)	Whole plant	Methanol	- Carrageenan	Khan et al., 2009

Table 2: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Model used	References
62	*Solanum melongena* Linn. (Solanaceae)	Leaves	Water	- Acetic acid	Mutalik et al., 2003
63	*Sonchus oleraceus* L. (Asteraceae)	Aerial parts	50% Ethanol	- Carrageenan - Cotton pellet	Vilela et al., 2010
64	*Strychnos nux-vomica* L. (Loganiaceae)	Brucine and Brucine N-oxide from Seeds	-	- Carrageenan - Formalin - Acetic acid - Hot plate	Yin et al., 2003
65	*Tabernaemontana catharinensis* DC. (Apocynaceae)	Bark	95% Ethanol	- Carrageenan - Acetic acid	Gomes et al., 2009
66	*Thespesia populnea* Soland ex. Correa. (Malvaceae)	Bark	95% Methanol	- Carrageenan - Histamine - Serotonin - Acetic acid - Hot plate	Vasudevan et al., 2007
67	*Trachelospermum jasminoides* Lindl. (Apocynaceae)	-	Water	- Acetic acid - Formalin - Carrageenan	Sheu et al., 2009
68	*Trichodesma indicum* Linn. (Boraginaceae)	Root	Chloroform	- Carrageenan - Histamine - Dextran - Serotonin - Cotton pellet	Perianayagam et al., 2006
69	*Vitex negundo* Linn. (Verbenaceae)	Leaves	Petroleum ether	- Carrageenan - Cotton pellet	Subramani et al., 2009
70	*Zingiber zerumber* L. (Zingiberaceae)	Rhizomes	Water, Ethanol	- Acetic acid	Somchit et al., 2005

Abbreviations

CMC-Na: Carboxymethylcellulose sodium, PGE2: prostaglandin E2, TPA: 12-*O*-tetradecanoylphorbol-13-acetate.

Table 3: List of medicinal plants, their family, part, solvent and hepatotoxicant used for hepatoprotective activity

Sr. No.	Plant (Family)	Part used	Solvent used	Hepatotoxicant used and parameters done	References
1	*Abutilon indicum* L. (Malvaceae)	Leaves	Water	**CCl_4, Acetaminophen** - Serum AST, ALT, ALP, TB, DB - Liver GSH	Porchezhian and Ansari, 2005
2	*Acanthopanax senticosus*	Stem bark	Water	**tert-butyl hydroperoxide (t-BHP)** - Liver SOD, CAT, GPx, GSH, GSSG - Histopathology	Wang et al., 2010
3	*Aloe barbadensis* Mill. (Liliaceae)	Aerial part	Water	**CCl_4** - Hexobarbitone induced narcosis - Zoxazolamine induced paralysis time - Serum AST, ALT, ALP, LDH, TB, TG - Liver GSH, LPO, TG	Chandan et al., 2007
4	*Andrographis lineate* Fam. (Acanthaceae)	Leaves	Methanol, Water	**CCl_4** - Serum ALT, AST, ALP	Sangameswaran et al., 2008
5	*Anoectochilus formosanus* Hayata. (Orchidaceae)	Whole plant	Water	**CCl_4** - Serum ALT - Liver LPO - Histopathology	Fang et al., 2008
6	*Apium graveolens* Linn. (Apiaceae) *Croton oblongifolius* Roxb. (Euphorbiaceae)	Seeds	Petroleum ether, Acetone, Methanol	**CCl_4** - Serum AST, ALT, ALP, TP, ALB	Ahmed et al., 2002
7	*Aralia continentalis* Kitagawa. (Araliaceae)	Root	Ethanol	**CCl_4** - Serum ALT, AST - Liver GSH, GST, LPO - Histopathology	Hwang et al., 2009
8	*Artemisia absinthium* L. (Asteraceae)	Aerial part	Water	**CCl_4** **Bacillus Calmette – Guerin and lipopolysaccharide** - Serum AST, ALT, TNF-α, IL-1 - Liver LPO, SOD, GPx - Histopathology	Amat et al., 2010

Table 3: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Hepatotoxicant used and parameters done	References
9	*Artemisia sacrorum* Ledeb. (Compositae)	Aerial part	Water	**Acetaminophen** - Serum ALT, AST - Liver LPO, GSH - Histopathology	Yuan et al., 2010
10	*Azadirachta indica* Juss. (Meliaceae)	Leaves	Fresh juice	**Acetaminophen** - Serum AST, ALT, ALP - Liver LPO, T-SH - Histopathology	Yanpallewar et al., 2002
11	*Bauhinia variegate* L. (Leguminosae)	Stem bark	Alcohol	**CCl_4** - Serum AST, ALT, ALP, TP, GGT, TL - Liver TP, TL - Histopathology	Bodakhe and Ram, 2007
12	*Berberis tinctoria* Lisch. (Berberidaceae)	Leaves	Methanol	**Acetaminophen** - Serum AST, ALT, ALP, TB, TP - Liver GSH, LPO, SOD, CAT - Histopathology	Murugesh et al., 2005
13	*Boerhaavia diffusa* Linn. (Nynctaginaceae)	Leaves	Ethanol	**Acetaminophen** - Serum AST, ALT, ALP, ALB, LDH - Liver AST, ALT, ALP, TB, LDH, LPO	Olaleye et al., 2010
14	*Camellia sinensis* Kuntze. (Theaceae)	-	Water	**Sodium oxalate** - Serum, liver and kidney AST, ALT, LPO, CAT, Vit-C	Oyejide and Olushola, 2005
15	*Cassia tora* L. (Caesalpiniaceae)	Ononitol monohydrate from Leaves	-	**CCl_4** - Serum ALT, AST, ALP - Liver CAT, SOD, GR, GPx, GST - Histopathology	Dhanasekaran et al., 2009
16	*Chamomile capitula* L. Rousch. (Asteraceae)	Whole plant	Ethanol	**Acetaminophen** - Serum AST, ALT, ALP, TP, TB - Liver GSH, LPO, GLY, TP, Na^+K^+-ATPase	Gupta and Misra, 2006

Table 3: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Hepatotoxicant used and parameters done	References
17	*Cistanche tubulosa* Wight. *(Orobanchaceae)*	Phenylethan-oid oligoglycosi-des (kankanosid-es) from Stem	Methanol	**D-galactosamine Lipopolysaccharide** - Serum ALT, AST	Morikawa et al., 2010
18	*Cistus laurifolius* L. (Cistaceae)	Isolation of flavonoid from Leaves	Ethanol	**Acetaminophen** - Plasma AST, ALT, LPO - Liver LPO	Kupeli et al., 2006
19	*Citrus limon* L. Burm. (Rutaceae)	Fruits	70% Ethanol	**CCl_4** - Serum AST, ALT, ALP, TP, ALB, TB, DB - Liver GSH, LPO, CAT, SOD - Histopathology	Bhavsar et al., 2007
20	*Cordia macleodii* Griff. (Boraginaceae)	Leaves	Ethanol	**CCl_4** - Serum ALT, AST, ALP, TB	Qureshi et al., 2009
21	*Coronopus didymus* Linn. (Bracicacea)	Whole plant	Water	**CCl_4** - Serum AST, ALT - Liver GSH, LPO, TP	Mantena et al., 2005
22	*Curcuma longa* Linn. (Zingiberaceae) *Glycyrrhiza glabra* L. (Leguminosae) *Moringa oleifera* Lam. (Moringaceae)	Rhizome	Ethanol, Water	**Diclofenac** - Serum ALT, ALB - Liver GSH, LPO, CAT, SOD - Histopathology	Hamza, 2007
23	*Cuscuta chinensis* Lam. (Convolvulaceae)	Seeds	Ethanol, Water	**Acetaminophen** - Serum AST, ALT, ALP - Liver LPO, CAT, GPx, SOD - Histopathology	Yen et al., 2007
24	*Cytisus scoparius* L. (Leguminosae)	Aerial part	Ethanol: Water (7:3)	**CCl_4** - Serum AST, ALT, LDH - Liver GSH, LPO, CAT, SOD, GPx, GST, GR	Raja et al., 2007

Table 3: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Hepatotoxicant used and parameters done	References
25	*Decalepis hamiltonii* Wight. (Asclepiadaceae)	Root	Water	**CCl_4** - Serum ALT, AST, ALP, LDH - Liver LPO, CAT, SOD, GPx, GST, GSH, GR - Histopathology	Srivastava and Shivanandappa, 2010
26	*Diospyros malabarica* Kostel. (Ebenaceae)	Bark	Methanol	**CCl_4** - Serum AST, ALT, ALP, TP, TB - Liver GSH, LPO, CAT	Mondal et al., 2005
27	*Enicostemma axillare* Raynal. (Gentianaceae)	Swertiamarin from Whole plant	Ethyl acetate	**D-galactosamine** - Serum AST, ALT, ALP, TG, TC, TB, TP, ALB, CR, GSH, LPO, CAT, SOD - Liver and kidney GSH, LPO, CAT, SOD	Jaishree and Badami, 2010
28	*Epaltes divaricata* L. (Compositae)	Whole plant	Water	**CCl_4** - Serum AST, ALT, ALP - Liver GSH - Histopathology	Hewawasam et al., 2004
29	*Euphorbia fusiformis* D.Don. (Euphorbiaceae)	Tubers	Ethanol	**Rifampicin** - Serum ALT, AST, ALP, GGT, TP, TB - Histopathology	Anusuya et al., 2010
30	*Glycyrrhiza glabra* L. (Leguminosae)	Glycyrrhizin from Root	Methanol	**CCl_4** - Serum AST, ALT - Liver LPO, GSH - Histopathology	Lee et al., 2007
31	*Grewia tiliaefolia* Vahl. (Tiliaceae)	γ-lactones from Stem bark	-	**CCl_4** - Serum AST, ALP, ALP, TP, TB - Liver GSH, LPO - Histopathology	Ahamed et al., 2010
32	*Halenia elliptica* (Gentianaceae)	Whole plant	70% Methanol	**CCl_4** - Serum AST, ALT, ALP, TB - Histopathology	Huang et al., 2010a

Table 3: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Hepatotoxicant used and parameters done	References
33	*Hedyotis corymbosa* Lam. (Rubiaceae)	Whole plant	Methanol	**Acetaminophen** - Serum AST, ALT, ALP, TB - Hexobarbitone induced sleeping time - Histopathology	Sadasivan et al., 2006
34	*Hibiscus sabdariffa* L. (Malvaceae) *Rosmarinus officinalis* L. (Lamiaceae) *Salvia officinalis* L. (Lamiaceae)	Flowers Leaves Leaves	Water	**Azathioprine** - Serum AST, ALT - Liver GSH, LPO, CAT, SOD	Amin and Hamza, 2005
35	*Hybrophila auriculata* Heine. (Acanthaceae)	Root	Water	**CCl_4** - Serum AST, ALT, ALP, LDH, TB, TP - Histopathology	Shanmugasundaram and Venkataraman, 2006
36	*Justicia simplex* D. Don. (Acanthaceae)	Whole plant (Isolated lignans)	Petroleum ether	**CCl_4** - Serum AST, ALT, ALP, LPO, TG, TP - Liver GSH, LPO	Jasemine et al., 2007
37	*Kyllinga nemoralis* L. (Cyperaceae)	Rhizome	Petroleum ether, Ethanol	**CCl_4** - Serum ALT, AST, ALP, ACP - Liver GSH, LPO, CAT, SOD	Somasundaram et al., 2010
38	*Laggera alata* (D. Don) Sch.-Bip.	Whole plant	-	**CCl_4** - Serum AST, ALP, TP, ALB - Histopathology	Wu et al., 2009
39	*Ligustrum robustum* Roxb. (Oleaceae)	Leaves	Water	**CCl_4** - Serum AST, ALT - Histopathology	Lau et al., 2002
40	*Lygodium flexuosum* (L.) Sw. (Lygodiaceae)	Whole plant	Hexane	**CCl_4** -Serum AST, ALT, LDH -Liver GSH, LPO -Histopathology	Wills and Asha, 2006
41	*Mamordica subangulata* Blume. (Cucurbitaceae) *Naragamia alata* W& A. (Meliaceae)	Leaves	Water	**Acetaminophen** - Serum AST, ALT, ALP	Asha, 2001

Table 3: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Hepatotoxicant used and parameters done	References
42	*Moringa oleifera* Lam. (Moringeaceae)	Seed	70% Ethanol	**CCl_4** - Serum AST, ALT, TP, ALB, GL - Liver LPO, SOD, TP	Hamza, 2010
43	*Nelumbo nucifera* Gaertn. (Nelumbonaceae)	Leaves	60% Ethanol	**CCl_4** - Serum ALT, AST, ALP, GGT, TB - Liver GSH, CAT, SOD, LPO	Huang et al., 2010b
44	*Operculina turpethum* L. (Convolvulaceae)	Root	Ethanol	**Acetaminophen** - Serum AST, ALT, ALP, TB - Histopathology	Suresh kumar et al., 2006
45	*Pergularia daemia* Forsk. (Asclepiadaceae)	Aerial part	Ethanol	**CCl_4** - Serum AST, ALT, ALP, TB, TP, TC, ALB - Histopathology	Suresh kumar and Mishra, 2006
46	*Petroselinum crispum* (Mill.) (Umbelliferae)	Leaves	Ethanol	**CCl_4** - Plasma AST, ALT, ALP, TB - Phenobarbital induced sleeping time - Histopathology	Al-Howiriny et al., 2003
47	*Phyllanthus amarus* Schum. et Thonn. (Euphorbiacae)	Aerial	Ethanol	**Aflatoxin B_1** - Liver LPO, GSH, GPx, GST, SOD, CAT - Histopathology	Naaz et al., 2007
48	*Phyllanthus niruri* L. (Euphorbiaceae)	Aerial part	Hexane	**Acetaminophen** - Serum ALT, AST, ALP, TB	Iqbal et al., 2007
49	*Phyllanthus niruri* L. (Euphorbiaceae)	Protein isolated from Leaves	-	**CCl_4** - Serum ALT, ALP - Liver LPO, GSH, CAT, SOD - Histopathology	Bhattacharjee and Sil, 2007
50	*Phyllanthus niruri* L.(Euphorbiaceae)	Leaves, Stem	PO_4 buffer	**Nimesulide** - Serum ALT, AST, ALP - Liver GSH, LPO, CAT, SOD, TP	Chatterjee and Sil, 2007

Table 3: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Hepatotoxicant used and parameters done	References
51	*Phyllanthus urinaria* L. (Euphorbiaceae)	Whole plant	80% Ethanol	**Acetaminophen** - In vitro cytochrome P_{450} CYP2E1 enzyme assay - Histopathology	Hau et al., 2009
52	*Physalis peruviana* L. (Solanaceae)	Whole plant	Water	**Acetaminophen** - Serum ALT, AST, ALP - Liver CAT, GPx, SOD	Chang et al., 2008
53	*Physalis peruviana* Linn. (Solanaceae)	Leaves	Hexane, Ethanol, Water	**CCl_4** - Serum AST, ALT, LDH - Liver GSH, LPO - Histopathology	Arun and Asha, 2007
54	*Picrorrhiza rhizoma* Benth. (Scrophulariaceae)	Whole plant	Water	**Poloxamer (PX)- 407** - Serum AST, ALT, LDL, HDL, TG, TC	Lee et al., 2006
55	*Piper chaba* Trel. & Yunck. (Piperaceae)	Amide constituents from Fruit	-	**D-galactosamine** - Serum ALT, AST	Matsuda et al., 2009
56	*Pittosporum neelgherrense* Wight & Arn. (Pittosporaceae)	Stem bark	Methanol	**CCl_4** **D- galactosamine** **Acetaminophen** - Serum AST, ALT - Histopathology	Shyamal et al., 2006
57	*Plantago major* L. (Plantaginaceae) *Diplotaxis acris* Boiss. (Compositae) *Schouwia thebaica* Webb. (Brassicaceae)	Seeds	Methanol	**CCl_4** - Serum AST, ALT, GGT, GL, TB, TG, TC, TP, ALB	Atta et al., 2006
58	*Platycodon grandiflorum* A. DC. (Campanulaceae)	Saponins derived from Root	Water	**CCl_4** - Serum ALT, AST - Liver LPO - Histopathology	Lee et al., 2008
59	*Polyalthia longifolia* var. pendula. (Annonaceae)	Leaves	Methanol	**Diclofenac** - Serum TP, ALB, GL, GLY, ALT, AST, TB	Tanna et al., 2009
60	*Polygala arvensis* Willd. (Polygalaceae)	Leaves	Chloroform	**D-galactosamine** - Serum AST, ALT, ALP, TP, ALB, TB, TG, TC, LDH	Dhanabal et al., 2006

Table 3: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Hepatotoxicant used and parameters done	References
61	*Pracparatum mungo*	Fermented product	-	**CCl_4** - Serum AST, ALT - Liver LPO, GSH, SOD - Histopathology	Kuo et al., 2010
62	*Pterocarpus marsupium* Roxb. (Papilionaceae)	Stem bark	Methanol, Water	**CCl_4** - Serum AST, ALT, ALP, TB, TP - Histopathology	Krishna et al., 2005
63	*Punica granatum* Linn. (Punicaceae)	Flowers	Water	**CCl_4** - Serum ALT, AST, ALP, LDH - GSH, MDA, GPx, SOD from different organ	Celik et al., 2009
64	*Quercus aliena* Blum. (Fagaceae)	-	Water, Ethanol, Methanol, Chloroform	**CCl_4** - Serum AST, ALT - Liver LPO - Histopathology	Jin et al., 2005
65	*Rhoicissus tridentata* Wild. (Vitaceae)	Root	Water	**CCl_4** - Serum AST, ALT - Liver LPO, G-6-Pase	Opoku et al., 2007
66	*Saccharomyces cerevisiae* Meyen. (Saccharomycetaceae)	Fermented substance	-	**CCl_4** - Serum AST, ALT, ALB - Liver LPO, CAT, GPx, GSH, SOD	Lai et al., 2009
67	*Sida acuta* Burm. f. (Malvaceae)	Root	Methanol	**Acetaminophen** - Hexobarbitone-induced sleeping time studies - Serum GOT, GPT, ALP, TB - Histopathology	Sreedevi et al., 2009
68	*Sophora flavescens* Aiton. (Leguminosae), *Glycyrrhiza glabra* L. (Leguminosae)	Matrine, Glycyrrhizin from root	-	**Acetaminophen** - Liver GSH	Xu-ying et al., 2009
69	*Strychnos potatorum* Linn. (Loganiaceae)	Seed	Water	**CCl_4** - Serum AST, ALT, ALP, TB - Liver GSH, LPO, SOD, GPx - Histopathology	Sanmugapriya and Venkataraman, 2006

Table 3: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Hepatotoxicant used and parameters done	References
70	*Syzygium cumini* L. (Myrtaceae)	Leaves	Water	**CCl_4** - Serum ALT, AST	Moresco et al., 2007
71	*Taraxacum officinale* (Asteraceae)	Root	12% Ethanol	**CCl_4** - Serum AST, ALT, ALP - Liver TP, SOD, HP - Histopathology	Domitrovic et al., 2010
72	*Terminalia arjuna* Bedd. (Combretaceae)	Bark	Water	**CCl_4** - Serum AST, ALT, ALP - Liver and kidney GSH, LPO, CAT, SOD, GST	Manna et al., 2006
73	*Terminalia catappa* L. (Combretaceae)	Leaves	Ethanol	**CCl_4** - Serum AST, ALT - Histopathology	Gao et al., 2004
74	*Thunbergia laurifolia* Linn. (Acanthaceae)	Leaves	Water	**Ethanol** - Determination of cell viability in primary cultures of rat hepatocytes - Serum AST, ALT, TG - Histopathology	Pramyothin et al., 2005
75	*Trichosanthes cucumerina* L. (Cucurbitaceae)	Whole plant	Methanol	**CCl_4** - Serum ALT, AST, ALP, TB, TP, ALB - Liver GSH, MDA - Histopathology	Sathesh Kumar et al., 2009
76	*Vernonia amygdalina* Delile. (Astereaceae)	Leaves	90% Methanol	**CCl_4** - Serum AST, ALP, GST - Liver LPO, CAT, SOD, GSH - Histopathology	Adesanoye and Farombi, 2010
77	*Vitis vinifera* L. (Vitaceae)	Leaves	80% Ethanol	**CCl_4** - Plasma AST, ALT, LPO - Liver GSH, LPO - Histopathology	Orhan et al., 2007

Table 3: Cont…

Sr. No.	Plant (Family)	Part used	Solvent used	Hepatotoxicant used and parameters done	References
78	*Zanthoxylum armatum* DC. (Rutaceae)	Bark	70% Ethanol	**CCl_4** - Serum ALT, AST, ALP, TP, TB - Liver GSH, LPO, CAT, SOD - Histopathology	Ranawat et al., 2010
79	*Zingiber officinale* Roscoe. (Zingiberaceae)	Rhizome	50% Ethanol	**Acetaminophen** - Serum ALT, AST, ALP - Liver GSH, LPO, CAT, SOD, GPx, GST - Histopathology	Ajith et al., 2007
80	*Ziziphus mauritiana* Lam. (Ramnaceae)	Leaves	80% Ethanol	**CCl_4** - Serum AST, ALT, ALP, TB - Liver GSH, LPO, Vit-E	Dahiru et al., 2005

Abbreviations

ACP: acid phosphatase, ALB: albumin, ALP: alkaline phosphatase, ALT: alanine transaminase, AST: aspartate transaminase, BUN: blood urea nitrogen, CAT: catalase, CR: creatinine, DB: direct bilirubin, G-6-Pase: glucose 6 phosphatase, GGT: gamma glutamyl transpeptidase, GL: globulin, GL: glucose, GLY: glycogen, GPx: glutathione peroxidase, GR: glutathione reductase, GSH: glutathione, reduced, GSSG: glutathione (oxidized form), GST: glutathione-S-transferase, HDL: high density lipoproteins, HP: hydroxyproline, IL-1: interleukin-1, K^+: potassium, LDH: lactate dehydrogenase, LDL: low density lipoproteins, LPO: lipid peroxidation, Na^+: sodium, PL: phospholipids, SOD: superoxide dismutase, TB: total bilirubin, TC: total cholesterol, TG: triglycerides, TL: total lipid, TNF-α: Tumor necrosis factor-α, TP: total protein, T-SH: total (protein and nonprotein) sulfhydryl groups, Vit-C: vitamin-C, Vit-E: vitamin-E.

Table 4: The reported activities of *Woodfordia fruticosa* Kurz. with part and solvent used

Sr. No.	Activity	Part used	Solvent used	References
1	Antibacterial	Flowers	Petroleum ether, Chloroform, Methanol Ethanol, Water	Kumaraswamy et al., 2008
2	Antibacterial	Flowers	Methanol	Parekh and Chanda, 2007b
3	Antibacterial	Leaves	Ethanol	Bajracharya et al., 2008
4	Antibacterial	Kutajarista- A ayurvedic preparation	-	Shenoy and Yoganarasimhan, 2009
5	Antidiarrhoeal	Kutajarista- A ayurvedic preparation	-	Shenoy and Yoganarasimhan, 2008
6	Antifertility	Flowers	50% Alcohol, Water	Khushalani et al., 2006
7	Antimicrobial	Leaves	Essential oil and Hexane, Methanol, Acetone	Kaur and Kaur, 2010
8	Antimicrobial	Flowers	Hexane, Chloroform, Acetone, Methanol, Water	Dabur et al., 2007
9	Antioxidant	Flowers	Petroleum ether, Chloroform, Methanol, Water	Kumaraswamy and Satish, 2008
10	Antitumor	Isolated compound from flowers	-	Yoshida et al., 1990
11	Antitumor	Isolated compound	-	Kuramochi-Motegi et al., 1992
12	Hepatoprotective	Flowers	50 % Alcohol	Brindha and Geetha, 2009
13	Hepatoprotective	Flowers	Water	Chandan et al., 2008
14	Immunomodulatory	Fermented product from flowers	-	Kroes et al., 1993
15	Immunostimulatory	Flowers	Ethanol	Shah and Juvekar, 2010
16	Pharmacognosy	Flowers	-	Shome et al., 1981

Chapter 3

MATERIAL AND METHODS

CHAPTER 3: MATERIAL AND METHODS

3.1. COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

The fresh flowers of *Woodfordia fruticosa* were collected from Junagadh (Girnar region), Gujarat in the month of March 2008. The plant was compared with voucher specimen (voucher specimen No. PSN303) deposited at Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India.

3.2. PHARMACOGNOSTIC STUDIES

3.2.1. Macroscopic characteristics

The plant was macroscopically examined for shape, size, surface characteristics, texture, color, consistency, odour, taste, etc. (Khandelwal, 2008).

3.2.2. Microscopic characteristics

Free hand sections of pedicel, calyx, sepal and ovary of fresh flower of *Woodfordia fruticosa* were taken. Sections were cleared with chloral hydrate and then stained with phloroglucinol and hydrochloric acid and mounted with glycerin. Same procedure was followed for microscopic characteristics of powdered material of *Woodfordia fruticosa* flower (Khandelwal, 2008).

3.3. PREPARATION OF THE EXTRACT

The flowers were washed under tap water, air dried, homogenized to fine powder and stored in airtight bottles. Ten grams of dried powder was first defatted with petroleum ether and then extracted with methanol by using Soxhlet apparatus (Lin et al., 1999). The solvent was evaporated to dryness and the dried crude extract was stored in air tight bottle at 4°C. The percentage yield of methanol extract was 36%. The methanol extract of *Woodfordia fruticosa* (WFM) was used for the entire study.

3.4. PHYSICOCHEMICAL ANALYSIS

The following physicochemical parameters were carried out (WHO, 2002; The Ayurvedic Pharmacopoeia of India 2008; Vaghasiya et al., 2008) in dried powder and or methanol extract of *Woodfordia fruticosa* flowers.

- Loss on drying
- Total ash
- Acid insoluble ash
- Water soluble ash
- Petroleum ether soluble extractive
- Hexane soluble extractive
- Ethyl acetate soluble extractive
- Acetone soluble extractive
- Methanol soluble extractive
- Water soluble extractive
- pH
- Melting point
- Solubility test
- Heavy metals analysis

3.4.1. Loss on drying

Two grams of crude powder of *Woodfordia fruticosa* flowers was taken in an evaporating dish and then dried in an oven at 105ºC till constant weight was obtained. The weight after drying was noted and loss on drying was calculated. The percentage was calculated on the basis of sample taken initially.

3.4.2. Total ash

Two grams of dried powder of *Woodfordia fruticosa* flowers was taken in a silica crucible and ignited it by gradually increasing the heat to 500°C until it was white, indicating the absence of carbon. Ash was cooled in a desiccator and weighed without

delay. The percentage of total ash was calculated on the basis of sample taken initially.

3.4.3. Acid insoluble ash

To the crucible containing total ash, 25 ml of hydrochloric acid (HCl, ~70g/l) was added; it was covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ashless filter paper and it was washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible; it was dried on a hot plate and ignited to constant weight. The residue was allowed to cool and then weighed without delay. The percentage of acid insoluble ash was calculated on the basis of sample taken initially.

3.4.4. Water soluble ash

To the crucible containing the total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected on an ashless filter paper. It was washed with hot water and ignited in a crucible for 15 minutes. The residue was allowed to cool and then weighed without delay. Weight of insoluble matter was subtracted from the weight of total ash. The percentage of water soluble ash was calculated on the basis of sample taken initially.

3.4.5. Determination of petroleum ether soluble extractive

Five grams of dried powder of *Woodfordia fruticosa* flowers was taken in 100 ml of petroleum ether in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. Thereafter, it was filtered and the filtrate was evaporated to dryness at 105°C till constant weight was obtained. The percentage of extractable matter was calculated with reference to the sample taken initially.

3.4.6. Determination of hexane soluble extractive

Five grams of dried powder of *Woodfordia fruticosa* flowers was taken in 100 ml of hexane in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. Thereafter, it was filtered and the filtrate was evaporated to dryness at 105°C till constant weight was obtained. The percentage of extractable matter was calculated with reference to the sample taken initially.

3.4.7. Determination of ethyl acetate soluble extractive

Five grams of dried powder of *Woodfordia fruticosa* flowers was taken in 100 ml of ethyl acetate in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. Thereafter, it was filtered and the filtrate was evaporated to dryness at 105°C till constant weight was obtained. The percentage of extractable matter was calculated with reference to the sample taken initially.

3.4.8. Determination of acetone soluble extractive

Five grams of dried powder of *Woodfordia fruticosa* flowers was taken in 100 ml of acetone in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. Thereafter, it was filtered and the filtrate was evaporated to dryness at 105°C till constant weight was obtained. The percentage of extractable matter was calculated with reference to the sample taken initially.

3.4.9. Determination of methanol soluble extractive

Five grams of dried powder of *Woodfordia fruticosa* flowers was taken in 100 ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. Thereafter, it was filtered and the filtrate was evaporated to dryness at 105°C till constant weight was obtained. The percentage of extractable matter was calculated with reference to the sample taken initially.

3.4.10. Determination of water soluble extractive

Five grams of dried powder of *Woodfordia fruticosa* flowers was taken in 100 ml of water in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. Thereafter, it was filtered and the filtrate was evaporated to dryness at 105°C till constant weight was obtained. The percentage of extractable matter was calculated with reference to the sample taken initially.

3.4.11. Determination of pH

The methanol extract of *Woodfordia fruticosa* flowers was dissolved in distilled water and was kept in a water bath for 20 min. It was then filtered and the pH of the filtrate was noted with the help of pH meter.

3.4.12. Determination of melting point

The melting point of methanol extract of *Woodfordia fruticosa* flowers was done at Department of Chemistry, Saurashtra University, Rajkot, by open capillary method (Sukhwal et al., 1995).

3.4.13. Determination of solubility

The quantitative solubility test of methanol extract of *Woodfordia fruticosa* flowers was determined in different solvents. 10 mg of extract was weighed for solubility test for different solvents. The extract was added in each solvent until saturated solution developed. Solubility was calculated in mg/ml.

3.4.14. Determination of heavy metals

The analysis of heavy metals like mercury, lead, chromium, arsenic and cadmium for crude powder and methanol extract of *Woodfordia fruticosa* flowers were done at Choksi Laboratries Limited, Baroda, Gujarat.

3.5. PHYTOCHEMICAL ANALYSIS

3.5.1. Qualitative phytochemical analysis

Preliminary chemical tests were carried out for crude powder and methanolic extract to identify different phyto-constituents (Harborne, 1973; Parekh and Chanda 2007c).

3.5.1.1. Alkaloids

The crude powder and methanol extract of *Woodfordia fruticosa* flowers was dissolved in 2 N HCl. The mixture was filtered and the filtrate was divided into 3 equal portions. One portion was treated with few drops of Mayer's reagent; one portion was treated with equal amount of Dragondroff's reagent and the other portion was treated with equal amount of Wagner's reagent. The creamish precipitate, orange precipitate and brown precipitate indicated the presence of respective alkaloids. A (+) score was recorded if the reagent produced only a slight opaqueness; a (++) score was recorded if a definite turbidity but no flocculation was observed and a (+++) score was recorded if heavy precipitate or flocculation was observed (Salehi-Surmaghi et al., 1992).

3.5.1.2. Flavonoids

3.5.1.2.1. Shinoda test

The presence of flavonoids was estimated by Shinoda test. The crude powder and methanol extract of *Woodfordia fruticosa* flowers were treated with few drops of concentrated HCl and magnesium ribbon. The appearance of pink or tomato red colour within few minutes indicated the presence of flavonoids (Somolenski et al., 1972).

3.5.1.2.2. Alkaline reagent test

The crude powder and methanol extract of *Woodfordia fruticosa* flowers was treated with few drops of diluted sodium hydroxide (NaOH) separately. Formation of intense

yellow color which turned colorless on addition of few drops of diluted HCl indicated presence of flavonoids.

3.5.1.3. Cardiac glycosides

Keller-kiliani test was performed for the presence of cardiac glycosides. The crude powder and methanol extract of *Woodfordia fruticosa* flowers was treated with 1 ml mixture of 5% $FeCl_3$ and glacial acetic acid (1:99 v/v). To this solution, few drops of concentrated H_2SO_4 were added. Appearance of greenish blue color within few minutes indicated the presence of cardiac glycosides (Ajaiyeobu, 2002).

3.5.1.4. Plobotannins

The crude powder and methanol extract of *Woodfordia fruticosa* flowers was boiled with 1% aqueous HCl. Deposition of red precipitate was taken as evidence for the presence of phlobatanins (Harborne, 1973).

3.5.1.5. Saponins

The presence of saponins was determined by Frothing test. The crude powder and methanol extract of *Woodfordia fruticosa* flowers was vigorously shaken with distilled water and was allowed to stand for 10 min and classified for saponin content as follows: no froth indicates absence of saponins and stable froth for more than 1.5 cm indicated the presence of saponins (Kapoor et al., 1969).

3.5.1.6. Steroids

Liebermann-Burchard reaction was performed for the presence of steroids. A chloroformic solution of the crude powder and methanol extract of *Woodfordia fruticosa* flowers was treated with acetic anhydride and few drops of concentrated H_2SO_4 were added down the sides of test tube. A blue green ring indicated the presence of steroids.

3.5.1.7. Tannins

The crude powder and methanol extract of *Woodfordia fruticosa* flowers was treated with alcoholic ferric chloride ($FeCl_3$) reagent. Blue color indicated the presence of tannins (Segelman et al., 1969).

3.5.1.8. Triterpenes

Chloroform extract of the crude powder and methanol extract of *Woodfordia fruticosa* flowers was treated with concentrated sulphuric acid (H_2SO_4). Appearance of reddish brown ring indicated the presence of triterpenes (Harborne, 1973).

3.5.2. Quantitative phytochemical analysis

3.5.2.1. Determination of total phenol content

The amount of total phenol content was determined by Folin-Ciocalteu reagent method (McDonald et al., 2001). 0.5 ml of extract and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) was mixed and incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate was added. The mixture was allowed to stand for 30 min at room temperature and the absorbance of the reaction mixture was measured at 760 nm. The total phenolic content is expressed in terms of gallic acid equivalent (mg/g of extracted compound).

3.5.2.2. Determination of flavonoid content

The amount of total flavonoid content was determined by Aluminium chloride method (Chang et al., 2002). The reaction mixture consisted of 1.0 ml extract, 1 ml methanol, 0.5 ml aluminium chloride (1.2%) and 0.5 ml potassium acetate (120 mM). The mixture was allowed to stand for 30 min at room temperature and the absorbance of the reaction mixture was measured at 415 nm. The flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compound).

3.6. ANIMALS

Wistar albino rats of both sexes (180-220 g) were used for the study. The animals were obtained from the animal house of Sarabhai Research Center (SRC), Baroda and Xcelris Labs Ltd., Ahmedabad. All the rats were kept in standard plastic rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were kept at the animal house of Department of Biosciences, Saurashtra University, Rajkot. The animals were facilitated with standard environmental condition of photoperiod (12:12 h dark: light cycle) and temperature (25 ± 2°C). They were provided with commercial rat and mice feed (Pranav Agro Industries Ltd., Baroda. Amruth Brand rat & mice pellet feed) and water given *ad libitum*. The use of these animals and the study protocols were approved by CPCSEA recognized local ethical committee.

3.7. SELECTION OF THE DOSES FOR ANIMAL STUDY

The dose considered for the experiment on rats was obtained from conversion of human dose of *Woodfordia fruticosa* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats (Ghosh 1984). Hence the calculated dose for the rats (considering human dose 5 g/kg) is 450 mg/kg. Thus, anti-inflammatory and hepatoprotective activity were done at two different doses 400 and 600 mg/kg body weight. Acute toxicity was done at three different doses 450, 1800, and 3600 mg/kg body weight.

3.8. ANTI-INFLAMMATORY STUDIES

3.8.1. Animal grouping for anti-inflammatory studies

The animals were divided into four groups (six animals in each group) for anti-inflammatory studies.

Group I: Vehicle treated control (distilled water)

Group II: Methanol extract of *Woodfordia fruticosa* - 400 mg/kg body weight (WFM-400)

Group III: Methanol extract of *Woodfordia fruticosa*- 600 mg/kg body weight (WFM-600)

Group IV: Diclofenac sodium- 10 mg/kg body weight (diclofenac- 10)

3.8.2. Carrageenan induced rat paw edema

Carrageenan induced rat paw edema was done by the method of Winter et al. (1962). Inflammation was induced by injection of 0.1 ml of freshly prepared carrageenan (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats. The different groups of rats were administered with WFM (400 and 600 mg/kg, p.o.) and diclofenac (10 mg/kg, p.o.). The control group received vehicle (distilled water, 10 ml/kg, p.o.). 1 h after drug treatment, paw edema was induced by the injection of carrageenan (an edematogenic agent). The paw volume was measured by a Plethysmometer. The measures were determined at 0 h (Vo: before edematogenic agent injection) and 1,2,3,4 and 5h intervals later (Vt). The difference between Vt and Vo was taken as the edema value. The percentage of inhibition was calculated according to the following formula:

$$\% \text{ inhibition} = \frac{(Vt - Vo)\text{control} - (Vt - Vo)\text{treated}}{(Vt - Vo)\text{control}} \text{ X } 100$$

3.8.3. Histamine induced rat paw edema

Inflammation was induced by injection of 0.1 ml of freshly prepared histamine (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats (Suleyman et al., 1999). The drug treatment and paw volume was measured in a similar manner to that of carrageenan induced paw edema model.

3.8.4. Dextran induced rat paw edema

Inflammation was induced by injection of 0.1 ml of freshly prepared dextran (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats (Winter and Porter, 1957). The drug treatment and paw volume was measured in a similar manner to that of carrageenan induced paw edema model.

3.8.5. Serotonin induced rat paw edema

Inflammation was induced by injection of 0.1 ml of freshly prepared Serotonin (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats (Mukherjee et al., 1997; Suleyman et al., 1999). The drug treatment and paw volume was measured in a similar manner to that of carrageenan induced paw edema model.

3.8.6. Formaldehyde induced rat paw edema

Inflammation was induced by injection of 0.1 ml of freshly prepared Formaldehyde (3%) underneath the plantar tissue of the right hind paw of rats (Brownlee, 1950). The test drug was administered consecutively for seven days to all the groups. On seventh day, after 1 h of drug administration, paw edema of the rat was induced by subplantar injection of formaldehyde solution. The paw volume was determined at 0 h and at 3, 24 and 48 h after formaldehyde injection as described in carrageenan model.

3.8.7. Cotton pellet induced granuloma in rats

The effect of methanol extract of *Woodfordia fruticosa* flowers on the chronic phases of inflammation was assessed in the cotton pellet induced granuloma rat model, as described by Swingle and Shideman (1972). Autoclaved cotton pellets weighing 100 mg each were implanted subcutaneously. One on each side of the abdomen of the animal, through a small ventral incision of rats anesthetized with ether. The different groups of rats were administered with WFM (400 and 600 mg/kg, p.o.) and diclofenac (10 mg/kg. p.o.) once daily for 7 consecutive days from the day of cotton pellet insertion. The control group received vehicle (distilled water, 10 ml/kg, p.o.). On the eighth day the animals were sacrificed and the cotton pellets were removed, dried at 60°C for 24 h and their mass was determined. The results are expressed as mg granulation tissue formed per 100 g body weight.

3.8.7.1. Biochemical analysis

On the eighth day, the animals were sacrificed under mild ether anesthesia and blood was collected in clean centrifuge tubes. The serum was obtained by centrifugation and

used for the estimation of various biochemical parameters. The absorbance of all the biochemical parameters was measured in a UV-VIS Spectrophotometer -1601 (Shimadzu, Tokyo, Japan).

3.8.7.1.1. Estimation of total protein content

The serum total protein was estimated by modified Biuret method (Yatzidis, 1977) using the total protein test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Biuret Reagent (Copper sulphate - 7 mM/L; sodium hydroxide - 200 mM/L; sodium potassium tartrate - 20 mM/L)

Reagent II: Protein standard (BSA - 6.5 g/dL)

Procedure

3.0 ml of Reagent I was added to all the test tubes. Thereafter, 0.03 ml serum was added for the test and 0.03 ml Reagent II was added for the standard, while in blank 0.03 ml of purified water was added. They were then mixed well and incubated at 37ºC for 5 minutes. The absorbance was read at 578 nm.

3.8.7.1.2. Estimation of albumin content

The serum albumin was estimated by the method given by Corcoran and Durnan (1977) using albumin test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Albumin reagent (Succinic acid - 37 mM/L; bromocresol green - 0.15 mM/L; sodium hydroxide - 1 mM/L; buffer pH - 3.68).

Reagent II: Albumin standard (BSA - 4 g/dL).

Procedure

3.0 ml of albumin reagent (Reagent I) was added to all the test tubes. Thereafter, 0.03 ml serum was added for the test and 0.03 ml Reagent II was added for the standard,

while in blank 0.03 ml of purified water was added. They were then mixed well and incubated at room temperature for 1 min. The absorbance was read at 630 nm.

3.8.7.1.3. Estimation of acid phosphatase (ACP) activity

The serum acid phosphatase activity was estimated by the method of King and Jagatheesan (1959) using ACP test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Buffered substrate, pH 4.9
Reagent II: Sodium hydroxide, 0.5 N
Reagent III: Sodium bicarbonate, 0.5 N
Reagent IV: 4-aminoantipyrine, 0.6%
Reagent V: Potassium ferricyanide, 2.4%
Reagent VI: Stock phenol standard, 10 mg%
Solution I: Reconstitute one vial of reagent I, buffered substrate with 2 ml of purified water.
Solution II: Dissolve reagent 4 in 25 ml of purified water
Solution III: Dissolve reagent 5 in 25 ml of purified water
Working standard: Dilute stock phenol standard 0.5 to 5 with purified water

Procedure

All the test tubes were marked properly as blank (B), standard (S), control (C) and test (T). 0.5 ml of solution I was added in control (C) and test (T). 0.5 ml of purified water was added in control (C) and test (T). 0.6 ml of purified water was added in standard (S) and 1.1 ml of purified water was added in blank (B). All the tubes were mixed well and incubated at 37ºC for 3 min. 0.1 ml of serum was added in test (T), 0.5 ml of working standard was added in standard (S). All the tubes were mixed well and incubated at 37ºC for 60 min. 0.5 ml of reagent II was added in all the tubes. 0.1 ml of serum was added in control (C). 0.5 ml of reagent III, 0.5 ml of solution II and 0.5 ml of solution III was added in all the tubes. All the tubes were mixed well and

absorbance was read at 510 nm. Serum acid phosphatase activity is expressed as KA units.

3.8.7.1.4. Estimation of alkaline phosphatase (ALP) activity

Alkaline phosphatase activity was estimated by the method of Kind and King (1954) using ALP test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Buffered substrate, pH 10.0
Reagent II: Chromogen reagent
Reagent III: Phenol standard, 10 mg%
Working solution: Reconstitute one vial of reagent I, buffered substrate with 2.2 ml of purified water.

Procedure

All the test tubes were marked properly as blank (B), standard (S), control (C), and test (T). 0.5 ml of working buffered substrate was added in clean tubes. 1.5 ml of purified water was added in all the tubes. They were mixed well and incubated at 37°C for 3 min. 0.05 ml of serum was added in test (T), 0.05 ml of reagent III (Phenol standard) was added in standard (S) and 0.05 ml of purified water was added in blank (B) tubes. All the tubes were mixed well and incubated at 37°C for 15 min. 1 ml of reagent II was added in all the tubes. 0.05 ml of serum was added in control (C). All the tubes were mixed well and absorbance was read at 510 nm. Serum alkaline phosphatase activity is expressed as KA units.

3.9. ANALGESIC STUDY

3.9.1. Formaldehyde induced paw licking response in rats

The effect of methanol extract of *Woodfordia fruticosa* flowers on formaldehyde induced paw licking response was evaluated by the procedure of Magali et al. (2000).

The test drug was administered once daily for seven consecutive days to all the groups. On seventh day, after 1 h of drug administration the subplantar injection of 0.1 ml of 3% formaldehyde solution in normal saline was injected. After the injection of formaldehyde, the animals were kept under observation for half an hour. The amount of time spent licking the injected paw was noted, and was considered to be indicative of pain. The time taken for the onset of paw licking was initially measured. The first of the nociceptive responses normally peaked 5 min after formaldehyde injection and the second phase 15-30 min after formaldehyde injection, representing the neurogenic and inflammatory pain (Hunskaar and Hole, 1987). Therefore the frequency of paw licking was measured in five intervals at 0-5 min., 6-10 min., 11-15 min., 16-20 min. and 21-30 min.

3.10. HEPATOPROTECTIVE STUDIES

3.10.1. Diclofenac induced hepatotoxicity

The rats were divided into five groups with six rats in each. Group I (Control) served as normal and received the vehicle alone (Sterile distilled water, 10 ml/kg, p.o.) for 5 days. Group II (Toxin control) animals received diclofenac (50 mg/kg i.p.) (Tanna et al., 2009) on the 3^{rd} and 4^{th} day. Group III (WFM-400) and IV (WFM-600) were treated with WFM at a dose level of 400 mg/kg and 600 mg/kg body weight p.o. per day respectively for 5 days and on the 3^{rd} and 4^{th} day diclofenac (50 mg/kg i.p.) was given 1 h after the treatment of the extract. Group V (Standard) was treated with standard drug silymarin (100 mg/kg p.o.) for 5 days and on the 3^{rd} and 4^{th} day diclofenac (50 mg/kg i.p.) was given 1 h after the treatment of the drug. The animals were sacrificed 48 h after the last injection of diclofenac under mild ether anesthesia. The blood was collected and allowed to stand for 30 min at 37°C and then centrifuged to separate the serum to estimate various biochemical parameters.

3.10.2. Carbon tetrachloride induced hepatotoxicity

The rats were divided into five groups with six rats in each. Group I (Control) served as normal and received the vehicle alone (Sterile distilled water, 10 ml/kg, p.o.) for 5 days. Group II (Toxin control) animals received CCl_4 (2 ml/kg, 1:1 in olive oil, i.p.)

(Jain et al., 2006) on the 3rd and 4th day. Group III (WFM-400) and IV (WFM-600) were treated with WFM at a dose level of 400 mg/kg and 600 mg/kg body weight p.o. per day respectively for 5 days and on the 3rd and 4th day CCl_4 (2 ml/kg, 1:1 in olive oil, i.p.) was given 1 h after the treatment of the extract. Group V (Standard) was treated with standard drug silymarin (100 mg/kg p.o.) for 5 days and on the 3rd and 4th day CCl_4 (2 ml/kg, 1:1 in olive oil, i.p.) was given 1 h after the treatment of the drug. The animals were sacrificed 48 h after the last injection of CCl_4 under mild ether anesthesia. The blood was collected and allowed to stand for 30 min at 37°C and then centrifuged to separate the serum to estimate various biochemical parameters.

3.10.3. Acetaminophen induced hepatotoxicity

The rats were divided into five groups with six rats in each. Group I (Control) served as normal and received the vehicle alone (Sterile distilled water, 10 ml/kg, p.o.) for 5 days. Group II (Toxin control) animals received acetaminophen (3 g/kg, p.o.) (Ajith et al., 2007) on the 4th day. Group III (WFM-400) and IV (WFM-600) were treated with WFM at a dose level of 400 mg/kg and 600 mg/kg body weight p.o. per day respectively for 5 days and on the 4th day acetaminophen (3 g/kg, p.o.) was given 1 h after the treatment of the extract. Group V (Standard) was treated with standard drug silymarin (100 mg/kg p.o.) for 5 days and on the 4th day acetaminophen (3 g/kg, p.o.) was given 1 h after the treatment of the drug. The animals were sacrificed 48 h after the dose of acetaminophen under mild ether anesthesia. The blood was collected and allowed to stand for 30 min at 37°C and then centrifuged to separate the serum to estimate various biochemical parameters.

In above three hepatoprotective models, various *in vivo* antioxidant parameters were estimated from liver.

3.10.4. Preparation of liver homogenate

The liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the liver was homogenized in chilled Tris-HCl buffer (0.025 M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged at 5,000 rpm for 10 min, supernatant was collected and used for analysis.

3.10.5. Biochemical analysis from serum

The absorbance of all the biochemical parameters was measured in a UV–VIS Spectrophotometer - 1601 (Shimadzu, Tokyo, Japan).

3.10.5.1. Estimation of total protein content

As described in section 3.8.7.1.1.

3.10.5.2. Estimation of albumin content

As described in section 3.8.7.1.2.

3.10.5.3. Estimation of blood urea nitrogen (BUN) content

The serum blood urea nitrogen was estimated by Enzymatic Urease (Berthelot) method (Fawcett and Scott, 1960) using Urea Berthelot test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Urease reagent (Phosphate buffer - 20 mM; urease - >20000 U/L; sodium nitroprusside - 3.2 mM/L; sodium salicylate - 60 mM/L)

Reagent II: Urea chromogen reagent (Sodium hypochlorite - 0.2 %; sodium hydroxide – 400 mM/L)

Reagent III: Urea standard, 50 mg/dL

Reagent IV: Purified water

Solution I: To Reagent I add 50 ml Reagent IV and mix gently.

Solution II: Dilute the contents of Reagent II, with 160 ml reagent IV.

Procedure

1.5 ml Solution I was added to clean test tubes. 0.01 ml serum was added for the test and 0.01 ml Reagent III was added for the standard. It was then mixed well and

incubated at 37ºC for 3 min; then 1.5 ml of Solution II was added. It was then mixed well and incubated at 37ºC for 5 min. The absorbance was read at 578 nm against reagent blank.

3.10.5.4. Estimation of alkaline phosphatase (ALP) activity

As described in section 3.8.7.1.4.

3.10.5.5. Estimation of aspartate aminotransferase (AST) activity

The serum aspartate aminotransferase was estimated by the method of Reitman and Frankel (1957) using AST test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Buffered aspartate - α-KG substrate, pH 7.4
Reagent II: DNPH (2,4- Dinitrophenyl hydrazine) colour reagent
Reagent III: Sodium hydroxide, 4 N
Reagent IV: Working pyruvate standard, 2 mM
Solution I: Dilute 1 ml of Reagent III up to 10 ml with purified water.

Procedure

0.25 ml of Reagent I was added in clean test tubes and incubated at 37ºC for 5 minutes. 0.05 ml of serum was added in the test, 0.05 ml Reagent IV was added in standard and 0.05 ml distilled water was added in the blank. They were mixed well and incubated at 37ºC for 60 minutes. Thereafter, 0.25 ml of Reagent II was added to all the tubes, mixed well and allowed to stand at room temperature for 20 min. Then 2.5 ml of Solution I was added to all the tubes, mixed well and allowed to stand at room temperature for 10 min. The absorbance of blank, standard and test were read at 505 nm.

3.10.5.6. Estimation of alanine aminotransferase (ALT) activity

The serum alanine aminotransferase was estimated by the method of Reitman and Frankel (1957) using ALT test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Buffered alanine - α-KG substrate, pH 7.4
Reagent II: DNPH (2,4- Dinitrophenyl hydrazine) colour reagent
Reagent III: Sodium hydroxide, 4 N
Reagent IV: Working Pyruvate Standard, 2 mM
Solution I: Dilute 1 ml of Reagent III up to 10 ml with purified water.

Procedure

0.25 ml of Reagent I was added in clean test tubes and incubated at 37ºC for 5 minutes. 0.05 ml of serum was added in the test, 0.05 ml Reagent IV was added in the standard and 0.05 ml distilled water was added in the blank. They were mixed well and incubated at 37ºC for 30 minutes. Thereafter, 0.25 ml of Reagent II was added to all the tubes, mixed well and allowed to stand at room temperature for 20 min. Then 2.5 ml of Solution I was added to all the tubes, mixed well and allowed it to stand at room temperature for 10 min. The absorbance of blank, standard and test were read at 505 nm.

3.10.6. Antioxidant parameters from liver homogenate

3.10.6.1. Estimation of total protein content

The total protein content was measured by the method of Lowry et al. (1951). The assay mixture contained 0.1 ml of liver homogenate, 0.9 ml of NaOH (0.1 N) and 5.0 ml of alkaline copper sulphate reagent. The reaction mixture was incubated for 15 min at room temperature and then 0.5 ml of Folin Ciocalteau reagent (1 N) was added. The reaction mixture was further incubated for 30 min at room temperature. The absorbance was measured at 660 nm. Bovine serum albumin was used as standard.

3.10.6.2. Estimation of glutathione reduced level

The glutathione reduced was estimated by the method of Ellman (1959). 1.0 ml of liver homogenate in Tris-HCl buffer (25 mM, pH 7.4) was added to 1.0 ml of TCA (10 %) and mixed thoroughly. The mixture was then centrifuged at 5000 rpm for 5 min at room temperature. The assay mixture consisted of 1.0 ml of this supernatant, 0.5 ml of Ellman's reagent (0.02% of 5,5' dithio(bis)nitrobenzoic acid (DTNB) in 1% tri sodium citrate) and 3 ml of phosphate buffer (200 mM, pH 8.0). The yellow colour developed was immediately measured at 412 nm. The values were calculated using molar extinction coefficient of chromophore (1.36×10^4 $M^{-1}cm^{-1}$).

3.10.6.3. Estimation of catalase activity

The catalase was colorimetrically assayed as described by Sinha (1972). The reaction mixture (1.5 ml) contained 1.0 ml of phosphate buffer (10 mM, pH 7.0), 0.1 ml of liver homogenate and the reaction was started by addition of 0.4 ml of H_2O_2 (2000 mM). The reaction mixture was incubated for 3 min at room temperature. The reaction was stopped by addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio), was incubated at 100°C for 2 min. The absorbance was measured at 620 nm. Catalase activity is expressed as μM H_2O_2 consumed/min/mg protein (U/mg protein). For control, 0.1 ml of Tris-HCl buffer (25 mM, pH 7.4) was used in place of the liver homogenate.

3.10.6.4. Estimation of glutathione peroxidase activity

The glutathione peroxidase activity was measured by the method described by Rotruck et al. (1973) and Mills (1959). The reaction mixture contained 1.0 ml of Tris-HCl buffer (400 mM, pH 7.0), 0.5 ml of sodium azide (10 mM), 0.5 ml liver homogenate, 0.5 ml glutathione (4 mM) and the reaction was started by addition of 1.0 ml of H_2O_2 (1.25 mM). The reaction mixture was incubated for 3 min at 37ºC. The reaction was stopped by addition of 1.5 ml TCA (10%) and centrifuged for 5 min at 5000 rpm. GSH in the protein free filtrates was determined by mixing 1.0 ml of supernatant with 2.0 ml of phosphate buffer (200 mM, pH 8.0) and 1.0 ml of DTNB solution (0.02% DTNB in 1% tri sodium citrate). The absorbance was read at 412 nm.

Glutathione peroxidase activity is expressed as µM of GSH utilized/min/mg protein (U/mg protein). For control, 0.5 ml of Tris-HCl buffer (25 mM, pH 7.4) was used in place of the liver homogenate.

3.10.7. Histopathological study

The liver was transferred to 4% formalin solution for fixation and later on processed for histopathological studies following the standard procedure described by Raghuramulu et al. (1983). The microtome sections were cut processed and stained with hematoxylin and eosin. The section thus obtained was scanned in Carl-Zeiss microscope (Germany) with photographic facility and photomicrographs were taken. Changes if any in the cytoarchitecture were noticed.

3.10.7.1. Fixation

Fixation is the process of preserving, hardening and preventing postmortem changes of the tissues. The tissues were excised out immediately after sacrificing, cleaned of extraneous matter, cut in to pieces of such thickness that the fixative readily penetrated throughout the tissue to be fixed. Tissue was transferred to the 4% formaldehyde solution and allowed to remain in it till they were taken up for processing.

3.10.7.2. Tissue processing

Tissue processing involves dehydration, clearing and infiltration of the tissue with paraffin. The usual dehydrating agent is ethyl alcohol; acetone and isopropyl alcohol can also be used. Following dehydration, the tissue was transferred to a paraffin solvent, which is miscible with the dehydrating agent as well. These are known as clearing agents such as chloroform and xylene. Tissue were thoroughly washed by placing them under running tap water and then conveyed through a series of the following solvents as per schedule for dehydration, clearing and paraffin infiltration.

Alcohol 70%	-	20 minutes
Alcohol 80%	-	20 minutes
Alcohol 90%	-	20 minutes
Alcohol 95% (2 changes)	-	20 minutes each
Isopropyl alcohol	-	20 minutes
Acetone (2 changes)	-	20 minutes each
Chloroform (3 changes)	-	20 minutes each
Melted paraffin wax (60°C) (3 changes)	-	30 minutes each

Then the tissues were embedded in paraffin wax to prepare tissue blocks, which were oriented so that sections could be cut in desired plane of the tissue. Tissues were then fixed to metal object holder after trimming them to suitable size.

3.10.7.3. Section cutting

A smear of 5% Mayer's egg albumin was prepared and smeared onto the slide and dried. The tissue sections of 6 µm thickness were cut with the help of Spencer type rotating microtome. The tissue sections were put on slide and then section were floated in water on slide at 55-60°C, water drained off and slide dried on hot plate at about 50°C for 30 minutes. This section was ready for staining.

3.10.7.4. Staining procedure

Reagents

1) Mayer's hematoxylin stain
2) Eosin stain, 2% w/v in alcohol

After fixing the sections on slides, they were stained by serially placing them in the following reagents:

Xylol (2 changes)	-	3 minutes
Acetone	-	3 minutes
Alcohol 95%	-	3 minutes

Haematoxyline stain	-	20 minutes
Running water	-	20 minutes
Eosin stain	-	5 minutes
Alcohol 95% (3 changes)	-	3 minutes each
Acetone (2 changes)	-	3 minutes each
Xylol (2 changes)	-	3 minutes each

After passing through all the above reagents and stains, the slides were mounted with D.P.X. (Diphenyl Phthalate Xylene) and cover slip was placed. Care was taken to avoid air bubbles while mounting the slide.

3.11. ACUTE TOXICITY STUDY

Acute oral toxicity (Ryu et al., 2004), study was performed as per OECD-404 guidelines (1987). 10 rats/group (5 males and 5 females) were used for the study. Group 1 was control group and other three groups were that of WFM at different doses (450, 1800, and 3600 mg/kg body weight). Single dose of the extract was administrated orally to each animal. Signs of toxicity, body weight, feed and water consumption of each animal was observed every day for 14 days.

3.11.1. Cage side observations

In acute toxicity study, the animals were observed prior to dosing. Thereafter, observations were made at every hour, for five hours and then at 24 h and then every day for 14 days. All observations were systematically recorded, with individual records being maintained for each animal. Cage side observations included evaluation of skin and fur; eyes; respiratory effect; autonomic effects, such as salivation, diarrhea, urination; and central nervous system effects, including tremors and convulsions, straub tail, ptosis, relaxation, changes in the level of activity, gait and posture, reactivity to handling, altered strength and stereotypy (Demma et al., 2007; Nair et al., 2009).

3.11.2. Feed and water consumption and body weight measurement

The animals were monitored daily for mortality, feed and water consumption and changes in body weight for 14 days in acute toxicity study. Feed intake was calculated as g/animal/day. Water intake was calculated as ml/animal/day. Individual animal body weight was recorded daily till the end of the experiment.

3.11.3. Hematological analysis

At the end of the study, all animals were fasted for 12 h and then under mild ether anesthesia, animals were sacrificed and blood samples were collected. Blood was collected immediately into tubes containing EDTA for analysis of hematological parameters *viz.* hemoglobin, total red blood cells (RBC), packed cell volume, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cells (WBC), neutrophils, lymphocytes, eosinophils, monocytes, basophiles, total platelet count (Theml et al., 2004) using hematology analyzer Sysmex XS800i (Sysmex corporation, USA).

3.11.4. Relative organ weight (ROW) analysis

Heart, liver, brain, kidneys, lungs, thymus glands, spleen, adrenal glands, testes and uterus were mopped with filter paper, weighed and the relative weights were calculated and expressed as g/100 g b.w.

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on sacrifice day (g)}} \text{ X } 100$$

3.12. STATISTICAL ANALYSIS

The data obtained from animal experiments are expressed as mean ± SEM (standard error of mean). For statistical analysis data were subjected to analysis of variance (ANOVA) followed by Student's t-test. Values are considered statistically significant at $F < 0.05$ for ANOVA and $P < 0.05$ for t-test.

Chapter 4

RESULTS

CHAPTER 4: RESULTS

4.1. PHARMACOGNOSTIC STUDIES

4.1.1. Macroscopic characteristics

The full-grown *Woodfordia fruticosa* shrub is about 3.0 m high, having long and spreading branches with fluted stems. The flowers are bright red, innumerable, arranged in dense axillary paniculate-cymose clusters (Figure 1), with short glandular pubescent pedicels. The inflorescence is deep red, 2-15 flowered, fascicled cymes. The calyx is long (11-33 mm), striated, covered with glandular dots, with a small campanulate base and a long slightly curved bright red tube. The petals are pink, papery, slightly longer than the calyx-teeth, narrowly linear, extended at the apex to a long fine point. The fruits are small capsules, ellipsoid and membranous, usually splitting the calyx near the base, and are irregularly dehiscent. The seeds are brown, numerous, very minute, smooth, shining, angular and obovate.

4.1.2. Microscopic characteristics

Pedicel

A transverse section of the pedicel flower shows a single-layered epidermis, with a fairly thick cuticle. Numerous unicellular trichomes arise from this layer. The epidermis is followed by a 7-8 layered cortex, differentiated into collenchyma and parenchyma with plenty of air spaces. The primary xylem is represented by uni- or bi-seriate groups of 3 or 4 tracheids arranged in a ring with phloem on either side of the xylem. The rosette and cluster crystals of calcium oxalate are found in the cortex (Figure 2).

Calyx

A transverse section of the calyx tube is circular in outline. The cells of the upper epidermis in surface showed scattered trichomes. The calyx tube consists of several

layers of ground tissue containing rosettes and cluster of calcium oxalate crystals and bounded on either side by upper and lower epidermis respectively. Anomocytic, actinocytic and anisocytic stomata are present. Vascular bundles are small, collateral and surrounded by bundle sheath. In sepals, the cells of the lower epidermis in surface view are broad, slightly irregular, thin walled in the upper region but thick walled in the basal region of the calyx. The tissue is differentiated into an adaxial palisade and an abaxial spongy parenchyma in the upper ¾ of the calyx tube (Figure 2).

Anther lobes

The anther lobes are tetrasporangiate and the walls separating the locules get disorganized. A transaction of a lobe shows an epidermis formed of large colourless cells followed by a fibrous layer, which appears crinkled (Figure 3).

Pollen grains

Pollen grains are 3-zonocolporate, oblate spheroidal shape and it's surface is psilate (Figure 3).

TS of ovary

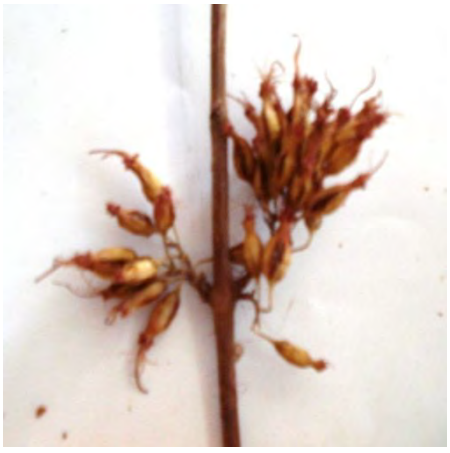
The ovary is bicarpellary and laterally flattened and as such appears elongated in transaction (Figure 3).

4.1.3. Powder characteristic

The crude powder of *Woodfordia fruticosa* flower was light brown in color, slightly bitter and astringent in taste. The specific characteristics determined from the powder study were (Figure 4):

- Epidermis in surface view showed straight wall cells.
- Few cells of fibrous layer observed in powder.
- Cluster crystals of calcium oxalate were present in powder.
- Pollen grains either in groups or singly found.

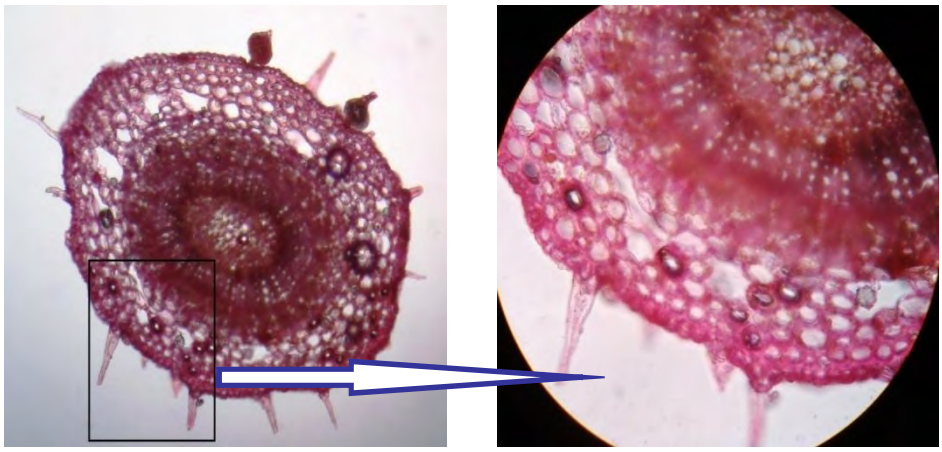
A twig of *Woodfordia fruticosa*



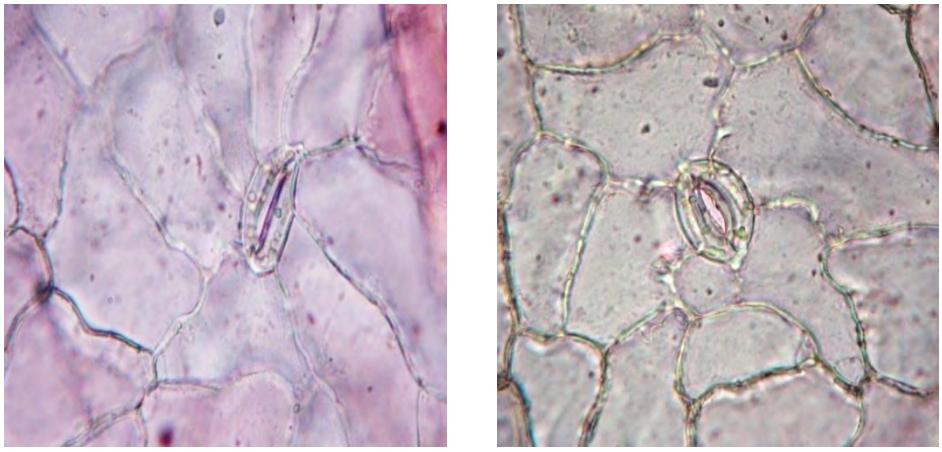
Inflorescence

From left to right three stages of development of bud, flower and fruit

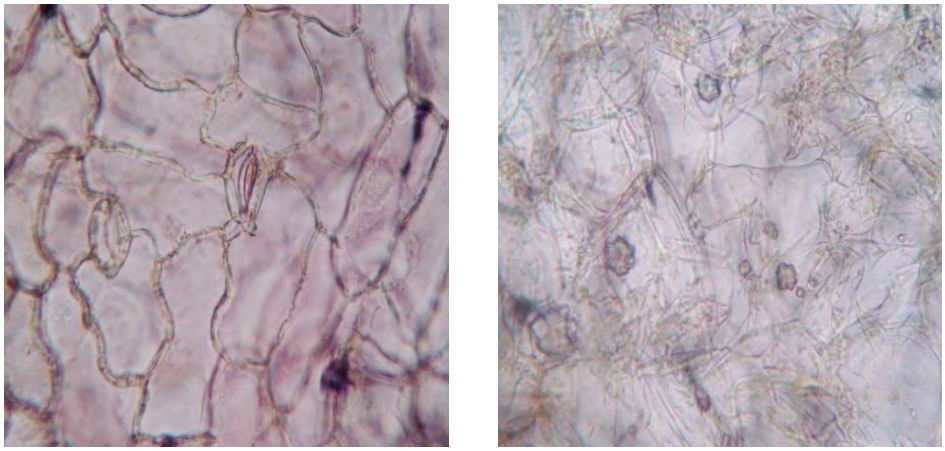
Figure 1: Macroscopic characteristics of *Woodfordia fruticosa.*



Pedicel **Pedicel with Trichome**

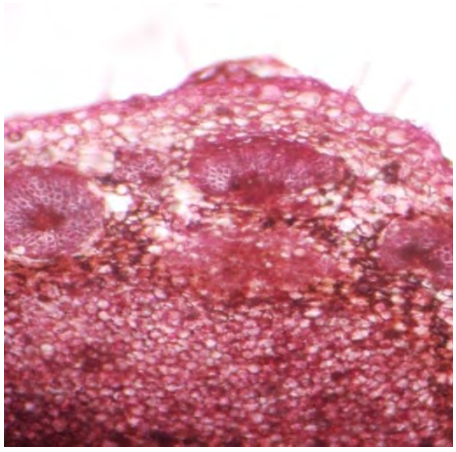


Actinocytic Stomata **Anisocytic Stomata**

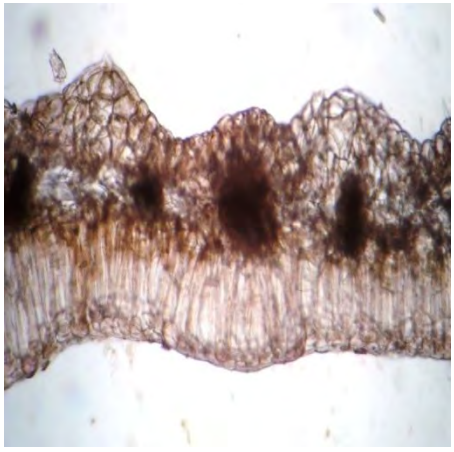


Anomocytic Stomata **Rosette and Cluster Crystal**

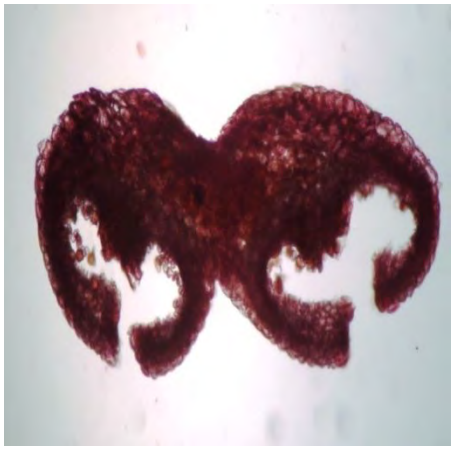
Figure 2: Microscopic characteristics of *Woodfordia fruticosa* flower.



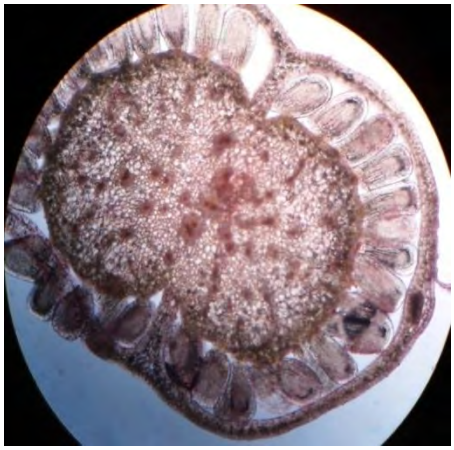
Calyx



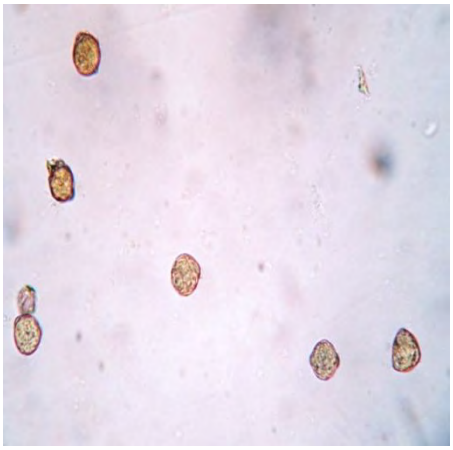
Sepal



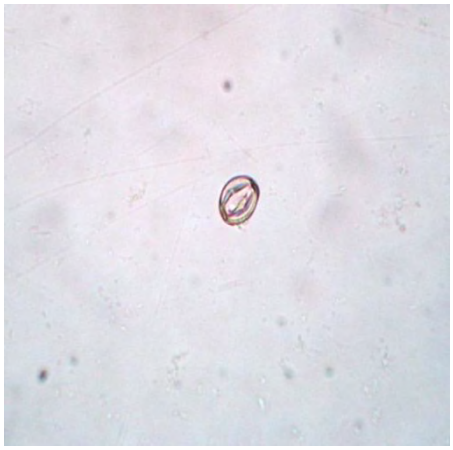
TS of Anther Lobe



TS of Ovary

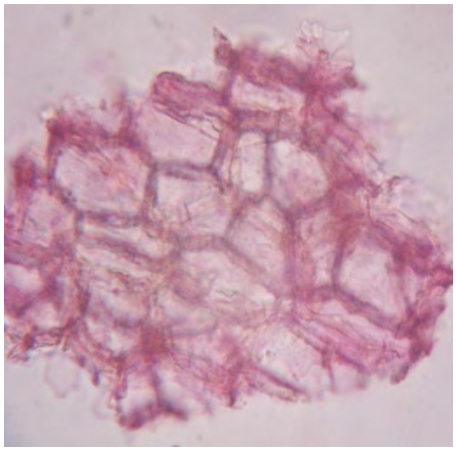


Pollen

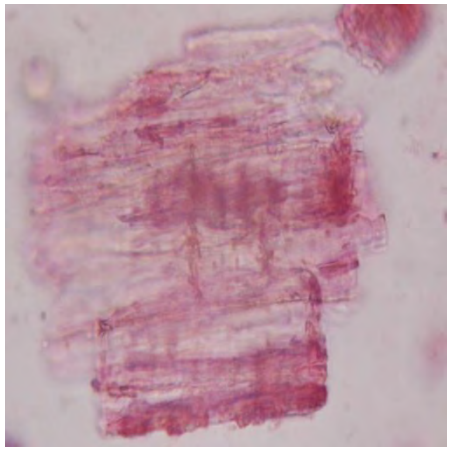


Palynogram of Pollen

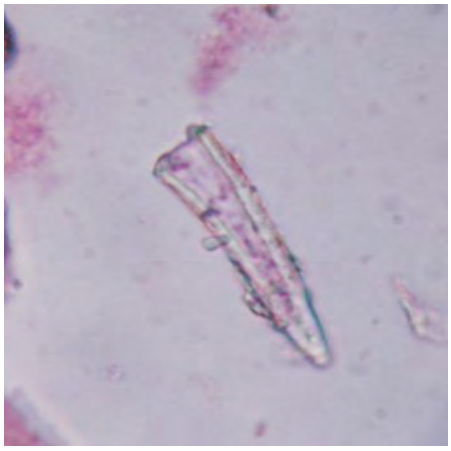
Figure 3: Microscopic characteristics of *Woodfordia fruticosa* flower.



Epithelial Cell

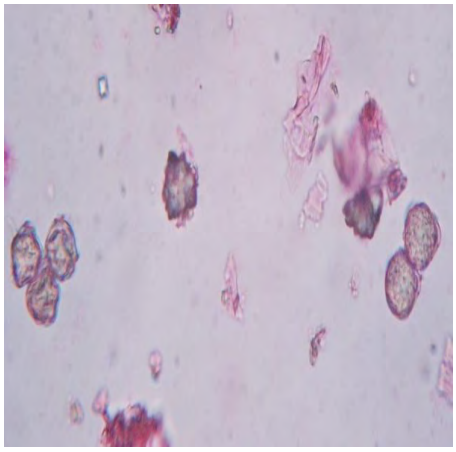


Fiber

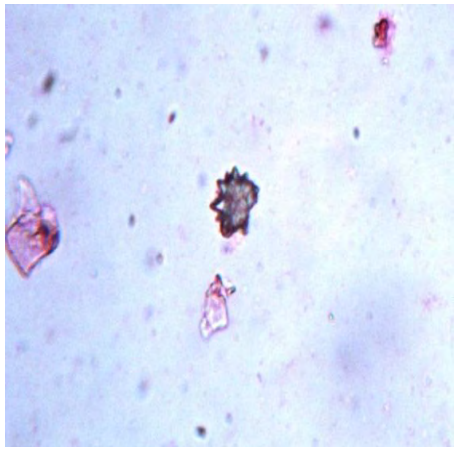


Unicellular Trichome

Annular Xylem Vessel



Pollen Grain



Cluster Calcium Oxalate Crystal

Figure 4: Powder characteristics of *Woodfordia fruticosa* flower.

- Simple covering unicellular trichomes are present.
- Annular xylem vessels were found.

4.2. PHYSICOCHEMICAL ANALYSIS

4.2.1. Proximate parameters analysis

The result of proximate analysis of crude powder of *Woodfordia fruticosa* flower is shown in Table 5. The average values are expressed as percentage of air-dried material. The loss on drying was 8%. Total ash was 5.45%, acid insoluble ash was 0.57% and water soluble ash was 2.47%. The extractive value of crude powder was maximum in water (41.59%), followed by methanol (32.77%) and minimum was in hexane (0.71%). pH and melting point of methanol extract was 3.5 and 114°C respectively.

Table 5: Proximate parameters of crude powder of *Woodfordia fruticosa* flowers

Parameters	**Value (w/w)**
Loss on drying	8.00%
Total ash	5.45%
Acid insoluble ash	0.57%
Water soluble ash	2.47%
Petroleum ether soluble extractive	0.74%
Hexane soluble extractive	0.71%
Ethyl acetate soluble extractive	1.80%
Acetone soluble extractive	8.08%
Methanol soluble extractive	32.77%
Water soluble extractive	41.59%
pH of methanol extract	3.5
Melting point methanol extract	114°C

4.2.2. Solubility test

The methanol extract of *Woodfordia fruticosa* flowers was evaluated for solubility in 10 solvents with varied polarities. The extract was highly soluble in dimethylformamide, methanol and dimethylsulphoxide but insoluble in hexane, petroleum ether and toluene solvents (Table 6).

Table 6: Solubility of methanol extract of *Woodfordia fruticosa* flowers in different solvents

Solvent	Solubility (mg/ml)
Acetone	6.0
Chloroform	3.0
Dimethylformamide (DMF)	119.0
Dimethylsulphoxide (DMSO)	109.8
Distilled water	34.2
Ethyl acetate	3.0
Hexane	-
Methanol	113.1
Petroleum ether	-
Toluene	-

(-): Not soluble

4.2.3. Heavy metals analysis

The crude powder of *Woodfordia fruticosa* flowers and its methanol extract were analysed for the presence of heavy metals. The results (Table 7) showed that crude powder and methanol extract contained 0.053 ppm and 0.05 ppm mercury; 0.12 ppm and 0.061 ppm arsenic respectively. Lead, chromium and cadmium were not detected in crude powder and methanol extract.

Table 7: Heavy metals in crude powder and methanol extract of *Woodfordia fruticosa* flowers

Plant extract	Heavy metals (ppm)				
	Mercury (Hg)	**Lead (Pb)**	**Chromium (Cr)**	**Arsenic (As)**	**Cadmium (Cd)**
Crude powder	0.053	NDT	NDT	0.12	NDT
Methanol extract	0.05	NDT	NDT	0.061	NDT

NDT: Not detected

4.3. PHYTOCHEMICAL ANALYSIS

4.3.1. Qualitative phytochemical analysis

The results of qualitative phytochemical analysis of the crude powder and the methanol extract of *Woodfordia fruticosa* flowers is shown in Table 8.

Table 8: Preliminary qualitative phytochemical analysis of *Woodfordia fruticosa* flowers

Phytochemical	**Test**	**Crude powder**	**Methanol extract**
Alkaloids	Dragondroffs test	++	+
	Mayers test	+	+
	Wagners test	++	+++
Flavonoids	Shinoda test	++	+
	Alkaline reagent test	+	+
Cardiac glycosides	Keller-kilianni test	-	-
Phlobotannins	HCl test	++	+
Saponins	Frothing test	+	++
Steroids	Liebermann-Burchard reaction	-	-
Tannins	$FeCl_3$ test	+++	+++
Triterpenes	H_2SO_4 test	++	+

(-): No presence, (+): Less presence, (++): Moderate presence, (+++): High presence

In crude powder and methanol extract maximum amount of tannins and alkaloids were present. Flavonoids, phlobotannins, saponins and triterpenes were present in moderate amount. Cardiac glycosides and steroids were absent in crude powder as well as in methanol extract.

4.3.2. Quantitative phytochemical analysis

The results of quantitative phytochemical analysis of the methanol extract of *Woodfordia fruticosa* flowers is shown in Table 9. The total phenol and flavonoid content was 64.24 mg/g and 13.07 mg/g respectively.

Table 9: Quantitative phytochemical analysis of methanol extract of *Woodfordia fruticosa* flowers

Total Phenol (mg/g)	Flavonoid (mg/g)
64.24 ± 0.93	13.07 ± 0.25

4.4. ANTI-INFLAMMATORY STUDIES

4.4.1. Carrageenan induced rat paw edema

The results of anti-inflammatory activity of methanol extract of *Woodfordia fruticosa* flowers on carrageenan induced paw edema is shown in Table 10. The lower dose i.e. WFM-400 showed inhibition at both early and late phase; though maximum inhibition was at late phase (69%, $P < 0.01$). The higher dose i.e. WFM-600 also showed maximum anti-inflammatory activity at late phase (42%) but this activity was less than that of WFM-400 at both early and late phase. The standard Diclofenac-10 showed maximum activity at early phase (59%, $P < 0.01$). In this model, the lower dose showed more inhibition of edema formation than standard diclofenac.

4.4.2. Histamine induced rat paw edema

The results of anti-inflammatory activity of methanol extract of *Woodfordia fruticosa* flowers on histamine induced paw edema is shown in Table 11.

Table 10: Anti-inflammatory activity of methanol extract of *Woodfordia fruticosa* flowers in carrageenan induced rat paw edema

Treatment group	% Increase in paw volume									
	After 1 h		After 2 h		After 3 h		After 4 h		After 5 h	
	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change
Control	16.49 ± 3.12		27.49 ± 3.49		34.37 ± 4.45		33.23 ± 5.64		32.08 ± 5.16	
WFM-400	8.05 ± 1.31*	51.16↓	12.06 ± 2.25**	56.11↓	15.16 ± 3.09**	55.90↓	12.87 ± 2.26**	61.26↓	9.93 ± 2.85**	69.04↓
WFM-600	10.76 ± 2.98	34.78↓	20.03 ± 3.46	27.15↓	25.22 ± 4.34	26.63↓	24.67 ± 4.72	25.77↓	18.46 ± 5.06	42.47↓
Diclofenac-10	9.00 ± 1.58	45.44↓	13.34 ± 3.86*	51.47↓	13.86 ± 2.33**	59.67↓	17.68 ± 3.55*	46.79↓	17.01 ± 4.39*	46.97↓
ANOVA - test			F < 0.05		F < 0.01		F < 0.05		F < 0.05	

Values are expressed as mean ± SEM, (n = 6). *P < 0.05; **P < 0.01. ↓ decreased

In this model, the *Woodfordia fruticosa* flower extract at both dose levels and standard diclofenac showed anti-inflammatory activity at late phase. A clear dose dependent inhibition of paw edema was observed. The percentage inhibition of WFM-400 was 36% ($P < 0.05$) while that of WFM-600 was 46% ($P < 0.01$). The later was nearer to standard Diclofenac-10 (55%, $P < 0.01$).

4.4.3. Dextran induced rat paw edema

The results of anti-inflammatory activity of methanol extract of *Woodfordia fruticosa* flowers on dextran induced paw edema is shown in Table 12. In this model, a clear dose dependent inhibition of paw edema was observed at both early and late phases. The higher dose showed distinctly more inhibition than lower dose at both phases ($P < 0.05$). The anti-inflammatory activity of higher dose was more at later phase (50%) than early phase. The standard diclofenac showed poor anti-inflammatory activity in this model.

4.4.4. Serotonin induced rat paw edema

The results of anti-inflammatory activity of methanol extract of *Woodfordia fruticosa* flowers on serotonin induced paw edema is shown in Table 13. The maximum paw thickness was observed at 3^{rd} h after sub-planter injection in all groups. The animals treated with lower and higher doses of WFM (400 and 600 mg/kg) produced statistically significant inhibition at 1^{st} h ($P < 0.05$; $P < 0.01$, respectively), 2^{nd} and 3^{rd} h ($P < 0.05$) and at 5^{th} h ($P < 0.01$). Standard drug diclofenac-10 showed significant decrease in paw volume at 1^{st}, 2^{nd} h ($P < 0.05$) and at 3^{rd}, 4^{th} and 5^{th} h ($P < 0.01$). The maximum decrease of paw volume in both the doses of WFM and diclofenac treated groups was found at 5^{th} h ($F < 0.001$). The percent inhibition of WFM-400 group at 5^{th} h was same as that of standard group. The extract effectively suppressed the inflammation produced by serotonin.

Table 11: Anti-inflammatory activity of methanol extract of *Woodfordia fruticosa* flowers in histamine induced rat paw edema

Treatment group	% Increase in paw volume									
	After 1 h		After 2 h		After 3 h		After 4 h		After 5 h	
	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change
Control	43.32 ± 3.93		48.81 ± 4.65		48.87 ± 5.75		47.32 ± 5.84		44.00 ± 4.38	
WFM-400	40.49 ± 2.77	6.51↓	36.78 ± 2.45*	24.65↓	31.93 ± 1.90*	34.66↓	29.91 ± 1.98*	36.79↓	28.00 ± 2.95*	36.35↓
WFM-600	37.13 ± 2.59	14.28↓	35.35 ± 4.46*	27.58↓	28.77 ± 3.71*	41.13↓	27.82 ± 3.58*	41.20↓	23.77 ± 3.45**	45.97↓
Diclofenac-10	30.31 ± 4.19*	30.02↓	28.38 ± 4.30**	41.86↓	27.46 ± 3.20**	43.82↓	21.74 ± 4.60**	54.05↓	19.83 ± 3.30**	54.93↓
ANOVA - test			F < 0.05		F < 0.01		F < 0.01		F < 0.001	

Values are expressed as mean ± SEM, (n = 6). *P < 0.05; **P < 0.01. ↓ decreased

Table 12: Anti-inflammatory activity of methanol extract of *Woodfordia fruticosa* flowers in dextran induced rat paw edema

Treatment group	% Increase in paw volume									
	After 1 h		After 2 h		After 3 h		After 4 h		After 5 h	
	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change
Control	44.80 ± 4.85		47.10 ± 6.08		41.90 ± 4.45		33.77 ± 5.82		25.43 ± 3.88	
WFM-400	33.71 ± 3.41	24.77↓	36.49 ± 3.56	22.53↓	30.07 ± 5.91	28.24↓	22.45 ± 3.01	33.53↓	20.75 ± 3.17	18.43↓
WFM-600	27.51 ± 3.66*	38.58↓	27.61 ± 4.18*	41.39↓	26.96 ± 1.59*	35.66↓	16.82 ± 3.09*	50.18↓	12.85 ± 3.23*	49.46↓
Diclofenac-10	42.21 ± 4.82	5.78↓	40.05 ± 4.60	14.98↓	36.41 ± 4.69	13.10↓	26.62 ± 4.68	21.17↓	23.91 ± 3.52	6.00↓
ANOVA - test	$F < 0.05$									

Values are expressed as mean ± SEM, (n = 6). *$P < 0.05$. ↓ decreased

Table 13: Anti-inflammatory activity of methanol extract of *Woodfordia fruticosa* flowers in serotonin induced rat paw edema

Treatment group	% Increase in paw volume									
	After 1 h		After 2 h		After 3 h		After 4 h		After 5 h	
	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change
Control	17.46 ± 2.88		22.71 ± 4.21		34.79 ± 5.45		27.50 ± 3.55		21.03 ± 3.57	
WFM-400	9.35 ± 1.05*	46.44↓	13.43 ± 2.79	40.88↓	18.31 ± 2.33*	47.37↓	14.77 ± 2.99*	46.30↓	4.79 ± 2.29**	77.22↓
WFM-600	6.89 ± 1.31**	60.52↓	14.52 ± 1.58	36.04↓	17.53 ± 3.71*	49.61↓	13.62 ± 4.56*	50.47↓	7.91 ± 2.00**	62.39↓
Diclofenac-10	6.45 ± 3.25*	63.05↓	8.53 ± 2.78*	62.45↓	10.82 ± 3.74**	68.91↓	6.95 ± 3.78**	74.73↓	4.81 ± 2.37**	77.13↓
ANOVA - test	F < 0.05		F < 0.05		F < 0.01		F < 0.01		F < 0.001	

Values are expressed as mean ± SEM, (n = 6). *P < 0.05; **P < 0.01. ↓ decreased

4.4.5. Formaldehyde induced rat paw edema

The results of anti-inflammatory activity of methanol extract of *Woodfordia fruticosa* flower in formaldehyde induced paw edema is shown in Table 14. Injection of formaldehyde subcutaneously into hind paw of rats produces localized inflammation. The administration of WFM-400, WFM-600 and diclofenac-10 daily for 7 days successfully significantly ($F < 0.001$) inhibited edema induced by formaldehyde. WFM-400 and WFM-600 group showed maximum decrease in paw volume at 3 h ($P < 0.05$ and $P < 0.01$ respectively). Diclofenac-10 group showed decrease in paw volume at 3 h (45.65%, $P < 0.001$) and the decreased in paw volume at 48 h was almost same (46.06%, $P < 0.01$).

Table 14: Anti-inflammatory activity of methanol extract of *Woodfordia fruticosa* flowers in formaldehyde induced rat paw edema

Treatment group	% Increase in paw volume					
	After 3 h		After 24 h		After 48 h	
	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change
Control	53.94 ± 3.02		57.94 ± 1.97		43.11 ± 2.23	
WFM-400	34.23 ± 5.23*	36.53↓	36.96 ± 2.89**	36.22↓	32.26 ± 5.13	25.16↓
WFM-600	33.44 ± 3.87**	38.01↓	37.52 ± 6.64*	35.24↓	30.31 ± 4.13*	29.69↓
Diclofenac-10	29.32 ± 1.53***	45.65↓	33.11 ± 3.39**	42.86↓	23.26 ± 1.95**	46.06↓
ANOVA - test	F < 0.001		F < 0.01		F < 0.01	

Values are expressed as mean ± SEM, (n = 6). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
↓ decreased

4.4.6. Cotton pellet induced granuloma in rats

The results of anti-inflammatory activity of methanol extract of *Woodfordia fruticosa* flower in cotton pellet induced granuloma is shown in Table 15. WFM-400 and WFM-600 groups showed 1.28% and 11.45% decrease in granuloma formation

respectively as compared to control group, while standard diclofenac-10 group showed significant decrease in granuloma formation (37.15%, $P < 0.001$).

Table 15: Anti-inflammatory activity of methanol extract of *Woodfordia fruticosa* flowers in cotton pellet induced granuloma in rats

Treatment group	**Pellet weight (g / 100 g b.w.)**	**% Change**
Control	0.107 ± 0.005	-
WFM-400	0.106 ± 0.005	1.28↓
WFM-600	0.095 ± 0.005	11.45↓
Diclofenac-10	0.067 ± 0.004***	37.15↓
ANOVA - test	F < 0.001	

Values are expressed as mean ± SEM, (n = 6). ***P < 0.001.

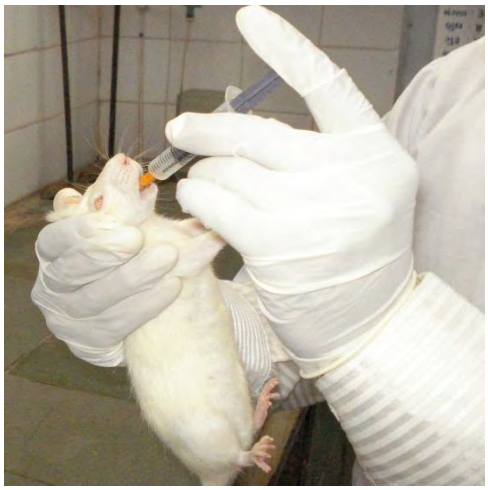
The results of changes in serum total protein and albumin levels in cotton pellet induced granuloma are given in Table 16. The total protein level increased at higher concentration ($P < 0.01$), and decreased at lower concentration of WFM, while diclofenac-10 group showed increase in total protein level as compared to control group. In standard and WFM-400 group, the albumin level was decreased as compared to control group.

Table 16: Effect of methanol extract of *Woodfordia fruticosa* flowers on serum biochemical parameters in cotton pellet induced granuloma in rats

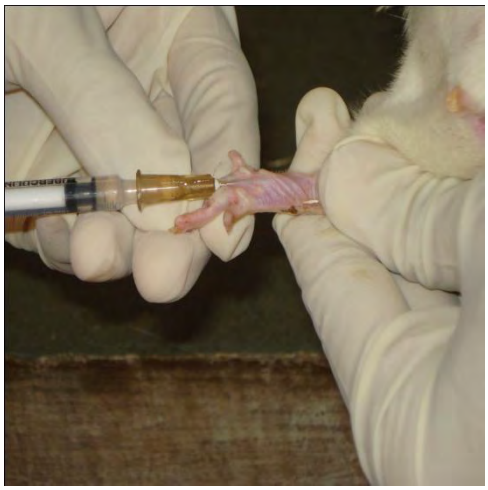
Treatment group	**Total protein (g/dl)**	**Albumin (g/dl)**	**ACP (KA units)**	**ALP (KA units)**
Control	6.23 ± 0.06	3.56 ± 0.04	13.03 ± 1.0	52.07 ± 7.12
WFM-400	6.15 ± 0.07	3.36 ± 0.03	13.26 ± 1.2	36.97 ± 6.26
WFM-600	6.54 ± 0.06**	3.57 ± 0.08	13.13 ± 0.61	43.69 ± 5.04
Diclofenac-10	6.35 ± 0.13	3.32 ± 0.36	18.41 ± 5.4	60.25 ± 13.08

Values are expressed as mean ± SEM, (n = 6). *P < 0.05; **P < 0.01.

The results of changes in serum ACP and ALP levels in cotton pellet induced granuloma are given in Table 16. The ACP level in both the doses of WFM was almost similar to that of control group. In standard group, the level of ACP was more as compared to control group. The ALP levels decreased in both the studied

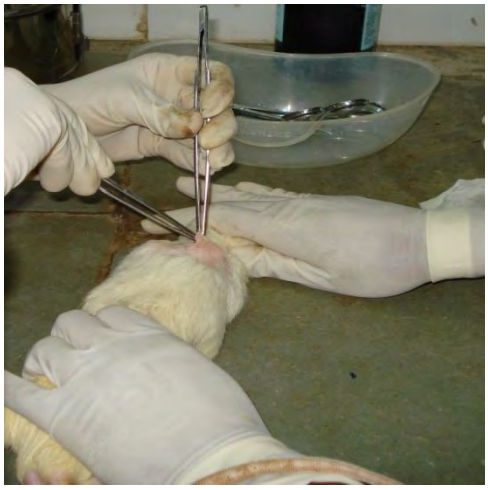


Drug administration (P.O.)

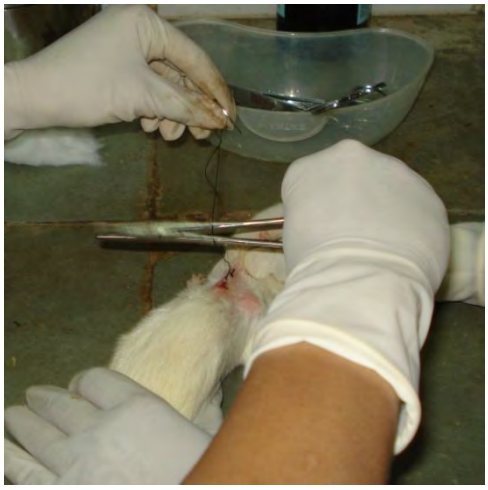


Subplantar injection

Measurement of paw volume



Implantation of cotton pellet



Stitching of incision

Figure 5: Photographs of anti-inflammatory study

concentrations, and the decrease in lower concentration was more than that of the higher concentration. In contrast, the ALP level in the standard group increased.

4.5. ANALGESIC STUDY

4.5.1. Formaldehyde induced paw licking test in rats

The result of formaldehyde induced paw licking test in rats of methanol extract of *Woodfordia fruticosa* flower is given in Table 17. The onset time of paw licking was measured after formaldehyde injection. WFM-400 and WFM-600 showed 82.72% ($P < 0.05$) and 45.06% increase in onset time respectively, while diclofenac-10 group showed 26.54% increase in onset time as compared to the control group. After formaldehyde injection, the frequency of paw licking was measured between 0-5 min, 6-10 min, 11-15 min, 16-20 min and 21-30 min. As compared with the control group, in the first phase (0-5 min), the extract reduced the paw licking up to 34.48% and 18.39% at WFM-400 and WFM-600 dose level respectively, while diclofenac-10 reduced the paw licking up to 21.84%. In second phase (16-20 min) both the extract and diclofenac reduced the dose dependent paw licking up to 27.42%, 47.96% and 49.33 and (during 21-30 min) 24.04%, 29.23% and 35.07% respectively.

Table 17: Analgesic activity of methanol extract of *Woodfordia fruticosa* flowers in formaldehyde induced paw licking test in rats

Treatment group	Onset time (Sec)	% Change	0-5 min		6-10 min		11-15 min		16-20 min		21-30 min	
			F	% Change	F	% Change	F	% Change	F	% Change	F	% Change
Control	27.00 ± 6.04		14.50 ± 3.79		2.67 ± 0.76		5.33 ± 1.86		12.17 ± 2.57		25.67 ± 4.59	
WFM-400	49.33 ± 4.11*	82.72↑	9.50 ± 0.72	34.48↓	2.00 ± 1.44*	25.09↓	1.33 ± 0.71	74.98↓	8.83 ± 2.15	27.42↓	19.50 ± 4.52	24.04↓
WFM-600	39.17 ± 6.81	45.06↑	11.83 ± 1.76	18.39↓	0.50 ± 0.22	81.27↓	1.50 ± 0.72	71.86↓	6.33 ± 1.69	47.96↓	18.17 ± 5.34	29.23↓
Diclofenac-10	34.17 ± 4.43	26.54↑	11.33 ± 1.76	21.84↓	0.00 ± 0.00*	100.00↓	1.67 ± 0.61	68.73↓	6.17 ± 1.47	49.33↓	16.67 ± 2.19	35.07↓

Values are expressed as mean ± SEM, (n = 6). *P ≤ 0.05, F = Frequency, ↑ Increase, ↓ decrease

4.6. HEPATOPROTECTIVE STUDIES

4.6.1. Diclofenac induced hepatotoxicity

The results of serum biochemical parameters in pre-treatment of WFM with respect to induction of hepatotoxicity using diclofenac are shown in Figure 6. The level of total protein and albumin depleted in the group treated with diclofenac (toxin control) and were significantly decreased ($P < 0.001$) when compared with the normal control group. The BUN and ALP levels increased significantly ($P < 0.01$, $P < 0.001$ respectively) in the group treated with diclofenac. The administration of diclofenac markedly increased serum AST and ALT levels which were significant as compared to normal control group ($P < 0.05$, $P < 0.01$ respectively).

The groups that received the pre-treatment of WFM at dose levels of 400 and 600 mg/kg body weight significantly controlled the change in the biochemical parameters. The extract at dose levels of 400 and 600 mg/kg exhibited significant increase ($P < 0.05$) in the serum total protein level as compared to toxin control group. The albumin level in lower as well as in higher dose group increased significantly ($P < 0.01$, $P < 0.001$ respectively) as compared to toxin control group and the effect was comparable with the standard group ($P < 0.01$) treated with silymarin. The BUN level decreased in both the dose groups significantly ($P < 0.05$) as compared to toxin control group. The ALP level also significantly decreased in WFM-400 ($P < 0.05$) as well as in WFM-600 group ($P < 0.001$). In WFM-600 group, the level of ALT and AST significantly decreased ($P < 0.05$), the result was comparable to that of standard group.

The results of relative liver weight, liver total protein, GSH and antioxidant enzymes in diclofenac induced hepatotoxicity are given in Figure 7. The relative liver weight in toxin control group increased significantly ($P < 0.001$) as compared to normal control group. The total protein and GSH levels from the liver homogenate decreased significantly ($P < 0.001$, $P < 0.01$ respectively) in toxin control group. The catalase (CAT) and GPx activity in the toxin control group was also significantly ($P < 0.001$, $P < 0.05$ respectively) depleted as compared to the normal control group. The mean relative liver weight decreased significantly in WFM-400 ($P < 0.001$) and WFM-600

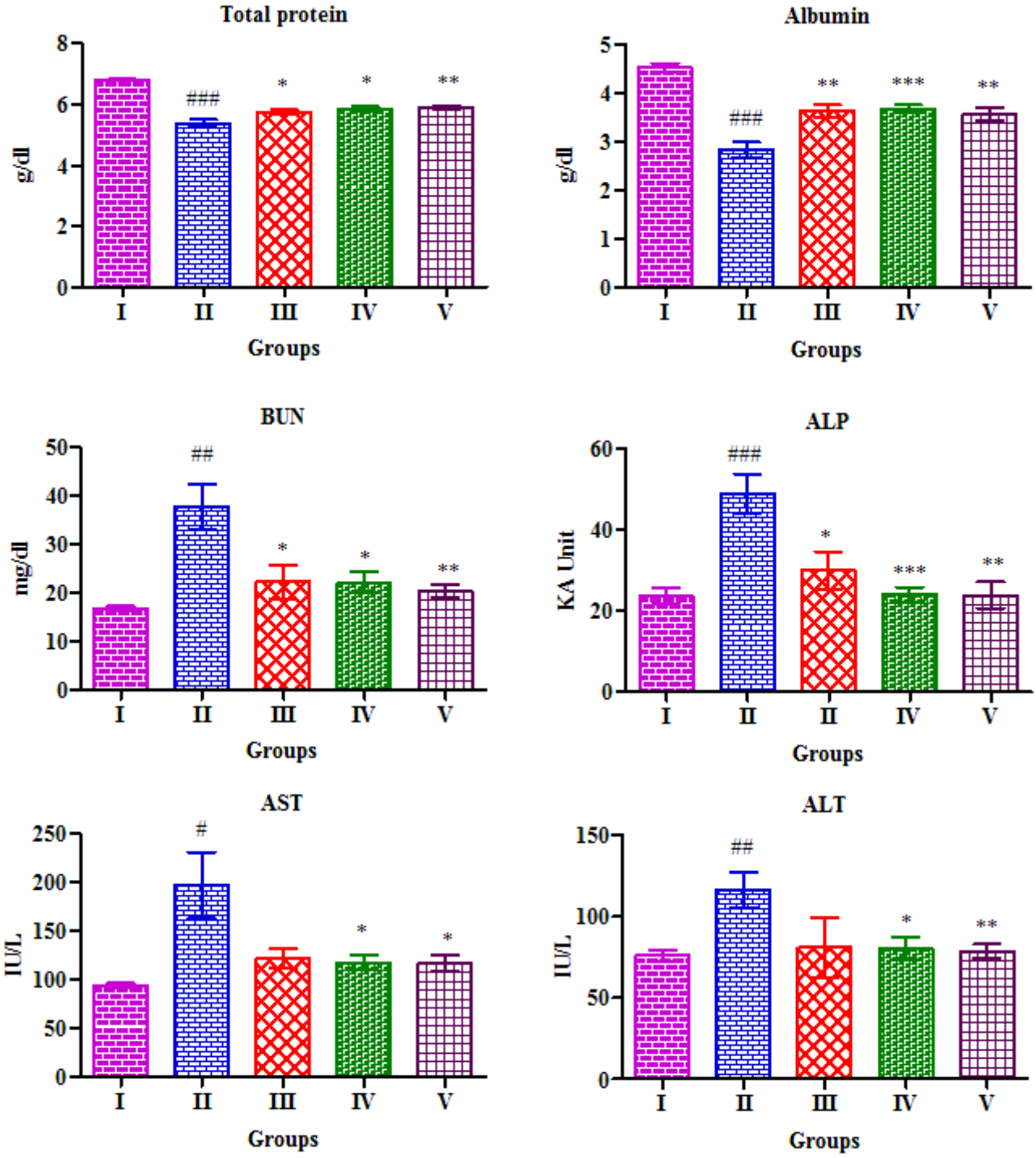


Figure 6: Effect of methanol extract of *Woodfordia fruticosa* flowers on different serum biochemical parameters in diclofenac (50 mg/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control diclofenac, Group III: WFM-400 mg/kg + diclofenac, Group IV: WFM-600 mg/kg + diclofenac, Group V: Silymarin-100 mg/kg + diclofenac. Results are expressed as mean ± SEM, (n = 6). $^{\#}P < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ as compared with normal control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.01$ as compared with toxin control group.

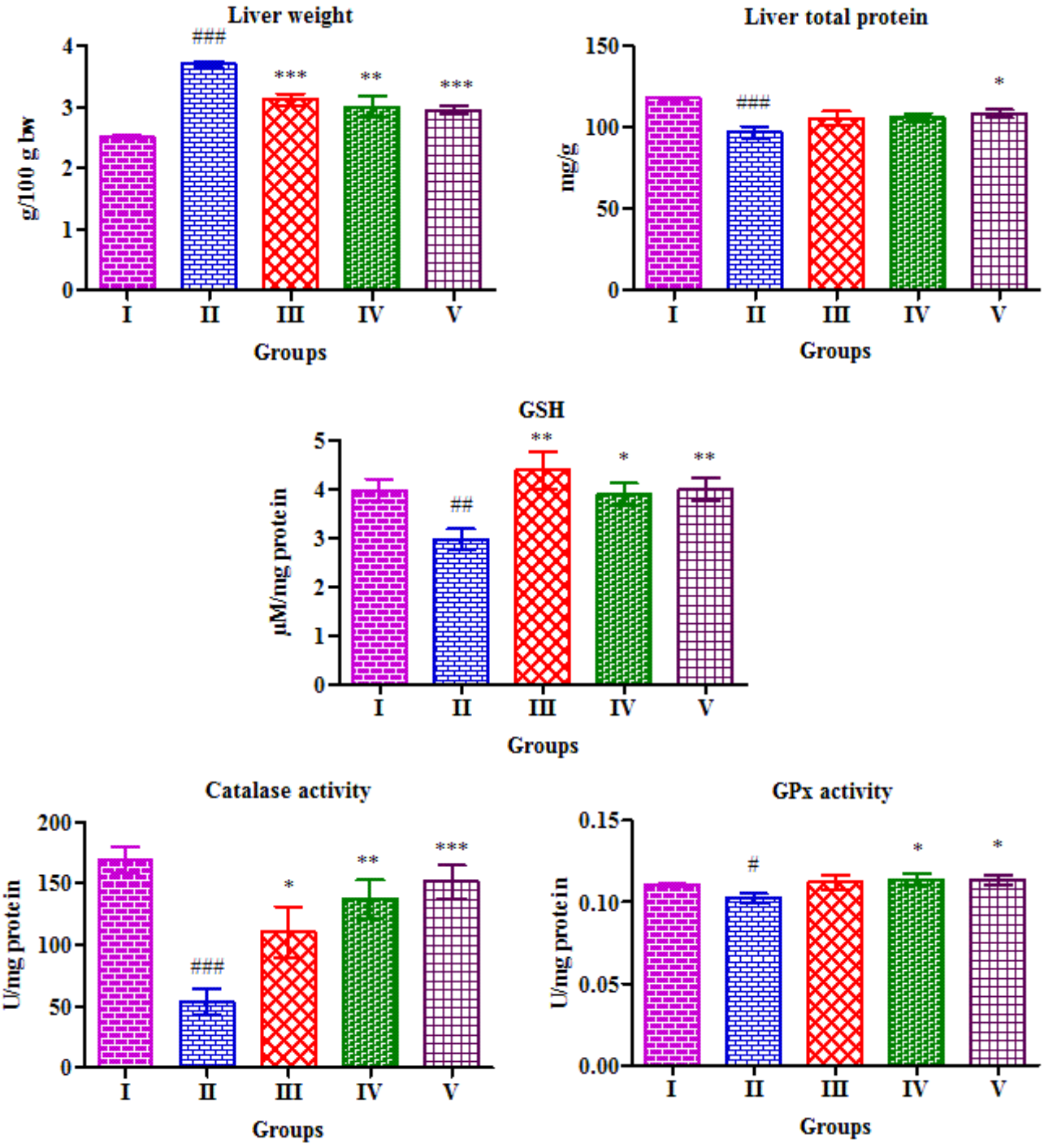


Figure 7: Effect of methanol extract of *Woodfordia fruticosa* flowers on relative liver weight, liver total protein and different liver antioxidants in diclofenac (50 mg/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control diclofenac, Group III: WFM-400 mg/kg + diclofenac, Group IV: WFM-600 mg/kg + diclofenac, Group V: Silymarin-100 mg/kg + diclofenac. Results are expressed as mean ± SEM, (n = 6). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ as compared with normal control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.01$ as compared with toxin control group.

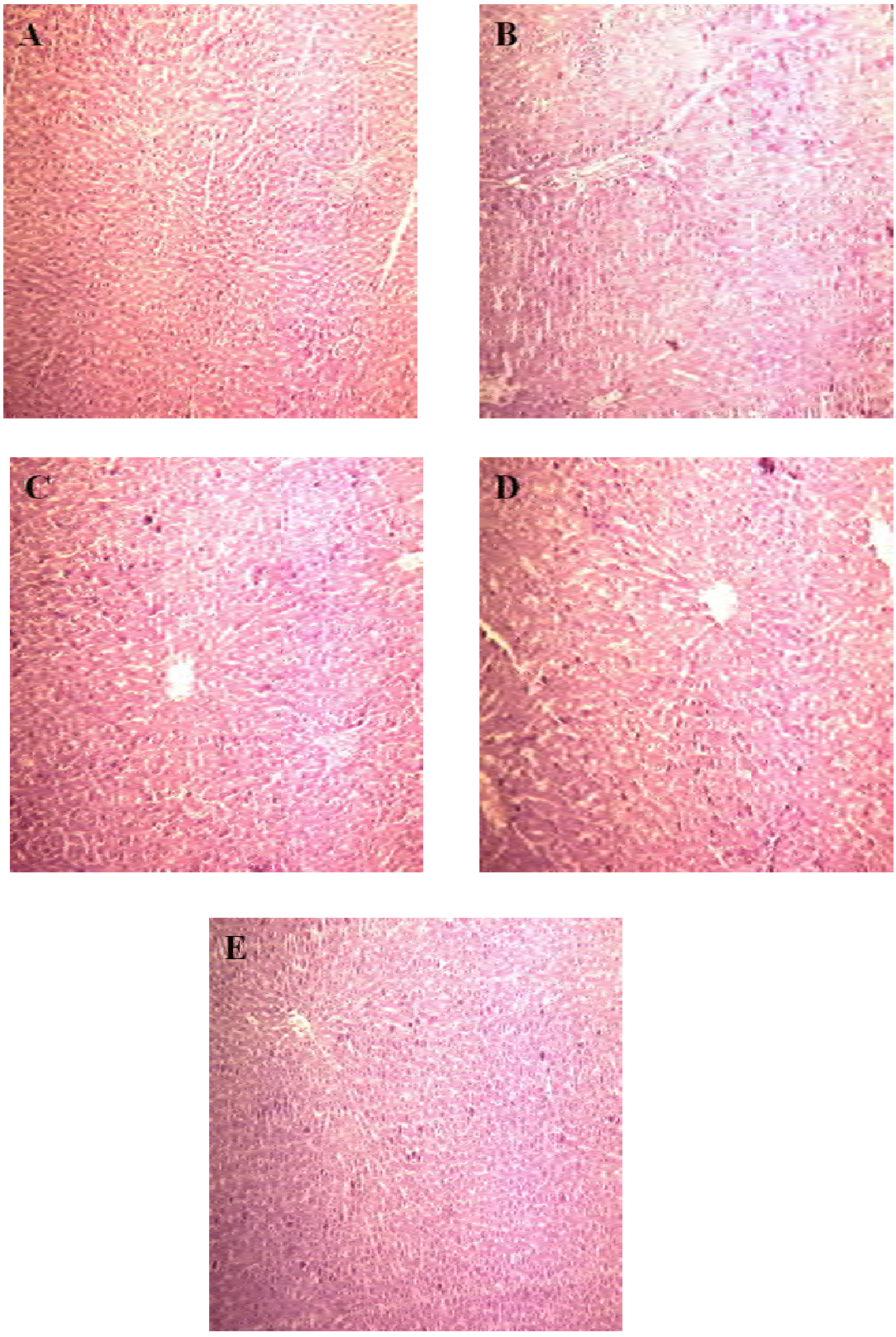


Figure 8: Photographs of liver sections of diclofenac (50 mg/kg) toxicity in rats (hematoxylin and eosin stained, 10x). (A) Normal control group, (B) Toxin control (diclofenac) group, (C) WFM-400 mg/kg + diclofenac, (D) WFM-600 mg/kg + diclofenac, (E) Silymarin-100 mg/kg + diclofenac.

($P < 0.01$) treated group as compared to the toxin control group. The result of the higher dose group was comparable to the standard drug treated group ($P < 0.001$). The total protein and GSH levels from liver homogenate in WFM treated groups elevated, but total protein level was not significant. However, pretreatment with WFM significantly recovered the diclofenac induced GSH depletion in lower and higher dose group ($P < 0.01$, $P < 0.05$ respectively). The catalase and GPx activity increased at both the dose levels; at higher dose WFM exhibited good activity ($P < 0.01$, $P < 0.05$ respectively). GPx activity of WFM-600 group was similar to that of standard drug treated group.

In normal control animal liver, histological sections showed hepatocytes that were well-preserved, uniform cytoplasm and sinusoidal spaces (Figure 8A). Compared with the normal control group, liver tissue in the rats treated with diclofenac revealed extensive liver injuries, characterized by severe hepatocellular degeneration, necrosis, inflammatory cell infiltration, sinusoidal dilatation and cytoplasmic vacuolation (Figure 8B). However, the histopathological hepatic lesions induced by administration of diclofenac was remarkably improved by the treatment with both the doses of WFM (Figure 8C & 8D), and showed marked protective effect by decreasing hepatocellular degeneration and necrosis. The protective effect was also observed in silymarin treated animals (Figure 8E). This was in good agreement with the results of serum aminotransferase activity and hepatic oxidative stress level.

4.6.2. Carbon tetrachloride induced hepatotoxicity

The results observed from serum biochemical parameters in pre-treatment of WFM with respect to induction of hepatotoxicity using CCl_4 are given in Figure 9. A marked reduction in total protein and albumin levels was observed in the group treated with CCl_4 and they were significantly decreased ($P < 0.05$) when compared with the normal control group. The BUN and ALP levels increased in the group treated with CCl_4 but not to a significant level. Rats treated with CCl_4 (toxin control) developed significant liver damage and it was well indicated by elevated levels of hepato specific enzymes like AST ($P < 0.01$) and ALT ($P < 0.001$) in serum.

The groups received the pre-treatment of WFM at dose levels of 400 and 600 mg/kg body weight significantly controlled the change in the biochemical parameters. The

extract at dose levels of 400 and 600 mg/kg exhibited significant increase ($P < 0.01$, $p < 0.05$ respectively) in the serum total protein level as compared to toxin control group and the effect was comparable with the standard group ($P < 0.01$) treated with silymarin (Sily-100). The albumin level also increased in drug treated groups but not to a significant level. The level of BUN was reduced in both the dose of WFM and standard drug treated groups, but it was not significant. The ALP ($P < 0.05$), AST ($P < 0.01$) and ALT ($P < 0.01$) levels significantly decreased in WFM-400 group as compared to toxin control group. WFM-600 group also showed significant decreased ($P < 0.05$) AST and ALP levels.

The result of relative liver weight, liver total protein, GSH and antioxidant enzymes in CCl_4 induced hepatotoxicity are given in Figure 10. The relative liver weight in toxin control group increased significantly ($P < 0.001$) as compared to normal control group. The total protein level in liver decreased significantly ($P < 0.001$) in toxin control group. The level of GSH in toxin control group decreased, but it was non-significant. The catalase (CAT) and GPx activities in the toxin control group depleted significantly ($P < 0.05$, $P < 0.001$ respectively) as compared to the normal control group. The mean relative liver weight in WFM at both the doses was slightly elevated as compared to the toxin control group. The total protein level in liver, in WFM treated as well as in the standard drug treated group, increased significantly ($P < 0.001$) as compared to toxin control group. GSH level increased significantly ($P < 0.01$) at higher dose as compared to toxin control group. Catalase activity increased at both the dose levels though not significantly, while in silymarin group, catalase activity decreased. Administration of WFM did not display effect of increase in the GPx activity.

The histopathological feature, as shown in Figure 11(A) indicated the normal liver lobular architecture and cell structure of the liver in the normal control animals. There were no pathological changes observed in normal control animals. In CCl_4 treated animals, there was a vacuolar degeneration of hepatocytes around central vein with moderate to severe hepatocyte necrosis due to CCl_4 toxicity (Figure 11B). The histological observations also supported results obtained from the serum enzyme levels.

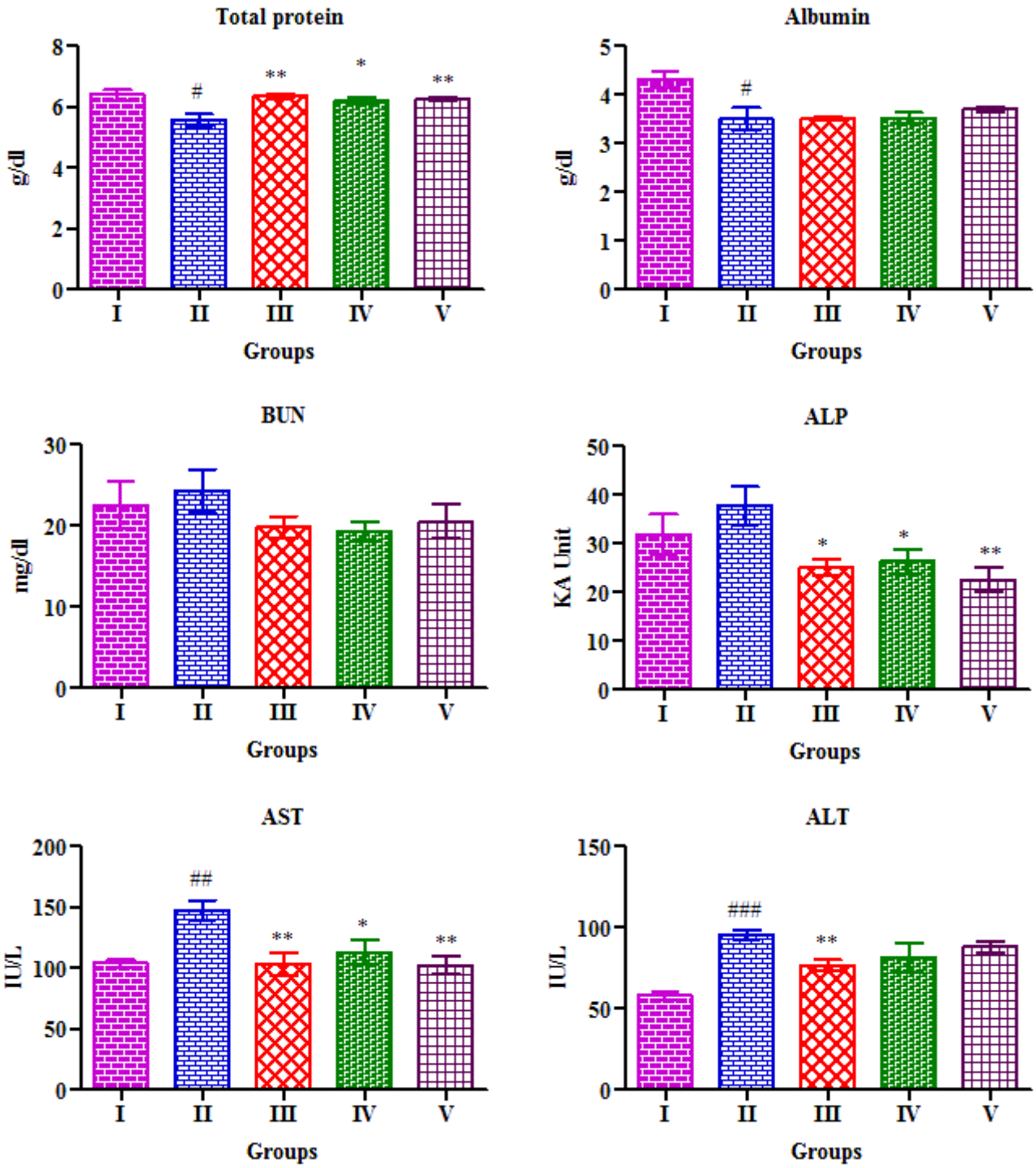


Figure 9: Effect of methanol extract of *Woodfordia fruticosa* flowers on different serum biochemical parameters in CCl_4 (2 ml/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control CCl_4, Group III: WFM-400 mg/kg + CCl_4, Group IV: WFM-600 mg/kg + CCl_4, Group V: Silymarin-100 mg/kg + CCl_4. Results are expressed as mean ± SEM, (n = 6). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ as compared with normal control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.01$ as compared with toxin control group.

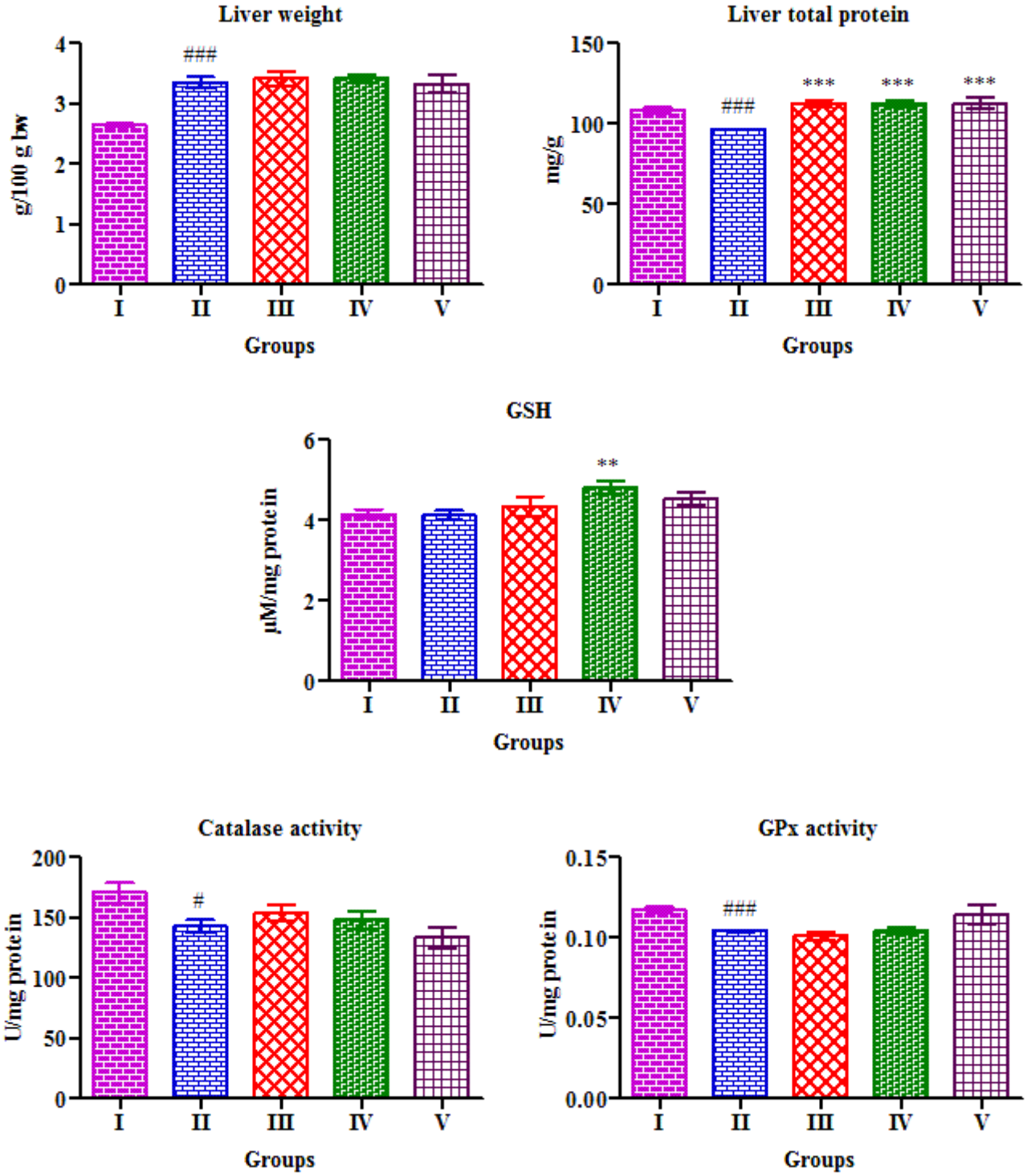


Figure 10: Effect of methanol extract of *Woodfordia fruticosa* flowers on relative liver weight, liver total protein and different liver antioxidants in CCl_4 (2 ml/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control CCl_4, Group III: WFM-400 mg/kg + CCl_4, Group IV: WFM-600 mg/kg + CCl_4, Group V: Silymarin-100 mg/kg + CCl_4. Results are expressed as mean ± SEM, (n = 6). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ as compared with normal control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.01$ as compared with toxin control group.

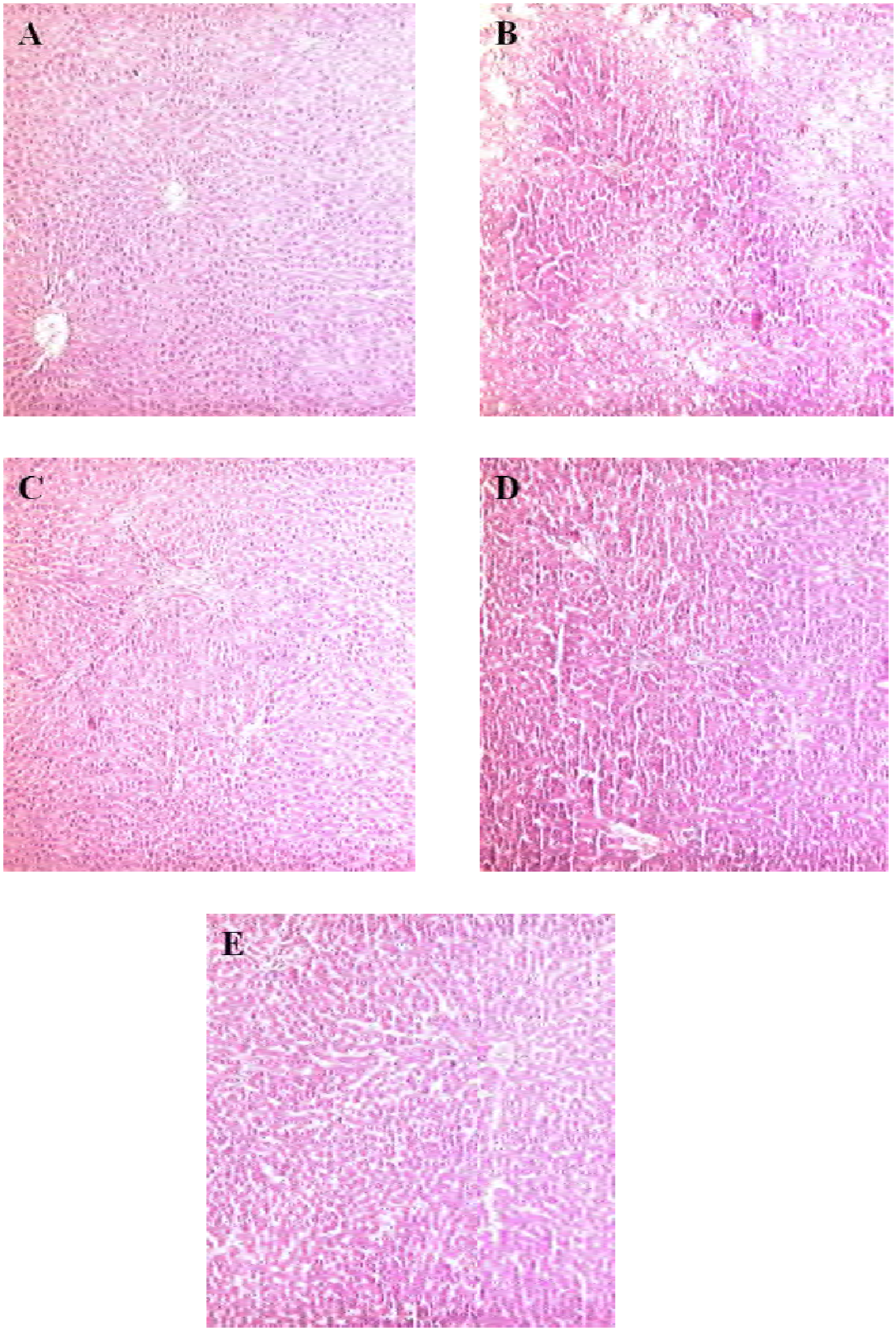


Figure 11: Photographs of liver sections of CCl_4 (2 ml/kg) toxicity in rats (hematoxylin and eosin stained, 10x). (A) Normal control, (B) Toxin control (CCl_4), (C) WFM-400 + CCl_4, (D) WFM-600 + CCl_4, (E) Silymarin-100 + CCl_4.

These histopathological changes were remarkably reversed in WFM treated animals, dose dependently with lesser vacuolar degeneration and hepatic necrosis (Figure 11C & 11D). Similar changes were also observed in the silymarin treated animals (Figure 11E).

4.6.3. Acetaminophen induced hepatotoxicity

Oral administration of Acetaminophen (APAP) caused significant liver damage as evidenced by altered biochemical parameters (Figure 12). APAP significantly ($P < 0.001$) decreased serum levels of total protein and albumin as compared to normal control group. APAP significantly ($P < 0.01$) enhanced BUN, ALP, AST and ALT levels in the blood circulation; about 3-fold increase was observed in AST and ALT levels in serum.

Treatment with WFM did not exhibit potential effect on recovery of total protein and albumin levels; while in standard drug treated group, the level of total protein and albumin levels increased significantly ($P < 0.01$). The BUN and ALP levels also decreased significantly in lower as well as higher dose of WFM ($P < 0.01$, $P < 0.05$ respectively) as compared to toxin control group. 400 and 600 mg/kg of WFM treated group showed significant ($P < 0.001$, $P < 0.01$ respectively) decrease in AST level as compared to toxin control group. The result of AST was similar to that of the standard drug treated group ($P < 0.001$). ALT level decreased in WFM treated groups towards normalization though not significantly.

The result of relative liver weight, liver total protein, GSH and antioxidant enzymes in APAP induced hepatotoxicity are given in Figure 13. The administration of APAP significantly increased the liver weight ($P < 0.001$) as compared to normal control group.

Significant decreased level was observed in hepatic total protein ($P < 0.001$). The administration of APAP significantly decreased the hepatic non-enzymatic antioxidant GSH contents ($P < 0.05$). The treatment of WFM decreased liver weight significantly ($P < 0.05$) at both the dose levels as compared to toxin control group.

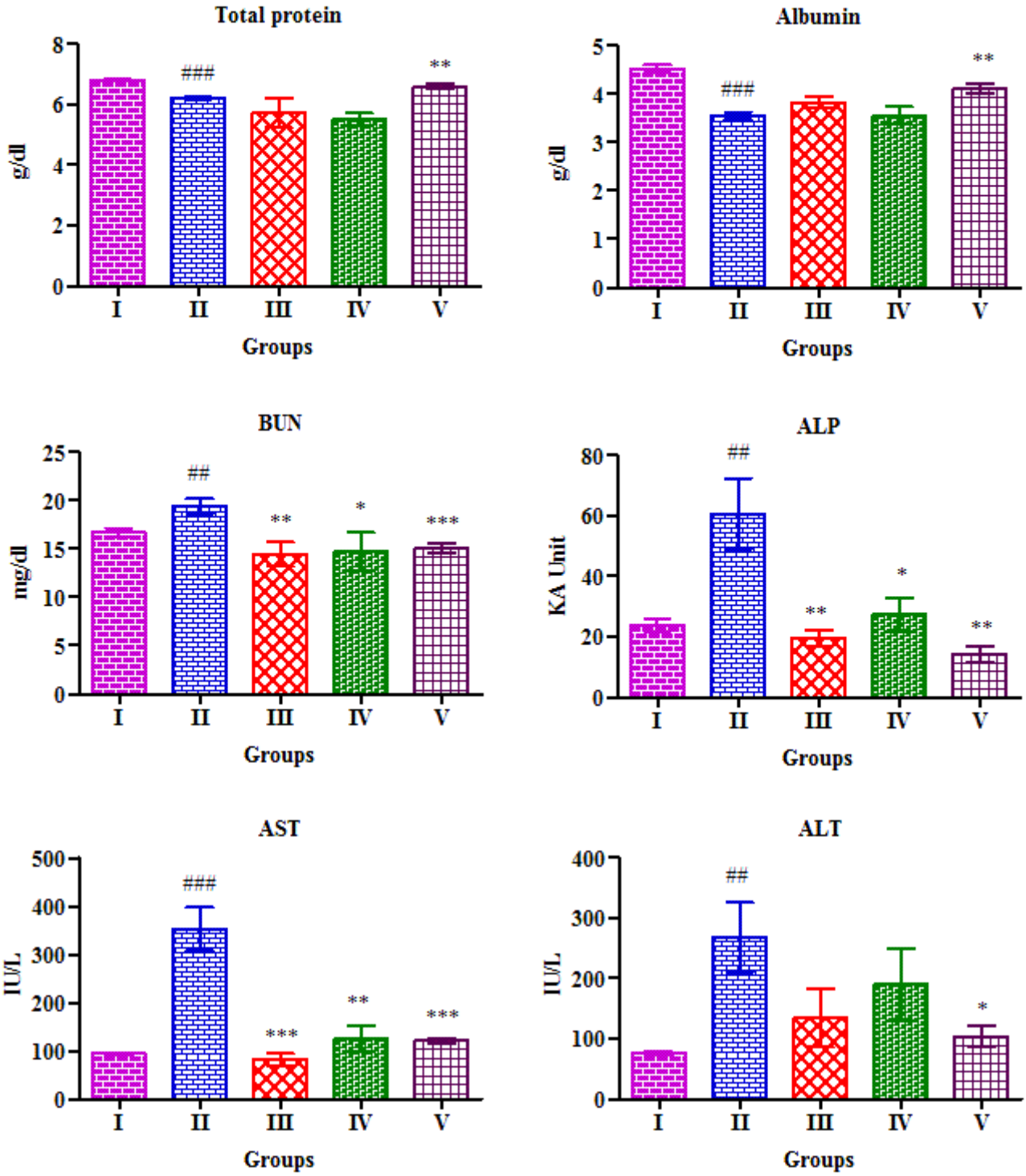


Figure 12: Effect of methanol extract of *Woodfordia fruticosa* flowers on different serum biochemical parameters in APAP (3 g/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control APAP, Group III: WFM-400 mg/kg + APAP, Group IV: WFM-600 mg/kg + APAP, Group V: Silymarin-100 mg/kg + APAP. Results are expressed as mean ± SEM, (n = 6). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ as compared with normal control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.01$ as compared with toxin control group.

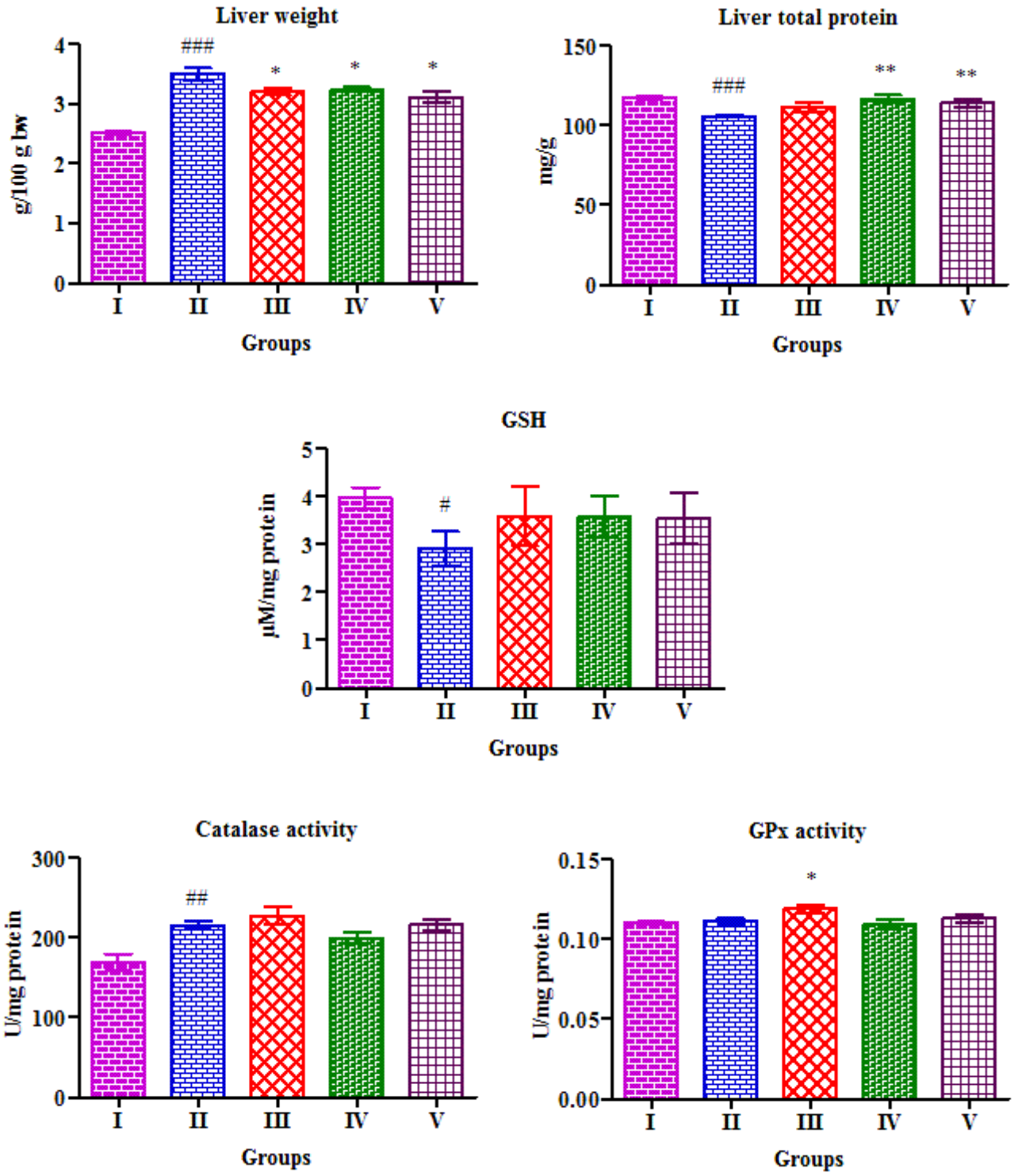


Figure 13: Effect of methanol extract of *Woodfordia fruticosa* flowers on relative liver weight, liver total protein and different liver antioxidants in APAP (3 g/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control APAP, Group III: WFM-400 mg/kg + APAP, Group IV: WFM-600 mg/kg + APAP, Group V: Silymarin-100 mg/kg + APAP. Results are expressed as mean ± SEM, (n = 6). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ as compared with normal control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.01$ as compared with toxin control group.

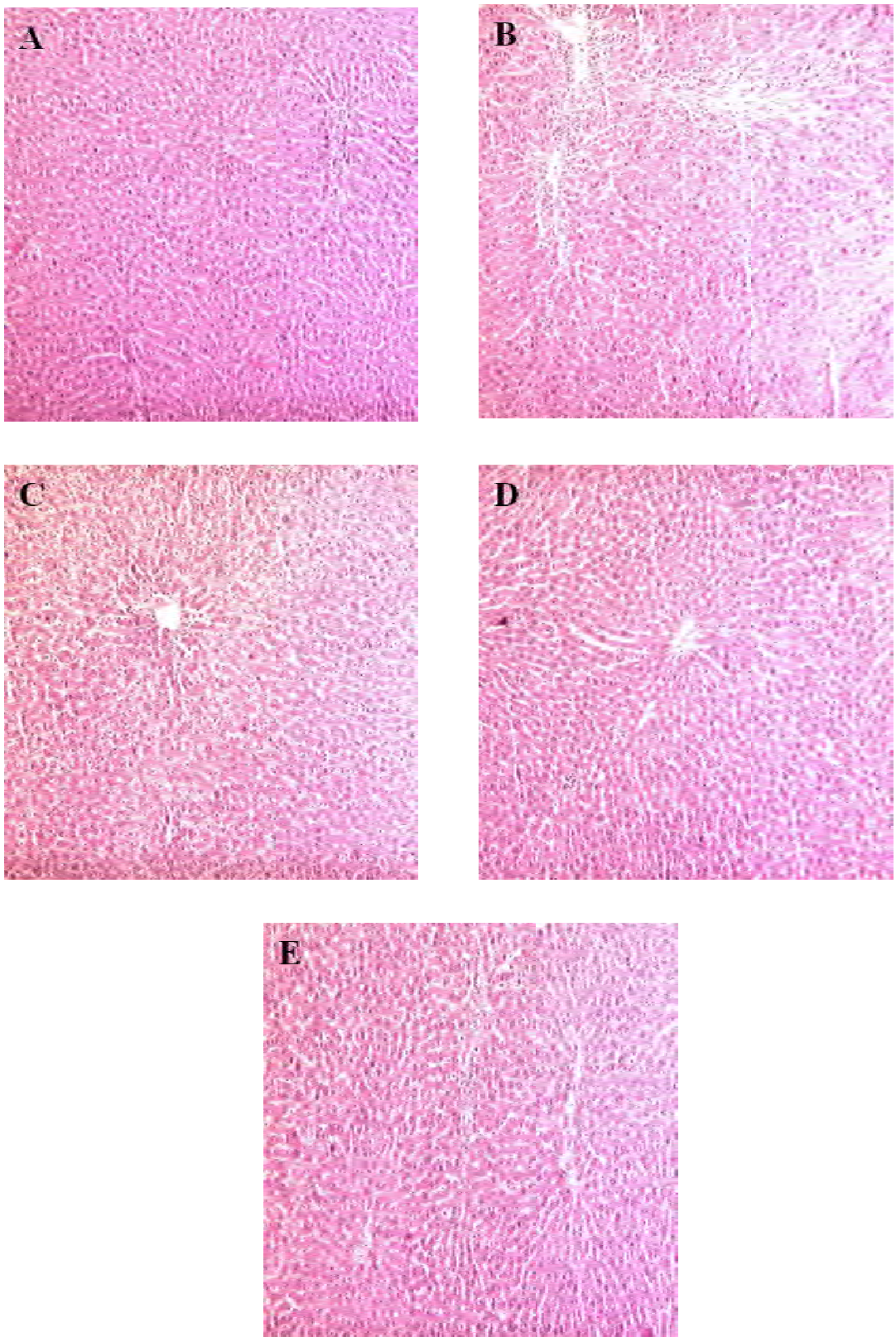


Figure 14: Photographs of liver sections of APAP (3 g/kg) toxicity in rats (hematoxylin and eosin stained, 10x). (A) Normal control, (B) Toxin control (APAP), (C) WFM-400 + APAP, (D) WFM-600 + APAP, (E) Silymarin-100 + APAP.

In higher dose, the level of hepatic total protein increased significantly ($P < 0.01$). The hepatoprotective efficacy of the WFM-600 was comparable with that of standard drug silymarin. WFM treatment enhanced the production of GSH towards normal control, but not to a significant level. Administration of APAP did not diminish the anti-oxidative status of hepatic catalase and GPx activity.

Liver histopathologic examination showed no histological abnormalities in normal control liver (Figure 14A); the hepatic lobular architecture was normal, connective tissue proliferation was not seen. In acetaminophen treated animals, the liver pathological changes were characterized by severe hepatocellular degeneration and necrosis along with periportal mononuclear cell infiltration due to acetaminophen toxicity (Figure 14B). WFM protected the liver tissue against acetaminophen toxicity (Figure 14C & 14D), with mild hepatocellular degeneration, necrosis, less inflammatory cell infiltration and well preserved hepatocytes were observed in most areas when compared with that of APAP group. The recovery from degeneration of hepatic cells of WFM treated animals was comparable to that of standard drug treated animals (Figure 14E).

4.7. ACUTE TOXICITY STUDY

In acute toxicity study, no adverse reactions or mortality were observed after administration of WFM (450, 1800, and 3600 mg/kg bw) and no behavioral changes were observed during the entire period of experimentation. Some alteration was noticed in daily feed and water intake in both male and female rats (Figure 15 & 16) treated with single dose of extract as well as in control animals. As compared to the control group, drug treated groups had several consecutive days of reduced/increased feed and water consumption at different times in the study. These periods of reduced/increased feed and water intake were not significant to the overall feed and water consumption rates. Individual body weights were recorded daily during the experimental period. Mean body weight gains were calculated for each group. In control and WFM treated groups, body weight of animals slightly increased during experimental period (Figure 17), but the increase was not significant.

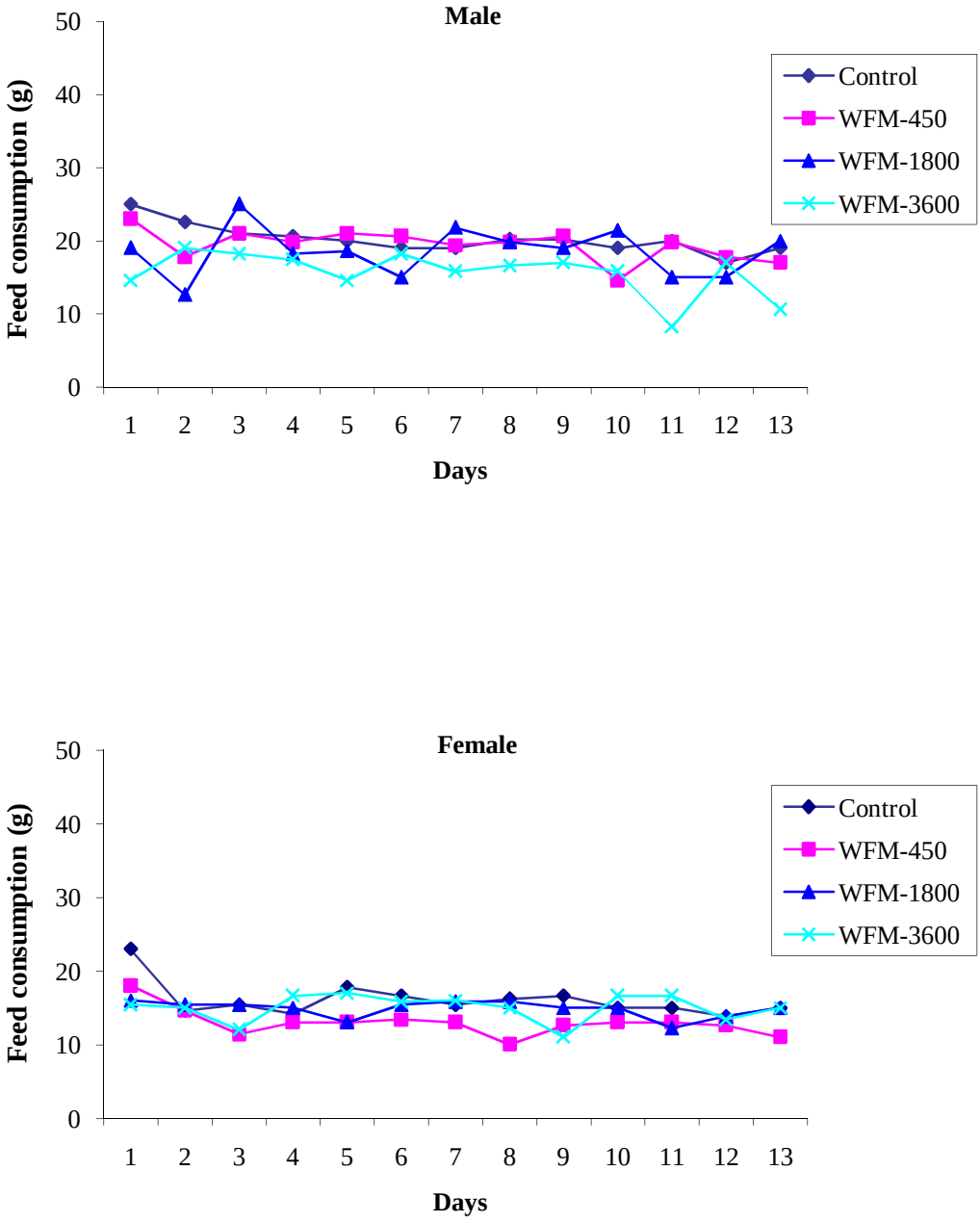


Figure 15: Changes in feed consumption (g/day/animal) of male and female rats during acute toxicity study.

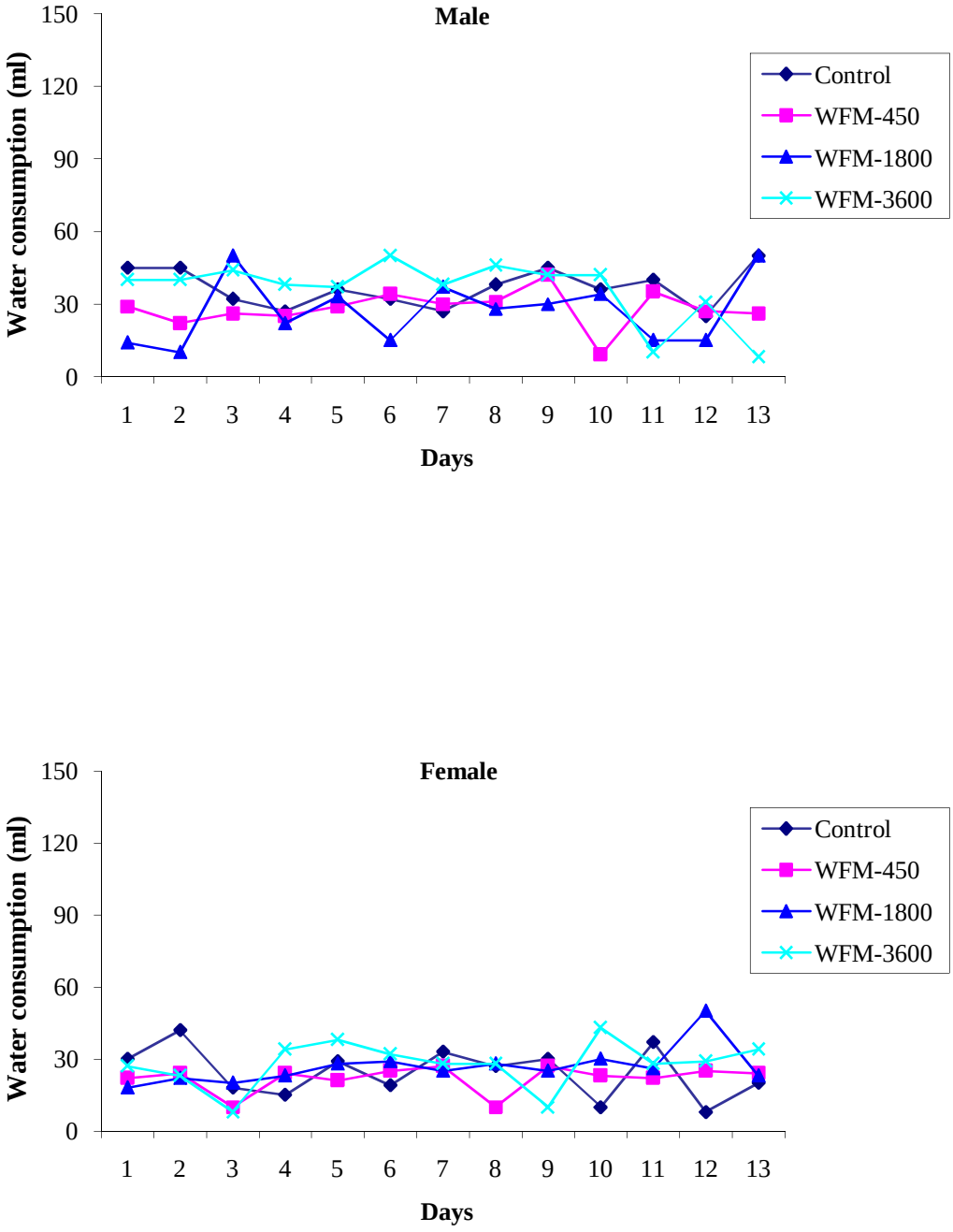


Figure 16: Changes in water consumption (ml/day/animal) of male and female rats during acute toxicity study.

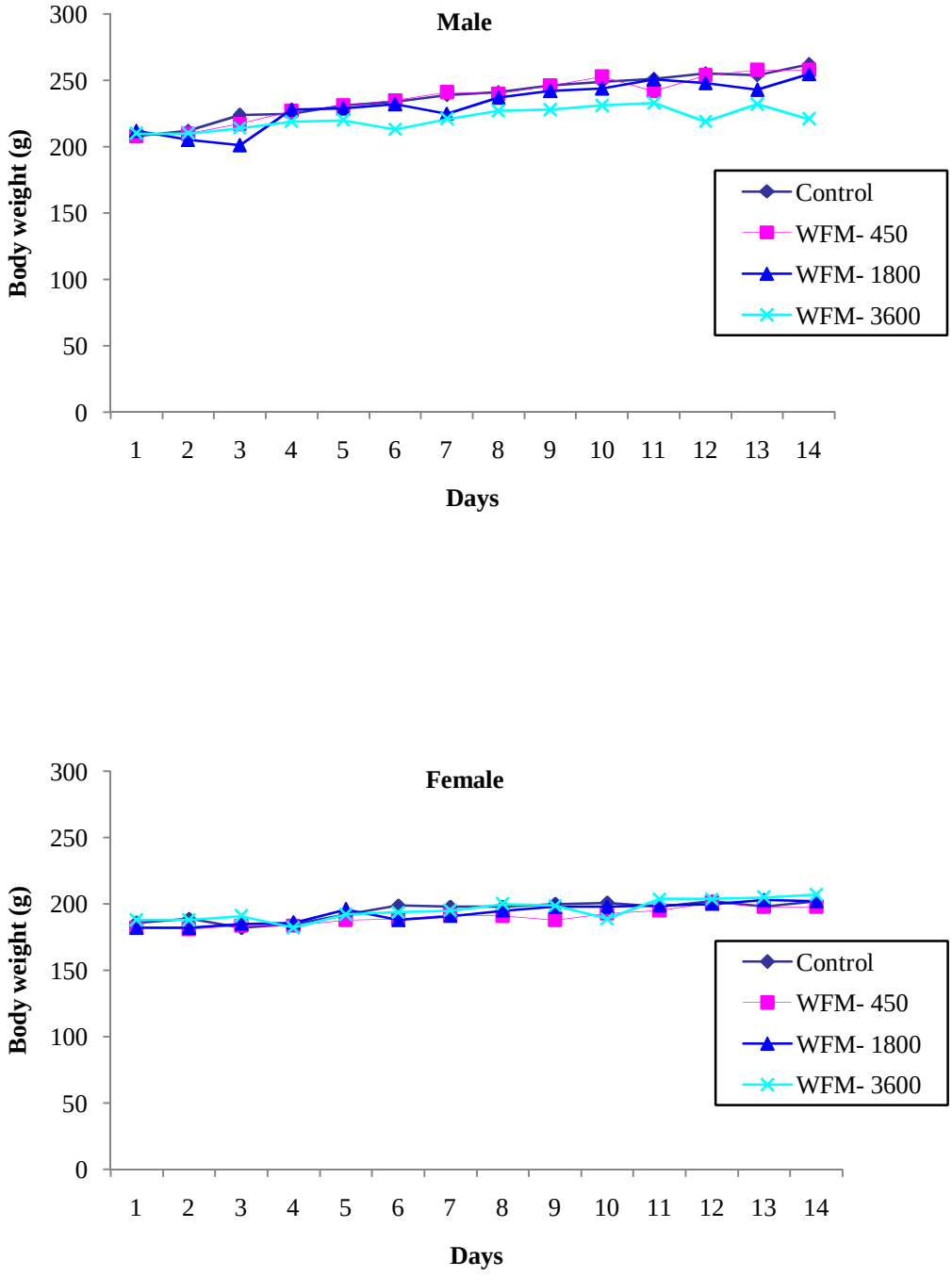


Figure 17: Changes in body weight (g) of male and female rats during acute toxicity study.

The relative organ weights of male and female rats are shown in Table 18 and 19 respectively. Gross examination of internal organs of all the rats revealed no detectable abnormalities. The organs like heart, liver, brain, thymus, spleen and adrenal of rats did not illustrate any significant change in their relative organ weights at any doses as compared to control. There were alterations in the weight of a few organs but this was not observed with all doses. The relative weight of kidney in male rats significantly increased ($P < 0.05$) at 450 mg/kg dose, while in female rats it significantly decreased ($P < 0.05$) at 1800 and 3600 mg/kg dose level.

Table 18: Relative organ weight (g/100 g body weight) of male rats treated with methanol extract of *Woodfordia fruticosa* flowers in acute toxicity study

Organs	**Control**	**WFM (mg/kg, b.w.) treated group**		
		450	**1800**	**3600**
Heart	0.324 ± 0.009	0.350 ± 0.012	0.334 ± 0.011	0.338 ± 0.013
Liver	2.556 ± 0.059	2.647 ± 0.090	2.711 ± 0.043	2.558 ± 0.070
Brain	0.652 ± 0.021	0.676 ± 0.057	0.734 ± 0.061	0.734 ± 0.050
Kidney	0.348 ± 0.009	0.388 ± 0.012*	0.364 ± 0.012	0.356 ± 0.011
Lung	0.575 ± 0.023	0.701 ± 0.027*	0.579 ± 0.031	0.781 ± 0.049*
Thymus	0.217 ± 0.012	0.205 ± 0.010	0.199 ± 0.007	0.195 ± 0.015
Spleen	0.243 ± 0.005	0.244 ± 0.012	0.243 ± 0.016	0.241 ± 0.013
Adrenal	0.0075 ± 0.000	0.0088 ± 0.001	0.0080 ± 0.000	0.0087 ± 0.001
Testis	0.507 ± 0.006	0.531 ± 0.005*	0.539 ± 0.019	0.555 ± 0.009*

Values are expressed as mean ± SEM (n = 5), *$P < 0.05$.

The relative weight of lung in male rats significantly increased ($P < 0.05$) at 450 and 3600 mg/kg dose level and in female rats it significantly increased ($p < 0.05$) at 3600 mg/kg dose level. In female rats relative lung weight at lower doses slightly increased but not to a significant level. The relative weight of testis significantly increased ($P < 0.05$) at 450 and 3600 mg/kg dose level, while at the same doses the relative uterus weight significantly ($P < 0.05$) decreased.

Table 19: Relative organ weight (g/100 g body weight) of female rats treated with methanol extract of *Woodfordia fruticosa* flowers in acute toxicity study

Organs	Control	WFM (mg/kg, b.w.) treated group		
		450	1800	3600
Heart	0.330 ± 0.01	0.332 ± 0.008	0.325 ± 0.005	0.316 ± 0.027
Liver	2.728 ± 0.059	2.589 ± 0.045	2.636 ± 0.052	2.475 ± 0.266
Brain	0.857 ± 0.051	0.851 ± 0.091	0.916 ± 0.059	0.787 ± 0.054
Kidney	0.376 ± 0.013	0.354 ± 0.015	0.338 ± 0.14*	0.318 ± 0.025*
Lung	0.720 ± 0.028	0.779 ± 0.023	0.846 ± 0.059	0.902 ± 0.062*
Thymus	0.226 ± 0.010	0.228 ± 0.023	0.223 ± 0.006	0.208 ± 0.019
Spleen	0.272 ± 0.010	0.298 ± 0.009	0.294 ± 0.008	0.247 ± 0.026
Adrenal	0.0117 ± 0.001	0.0122 ± 0.001	0.0131 ± 0.001	0.0134 ± 0.001
Uterus	0.390 ± 0.023	0.304 ± 0.021*	0.327 ± 0.031	0.239 ± 0.015*

Values are expressed as mean ± SEM (n = 5), *$P < 0.05$.

The hematological parameters of male and female rats are shown in Table 20 and 21 respectively. In male rats the RBC count, Hb and PCV increased significantly ($P < 0.05$) at 3600 mg/kg dose level. In female rats at dose level of 1800 and 3600 mg/kg, the Hb level significantly ($P < 0.05$) increased. The WBC count in both sexes decreased at lower dose, in male rats it was significant ($P < 0.05$) while in female rats it was not significant. This increase and decrease level of some parameters was not dose dependent. There were no significant changes observed in MCV, MCH, MCHC, platelet count and differential leukocyte counts of the treated animals as compared to control animals.

Table 20: Hematological parameters of male rats treated with methanol extract of *Woodfordia fruticosa* flowers in acute toxicity study

Parameters	Control	WFM (mg/kg, b.w.) treated group		
		450	1800	3600
RBC (10^6/µl)	8.48 ± 0.12	8.544 ± 0.14	8.616 ± 0.17	9.078 ± 0.17*
Hb (g/dl)	15.52 ± 0.24	15.34 ± 0.26	15.5 ± 0.31	16.4 ± 0.16*
PCV (%)	43.98 ± 0.58	43.66 ± 0.77	44.44 ± 0.84	46.34 ± 0.63*
MCV (F1)	51.86 ± 0.19	51 ± 0.24	51.6 ± 0.19	51.06 ± 0.52
MCH (pg)	18.3 ± 0.09	17.96 ± 0.13	17.96 ± 0.13	18.08 ± 0.24
MCHC (g/dl)	35.26 ± 0.24	35.18 ± 0.19	34.84 ± 0.20	35.15 ± 0.23
Platelet Count (10^3/µl)	940.2 ± 175.6	1299.4 ± 30.8	1172.8 ± 92.0	1254.0 ± 49.7
WBC (10^3/µl)	11.70 ± 0.70	9.68 ± 0.20*	9.02 ± 1.02	12.98 ± 1.19
Neutrophils (%)	22.4 ± 2.01	18.2 ± 2.13	22.2 ± 2.22	24.4 ± 3.98
Lymphocytes (%)	77.4 ± 2.06	81.4 ± 2.16	77.6 ± 2.16	75.4 ± 3.89
Eosinophls (%)	0.2 ± 0.00	0.4 ± 0.24	0.2 ± 0.00	0.2 ± 0.00
Monocytes (%)	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Basophils (%)	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

Values are expressed as mean ± SEM (n = 5), *P < 0.05.

RBC - red blood cell; Hb - hemoglobin; PCV - packed cell volume; MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular haemoglobin concentration; WBC - white blood cell

Table 21: Hematological parameters of female rats treated with methanol extract of *Woodfordia fruticosa* flowers in acute toxicity study

Parameters	Control	WFM (mg/kg b.w.) treated group		
		450	1800	3600
RBC (10^6/µl)	7.864 ± 0.28	7.688 ± 0.13	8.246 ± 0.25	8.286 ± 0.10
Hb (g/dl)	14.7 ± 0.28	14.66 ± 0.22	15.98 ± 0.39*	16.16 ± 0.22*
PCV (%)	39.9 ± 1.22	38.92 ± 0.62	42.48 ± 1.05	42.84 ± 0.60
MCV (F1)	50.78 ± 0.47	50.64 ± 0.54	51.54 ± 0.31	51.7 ± 0.29
MCH (pg)	18.74 ± 0.49	19.08 ± 0.27	19.4 ± 0.21	19.52 ± 0.18
MCHC (g/dl)	36.9 ± 0.70	37.7 ± 0.28	37.64 ± 0.24	37.58 ± 0.70
Platelet Count (10^3/µl)	1041.2 ± 159.0	1018.0 ± 217.8	1119.6 ± 207.0	981.4 ± 187.2
WBC (10^3/µl)	9.52 ± 0.92	8.10 ± 0.72	9.20 ± 0.97	8.34 ± 1.36
Neutrophils (%)	17.8 ± 1.98	13.6 ± 1.36	20 ± 3.08	23.4 ± 2.11
Lymphocytes (%)	81.8 ± 2.03	86.8 ± 1.66	79.4 ± 3.03	76 ± 2.30
Eosinophls (%)	0.4 ± 0.00	0.4 ± 0.00	0.6 ± 0.00	0.6 ± 0.00
Monocytes (%)	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Basophils (%)	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

Values are expressed as mean ± SEM (n = 5), *P < 0.05.

RBC - red blood cell; Hb - hemoglobin; PCV - packed cell volume; MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular haemoglobin concentration; WBC - white blood cell

Chapter 5

DISCUSSION

CHAPTER 5: DISCUSSION

5.1. PHARMACOGNOSTIC STUDIES

The pharmacognostical study is a major and reliable criterion of identification of plant drugs. The pharmacognostic parameters are necessary for confirmation of the identity and determination of quality and purity of a crude drugs (Bhattacharya and Zaman, 2009). To ensure reproducible quality of herbal products, proper control of starting material is utmost essential (Venkatesh et al., 2004). Thus, in recent years there has been an emphasis on standardization of medicinal plants, and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means. Pharmacognostic studies on different plants has been done by various workers (Khatoon et al., 2006; Abere et al., 2007; Dave et al., 2010; Essiett et al., 2010; Sandhya et al., 2010). According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken (WHO, 2002). The flowers of *Woodfordia fruticosa* have three types of matured stomata viz. anomocytic, actinocytic and anisocytic. Stomata is the main factor responsible for the physiological activities of the plant, abnormal stomata is responsible for behavior and hormonal imbalance in plants (Kridemann et al., 2000). In calyx both rosette and cluster type of calcium oxalate crystals were found; these could be used to distinguish the species.

5.2. PHYSICOCHEMICAL ANALYSIS

The physical constant evaluation of the powder is an important parameter in detecting adulteration or improper handling of drugs. The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis. Therefore, the loss on drying of plant materials should be determined and the water content should also be controlled. The moisture content of dry powder of *Woodfordia fruticosa* flowers was 8 % which is not very high, hence it would discourage bacteria fungi or yeast growth (Bhattacharya and Zaman, 2009). The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter

such as metallic salts and/or silica (Musa et al., 2006; Chanda et al., 2010). Low amount of total ash, acid insoluble ash and water soluble ash indicate that the inorganic matter and non-physiological matter such as silica is less in *Woodfordia fruticosa* flowers. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. The variation in extractable matter in various solvents is suggestive of the fact that the formation of the bioactive principle of the medicinal plants is influenced by number of intrinsic and extrinsic factors. High alcohol soluble and water soluble extractive values reveal the presence of polar substance like phenols, tannins and glycosides, as reported by Sharma et al. (2009) and Baravalia et al. (2010).

Heavy metals like lead, chromium and cadmium were absent, while mercury and arsenic were present in very low amount, which was within the permissible limits of heavy metals (The Ayurvedic Pharmacopoeia of India, 2008). Therefore it can be stated that the extract was free from heavy metal contamination.

5.3. PHYTOCHEMICAL ANALYSIS

The phytoconstituents are known to play an important role in bioactivity of medicinal plants. In qualitative phytochemical analysis, tannins and alkaloids were present in high amount as compared to other phytoconstituents analyzed. In quantitative phytochemical analysis, phenolic content was much more than flavonoid content. The presence of alkaloids, phenolic compounds, tannins, flavonoids have been associated with various degrees of anti-inflammatory, analgesic (Wang JR et al., 2008) and antioxidant activities (Molina et al., 2003; Gholivand et al., 2010). Therefore, the anti-inflammatory, analgesic and hepatoprotective effects observed in this study may be due to the activity(s) of one or a combination of some of the classes of compounds present in *Woodfordia fruticosa* flowers.

5.4. ANTI-INFLAMMATORY STUDIES

It is believed that current anti-inflammatory drugs such as opioids and non-steroidal anti-inflammatory drugs are not useful in all cases because of their side effects and

low potency (Jaishree et al., 2009). As a result, search for other alternatives became necessary and imperative. Novel anti-inflammatory agents could be discovered from medicinal plants containing a wide variety of phytoconstituents. Traditional medicine for the treatment of various diseases is becoming more popular. Many medicinal plants provide relief of symptoms comparable to that of conventional medicinal agents. Therefore, the present study was aimed at evaluating the scientific basis for the traditional use of *Woodfordia fruticosa* flowers using *in vivo* anti-inflammatory models.

5.4.1. Carrageenan induced rat paw edema

Carrageenan has been widely used as a harmful agent able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. This phlogistic agent, when injected locally into the rat paw, produced a severe inflammatory reaction, which was discernible within 30 min (John and Nodine, 1999; Marzouk et al., 2010). Carrageenan induced rat paw edema is a suitable *in vivo* model to predict the value of anti-inflammatory agents, which act by inhibiting the mediators of acute inflammation (Morebise et al., 2002). Carrageenan-induced hind paw edema in rat is a biphasic event. The early phase (90 - 180 min) of the inflammation is due to the release of histamine, serotonin and similar substances; and the later phase (270–360 min) is associated with the activation of kinin-like substances, i.e., prostaglandins, proteases and lysosome (Erdemoglu et al., 2009; Thomazzi et al., 2010). The methanol extract of *Woodfordia fruticosa* flowers inhibited the carrageenan induced rat paw edema formation, at both early and later phase. This result tends to suggest that the inhibitory effect of the extract on edema formation is probably due to the inhibition of the synthesis and/or release of the inflammatory mediators, especially the cyclooxygenase products. The carrageenan induced paw edema test is effectively controlled with the arachidonate cyclooxygenase (COX) inhibitors due to its COX-dependent mechanism, thus, it is suggested that the WFM may possess arachidonate COX inhibitory property.

5.4.2. Histamine induced rat paw edema

Histamine is another pro-inflammatory mediator involved in exudation and cell chemotaxis (Jutel et al., 2005). The histamine is a basic amine related with

inflammatory and allergic process causing, among several effects, both vasodilatation and increase of vascular permeability (Rang et al., 2001). Edema was reduced by WFM in a dose dependent manner till the end of 5th hour. The antihistaminic activity may be related to the inhibition of inflammation mediator formation. The extract may also inhibit histamine release from mast cells and/or block histamine receptors.

5.4.3. Dextran induced rat paw edema

Dextran is a polysaccharide of high molecular weight that induces anaphylactic reaction after injection in rats extremities, which is characterized by extravasation and edema formation, as a consequence of liberation of histamine and serotonin from mast cells (Van Wauwe and Goossens, 1989; Prakash et al., 2009). Thus, carrageenan and dextran induced models are suitable test procedure to screen anti-inflammatory agents. In this study, WFM exhibited dose-dependent inhibitory effect in dextran induced paw edema and was capable to reduced the inflammation up to 5 h. The ability of the extract to reduce the edema volume suggests that the phytochemicals present in the extract may block or counteract the release of any of those mediators, alone or in combination.

5.4.4. Serotonin induced rat paw edema

The extract effectively suppressed the inflammation produced by serotonin. The extract of *Woodfordia fruticosa* was able to significantly reduce paw edema, and these effects were similar to those exhibited by the group of rats treated with diclofenac. So it may be suggested that its anti-inflammatory activity is possibly backed by its anti-serotonin activity.

5.4.5. Formaldehyde induced rat paw edema

It is well known that inhibition of formaldehyde induced paw edema in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis (Greenwald, 1991). Thus formaldehyde induced paw edema is a model used for the evaluation of an agent with antiproliferative activity (Banerjee et al. 2000). Injection of formaldehyde

subcutaneously into hind paw of rats produces localized inflammation. In the present study, the methanol extract of *Woodfordia fruticosa* flowers significantly inhibited paw edema induced by formaldehyde. WFM showed significant decrease in paw volume till 48 h with both doses, which suggests its long duration of action.

5.4.6. Cotton pellet induced granuloma in rats

Cotton pellet-induced granuloma formation is a typical feature of an established chronic inflammatory reaction and can serve as a subchronic and chronic inflammatory test model for investigation of anti-arthritic substances (Panthong et al., 2004). This model has been employed to assess the transudative and proliferative components of chronic inflammation. The fluid adsorbed by the pellet greatly influences the wet weight of the granuloma whereas the dry weight correlates well with the amount of granulomatous tissue formed. The extract showed decrease in granuloma formation. This reflected its efficacy to reduce an increase in the number of fibroblasts and synthesis of collagen with mucopolysaccharide, which are natural proliferative events of granulation tissue formation.

At higher concentration of extract the protein level increased. Increase in protein level at higher concentration of methanol extract of *P. longifolia* was also reported by Tanna et al., (2009). The rise in protein and albumin levels at high doses suggests stabilization of the endoplasmic reticulum, leading to protein synthesis (Mondal et al., 2005).

5.5. ANALGESIC STUDY

5.5.1. Formaldehyde induced paw licking test in rats

The formaldehyde test has been described as a convenient method for producing and quantifying pain in rats (Dubuisson and Dennis, 1977). The test employs an adequate painful stimulus to which the animals show a spontaneous response and it is sensitive to commonly used analgesics. The pain stimulus, a continuous rather than a transient one, may have resemblance to some kinds of clinical pain and observations are made on animals which are restrained only lightly or not at all (Hunskaar et al., 1985;

Ghannadi et al., 2005). The advantage of the formaldehyde model of nociception was that it could discriminate between central and peripheral pain components. The test consists of two different phases which could be separated in time: the first one that occurs on the first 5 min after the formaldehyde injection was generated in the periphery through the activation of nociceptive neurons by the direct action of formaldehyde and the second phase that occurs between the 15^{th} and 30^{th} minute after formaldehyde injection, occurred through the activation of the ventral horn neurons at the spinal cord level (Tjolsen et al., 1992; Li et al., 2010). In the present study, WFM orally administered 1 h before formaldehyde injection, was capable of inhibiting the paw licking process when compared with the control group. It was observed that rats treated with WFM showed nociceptive reaction, which was dose-related and inhibited in second phase of formaldehyde test. Drugs that act primarily on the central nervous system inhibit both phases equally, while peripherally acting drugs inhibit the second phase only (Shibata et al., 1989). The second phase is an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs (Hunskaar and Hole, 1987; Rosland et al., 1990). The experimental results show that WFM produced better inhibitory effect during the second phase of the formaldehyde test. This experimental evidence suggests that the analgesic effect produced by WFM was involved in its peripheral action.

The anti-inflammatory activity and anti-nociceptive activity of WFM, justifies the traditional uses of *Woodfordia fruticosa* for the treatment of pain and inflammatory related ailments. Previous studies have revealed that the NSAIDs were capable of inhibiting this test (Vinegar et al., 1969; Rosa et al., 1971); Therefore mechanism of anti-inflammation of WFM may be similar to the mechanism exerted by the NSAIDs. Since prostaglandins are known to take part in the inflammatory and nociceptive processes (Marieb, 2000; Katzung, 2005; Zakaria et al., 2010), the anti-inflammatory and anti-nociceptive activities of the WFM could be due to the modulation of the COX or prostaglandins actions.

5.6. HEPATOPROTECTIVE STUDIES

Hepatic fibrosis is usually initiated by hepatocyte damage. Biologic factors such as hepatitis virus, bile duct obstruction, cholesterol overload, etc. or chemical factors

such as CCl_4 administration, alcohol intake are known to contribute to liver fibrosis. The incidence of chronic fibrosis is high, but there are no satisfactory agents with ascertained effectiveness and with fewer side effects on liver. So, finding effective ways to inhibit liver fibrosis and prevent the development of cirrhosis are of great significance (Wang et al., 2009). The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms which have been disturbed by a hepatotoxic agent is the index of its protective effect (Yadav and Dixit, 2003).

5.6.1. Diclofenac induced hepatotoxicity

Hepatotoxicity is currently a class warning for NSAIDs and infrequent hepatic injury has been observed for nearly all NSAIDs currently on the market. There are 3 drugs that have more commonly been associated with liver disease: diclofenac, sulindac, and aspirin (Purcell et al., 1991; Bjorkman, 1998).

Diclofenac, undergoes similar hepatic metabolism both in rat and in humans. Major metabolic pathways are the hydroxylation in position 4 and 5 and to a much lesser extent the formation of 3′-hydroxy- (humans) and 4′,5-dihydroxydiclofenac (rat and humans). Diclofenac and its metabolites undergo extensive conjugation with glucuronic acid and sulfate (Riess et al., 1978; Stierlin et al., 1979). The major constitutive P_{450} form involved in diclofenac hydroxylation in man is cytochrome P_{450}2C9, the human orthologous form of rat 2C11. Diclofenac forms selective protein adducts in livers of treated mice (Pumford et al., 1993; Kretz-Rommel and Boelsterli, 1994). This is caused by a transacylation reaction of its glucuronide conjugate. This mechanism has been proposed to explain both the allergic and intrinsic hepatotoxicity of the drug.

Since unwanted side effects of diclofenac in man and other mammals was reported to occur particularly in the liver (Ramesh et al., 2002; Triebskorn et al., 2004) it was thought of interest to evaluate WFM for its hepatoprotetive property in diclofenac induced hepatic damage in rats. In the present study, the administration of diclofenac to rats decreased the total protein and albumin level and increased the BUN level significantly. The pretreatment of WFM at two different dose levels restored the level

of protein, albumin and BUN towards normalization. Hepatocellular injury from metabolic inhibition, oxygen radical toxicity, immunologically mediated damage, or some other mechanism results in predominant elevations of aminotransferase and alkaline phosphatase (Manoukian and Carson, 1996). The ALT, AST and ALP levels were significantly elevated when rats were administered with diclofenac indicating hepatocellular damage. The increased levels of these enzymes were significantly decreased by pretreatment with WFM in dose dependent manner. This is the indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by diclofenac. The hepatoprotetive property of *Polyalthia longifolia* (Tanna et al., 2009), *Curcuma longa*, *Glycyrrhiza glabra* and *Moringa oleifera* (Hamza, 2007) in diclofenac induced hepatic damage in rats was reported.

The significant increased liver weight of diclofenac exposed animals seems to be due to toxic potential of diclofenac. The significant increase in weight of liver was, however, found to be associated with concomitant increase of serum AST and ALT enzyme levels. It is important to note that the elevated activity of serum AST and ALT recorded in this study may be due to loss of enzymes of liver tissue. Pretreatment of WFM decreased the liver weight significantly indicating recovery of liver tissue from damage. Significant decrease in total protein of the liver contents is a reflection of hepatic toxicity (Gatsing et al., 2005; Adebayo et al., 2010). The significant reductions of protein in diclofenac intoxicated group indicate depletion in the protein reserve and thus suggest hepatic toxicity. WFM administration increased the total protein content leading to normalization. GSH is an extremely efficient intracellular buffer for oxidative stress and GSH acts as a non-enzymatic antioxidant that reduces H_2O_2, hydroperoxides (ROOH) and xenobiotic toxicity (Kadiiska et al., 2000). The level of GSH depleted when animals were injected with diclofenac. The depleted level of GSH raised with the pretreatment of WFM. The catalase and GPx are enzymatic antioxidants widely distributed in all animal tissues that decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. Therefore, the reduction in the activity of these two enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. In the present study, WFM significantly restored the hepatic catalase and GPx activity, which indicated that WFM could scavenge reactive free radicals that eventually lessen the oxidative damage to the tissues and subsequently improved the

activities of these antioxidant enzymes. The preventive effect of WFM was also confirmed by the results of histopathological study, as evidenced by a dose related decrease in the incidence and severity of histopathological hepatic lesions.

Woodfordia fruticosa extract pretreatment prevented the reduction in the antioxidant enzyme activities and consequent oxidative damage to the liver. In fact, the multiple dose pretreatment of *W. fruticosa* extract alone significantly boosted the antioxidant enzyme activities**.** Molina et al. (2003), Srivastava and Shivanandappa (2010) also reported good hepatoprotective activity in their studies; and they suggested that the hepatoprotective activity of plant extract could be a result of boosting the antioxidant capacity of the liver.

5.6.2. Carbon tetrachloride induced hepatotoxicity

CCl_4 is a well-known hepatotoxic agent and the preventive action of liver damage by CCl_4 has been widely used as an indicator of liver protective activity of drugs in general (Clawson, 1989). Hepatotoxicity induced by CCl_4 is the most commonly used model system for the screening of hepatoprotective activity of plant extracts/drugs (Srivastava and Shivanandappa, 2010). The changes associated with CCl_4-induced liver damage are similar to that of acute viral hepatitis (Rubinstein, 1962). Toxicity begins with the changes in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structure (Recknagel, 1983).

CCl_4 is a xenobiotic that produces hepatotoxicity in various experimental animals. CCl_4 is metabolized by cytochrome P_{450} to form a reactive trichloromethyl radical (CCl_3) and a trichloromethyl peroxyl radical (CCl_3O_2). Both radicals are capable of binding to DNA, lipids, proteins or carbohydrates, leading to lipid peroxidation, cell necrosis, excessive deposition of collagen in liver, and liver fibrosis (Sheweita et al., 2001; Weber et al., 2003). The effect of CCl_4 is generally observed after 24 h of its administration. Hence the withdrawal of the blood for biochemical parameters should be carried out only after 24 h of CCl_4 intoxication (Sureshkumar and Mishra, 2006).

The total protein and albumin levels decreased due to the hepatotoxin intoxication. The reduction is attributed to the damage produced and localized in the endoplasmic

reticulum which results in the loss of P_{450} leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides. In the present study, CCl_4 intoxication reduced the serum total protein and albumin levels. The pre-treatment of WFM restored the total protein and albumin levels. The rise in protein and albumin level suggests the stabilization of endoplasmic reticulum leading to protein synthesis (Sureshkumar and Mishra, 2006).

The liver marker enzymes (AST, ALT and ALP) are cytoplasmic in nature; upon liver injury these enzymes enter into the circulatory system due to altered permeability of membrane (Zimmerman and Seeff, 1970). In this study, significant increase in AST and ALT levels in the serum was observed after administration of CCl_4. ALP level also increased after CCl_4 administration. The increased levels of these enzymes significantly decreased by pretreatment with WFM extract. Reduction in the levels of AST, ALT and ALP towards the normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by CCl_4 (Ranawat et al., 2010).

Many studies have demonstrated that the hepatoprotective effect of plant extracts may be related to its antioxidant capacity to scavenge reactive oxygen species (Naik and Panda, 2007; Tsai et al., 2009). CCl_4 intoxication reduced the total protein level in liver homogenate, which restored significantly with the pretreatment of WFM. Liver cells possess antioxidant defense system consisting of antioxidants such as GSH and antioxidant enzymes such as catalase and GPx to protect own cells against oxidative stress, which causes destruction of cell components and cell death. GSH is widely distributed among living cells and is involved in many biological functions, acting as an essential intracellular reducing agent for maintenance of intracellular redox status. It is also the most important biomolecule protecting against chemically induced cytotoxicity, by participating in the elimination of reactive intermediates by conjugation and hydroperoxide reduction, or by direct quenching of free radicals (Jewell et al., 1986; Wang et al., 2000). CCl_4 intoxication slightly reduced the level of GSH, which was significantly restored in WFM treated (higher dose) rats. Trichloromethyl peroxy radical, the metabolic product of CCl_4 binds covalently to the macromolecules and causes peroxidative degradation of cellular membrane leading to the necrosis of hepatocytes (Brattin et al., 1985; Ranawat et al., 2010). The hepatic

antioxidant enzymatic activity of catalase and GPx significantly decreased in CCl_4-intoxicated rats as compared with control rats. The decreased enzymatic activity would result in an increased steady-state level of oxidants, contributing to cell injury. The catalase level was elevated by administration of WFM to CCl_4 intoxicated rats suggesting that it has the ability to restore the enzyme activity towards normalization in CCl_4 damaged liver. However, administration of WFM to CCl_4 intoxicated rats had no effect in hepatic GPx activity and relative liver weight as compared to the CCl_4 treated toxin control group. This result suggests that WFM markedly inhibited CCl_4 induced liver damage by elevated hepatic antioxidant enzymatic system such as catalase and GSH.

The rise in marker enzymes level in CCl_4 treated animals has been attributed to damaged structural integrity of the liver. Administration of the WFM preserved the structural integrity of the hepatocellular membrane as evidenced from attenuation of the marker enzymes level when compared to CCl_4 treated animals. It was further confirmed by the histopathological assessment of the liver tissue.

5.6.3. Acetaminophen induced hepatotoxicity

Acetaminophen (APAP), a frequently used analgesic and antipyretic drug, is known to be hepatotoxic in higher doses, which is primarily metabolized by sulfation and glucuronidation to unreactive metabolites, and then activated by the cytochrome P_{450} system to produce liver injury. It is established that acetaminophen is bioactivated to a toxic electrophile, N-acetyl p- benzoquinone imine (NAPQI), which binds covalently to tissue macromolecules, and probably also oxidizes lipids, or the critical sulphydryl groups (protein thiols) and alters the homeostasis of calcium (Lin et al., 1997). The massive production of reactive species may lead to depletion of protective physiological moieties (glutathione and α-tocopherol, etc.), ensuing wide-spread propagation of the alkylation as well as peroxidation, causing damage to the macromolecules in vital biomembranes (Aldridge, 1981; Gilani et al., 2005). The experimental evidence suggests that during metabolism of this type of drug, different reactive metabolites are produced that covalently modify proteins (Bernareggi, 1998), impose oxidative stress (Berson et al., 1991; Ritter and Malejka-Giganti, 1998) and causes mitochondrial injury (Mingatto et al., 2000).

In the present study, a reduction in total serum protein including albumin levels observed in the APAP treated rats may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein. But the treatment with WFM did not have protective effects in regards to total protein and albumin levels. Blood urea nitrogen (BUN) is also a marker of liver and renal functions, which is used to diagnose acute and chronic diseases related to liver and kidney. APAP administration to the rats increased the BUN level. The increase in BUN after APAP administration was prevented by WFM.

The hepatic cells consist of higher concentrations of AST, ALT and ALP in cytoplasm and AST in particular exists in mitochondria (Wells, 1988). Due to the damage caused to hepatic cells, the leakage of plasma cause an increased level of hepatospecific enzymes in serum (Zimmerman and Seef, 1970). The elevated serum enzyme levels are indicative of cellular leakage and functional integrity of cell membrane in liver (Drotman and Lawhorn, 1978; Sathesh Kumar et al., 2009). The hepatoprotective index of a drug can be evaluated by its capability to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been induced by a hepatotoxin. The measurement of serum AST, ALT and ALP levels serve as a means for the indirect assessment of condition of liver. The level of these enzymes significantly increased in serum, when animals were administered with APAP. The pre-treatment of the animals with WFM with respect to intoxication with APAP controlled the AST, ALT and ALP levels when compared with the toxic group.

The relative liver weight significantly increased in APAP intoxicated animals indicating toxic effect of APAP. Pretreatment of WFM decreased the liver weight significantly indicating recovery of liver tissue from damage. GSH, a major known protein thiol in living organisms plays a central role in coordinating the body's antioxidant defense process (Boyd et al., 1981). Excessive peroxidation causes increased GSH consumption. GSH is a scavenger of toxic metabolites, including NAPQI, which is a metabolite of APAP (Hwang et al., 2008; Yuan et al., 2010). GSH, plays a major protective role as a scavenger of free radicals that combine with non-protein thiols at the GSH reactive center to abolish free radical toxicity (Vane et al., 1994; Swierkosz et al., 1995). Anti-oxidation by GSH protects the body from many diseases and conditions such as damage by H_2O_2, ethanol and numerous other toxins

(Choi et al., 2009). Because GSH plays an important role in the antioxidant defense system (Hsu et al., 2008), it becomes the key determinant in the APAP-induced hepatotoxicity. In the present study, the contents of liver protein and GSH in the APAP group decreased significantly after APAP administration, when compared with the control group. Pretreatment with WFM restored the total protein and GSH levels towards normalization. Administration of APAP as well as WFM did not have any effects in catalase and GPx activity.

Histopathological observations after APAP administration showed severe damage in hepatocytes, which basically supported the alterations observed in biochemical analysis. Hepatocellular necrosis, infiltration of periportal mononuclear cell of liver cells were characteristic alterations occurred due to acetaminophen intoxication. Treatment of WFM decreased focal necrosis, vacuolation and reduced the lymphocytic infiltration in liver and presented regenerative effects. This can be considered as an expression of the functional improvement of hepatocytes, which might be due to accelerated regeneration of parenchymal cells or little damage of cells.

5.7. ACUTE TOXICITY STUDY

Toxicology is a science to study adverse-effects of chemicals or physical agents on biological system and preclinical toxicology is a science to evaluate safety of a drug (mostly) in animals to decide if the drug is safe for human use or not. Plants, vegetables and herbs used as food and in the folk treatment have been accepted currently as one of the main source of drug discovery and development, but only a few of them have been scientifically investigated, especially regarding their toxic aspects (Pereira et al., 2010).

Acute toxicity studies in animals are usually necessary for any pharmaceutical intended for human use. The information obtained from these studies is useful in choosing doses for repeat-dose studies, providing preliminary identification of target organs of toxicity and occasionally, revealing delayed toxicity. Acute toxicity studies may also aid in the selection of starting doses for Phase 1 human studies, and provide

information relevant to acute overdosing in humans. It could also be used to estimate the therapeutic index (LD_{50}/ED_{50}) of drugs (Rang et al., 2001; Maikai et al., 2008).

In the present study, acute toxicity test was done to establish if any adverse effects of the administration of the methanol extract of *Woodfordia fruticosa* on some observable and hematological parameters. The results indicate no abnormal symptoms and no death of the rats. Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Hilaly et al., 2004; Mukinda and Eagles, 2010). In the present study, no significant changes were observed in the general behavior, body weight, feed and water intake of rats in the treated groups as compared to the control group, suggesting that at single oral doses administered, methanol extract of *Woodfordia fruticosa* flowers had no effect on the normal growth of rats.

Organ weight changes have long been accepted as a sensitive indicator of chemically induced changes to organs and in toxicological experiments, comparison of organ weights between control and treated groups have conventionally been used to predict toxic effect of a test material (Pfeiffer, 1968; Nisha et al., 2009). In acute toxicity study in male rats at lower dose kidney weight increased while in female rats at higher dose kidney weight decreased significantly. The weight of lung in acute toxicity study increased in both male and female rats; but this was not associated with morphological changes and no evidence of toxicity was found. Increased testis weight and decreased uterus weight in treatment groups cannot be considered as a manifestation of toxicity due to the variability attributable to its small size and physiological factors unrelated to treatment like estrus cycle and relative infrequency of these organs as target organs of toxicity. The absence of significant changes in other organs in the present study points to the fact that ingestion of *Woodfordia fruticosa* methanol extract did not induce any anomalous growth or inflammation to these organs which would otherwise have resulted in higher relative organ weights in the treatment groups.

The hematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animal (Mukinda and Syce, 2007). The various hematological parameters investigated in this study are useful indices of evaluating the toxicity of plant extract in animals (Toyin et

al., 2008). Assessment of hematological parameters are not only used to determine the extent of deleterious effect of extracts on the blood of animals, but it can also be used to explain blood relating functions of a plant extract or its products (Toyin et al., 2007). Analysis of blood parameters is relevant in risk evaluation as changes in the haematological system have higher predictive value for human toxicity when the data are translated from animal studies (Olson et al., 2000; Adebayo et al., 2010).

The hematological studies revealed increase in RBC count and PCV ($P < 0.05$) at higher dose in male rats and Hb ($P < 0.05$) in both male in female rats during acute toxicity study. It also revealed a fall ($P < 0.05$) in WBC level in lower dose during acute toxicity study in male rats. The general decrease in the values of these hematological parameters can be due to direct destruction of mature circulating cells or loss of cells from the circulation by hemorrhage, or leakage through capillary walls and reduced cell production (Nunia et al., 2007). However, this was not considered to be adverse or related to exposure to the WFM because similar differences were not observed in both sexes.

CONCLUSIONS

CONCLUSIONS

In pharmacognostic study, *Woodfordia fruticosa* flowers showed the presence of unicellular trichomes, rosettes and cluster of calcium oxalate crystals; and anomocytic, actinocytic and anisocytic stomata. In physicochemical analysis, crude powder and methanol extract of *Woodfordia fruticosa* flowers were free from heavy metals. The highest extractive value was obtained from water and methanol. The solubility of the extract was maximum in polar solvents like DMF, methanol and DMSO; the extract was acidic in nature. In qualitative phytochemical analysis tannins and alkaloids were present in higher amount, while cardiac glycosides and steroids were totally absent. In quantitative analysis of phytoconstituents, total phenol content was higher than flavonoid content. Hence, the determination of pharmacognostical and phyto-physicochemical profile of *Woodfordia fruticosa* Kurz. flowers may be useful to supplement information in respect to its identification, authentication and standardization of herbal drugs. In other words, the pharmacognostic features examined in the present study may serve as tool for identification of the plant for validation of the raw material and for standardization of its formulations at herbal industrial level in the coming days.

In anti-inflammatory studies the methanol extract of *Woodfordia fruticosa* flowers inhibited the carrageenan induced paw edema at both early and late phase. The action of early phase may be due to the inhibition of histamine and serotonin. The action of later phase may be due to the inhibition of prostaglandins, proteases and lysosome. Dose dependent inhibition of inflammation was observed in histamine and dextran induced paw edema. This may be due to antihistaminic activity of WFM. The extract also significantly reduced the paw volume in serotonin induced edema, and the activity of WFM-400 was almost similar to that of standard diclofenac treated group. The subcutaneous injection of formaldehyde into paw of rats produces localized inflammation. WFM and standard diclofenac administered continuously for 7 days successfully inhibited edema induced by formaldehyde. From the results of acute inflammatory models, it can be concluded that the WFM showed antiedematogenic effects on carrageenan, histamine, dextran, serotonin and formaldehyde induced edema, which may be related to inhibition of inflammatory mediators formation. In

chronic cotton pellet induced granuloma model, WFM reduced the granuloma formation in dose dependent manner. In analgesic study, WFM effectively reduced the frequency of paw licking in formaldehyde induced paw licking test at both early (0-5 min) and late (15-30 min) phases. But better analgesic effect of WFM was observed at later phase.

In hepatoprotective studies, the induced diclofenac toxicity elevated levels of serum marker enzymes ALT, AST, ALP and the level of BUN along with the decrease in total protein and albumin levels. It also increased the relative liver weight and decreased the level of liver total protein and GSH. The activity of catalase and GPx significantly decreased in diclofenac intoxicated animals. The pre-treatment of methanol extract of *Woodfordia fruticosa* at dose levels of 400 and 600 mg/kg had restored the ALT, AST, ALP and BUN levels towards normalization and the effects were comparable with standard drug (Silymarin 100 mg/kg). The total protein, albumin, GSH levels and catalase, GPx activity increased significantly in the animals received pre-treatment of the WFM. The histopathological study showed the reduction of hepatic damage in WFM treated animals.

In CCl_4 and acetaminophen induced hepatotoxicity models, the serum biochemical parameters and liver antioxidants were altered when animals were intoxicated with CCl_4 and acetaminophen. The treatment with WFM restored the level of serum biochemical parameters as well as liver antioxidants in both the animal models. The administration of acetaminophen and WFM did not have any effect in serum total protein level, catalase and GPx activity. The treatment with WFM prevented the structural damage of hepatocellular membrane.

In acute toxicity study, the methanol extract of *Woodfordia fruticosa* flowers had no mortality and observable acute toxic effect on the experimental animals and therefore can be considered as non-toxic. However, acute toxicity data sometimes is of limited clinical application since accumulative toxic effect may not be seen in short period with a single dose application. Hence, sub acute and chronic evaluation of the extract should be carried out in evaluating the safety profile of *Woodfordia fruticosa*.

These studies have shown that the methanol extract of flowers of *Woodfordia fruticosa* contain some active ingredients with the potential of being good anti-inflammatory, analgesic and hepatoprotective agents. NSAIDs like diclofenac, used as standard drug in anti-inflammatory study, is having good anti-inflammatory and analgesic property, but is also having side effects on liver. Therefore, *Woodfordia fruticosa* may become the alternative to the NSAIDs. For that, further study for detailed investigation of the mechanism of action of WFM is needed.

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SUMMARY

SUMMARY

Inflammation is a major threat to human health and plays an important role in the development of various infectious and non-infectious diseases such as Alzheimer's, heart disease, asthma, rheumatoid arthritis, etc. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever. Clinical treatment of inflammatory diseases is dependent on drugs, which belong either to the non-steroidal or to the steroidal chemical groups. The use of non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of diseases associated with inflammatory reactions has potent activity, but long term uses of these drugs have various and severe adverse effects on liver, gastrointestinal tract, etc. Hence, new anti-inflammatory and analgesic drugs lacking such effects are being searched for as alternatives to NSAIDs.

Owing to safety concerns associated with the use of synthetic anti-inflammatory and analgesic agents, generally the people prefer to take natural anti- inflammatory and analgesic treatments from edible materials such as fruits, spices, herbs and vegetables. Therefore, the development and utilization of more effective anti-inflammatory and analgesic agents with fewer side effects from natural origin are desired.

Liver diseases are a serious health problem. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play an important role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional system of medicine in India. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effect. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory and hepatoprotective agents, should therefore be viewed as a fruitful and logical research strategy in search for new drugs.

In the present study, the prime objective was to select a medicinal plant which can be used as anti-inflammatory and hepatoprotective agent with fewer/without side effects. On the basis of literature survey, medicinal uses and availability of the plant, *Woodfordia fruticosa* Kurz. flowers were selected for evaluation of anti-inflammatory and hepatoprotective property.

Woodfordia fruticosa Kurz. belongs to the family Lythraceae, is a much branched beautiful shrub. It is the plant of tropical and subtropical regions with a long history of medicinal use. English names of the plant are Fire Flame Bush and Shiranjitea. The plant is abundantly present throughout India. Locally (In Gujarat) it is known as Dhavdi. All parts of this plant possess valuable medicinal properties viz. anti-inflammatory, anti-tumor, hepatoprotective and free radicals scavenging activity, but flowers are in maximum demand. The flowers are being used in the preparation of Ayurvedic fermented drugs called 'Aristha's and 'Asava's, and very popular in the Indian subcontinent as also in other South Asian countries.

For the identification of plant, macroscopic and microscopic study of *Woodfordia fruticosa* flowers was done by evaluating different characteristics from free hand sections of fresh flower and dried powder. The T.S of the pedicel showed single layered epidermis, with a fairly thick cuticle. The unicellular trichomes were present in this layer. The rosette and cluster crystals of calcium oxalate were found in pedicel and calyx. Anomocytic, actinocytic and anisocytic stromata were also found in calyx. The anther lobes were tetrasporangiate; the T.S of anther lobe showed large colourless cells followed by a fibrous layer. The dried powder of *Woodfordia fruticosa* flowers was light in colour, slightly bitter and astringent in taste. Microscopic observation of dried powder showed the presence of straight wall cells of epidermis, cluster crystals of calcium oxalate and unicellular trichomes. The pollen grains were present either singly or in a group.

Woodfordia fruticosa flowers were collected in March, 2008 from Girnar region, Junagadh, dried, powdered and stored in air tight bottles. The dried powder was defatted with petroleum ether and then extracted in methanol in soxhlet apparatus. In all further studies methanol extract was used (WFM).

The result of physicochemical analysis showed 8% loss on drying. It contained 5.45% total ash, 0.57% acid insoluble ash and 2.47% water soluble ash. The maximum percentage of extractive value was obtained in water (41.59%) followed by methanol (32.77%). The extract was acidic in nature and maximum solubility was in DMF, methanol and DMSO. Mercury and arsenic were present in very minor amount in crude powder and extract but they were within the permissible limits reported by The Ayurvedic Pharmacopoeia of India; lead, chromium and cadmium were not detected.

The preliminary qualitative phytochemical analysis of crude powder and WFM revealed the presence of alkaloids, flavonoids, phlobotannins, saponins, tannins and triterpenes. Tannins and alkaloids were present in more amount as compared to other phytoconstituents. Quantitatively estimated total phenol content was higher than that of flavonoid content in the methanol extract.

Anti-inflammatory studies of WFM was done using carrageenan, histamine, dextran, serotonin and formaldehyde induced rat paw edema acute models; cotton pellet induced granuloma chronic model and formaldehyde induced paw licking test as analgesic model. Among these models, carrageenan induced rat paw edema is most widely used for evaluation of anti-inflammatory properties of medicinal plants. WFM inhibited the carrageenan induced paw edema at both early and late phase. In histamine and dextran induced paw edema, dose dependent inhibition of paw volume was observed at both early and late phase. WFM also inhibited the increase in paw volume in serotonin and formaldehyde induced edema. In chronic inflammatory model, WFM dose dependently reduced the granuloma tissue formation. WFM effectively reduced the frequency of paw licking at both early and late phase in formaldehyde induced paw licking test. Thus, WFM showed promising activity in all the studied anti-inflammatory models, but it was more effective in acute models than in chronic model.

Hepatoprotective study of WFM was done by diclofenac, carbon tetrachloride and acetaminophen (APAP) induced hepatotoxicity in rats. In all the three models, different serum biochemical parameters (viz. total protein, albumin, blood urea nitrogen, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase), liver total protein, liver antioxidant glutathione reduced, and antioxidant enzymes viz. catalase and glutathione peroxidase and liver histopathological study were carried out.

Diclofenac, is one of the most widely used NSAID for the treatment of inflammatory diseases and analgesia. However, long term use of diclofenac causes hepatotoxicity. In the present study, administration of diclofenac to the animals caused significant changes in the serum biochemical parameters and liver antioxidants as compared to the normal control group. Treatment with WFM restored the level of serum

biochemical parameters and liver antioxidants towards normalization; and the effect of WFM on these parameters was comparable to that of standard silymarin.

CCl_4 induced hepatotoxicity is frequently used model for evaluating the hepatoprotective properties of medicinal plants. APAP has been widely used as a medicine for pain and fever relief. Since APAP can be purchased easily from any pharmaceutical outlet and even from supermarkets, without prescriptions from clinicians, it is commonly considered as a ‘‘safe drug’’ when taken within the suggested therapeutic dose. However, APAP can be hepatotoxic when an overdose is administered. The administration of CCl_4 and APAP to the animals caused the alteration in serum biochemical parameters and liver antioxidants as compared to the normal control group. Treatment with WFM restored the serum biochemical parameters and liver antioxidants towards normalization. The hepatoprotective effect of WFM was also confirmed by histopathological study. The severe hepatocytes necrosis, periportal mononuclear cell infilteration, cytoplasmic vacuolation were seen in diclofenac, CCl_4 and APAP intoxicated animals. Administration of WFM preserved the structural integrity of the hepatocellular membrane. The results of histopathological study supported the result obtained from serum biochemical and liver antioxidants parameters.

Any drug whether promising or more promising without toxicity evaluation, loses its importance because non toxic nature of the drug is more important than efficacy. Therefore, acute toxicity study was done to check toxicity profile of the WFM. No adverse reaction or mortality was observed in rats up to highest dose (3600 mg/kg, b.w.) studied. No significant change was observed in feed and water consumption and body weight of animals treated with WFM as compared to normal control group. No significant changes were recorded in organ weight of the animals. Hematological parameters were also not affected.

Overall it can be concluded that methanol extract of *Woodfordia fruticosa* flowers had good potential as anti-inflammatory, analgesic and hepatoprotective agent and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals.