THE EFFECT OF PROCESSING ON THE EFFICACY AND SAFETY OF *SOLANUM ACULEASTRUM* Dunal. BERRIES.

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SEPTEMBER, 2009

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SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY: BOTANY (PHYTOMEDICINE)

DEPARTMENT OF BOTANY
FACULTY OF SCIENCE AND AGRICULTURE
UNIVERSITY OF FORT HARE

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SEPTEMBER, 2009

DEDICATION

TO GOD

WHO HAS BEEN MY HELP IN AGES PAST AND WHO IS THE HOPE OF THE YEARS TO COME

YOU ARE A FAITHFUL FATHER

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GENERAL ABSTRACT

General Abstract

There has been a steady increase in the use of medicinal plants in the last two decades in both developed and developing countries for prevention, management and treatment of diseases. This increase has been due to reasons such as ease of access, better cultural acceptability and compatibility, cost effectiveness and also the bid to "go natural". Unfortunately, the notion that herbal medicines are relatively safe because they are natural has led to serious and potentially fatal consequences in phytotherapy. The lack of rigorous research to prove the effectiveness and safety of many medicinal plants is of great concern to the health care system.

This thesis therefore addresses not just the efficacy, but also the safety of the extracts of the berry of *Solanum aculeastrum* - a medicinal plant used, among other things, for the treatment of breast cancer in the Eastern Cape Province of South Africa. Particular attention was paid to the possible effect of different processing methods of the berry extracts on inflammation, cytotoxicity, and toxicity. In studying the comparative effects of various processing methods, four different preparations of the extracts were investigated. These include fresh, dried, boiled fresh, and boiled dried berries. While the effect of processing on the anti-inflammatory properties of the extracts was not dose dependent, the percentage reduction in inflammation was highly significant and more prominent in both concentrations of the boiled fresh berries than the reference drug, indomethacin. Furthermore, the four extracts varied in their ability to act either centrally or peripherally in their effect on pain. Assessment of the analgesic response using the formalin test showed that, at both concentrations tested, none of the extracts inhibited the first phase of the formalin test. Furthermore, it was observed that boiling had differing results on the activity of the

fresh and dried extracts. While boiling of the dried berries reduced pain in the rats, the opposite trend was observed with the boiled fresh berries. Results of the influence of processing of the berries on cytotoxicity indicated that the extracts are potent inhibitors of human breast, cervical and colonic carcinoma cells and the non-cancerous cells (both the actively dividing and confluent Chang liver cells). Although, in terms of relative potency, the fresh berries appeared to be the most active of the extracts, processing of the berries caused an increase in apoptotic cells and a subsequent decrease in the necrotic cells. The effect of processing on the safety of the berry of *S. aculeastrum* on the rats fed for 28 days was also investigated. The various doses (1, 10 and 25 mg/kg body weight) of all the four extracts did not alter the activity and the weight of the animals throughout the period of treatment. A reduction in organ to body weight ratio of the heart, kidney, liver and spleen was observed in all the extracts. Regarding the haematological parameters, different patterns of effect were observed between the extracts and within the treated doses. The observed alterations in the biochemical parameters by the various extract of *Solanum aculeastrum* berries at all the doses may have consequential effects on the normal functioning of these organs.

In conclusion, this study has shown that there is some justification for the traditional use of the berries of *Solanum aculeastrum* in the treatment of inflammation related ailments and cancer. However, the medicinal use of the plant also poses considerable health risks. Investigation conducted into the plant's safety revealed that the berry extracts were nephrotoxic, hepatotoxic, haematotoxic and at higher doses, fatal. Another concern with regard to the plant's safety is the non-selectivity of its extracts in the inhibition of carcinoma, actively dividing and un-dividing cells. Assessment of the effect of the processing on the berry's efficacy and safety as herbal

remedy produced mixed results. On the one hand, processing seemed to improve the extract's anti-inflammatory and analgesic activity, while reducing its cytotoxic potential. On the other hand, a reduction in the toxicity was observed on the processed extracts compared to the fresh ones. This may be an indication that processing has an overall beneficial effect on the medicinal properties of the plant and should thus be considered as a method of making the berries of *Solanum aculeastrum* safer for use as a herbal remedy.

Keywords: Medicinal plants, *Solanum aculeastrum*, efficacy, safety, processing, cytotoxicity, toxicity.

INTELLECTUAL PROPERTY AGREEMENT STATEMENT

All the elderly and the traditional healers who contributed one information or the other during the preliminary investigation on the folkloric use of *Solanum aculeastrum* were adequately financially rewarded with further verbal agreement and understanding that this research shall not be for commercial purposes but to serve as an enlightenment information to the community and the entire Eastern Cape Province on the efficacy, safety and toxicity of this plant.

ETHICAL COMMITTEE APPROVAL

The study involving the use of animals in this project was carried out following the approval of the Ethical Committee on Animal Use and Care of the University of Fort Hare.

COMPLIANCE STATEMENT

No part of this study in any form has been commercialized. The thesis is meant to be used for information dissemination on the medicinal potentials of *Solanum aculeastrum* to the immediate community and the entire Eastern Cape Province of South Africa.

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Supervisor signature Student signature

CHAPTER 1

General Introduction

GENERAL INTRODUCTION

1.0 Introduction

1.1 General overview of cancer and its treatment

The human adult is comprised of about 10^{15} cells, many of which are required to divide and differentiate in order to repopulate organs and tissues which require cell turnover (Bertram, 2001). The ability of the body to control cell multiplicity is achieved by a network of overlapping molecular mechanisms which direct cell proliferation and death. Any alteration in this balance (birth and death of cells), has a potential, if uncorrected, to alter the number of cells in an organ or tissue. Such changes may result in cancer, a disease that is manifested in many forms depending primarily on the organ from which it evolves. Characteristically, cancer is defined as the uncontrolled proliferation of cells which become structurally abnormal and possess the ability to detach themselves from a tumor and establish a new tumor at a remote site within the host (National Cancer Institute (NCI), 2009).

Globally, cancer is one of the leading causes of death. According to the American Cancer Society (ACS), an estimation of about 1,500,000 new cases and over 500,000 deaths are expected to be recorded in the US in 2009 (ACS, 2009). South Africa experiences one of the highest incidence rates of cancer in Africa (Mqoqi et al., 2004). Every one in four males and six females have the potential of developing cancer. The current statistics by the National Cancer Registry of South Africa indicate that cancers of the bladder, colon, breast, cervix, lungs and melanoma are among the most common (Mqoqi et al., 2004).

The existing strategy of eradicating cancer after detection has resulted in mortality that may have been preventable if caution was taken against the causative agents (Doll and Peto, 1981). Although, the etiology of cancer remains unknown to an extent, epidemiology has suggested the hypotheses that multiple causative factors may be operating. These factors (exogenous and endogenous) exert their specific effects at different times in the life of the patient. The impact of such effects might be cumulative or synergistic. The main predictors of the incidence of cancer fall largely into two broad categories: environmental and positive family history (Parkin et al., 2005). A number of other risk factors exist from a wide range of studies in various populations and geographic locations.

The progress in research on the etiology of cancer has revealed the evidence that dietary patterns, nutrients and food constituents are closely associated with the risk of several types of cancer (Doll and Peto, 1981). Fat has been the focus of nutritional studies on cancers of the prostate, breast and colon more than any other dietary component (National Research Council, 1989). Several studies in countries consuming high fat diets have consistently shown higher incidence and mortality rates for breast, colon and prostate cancers (National Research Council, 1989; Hursting et al., 1990). Studies of specific environmental influences have suggested an increased risk of developing various forms of cancer with exposure to particulate air pollutants and fertilizers. Substances such as asbestos, aniline dye, uranium and nickel have been implicated as environmental carcinogens (Monson and Christiani, 1997).

1.2 The role of inflammation in the initiation of cancer

The association between inflammation and tumor has long been known (Balkwill and Mantovani, 2001). Since then, inflammation is increasingly recognized as an important component of several cancers, although the mechanisms involved are not fully understood (Ben-Baruch, 2006). A vast body of evidence has indicated that inflammatory leucocytes contribute to cancer development either directly by the release of vesicle stored growth and survival factors and diverse proteolytic enzymes, or indirectly via the activation of cell signaling cascades as a result of altered pericellular matrix remodelling activity (van Kempen et al., 2006). Products of inflammation such as growth factors, cytokines and transcription factors, like nuclear factor-kappa B (NF-κB), control the expression of cancer genes and key inflammatory enzymes such as inducible nitric oxide (iNOS) and cyclooxygenase-2 (COX-2) (Hofseth and Ying, 2006).

Bacterial, viral and parasitic infections, chemical irritants and non-digestible particles are some of the causes of chronic inflammation. The longer this inflammation persists, the higher the risk of associated carcinogenesis (Shacter and Weitzman, 2002). Chronic inflammation occurs due to environmental stress around the tumor, thus generating a shield protecting the tumor from the immune system (Assenat et al., 2006). Recent demonstrations have shown that micro-environment of tumors highly resemble an inflammation site, with a significant tendency for tumor progression (Assenat et al., 2006). In addition, this micro-environment apart from its significant role in cancer progression and protection, has a considerable adverse effect on the success of the various current cancer treatments (Assenat et al., 2006). The pro-cancerous outcome of chronic inflammation are increased DNA damage, increased DNA synthesis, cellular

proliferation, the disruption of DNA repair pathways and cellular milieu, the inhibition of apoptosis, the promotion of angiogenesis and invasion (Hofseth and Wargovich, 2007). Therefore, inflammation plays a major role in the initiation and progression of cancers.

Inflammatory-related ailments are treated mainly with non-steroidal anti-inflammatory drugs (NSAIDs). These drugs are used to reduce the consequences of inflammation (Vane and Botting 1996). Indomethacin, an NSAID, for example has been found to block carcinogenesis in animals by reducing the production of inflammatory cytokines (Federico et al., 2007). A lower risk of cancer incidence has also been found in people regularly taking NSAIDs (Fosslien, 2000).

1.3 Treatment options for cancer

The treatment option for cancer is influenced by several factors, such as the specific nature of the cancer; the status of the patient (age and health); and whether the goal of treatment is eradication of the tumor, control of the local tumor growth, prolongation of survival or palliation of cancer symptoms (NCI, 2009). Depending on these factors, treatment options such as surgery, chemotherapy, radiation and hormonal therapy could be used. More than half of all people diagnosed with cancer are treated with chemotherapy because it is considered a systemic treatment. The cancer-fighting drugs circulate in the blood to parts of the body where the cancer may have spread and can kill or eliminate cancers cells at sites of great distances from the original cancer. The side effects observed with these treatments may be severe, thus reducing the quality of life, compromising treatment and sometimes limiting the chance for an optimal outcome from treatment. Common side effects includes anaemia, depression, fatigue, hair loss,

infections, low blood counts, nausea and vomiting and long term effects such as cardiac toxicity, growth problems and sterility (NCI, 2009).

1.3.1 Phytotherapy for cancer treatment

Despite the major scientific and technological progress in the treatment and management of cancer, no reliable and definitive cure has been found (Richardson, 1999). This has led to an increase in the dependence of patients on unconventional medical therapies (Alschuler et al., 1997). All over the world, the traditional use of plants in the treatment of ailments has been on the increase especially in developing countries where there is invariably poor availability of primary health care (Alschuler et al., 1997).

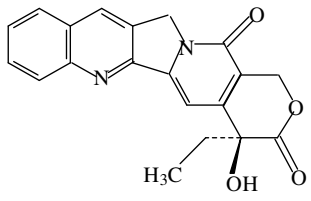
Plants are a viable source of biologically active natural products which have served as commercial drugs or as lead structures for the development of modified derivatives possessing enhanced activity (Cordell et al., 1991). Extracts of plants have a long history of use in the treatment of cancer (Hartwell, 1982). Over 60% of the currently used anticancer agents are derived in one way or the other from natural sources including plants and marine organisms (Cragg and Newman, 2005). For example, the breakthrough for cancer treatment was achieved by the discovery and development of the vinca alkaloids, vincristine and vinblastine isolated from *Catharanthus roseus* in the early 1950's (Cragg et al., 1994). The discovery of these chemicals led to other research where compounds such as podophyllotoxin derivatives, etoposide and teniposide from the root of various *Podophyllum* species (Gurib-Fakim, 2006) and paclitaxel from the bark of *Taxus brevifolia* (Kingston, 2005) were isolated (Fig 1). Other examples include the camptothecin derivatives (topotecan, irinotecan and 9- aminocamptothecin) isolated from

Camptotheca acuminata, homoharringtonine from *Cephalotaxus harringtonia* var drupaceae and elliptinium from several genera in the Apocyanaceae family (Wall, 1998; Kuo and King, 2001). Since then, various studies have been undertaken to discover more natural sources of drugs for the treatment of cancer.

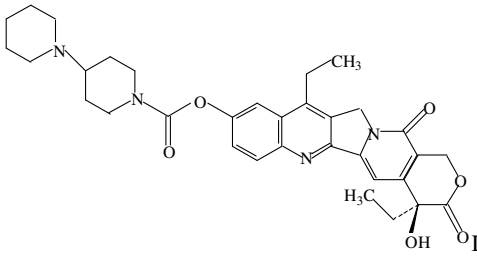
1.4 Efficacy and safety of medicinal plants

The traditional use of plants in the treatment of ailments has been on the increase both in developing countries, where there is poor availability of primary health care, and also in the developed world. Herbal medicines are in great demand in the developing world for primary health care not only because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. This is primarily due to the general belief that herbal medicines are relatively safe because they are natural (Gesler, 1992).

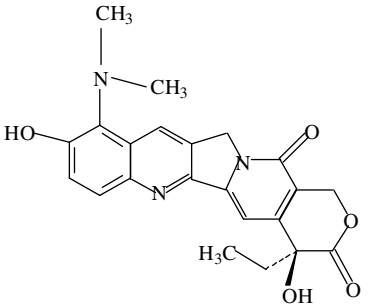
The knowledge of the healing virtues of medicinal plants has been passed on from ancient times. History tells of medicinal plants such as *Catharanthus roseus* G. Don*, Digitalis purpurea* Linn, *Rauwolfia serpentina* Plum ex Linn, Willow (*Salix* species), *Physostigma venenosum* Balf. and a host of other plants which have been used for centuries for the treatment of diseases such as cancer, cardiovascular diseases, hypertension, depression, pain, glaucoma and an array of other diseases that have plagued the world. Since then, plants have served as viable sources of biologically active natural products which are either used as commercial drugs or as lead structures for the development of modified derivatives possessing enhanced activity (Cordell et al., 1991). Modern medicine as a result of civilization led to the reduced importance of medicinal plants to human survival. This was not because these plants were ineffective but because they



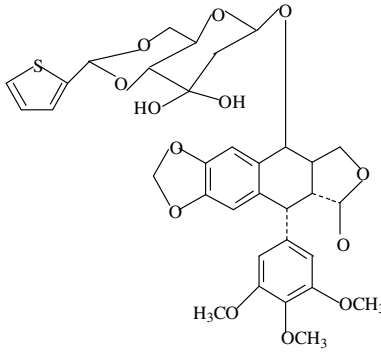
Campthothecin



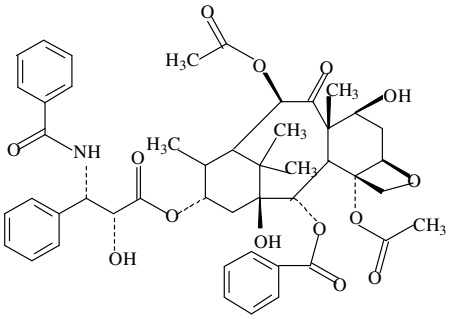
Irinothecin



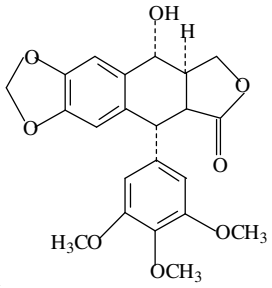
Tenoposide



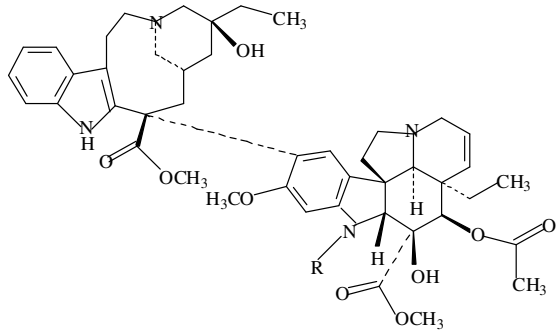
Topothecin



Paclitaxel

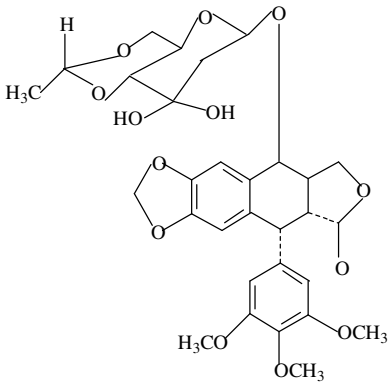


Podophyllotoxin



Vincrisitine R = CHO

Vinblastine R = CH_3



Etoposide

Cragg et al., 1994; Gurib-Fakim, 2006; Kingston, 2005; Kuo and King, 2001; Wall, 1998.

Figure 1: Plant derived anticancer agents currently in clinical use

were not economically profitable as the newer synthetic drugs (Tyler, 1999). However, in recent times, the concerns over the serious adverse effect of conventional drugs and the movement towards a more natural living has brought about a resurgence in the use of herbal products (Pal and Shukla, 2003).

The number of patients seeking herbal approaches for therapy has grown exponentially (Alschuler et al., 1997). This has, for example increased the number of medical doctors in France and Germany that regularly prescribe herbal medicine to 70% (Murray and Pizzorno, 2000). Available records have illustrated the growth of the herbal medicine market in the European Union countries. In 1991, sales were about $ US 6 billion, with Germany accounting for $ US3 billion, France $ US 1.6 billion and Italy $ US 0.6 billion while in the US, herbal medicine market was about $ 4 billion in 1996 (Pal and Shukla, 2003). India boasts about $ US 80 million for the exportation of herbal crude extracts (Kamboj, 2000). The resurgence and popularity of herbal medicines have led to an increase in the number of medicinal plant products in the market (Gupta and Raina, 1998). Unfortunately, the increased dependence on phytotherapy, without concern for efficacy and safety has resulted in preventable serious adverse effects (Gurib-Fakim, 2006).

1.4.1 Efficacy of medicinal plants

With the slight increase in the randomized controlled trials to evaluate the efficacy of herbal medicines, an estimate of about 39% of all 520 new approved drugs were natural products or

derived from natural products in 1983-1994 (Cragg et al., 1997). The study of Harvey (1999), reported that 60-80% of antibacterial and anticancer drugs were derived from natural products. The antimalaria quinine from *Cinchona officinalis*, analgesics codeine and morphine from *Papaver somnifera*, antihypertensive reserpine from *Rauwolfia serpentina* and cardiac glycoside digoxin from *Digitalis pupurea* are some of the many drugs derived from medicinal plants that have been in use. The fact remains that plant substances constitute the basis for a very large proportion of medications used today for the treatment of diseases of the liver and heart, cancer, hypertension, depression and other ailments. This is the result of an increase in the scientific studies carried out to validate the traditional claims of these plants (Gurib-Fakim, 2006).

1.4.2 Safety of medicinal plants

Recent findings indicate that herbal medicines may not be safe and severe consequences have arisen from the use of certain products (Gurib-Fakim, 2006; Bush et al., 2007). Information obtained from health centres and hospital emergency rooms have shown that 5 % of patients receiving complementary therapies report side effects (Molassiotis et al., 2005). The true frequency of the incidence of side effects from herbal remedies may be several folds higher than this (Ernst, 2004), because the lack of surveillance systems which are less extensive than for conventional drugs have limited these reports (Bent and Ko, 2004). For example, acute poisoning as a result of herbal medicines is estimated to cause anywhere from 8,000 to 20,000 deaths annually in South Africa (Thomson, 2000). These side effects may occur through several different mechanisms, including direct toxic effects of the herbs, effects of contaminants, and interactions with drugs or other herbs (Niggemann and Gruber, 2003; Bent and Ko, 2004; Ernst, 2004). The risk of herbal remedies producing side effects depends not only on the herb and the

dose consumed, but also on the health status and age of the patient and the concurrent use of other drugs (De Smet, 1995).

1.4.2.1 Direct toxic effects of medicinal plants

Serious and potentially fatal adverse effects can occur with the use of herbal remedies. Unfortunately, patients who experience such side effects may not associate them with their use of herbal medicines (Pal and Shukla, 2003). This is further complicated by the fact that the majority of these drugs are self-prescribed and never mentioned when patients consult the doctors (Bent and Ko, 2004), resulting in the under-reporting of adverse reactions associated with herbal preparations.

Gupta and Raina, (1998) reviewed the side effects of some medicinal plants. Their study listed 21 popular medicinal plants such as garlic, licorice, senna and digitalis used in the treatment of various diseases. Toxic symptoms such as respiratory distress, raised blood pressure, paralysis of the central nervous system, dermatitis, nephrotoxicity, hepatotoxicity and ultimately death were recorded from the use of these plants. Hepatic failure and even death, following ingestion of herbal medicines was also reported by Chattopadhyay, (1996). Studies have shown that 25% of corneal ulcers in Tanzania and 26% of childhood blindness in Nigeria and Malawi were associated with the use of traditional eye medicine (Harries and Cullinan, 1994). Vickers and Zollman, (1999) reported the development of rapidly progressive interstitial renal fibrosis by several women after taking Chinese herbs prescribed by a slimming clinic. Doctors in Belgium discovered that a Chinese herb, *Aristolochia fangchi* was not only linked to kidney failure, but

also cancer (Kew et al., 1993). Table 1 shows a summary of some commonly used medicinal plants with adverse effects.

1.4.2.2 Effect of contaminants

Side effects due to contamination in herbal products have mainly been recorded in Asian herbal remedies. Kew et al., (1993) and De Smet, (1997) reported the presence of mercury, lead, arsenic, corticosteroids and poisonous organic substances in harmful amounts in herbal remedies. These results were also observed when Ko, (1998), examined 260 of the patent drugs in Asia and found that 25% contained high level of heavy metals such as mercury, lead or arsenic and 7% contained undeclared pharmaceutical drugs to enhance the desired effects. A good example is PC-SPES (a combination of 8 medicinal plants), a formulated drug for the treatment of prostate cancer. Investigations into this remedy resulted in the detection of varying amount of synthetic drugs such as warfarin, indomethacin, diethylstilbestrol and ethinyl estradiol which ultimately led to its removal from the herbal market (Ko et al., 2003).

1.4.2.3 Interaction with other herbs and drugs

A typical example of a herbal remedy which, on interaction with other herbs or drugs, caused varying levels of toxicity is St. John's wort (*Hypericum perforatum*) (Ernst, 2004). Extracts of this plant have been used traditionally for the treatment of wounds and burns, or taken orally as an infusion or tea to treat fevers and nervous conditions including depression (Tonbridge, 1999). Preparations of St John's wort in Europe and the United States are bought over-the-counter for the treatment of a variety of conditions. However, recent indications of side effects and also interactions with other drugs, resulting in potentially serious adverse effects, have put questions marks on its safety. Adverse effects such as gastrointestinal symptoms, allergic reactions,

dizziness, confusion, tiredness, sedation and dry mouth have been associated with this remedy (Ernst et al., 1998; Woelk et al., 1994). The interaction between St. John's wort and drugs such as warfarin or phenoprocumon, cyclosporine, theophylline, digoxin, HIV protease inhibitors (indinavir and lamivudine) and oral contraceptives have been found to cause clinically significant consequences such as the rejection of a transplanted organ, reduction in the efficacy of these drugs and failure in treatment (Dasgupta and Bernard, 2006). Other common herbal remedies worth mentioning include garlic, ginger, ginseng, ginkgo and feverfew which interact with warfarin leading to changes in its effectiveness (Dasgupta and Bernard, 2006).

1.5 Aims and objectives of this study

The primary aim of this work is to examine the effect of processing on the efficacy and safety of *Solanum aculeastrum* berries as remedy for inflammation and cancer. The specific objectives include:

1. *In vivo* anti-inflammatory and analgesic activity
2. *In vitro* anticancer activity based on the following parameters: cytotoxic assay (cell growth inhibition), apoptosis determination, cell cycle/cell death analysis and
3. Toxicological and histopathological evaluation of the extracts from the berries of this plant.
4. Investigation into the effect of processing on the efficacy (as an anti-inflammatory and anti-cancer agent) and safety of *Solanum aculeastrum* berries.

Table 1: Common medicinal plants and their adverse effects

Medicinal plant	Common uses	Side effects	References
Cassia senna	Laxative	Carcinogenic, hepatotoxicity, hepatitis	Dasgupta & Bernard, 2006; Gupta & Raina, 1998
Urtica dioica	Genitourinary ailments	Uterine-stimulant effect, induction of abortion	De Smet, 2002.
Matricaria chamomilla	Migraine, bronchitis, fever, Gastrointestinal tract ailments	Allergic reactions and conjunctivitis	De Smet, 2002; Gupta & Raina, 1998
Allium sativum	Hypercholesterolemia, cough, asthma, chronic bronchitis	Death at a dose of 10mg/100g body weight intragastrically to rats, hepatotoxicity	Dasgupta & Bernard 2006; Gupta & Raina, 1998
Piper methysticum	Sleeping aid, anti-anxiety	Hepatotoxicity, kidney damage, loss of muscle control	Dasgupta & Bernard 2006; Ernst, 2004.
Glycyrrhiza glabra	Bronchitis, chronic gastritis, peptic ulcer	Heart failure, hypertension, sodium and water retention, weight gain	Dasgupta & Bernard 2006; Gupta & Raina, 1998
Larrea tridentate	Diabetes, rheumatism, infertility, kidney and gall	Hepatotxicity, nephrotoxicity, carcinogenic	Dasgupta & Bernard 2006; O'Hara et al., 1998

	bladder stones, anticancer		
Catharanthus roseus	Diabetes, cancer chemotherapeutic	Male reproductive system toxicity, DNA damage and chromosomal damage	Fennell et al., 2004 Gupta & Raina, 1998
Callilepis laureola	Stomach complaints, induce fertility impotence, cough remedy	Liver necrosis, nephrotoxicity, carcinogenic, death	Popat et al., 2001
Aristolochia fangchi	Weight loss, rheumatism, oedema	Nephrotoxicity, carcinogenic	Niggemann & Gruber, 2003
Datura stramonium	Anaesthesia, eye drops, spasm, motion sickness, parkinsonism	Blurred vision, delirium, incoherence, coma, death	Kurzbaum et al., 2001
Atropa belladonna	Relaxant, antiulcer	Anticholinergic, central nervous system and respiratory depression	O'Hara et al. 1998;
Ephedra sinica	Anorectic, stimulant, bronchodilator	Associated with hypertensive stroke, palpitations, and nerve damage, death	Dasgupta & Bernard, 2006 O'Hara et al., 1998
Viscum album	Antihypertensive, antitumor	Central nervous system and cardiac toxic reaction, gastrointestinal bleeding	O'Hara et al., 1998; Gupta & Raina, 1998

1.6 Scope of the study

This study starts with the review of previous studies done on *S. aculeastrum*. It includes the investigation of the anti-inflammatory and analgesic potential of fresh and processed (dried, boiled dried and boiled fresh) extracts of the plant's berries *in vivo*. This is important because anti-inflammatory treatments have been found to block carcinogenesis (Federico et al., 2007) leading to lower risk of cancer incidence in people regularly taking them (Fosslien, 2000). The cytotoxic effect of these extracts on human tumours such as HeLa, MCF-7, HT-29, as well as healthy Chang liver cell line using the MTT assay was also studied. In addition, the induction of apoptosis and cell cycle arrest was monitored using Annexin V–FITC Apoptosis Detection Kit. Toxicological studies, specifically the effect of the extracts on the organs as well as on the haematological and biochemical parameters of male Wistar rats were studied for 14 and 28 days respectively.

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CHAPTER 2

Overview of the study plant: *Solanum aculeastrum*

Chapter 2

OVERVIEW OF THE STUDY PLANT: *Solanum aculeastrum*

2.0 Introduction

The choice of this plant (*Solanum aculeastrum*) for the present study emanated from the conclusions derived from the PhD thesis of Koduru (2006), who validated the traditional use of the plant in the treatment of cancer *in vitro*. In 2005, this author conducted an ethnobotanical survey of the plants used in the treatment of cancer by the people of the Eastern Cape Province in South Africa and one of such plants was *S. aculeastrum*. He further worked and reported on its antimicrobial, antioxidant and antiproliferative potentials. However, that study did not investigate the possible effect of any form of processing on the efficacy of the plant as well as its safety *in vivo*.

Solanum aculeastrum Dunal commonly known as *umthuma* or goat bitter-apple belongs to the family Solanaceae (Fig 2). This plant is widely distributed in southern Africa (Watt and Breyer-Brand wijk, 1962). It is a thorny perennial plant that grows up to 2–3 m in height with white flowers and lemon shaped berries that become yellow-green when ripe (Wanyonyi et al., 2002). The extremely bitter berries and leaves are used for the treatment of various diseases in humans and domestic animals (Hutchings et al., 1996). The bitter fruits of *S. aculeastrum* is used fresh, dried, ashed or boiled for the treatment of jigger wounds and gonorrhea (Agnew and Agnew, 1994), rheumatism as well as ringworm in cattle and horses (Watt and Breyer-Brandwijk, 1962). An ethnobotanical survey indicated that the berries are used in the treatment of breast cancer in the Eastern Cape Province of South Africa (Koduru et al., 2006c).

Figure 2. *Solanum aculeastrum.* Note both the ripe and unripe fruits.

In the last few years, several researchers have reported the effect of both the crude extracts and the isolated compounds of *S. aculeastrum* in various biological systems, as antitumor, antimicrobial, anthelmintic, antioxidant as well as molluscicidal agent (Nfi et al., 1999; Wanyonyi et al., 2002; Wanyonyi et al., 2003; Koduru et al., 2006a, b, c, d, e; Koduru et al., 2007a, b; Steenkamp et al., 2007a, b).

2.1 Chemistry of the plant

The main constituents of *Solanum aculeastrum* which have been implicated in the pharmacological activities of this plant are steroidal alkaloids. These include β-solamarine, solamargine, solasonine, solasodine, solaculine, tomatine and tomatidine (Drewes and van-Staden, 1995; Wanyonyi et al., 2002; Koduru et al., 2007a). Steroidal alkaloids have been the

target of pharmacological investigations because of their structural similarities with anabolic steroids, steroidal hormones and corticosteroids (Gurib-Fakim, 2006).

The leaves and berries of *S. aculeastrum* have also been found to contain mainly straight- chain aliphatic hydrocarbons (Koduru et al., 2006a). These authors reported the isolation of volatile compounds such as alkanes (64.5%), phthalic acid (14.9%) and fatty acid esters (7.7%) from the berries, while the leaves possessed 17.5% alkanes, 17% aldehydes, 15.2% aromatic hydrocarbons, 11.8% phthalic acid, 2.3% methyl salicylate and 6.5% terpenoids.

2.2 Antimicrobial activity

The antibacterial activity of the methanol and aqueous extracts of the berries have been studied extensively using various methods. Wanyonyi et al., (2003) showed that these extracts were moderately active against *Staphylococcus aureus, Escherichia coli, pseudomonas aureginosa* and *Bacillus subtilis.* Koduru et al., (2006d) reported that while the aqueous extracts of the berries were not active against all tested bacteria, the methanol extract inhibited these organisms with minimum inhibitory concentrations ranging from 4 to10 mg/ml. However, Steenkamp et al., (2007a) observed that the aqueous and methanol extracts of the fruits of *S. aculeastrum* were not active against *Staphylococcus epidermis* and *Staphylococcus aureus.*

Koduru et al., (2006d) also studied the effect of the aqueous and methanol extracts of *S. aculeastrum* berries against various fungi. Their results showed that these extracts completely inhibited the growth of *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Penicillium notatum* at 5 mg/ml, however they were inactive against *Candida albicans*. Steenkamp et al.,

(2007b) also reported that the methanol and aqueous extracts of the berries inhibited 5 clinical isolates and 1 standard strain of *Candida albicans* at concentrations ranging from 0.04 to 1.50 mg/ml and 1.88 to 8.50mg/ml respectively.

2.3 Molluscicidal activity

The molluscicidal property of the aqueous and methanol extracts of the berries and the root bark against *Biomphalaria pfeifferri* was determined by Wanyonyi et al., 2002. The aqueous extracts of the berries were more potent against the test snails than the root bark. Further studies on the activity of four alkaloid-rich fractions obtained from the crude methanolic extract showed 100% mortality in all the fractions at concentrations ranging from 20 to 40 ppm (Wanyonyi et al., 2003).

2.4 Anthelminthic activity

Nfi et al., (1999) studied the anthelminthic property of *S. aculeastrum* along with other plants used for ethnoveterinary medicine in Cameroon. The roots and leaves of the *S. aculeastrum* was found to possess 34.4% anthelminthic activity compared with *Khaya anthoteca* (55.8%) and *Vernonia amygdalina* (52.4%).

2.5 Antioxidant activity

Koduru et al., (2006b) examined the effect of the extracts of the berries of *Solanum aculeastrum* on free radicals. While the acetone extract did not demonstrate any significant antioxidant activity at the tested concentrations, the aqueous extracts possessed higher activity than the methanol extracts. The higher antioxidant activity of the aqueous extract was attributed to the substantial amount of polar constituents present in the plant.

2.6 Anticancer property

The antiproliferative activity of the aqueous, methanol and acetone extracts of the leaves and berries of *S. aculeastrum* against HeLa, MCF-7 and HT-29 human cancer cell lines was reported by Koduru et al., (2006e). Their study showed that the extracts of the leaves showed no anticancer property while the methanol and aqueous extracts of the berries possessed the highest antiproliferative activity, with IC_{50} ranging between 17.1 and 48.5 µg/ml.

The effect of solasodine and tomatidine, steroidal alkaloids isolated from the berries of *S. aculeastrum* were also tested on these human cancer cells (Koduru et al., 2007a). Reports from this study indicated that pronounced activity was evident from the combined compounds rather than the individual compounds. The IC_{50} of the combined compounds ranging from 126.9 to 149.3 µM was not comparable to cisplatin (2.5 µM). Also, the compounds, both individually and combined, possessed low apoptotic index (0.3 – 4.2%) when compared to cisplatin (9.6%).

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CHAPTER 3

Anti-nociceptive and anti-inflammatory activities of the aqueous extract of fresh *Solanum aculeastrum* Dunal. berries in male Wistar rats.

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*This chapter has been submitted for publication in this format to the **Journal of Medicinal Plant Research**

Anti- nociceptive and anti-inflammatory activities of the aqueous extract of fresh *Solanum aculeastrum* Dunal. berries in male Wistar rats.

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Abstract

The berry of *Solanum aculeastrum* is used among other remedies, for treating inflammatory-related ailments in South Africa. The aqueous extract of the fresh berries at 25, 50 and 75 mg/kg body weight was evaluated for anti-inflammatory and analgesic effects in rats using histamine and carrageenan-induced paw oedema as well as formalin and tail immersion tests. The result of the phytochemical screening indicated that the berries possess alkaloids, saponins, phenolics, flavonoids, cardenolides and dienolides. Oral administration of the extract to Wistar rats significantly reduced the formation of oedema induced by carrageenan and histamine after 3 h. The extracts also prolonged the reaction time in the tail immersion-induced pain 60 min after administration. In addition, the extract significantly suppressed the nociceptive response in the early and late phases of the formalin induced pain in a dose-dependent manner with more pronounced effect on the late phase. These results also compared well with those of indomethacin, the reference drug used in this study. This study therefore gives credence to the traditional uses of *Solanum aculeastrum* in the treatment of certain conditions associated with inflammatory pain.

Key words: *Solanum aculeastrum***,** anti-inflammatory, analgesic.

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3.1 Introduction

Inflammation is a process which involves the response of cellular tissues to foreign bodies such as pathogenic micro-organisms, toxic chemicals, parasites and injury (van Kempen et al., 2006). Several studies have established a role of inflammation in the initiation of diseases such as diabetes, rheumatoid arthritis and cancer (Balkwill and Mantovani, 2001; Coussens and Werb, 2002). Inflammatory-related ailments are treated mainly with non-steroidal anti-inflammatory drugs (NSAIDs). These drugs are used to reduce the consequences of inflammation (Vane and Botting, 1996). For example, indomethacin has been found to block carcinogenesis in animals by reducing the production of inflammatory cytokines (Federico et al., 2007). However, prolonged use of these drugs has been reported to produce frequent adverse effects such as dyspepsia and severe gastrointestinal complications (Bures et al., 2002). Therefore, there is a need for potent drugs with fewer side effects. As a result, the search for other alternatives have become necessary.

Plants possess biologically active compounds of medicinal value (Talhouk et al., 2007). Extracts of many plants have been used to treat inflammation-related ailments in the local communities of developing countries. *Solanum aculeastrum* Dunal. (Solanaceae) commonly known as *Umthuma* by the Xhosa speaking people in South Africa is one of such plants. This plant, also known as *goat bitter apple*, is widely distributed in southern Africa (Watt and Breyer-Brand wijk, 1962). It is a thorny perennial plant that grows up to 2–3 m in height with white flowers and lemon shaped berries that become yellow-green when ripe (Wanyonyi et al., 2002). The bitter fruits of *S. aculeastrum* is used fresh, dried, ashed or boiled for treating jigger wound and gonorrhea (Agnew and Agnew, 1994), rheumatism as well as ringworm in cattle and horses (Watt and Breyer-Brand wijk, 1962). Pharmacological studies have revealed the antimicrobial and

anticancer properties of the leaves and berries (Koduru et al., 2006a, b). Steroidal alkaloids, the major components of the berries of *S. aculeastrum* produced molluscicidal and anticancer effects *in vitro* (Koduru et al., 2007b; Wanyonyi et al., 2002; Drews and Van Staden, 1995).

The main objective of the present study was to evaluate the anti-inflammatory and anti-nociceptive potentials of *S. aculeastrum* berries in animal models since the berry is used for the local treatment of some painful inflammatory conditions.

3.2 Materials and methods

3.2.1 Collection of plant materials and authentication

Fresh berries of *S. aculeastrum* collected from Kayalethu village in the Eastern Cape province of South Africa, was authenticated by Prof. D.S Grierson at the Department of Botany, University of Fort Hare. A voucher specimen (SA/Med 01) was deposited at the Giffens herbarium of the University.

3.2.2 Extraction of plant materials

The berry (500 g), cut into small pieces, was extracted in 1000 ml of distilled water for 48 h on a mechanical shaker (Stuart Scientific Orbital Shaker SO1, United Kingdom). The extract was filtered using a Buchner funnel and Whatman No 1 filter paper. The resulting filtrate was freeze dried (Vir Tis benchtop K, Vir Tis Company, Gardiner, NY) to give a yield of 7.50 g. This was reconstituted in distilled water to give the required doses for each experiment.

3.2.3 Animals

Male rats (*Rattus norvegicus*) of Wistar strains weighing between 120 and 180 g were obtained from the Experimental Animal House of the Agricultural and Rural Development Research Institute, University of Fort Hare. The animals were housed in clean metabolic cages

placed in well ventilated house with optimum condition (temperature 28 ± 1^{o}C; photoperiod: 12 h natural light and 12 h dark; humidity: 45-50%). They were also allowed free access to food (Balanced Trusty Chunks (Pioneer Foods (Pty) Ltd, Huguenot, South Africa) and water. All experimental protocols were in compliance with University of Fort Hare Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

3.2.4 Chemicals

Carrageenan, Tween-80, indomethacin and histamine were obtained from Sigma-Aldrich Chemie Gmbh, Steinheim, Germany. All other chemicals used were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

3.2.5 Phytochemical screening

The screening for some chemical constituents of the extract was carried out as described for tannins and triterpenes (Odebiyi and Sofowora, 1978), alkaloids (Harbourne, 1973), phenolics and flavonoids (Awe and Sodipo, 2001), cardiac glycosides, saponins and steroids (Edeoga et al., 2005), cardenolides, dienolides and anthraquinones (Trease and Evans, 1989).

3.2.6 Anti-inflammatory activity

3.2.6.1 Carrageenan-induced paw oedema

Thirty rats were grouped into five of six animals each. While the first group served as the negative control, the second, third and fourth groups received indomethacin (10 mgkg^{-1} b.wt) and extract at 25, 50 and 75 mgkg^{-1} body weight respectively. The extract was dissolved in normal saline while indomethacin was suspended in 3% Tween 80 in normal saline. Carrageenan

solution (0.1 ml of 1%) was injected into the sub plantar region of right hind paw of the rats, 1 h after intraperitoneal administration of normal saline, indomethacin and the extract (Moody et al., 2006). The paw volume was measured at 0.5, 1, 2, 4 and 6 h after administration of drug and extract using a micrometer screw gauge (SMC-20326, Sterling Manufacturing Company, Ambala Cantt, India).

The anti-inflammatory effect of the extract was calculated using the expression: anti-inflammatory activity (%) = (1-D/C) X 100, where D represents the average paw volume after extract was administered to the rats and C was the average paw volume of the negative control animals. The percentage inhibition of inflammation was calculated from the expression: inhibition % = D_0-D_t/$D0$ X 100, where D_0 was the average inflammation (hind paw oedema) of the control group at a given time; and D_t was the average inflammation of the drug treated (i.e. extracts or reference indomethacin) rats at the same time (Gupta et al., 2005, Sawadogo et al., 2006).

3.2.6.2 Histamine induced paw oedema

Using the method of Perianayagam et al., (2006), paw oedema was produced by sub-plantar administration of 0.1% freshly prepared solution of histamine into the right hind paw of the rats. The paw volume was recorded at 0 and 1 h after histamine injection. Different groups of the rats were pretreated with extract (25, 50 or 75 mg/kg body weight) or with 2 ml/kg b.wt of normal saline (vehicle control) or 10 mg/kg body weight indomethacin (reference drug). These were administered intraperitoneally 1 h before eliciting paw oedema. The anti-inflammatory activity was calculated as described for carrageenan-induced oedema.

3.2.7 Analgesic activity

3.2.7.1 Tail immersion test

Acute nociception was assessed using the tail immersion test. This method involves immersing extreme 3 cm of the rat tail in a waterbath (Buchi waterbath B480, Buchi Switzerland) containing water at a temperature of 55 ± 0.5^0C. The time the animal's tail spent in the water before reacting to the pain is recorded with a stop watch. Each animal served as its own control. The average of the values was recorded as the initial reaction time (Tb). The test groups were given the extracts (25, 50 or 75 mg/kg body weight), indomethacin (10 mg/kg b.wt) or distilled water. The reaction time (Ta) for the test groups were taken at 30, 60 120, 240 and 360 min after a latency period of 30 min, following administration of the extract and reference drug (Vogel and Vogel, 1997). The percentage analgesic activity was computed from the expression:

$$\text{Percentage analgesic activity} = \frac{(Ta - Tb)}{Tb} \times 100$$

3.2.7.2 Formalin induced pain test

The formalin induced pain test was carried out according to the procedure described by Correa and Calixto, (1993). Briefly, 0.05 ml of 2.5% formalin solution was injected into the sub-plantar of the right hind paw. The number of times the injected paw was licked was recorded and was considered as indicative of pain. The animals were pretreated with normal saline, indomethacin and extracts, 30 min before the administration of formalin and the response was observed for 30 min.

3.2.8 Statistical analysis

Data were expressed as means of four replicates. Statistical difference between the control and the treated groups were tested by Student's t-test. Values were considered statistically significant at $p < 0.05$.

3.3 Results

Phytochemical screening of the aqueous extract of *Solanum aculeastrum* berries showed the presence of alkaloids, saponins, phenolics, flavonoids, cardenolides and dienolides.

In the carrageenan-induced inflammatory model, oedema was reduced by the aqueous extract of *S. aculeastrum* at concentrations of 25, 50 and 75 $mgkg^{-1}$ in a dose-dependent manner. Maximum inhibition of paw oedema was observed at the end of 6 h (Table 1). The percentage reduction in inflammation diameter was highly significant in the group treated with 75 $mgkg^{-1}$ of the extract after 3 h compared to the control.

The sub-acute inflammation test with histamine showed an inhibition of the paw oedema by indomethacin and the extract in a dose-dependent manner, while an increase in paw volume of the negative control group was observed. The extract at 75 $mgkg^{-1}$ body weight and indomethacin exhibited comparable activity at 6 h.

All the doses of the extract (25, 50 and 75 $mgkg^{-1}$ b.wt), had analgesic effect on both early and late phases of the formalin test as shown on Table 3. These phases correspond to neurogenic and inflammatory pains respectively. Inhibition of pain resulting from inflammation was higher than the neurogenic-induced pain at all concentrations tested. The animals treated with the highest dose (75 $mgkg^{-1}$ b.wt) exhibited more prominent response to the pain at both phases compared to indomethacin.

Following 30 min latency period, 50 and 75 $mgkg^{-1}$ of orally administered extract caused reduction of the painful sensation due to tail immersion in warm water (Fig. 1). The antinociceptive property of the extract at 75 $mgkg^{-1}$ was more pronounced than that of indomethacin at the 60 min time point.

3.4 Discussion

The study indicated that the aqueous extract of fresh *Solanum aculeastrum* berry possess both peripheral and central analgesic properties as well as anti-inflammatory property.

The ability of anti-inflammatory agents to inhibit mediators of acute inflammation is the basis of the carrageenan-induced inflammation test. These mediators include histamine, serotonin and bradykinnins, which induce the first phase of inflammation (Kasahara et al., 2002). The second, late phase of inflammation is induced mainly by prostaglandins which are released from the third hour (Di-Rosa et al., 1971). The pronounced anti-inflammatory effect of *S. aculeastrum* berry observed in both the carrageenan and histamine-induced oedema test from 4 h is an indication of the potential of the extract to inhibit the release of prostaglandins (Di-Rosa et al., 1971). These results indicate the effectiveness of *Solanum aculeastrum* on acute inflammatory conditions.

Phytochemical screening of the extract shows that the berries possess alkaloids, saponins, phenolics, flavonoids, cardenolides and dienolides. Of these, flavonoids are the main compounds possessing anti-inflammatory activities (Talhouk et al., 2007). Many studies have also demonstrated the anti-inflammatory and analgesic properties of alkaloids, phenols (Ahmadiani et al., 2001) and saponins (Peana et al., 1997). The study of Filderman and Kovacs, (1969), showed that the oral administration of tomatine, one of the steroidal alkaloids found in *Solanum aculeastrum*, exerted significant dose dependent inhibitory activity on oedema formation.

Therefore, it could be suggested that the anti-inflammatory property of the berries of this plant may be due to the presence of these active constituents.

The formalin test is a more valid model for the assessment of clinical pain (Tjolsen et al., 1992) producing distinct biphasic responses (Vasudevan et al., 2007). Various analgesics differ in their response to the formalin test. Drugs such as opioids inhibit both phases of the formalin test because they act on the central nervous system (Shibata et al., 1989), while aspirin, hydrocortisone and dexamethazone act peripherally, thereby inhibiting only the second phase (Vasudevan et al., 2007). The first (neurologic) phase results from the stimulation of nociceptors while the second phase is of an inflammatory pain origin. Assessment of the anti-nociceptive response using the formalin test showed that all the doses in this study inhibited both phases of the formalin test. The highest dose exhibited more prominent response to the pain at both phases compared to indomethacin (Table 3). Therefore, the result of this study has suggested that the *Solanum aculeastrum* extract acted centrally and peripherally in the formalin test.

The study also showed that the extract of this plant possesses centrally acting protective effect in the tail immersion test. The result indicated that after 30 min latency period, 50 and 75 $mgkg^{-1}$ of orally administered extract caused reduction of the painful sensation by the warm water. The protective effect at 75 $mgkg^{-1}$ b.wt was more pronounced than indomethacin, which was inactive.

In conclusion, the anti-inflammatory and analgesic properties of *Solanum aculeastrum* berry observed in these models might, in part, be due to the presence of compounds such as alkaloids, saponins, phenolics and flavonoids. The results also suggest a rationale for the traditional uses of this plant in southern Africa for some painful inflammatory conditions.

Acknowledgement

This research was supported by the National Research Foundation of South Africa.

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Table 1: Effect of oral administration of *S. aculeastrum* berries on carrageenan-induced paw oedema in Wistar rats (n = 6; $\overline{X}$ ± S.D)

Treatment	Dose (mg/kg)	Differences in right hind paw (in mm) 1 h	2 h	3 h	4 h	6 h
Control	0	0.72 ± 0.08[a]	2.08 ± 0.03[a]	2.36 ± 0.01[a]	2.18 ± 0.02[a]	1.56 ± 0.02[a]
SAB	25	0.58 ± 0.09[a] (11.9)	0.86 ± 0.1 [b] (49.5)	1.27 ± 0.20[c] (35.5)	0.90 ± 0.09[b] (49.2)	0.28 ± 0.06[d] (77.7)
	50	0.62 ± 0.06[a] (7.3)	1.00 ± 0.08[c] (43.0)	0.67 ± 0.09[d] (63.9)	0.52 ± 0.08[d] (69.7)	0.26 ± 0.07[c] (79.7)
	75	0.36 ± 0.04[b] (46.5)	0.94 ± 0.04[d] (47.4)	0.93 ± 0.05[e] (52.8)	0.65 ± 0.07[c] (63.9)	0.08 ± 0.01[e] (96.9)
Indomethacin	10	0.45 ± 0.09[a] (40.4)	0.80 ± 0.05[d] (59.6)	1.33 ± 0.30[b] (42.3)	0.51 ± 0.07[e] (74.1)	0.29 ± 0.09[b] (80.2)

Values carrying superscripts different from the control down the group for each hour are significantly different ($P < 0.05$)

Table 2: Effect of oral administration of S. aculeastrum berries on histamine-induced paw oedema in Wistar rats (n = 6; $\overline{X}$ ± S.D)

Treatment	Dose (mg/kg)	Differences in paw volume (mm) 1 h	2 h	3 h	4 h	6 h
Control	0	0.78 ± 0.06[a]	0.86 ± 0.06[a]	1.03 ± 0.01[a]	1.16 ± 0.03[a]	1.24 ± 0.11[a]
SAB	25	0.71 ± 0.06[a] (5.1)	0.72 ± 0.05[b] (12.2)	0.65 ± 0.09[b] (31.5)	0.48 ± 0.10[b] (53.2)	0.33 ± 0.09[b] (69.4)
	50	0.69 ± 0.17[a] (9.9)	0.73 ± 0.11[a] (12.3)	0.34 ± 0.08[c] (63.5)	0.35 ± 0.07[d] (66.3)	0.35 ± 0.09[c] (73.1)
	75	0.55 ± 0.07[a] (26.9)	0.40 ± 0.07[c] (49.8)	0.39 ± 0.07[c] (58.0)	0.16 ± 0.09[d] (84.1)	0.12 ± 0.04[d] (92.0)
Indomethacin	10	0.54 ± 0.09[a] (19.2)	0.51 ± 0.07[b] (29.7)	0.28 ± 0.08[d] (66.8)	0.20 ± 0.09[c] (77.8)	0.14 ± 0.08[d] (86.0)

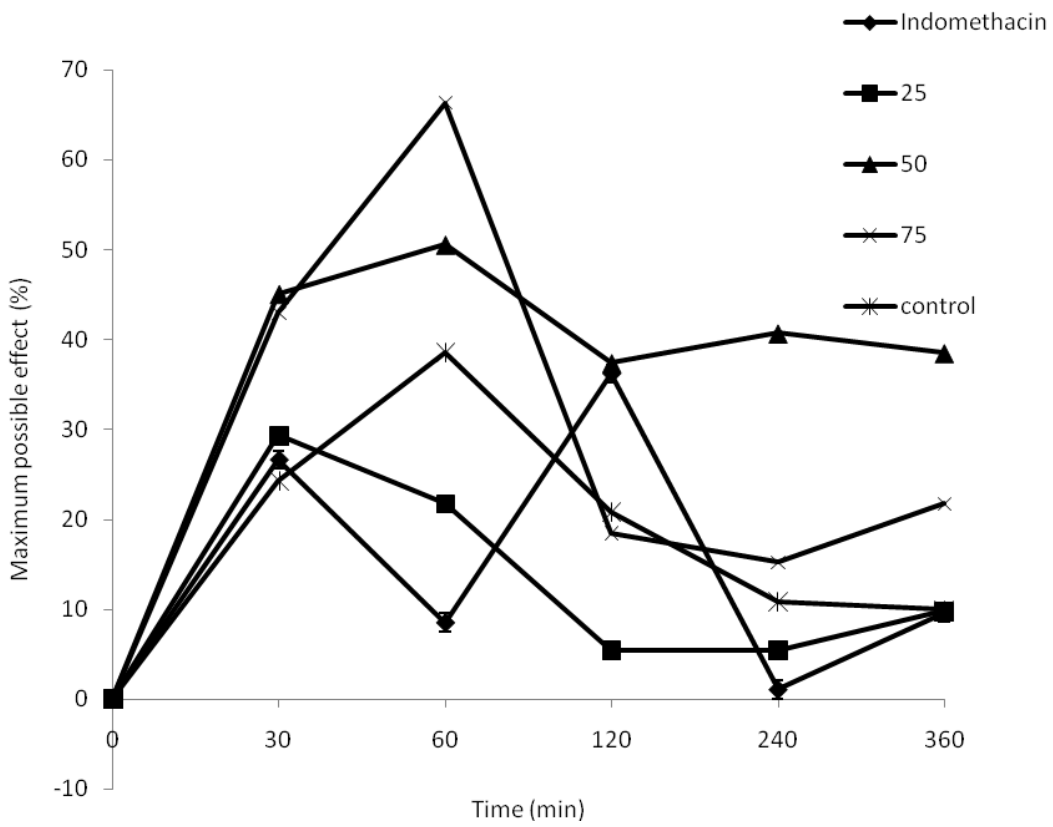
Values carrying superscripts different from the control down the group for each hour are significantly different ($P < 0.05$)

Table 3: Effect of oral administration of Solanum aculeastrum berries on formalin nociception response in Wistar rats (n = 6; $\overline{X}$ ± S.D)

		Number of times licked (mean ± SD)			
Treatment	Dose ($mgkg^{-1}$)	Phase 1	% Inhibition	Phase 2	% Inhibition
Control	0	14.00 ± 1.41^{a}	-	32.25 ± 8.42^{a}	-
Extract	25	10.67 ± 3.21^{a}	23.8	15.50 ± 7.78^{a}	51.9
	50	6.00 ± 1.63^{b}	57.1	5.67 ± 2.31^{c}	82.4
	75	5.00 ± 1.41^{c}	64.3	4.00 ± 2.65^{c}	87.6
Indomethacin	10	5.25 ± 1.71^{b}	62.5	8.00 ± 2.16^{b}	75.2

Values carrying superscripts different from the control down the group for each hour are significantly different ($P < 0.05$)

Figure 1: Effect of oral administration of *S. aculeastrum* berries on tail immersion nociception response in male Wistar rats (n = 6; $\overline{X}$ ± S.D)



CHAPTER 4

Comparative studies on the anti-inflammatory and analgesic activity of the fresh and boiled extracts of *Solanum aculeastrum* Dunal.

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Comparative studies on the anti-inflammatory and analgesic activity of the fresh and boiled extracts of *Solanum aculeastrum* Dunal.

*This chapter has been submitted in this format and is under review with **African Journal of Biotechnology**

Comparative studies on the anti-inflammatory and analgesic activity of the aqueous extracts from fresh, dried and boiled berries of Solanum aculeastrum Dunal.

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Abstract

The berry of *Solanum aculeastrum* Dunal. is used for treating diseases such as rheumatism, gonorrhea, breast cancer and other inflammatory-related ailments in South Africa. The aqueous extracts of the fresh, dried and boiled berries at doses of 1 and 10 mg/kg body weight was evaluated for anti-inflammatory and analgesic effects in male Wistar rats using carrageenan-induced paw oedema as well as formalin, acetic acid induced writhing and tail immersion tests. Oral administration of the extract showed some inhibition of the paw oedema that was not dose dependent. The percentage reduction in inflammation diameter was more prominent in both concentrations of the boiled fresh berries than indomethacin. The extracts at 10 mg/kg prolonged the reaction time in the tail immersion-induced pain 60 min after administration. Although, only the extracts of the boiled dried berries (10 mg/kg) suppressed pain in the formalin test at the late phase, a more pronounced effect of all the extracts in a dose-dependent manner was observed in the late phase than the early phase. The results of the acetic acid induced writhing test showed that all the extracts possessed analgesic effect at the tested doses (1 and 10 mg/ml). In conclusion, the extracts of *Solanum aculeastrum* berry did not show considerable anti-inflammatory and analgesic effects in comparison to indomethacin. This observation in these models might in part be due to the low doses fed to the rats in this experiment.

Key words: *Solanum aculeastrum*, anti-inflammatory, analgesic.

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1.0 Introduction

Inflammation is the way the body deals with infections and tissue damage (Simmons, 2006). Uncontrolled, inflammation can lead to a cascade of diseases such as rheumatoid arthritis, chronic asthma, psoriasis, cancer, multiple sclerosis and a host of other diseases (Balkwill and Mantovani, 2001; Coussens and Werb, 2002; Simmons, 2006). Various studies have shown that inflammation may be an initiator of carcinogenesis via genotoxic and cytotoxic effects (Platz and De Marzo, 2004). The longer inflammation persists, the higher the risk of cancer (Federico et al., 2007). Cancers of infectious etiology such as stomach, bladder, colon, liver and bile duct have been linked to inflammation (Coussens and Werb, 2002). Non-steroidal anti-inflammatory drugs (NSAIDs) are the main drugs used to reduce the consequences of inflammation (Vane and Botting, 1996). Indomethacin, an NSAID, for example has been found to block carcinogenesis in animals by reducing the production of inflammatory cytokines (Federico et al., 2007). A lower risk of cancer incidence has also been found in people regularly taking NSAIDs (Fosslien, 2000). Although, these drugs have been found to be effective, prolonged use has been reported to produce frequent adverse effects such as dyspepsia and severe gastrointestinal complications (Bures et al., 2002). This has therefore led to various researches ~~to~~ for other effective drugs with less/no side effects.

Medicinal plants have been in use since ancient time in the treatment of various ailments. Plants such as *Catharanthus roseus, Digitalis purpurea*, *Rauwolfia serpentina*, Willow (*Salix* species), *Physostigma venenosum* have been used for centuries for the treatment of diseases. Since then, plants have served as sources of biologically active natural products which are either used as commercial drugs or as lead structures for the development of modified derivatives (Cordell et

al., 1991). Extracts of *Solanum aculeastrum* Dunal. (Solanaceae) have been used to treat inflammation-related ailments in the local communities of South Africa. This plant also known as goat bitter apple is traditionally used by the Xhosa speaking people of South Africa in the treatment of breast cancer (Koduru et al., 2007a). *S. aculeastrum* is widely distributed in southern Africa (Watt and Breyer-Brand wijk, 1962) and the bitter fruits are either used fresh, dried, ashed or boiled for treating jigger wound and gonorrhea (Agnew and Agnew, 1994), rheumatism as well as ringworm in cattle and horses (Watt and Breyer-Brand wijk, 1962). Pharmacological studies have revealed the antimicrobial and anticancer properties of the leaves and berries (Koduru et al., 2006a, b).

Previously, the authors studied the anti-inflammatory and analgesic effect of the fresh berries of *Solanum aculeastrum*. Although the extract was found to be active at the tested doses, toxicological studies indicated that those concentrations were not completely safe for use. Therefore, the main objective of the present study was to compare the anti-inflammatory and anti-nociceptive potentials of the fresh and boiled extracts of the berries in animal models in view of its use in the local treatment of some painful inflammatory conditions.

2.0 Materials and methods

2.1 Collection of plant materials and authentication

Fresh berries collected from Kayalethu village in the Eastern Cape province of South Africa, was authenticated by Prof. D.S Grierson at the Department of Botany, University of Fort Hare. A voucher specimen (SA/Med 01) deposited at the Giffens Herbarium of the University.

2.2 Extraction of plant materials

The fresh berries collected were washed, cut into pieces and divided into two portions. The first portion was oven dried at 40^{o}C , a part of which (400 g) was boiled with distilled water (BDB) and the other (300 g) soaked with water (DB) by shaking for 24 h on an orbital shaker. The second portion of fresh berries (1500 g) was soaked (FB) and boiled (2000 g) separately with distilled water (BFB). The extracts were filtered using a Buchner funnel and Whatman No 1 filter paper. The filtrates were freeze dried (Vir Tis benchtop K, Vir Tis Company, Gardiner, NY) to give yields of 2.03 g (BFB), 2.01 g (FB), 11.85 g (BDB), 13.16 g (DB) per 100g of plant material. The crude extracts were then reconstituted in distilled water to give the required doses for each experiment.

2.3 Animals

Male rats (*Rattus norvegicus*) of Wistar strains weighing between 120 and 180 g were obtained at the Experimental Animal House of the Agricultural and Rural Development Research Institute (ARDRI), University of Fort Hare. The animals were housed in clean metabolic cages placed in well ventilated house with optimum condition (temperature 28 ± 1^{o}C; photoperiod: 12 h natural light and 12 h dark; humidity: 45-50%). They were also allowed free access to food (Balanced Trusty Chunks (Pioneer Foods (Pty) Ltd, Huguenot, South Africa) and water. All experimental protocols were in compliance with University of Fort Hare Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

2.4 Chemicals

Carrageenan, Tween-80, indomethacin and histamine were obtained from Sigma-Aldrich Chemie Gmbh, Steinheim, Germany. All other chemicals used were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

2.5 Anti-inflammatory activity

2.5.1 Carrageenan-induced paw oedema

The animals were assigned into ten groups (A-J), each consisting of six rats. Group A (control) received only 0.5 ml of normal saline (the vehicle), group B received indomethacin (10 mg/kg) while groups C to J were treated with the same volume of the extract preparation corresponding to 1 and 10 mg/kg body weight of the extracts respectively. The extracts were dissolved in normal saline while indomethacin was suspended in 3% Tween 80 in normal saline. Carrageenan solution (0.1 ml of 1%) was injected into the sub plantar region of right hind paw of the rats, 1 h after intraperitoneal administration of normal saline, indomethacin or the extract (Moody et al., 2006). The paw volume was measured at 1, 3 and 5 h after administration of drug and extract using a micrometer screw gauge (SMC-20326, Sterling Manufacturing Company, Ambala Cantt, India).

The anti-inflammatory effect of the extract was calculated using the expression: anti-inflammatory activity (%) = (1-D/C) X 100, where D represents the average paw volume after extract was administered to the rats and C was the average paw volume of the negative control animals. The percentage inhibition of inflammation was calculated from the expression: % inhibition = D_0-D_t/D_0 X 100, where D_0 was the average inflammation (hind paw oedema) of the control group at a given time; and D_t was the average inflammation of the drug treated (i.e. extracts or reference indomethacin) rats at the same time (Gupta et al., 2005, Sawadogo et al., 2006).

2.6 Analgesic activity

2.6.1 Tail immersion test

Acute nociception was assessed using the tail immersion test described by Vogel and Vogel (1997). Briefly, this method entails immersing the extreme 3 cm of the rat's tail in a water bath (Buchi water bath B-480, Buchi, Switzerland) maintained at a temperature of 55.00 ± 0.5°C. The time spent by the animal before reacting to the pain was measured with a stop watch as the initial reaction time (Tb). The various groups of the animals were orally administered with the extracts (25, 50 and 75 mg/kg body weight), indomethacin (10 mg/kg body weight) and distilled water. The response latency between the onset of immersion and the withdrawal of the tail (Ta) following the administration of the extract and the reference drug was recorded at 30, 60 120, 240 and 360 min after a latency period of 30 min. The percentage analgesic activity was computed from the expression:

Percentage analgesic activity = (Ta – Tb/Tb) x 100

2.6.2 Formalin induced pain test

The formalin induced pain test was carried out according to the procedure ~~as~~ described by Correa and Calixto, (1993). Briefly, 0.05 ml of 2.5% formalin solution was injected into the sub-plantar of the right hind paw. The number of times the rat licked the injected paw was recorded and was considered as indicative of pain. The animals were pretreated with normal saline, indomethacin and extracts, 30 min before the administration of formalin, and the responses were observed for 30 min.

2.7 Statistical analysis.

Data were expressed as mean of six replicates and were subjected to one way analysis of variance (ANOVA). Means were separated by the Duncan multiple range test using SAS and complemented with student's t-test. Values were considered statistically significant at $p < 0.05$.

3.0 Results

The sub-acute inflammation test with the carrageenan-induced inflammatory model showed that the inhibition of the paw oedema by all the extracts was not dose dependent (Table 1). The percentage reduction in inflammation diameter was highly significant ($p < 0.05$) and more prominent in both concentrations of the boiled fresh berries than indomethacin. At 1 and 5 h (1 mg/kg) for dried berry and 5 h (1 and 10 mg/kg) fresh berry, significant reduction in the inflammation diameter of the rats treated with these extracts was achieved.

Regarding the inhibition of pain in the formalin nociception test, all the extracts at 1 mg/ml did not reduce pain to a significant level compared to the control (Table 2). The boiled dried berry extract showed analgesic effect in both the early and late phase of the test, while the extracts of the dried, fresh and boiled fresh berries failed to demonstrate any significant analgesic effect. Inhibition of pain resulting from inflammation was higher in all the animals compared to the neurogenic induced pain.

The 30 min latency period following the oral administration of the aqueous extracts of the dried, fresh, boiled dried and boiled fresh extracts of the berry showed that the extracts and indomethacin at 10 mg/kg significantly increased the reaction time. Indomethacin and the extract

of the boiled dried berries at 10 mg/kg showed their maximal analgesic effect at 1 h post-treatment with percentage inhibitions of 28.04 and 25.11 % respectively. At 1 mg/kg, only the animals fed with the boiled extracts of the fresh berries demonstrated any significant effect on pain (24.08%) in the tail immersion test at the fifth hour (Table 3).

4.0 Discussion

The results of the present study showed the effect of processing on the activity of the aqueous extracts of *Solanum aculeastrum* berries. The four extracts varied in their potential to act either centrally or peripherally in their effect on pain. The peripheral analgesic activity of this plant can be deduced from its inhibitory effect on chemically induced nociceptive stimuli. Only the boiled extracts of the dried and fresh berries exhibited both peripheral and centrally mediated protective effect on the animals.

Assessment of the analgesic response using the formalin test showed that at both concentrations tested, none of the extracts inhibited the phase of the formalin test which produces a biphasic response in agreement with the observations of Vasudevan et al., 2007. The study showed the extracts exerted significant effect in the second phase (resulting from an inflammatory pain) than in the first phase (caused by the stimulation of nociceptors) of the formalin test corroborating the studies of Coderre and Melzack, 1992 and Abbadie et al., 1997. Also boiling had differing effects on the activity of the fresh and dried extracts. While inhibition of pain increased when the dried extracts were boiled, the opposite trend was observed with the boiled, fresh extracts. Conversely, the tail immersion test showed that boiling had similar, positive effects on the inhibitory performance of both fresh and dry extracts.

The carrageenan induced oedema results from the ability of anti-inflammatory agents to inhibit mediators of acute inflammation. This study showed that the effect of the extracts on this model was not dose dependent. At 10 mg/kg, extracts of the dried, boiled dried and boiled fresh berries possessed higher inhibitory activity than the fresh extracts at all the measurement intervals. This implies that processing enhanced the anti-inflammatory potential of the berries of *S. aculeastrum*.

In conclusion, the extracts of *Solanum aculeastrum* berry did show comparable anti-inflammatory and analgesic effects to indomethacin. Also, in all but the formalin test, processing improved anti-inflammatory and analgesic activity of the berry extracts.

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Table 1: Effect of aqueous extract of *Solanum aculeastrum* berries and indomethacin on carrageenan-induced edema paws volume in male Wistar rats. n = 6, $\overline{X}$ ± SD

Treatment Groups	Doses (mg/kg body weight)	Right hind paw volume (mm)		
		1 h	3 h	5 h
Control	0	1.26 ± 0.28^{a}	1.83 ± 0.56^{a}	1.85 ± 0.50^{a}
Indomethacin	10	0.53 ± 0.09^{a} (58.3)	0.65 ± 0.09^{ba} (62.6)	0.55 ± 0.02^{ba} (68.8)
Dried berries	1	0.22 ± 0.67^{b} (80.3)	1.03 ± 0.61^{bcd} (39.5)	0.19 ± 0.13^{c} (87.8)
	10	0.49 ± 0.23^{b} (56.4)	0.76 ± 0.25^{c} (52.2)	0.73 ± 0.04^{c} (54.6)
Boiled dried berries	1	0.59 ± 0.55^{b} (45.3)	1.17 ± 0.17^{bc} (25.7)	0.52 ± 0.18^{c} (64.8)
	10	0.55 ± 0.12^{b} (50.4)	0.78 ± 0.17^{c} (49.6)	0.47 ± 0.09^{dc} (68.8)
Fresh berries	1	0.49 ± 0^{b} (55.3)	0.84 ± 0.33^{cd} (46.2)	0.39 ± 0.21^{c} (74.0)
	10	0.73 ± 0.30^{b} (37.2)	1.00 ± 0.40^{bc} (39.1)	0.31 ± 0.20^{dc} (79.4)
Boiled fresh berries	1	0.44 ± 0.31^{b} (60.5)	0.65 ± 0.32^{d} (86.1)	0.73 ± 0.42^{cb} (95.2)
	10	0.58 ± 0.06^{b} (53.2)	0.56 ± 0.08^{c} (66.9)	0.07 ± 0.13^{d} (95.9)

Values in brackets denote percentage inhibition of the edema paw volume.
[a-d] Values carrying superscripts indicate difference ($P<0.05$).

Table 2: Effect of oral administration of Solanum aculeastrum berries on formalin nociception response in rats (n = 6), $\overline{X}$ ± SD

		Number of times licked (mean ± SD)			
Treatment	Dose ($mgkg^{-1}$)	Phase 1	% Inhibition	Phase 2	% Inhibition
Control	0	16.0 ± 4.00[a]	-	28.3 ± 7.64[a]	-
Dried berries	1	19.0 ± 5.57[a]	-18.8	21.3 ± 3.21[ba]	24.7
	10	16.3 ± 2.52[ba]	-2.08	20.3 ± 0.58[ba]	28.2
Boiled dried berries	1	14.3 ± 8.08[a]	10.4	14.0 ± 7.81[b]	50.6
	10	9.0 ± 1.73[bc]	43.8	12.7 ± 2.52[b]	55.3
Fresh berries	1	17.3 ± 1.53[a]	-8.3	19.0 ± 1.73[b]	32.9
	10	15.3 ± 3.21[ba]	4.2	17.3 ± 0.58[b]	38.8
Boiled fresh berries	1	20.0 ± 1.0[a]	-25.0	22.3 ± 1.53[ba]	21.2
	10	20.3 ± 8.96[ba]	-27.1	21.0 ± 9.64[ba]	25.9
Indomethacin	10	3.0 ± 1.0[b]	81.3	1.7 ± 0.58[c]	94.1

[a-c] Values carrying superscripts indicate difference ($P<0.05$).

Table 3: Effect of oral administration of aqueous extracts of *S. aculeastrum* berries on tail immersion nociception response in rats (n = 6), $\overline{X}$ ± SD.

Treatment Groups	Doses (mg/kg body weight)	0 h	1 h	3 h	5 h
		Time (sec)			
Control	0	2.83 ± 0.45^{a}	3.77 ± 1.17^{a}	4.08 ± 1.76^{a}	2.88 ± 0.50^{a}
Indomethacin	10	2.28 ± 0.49^{ba}	2.92 ± 1.07^{ba} (28.04)	2.50 ± 0.39^{b} (9.96)	2.51 ± 0.31^{ba} (10.32)
Dried berries	1	2.98 ± 1.01^{a}	2.22 ± 0.42^{bc} (-25.42)	1.99 ± 0.70^{b} (-33.03)	2.57 ± 0.72^{ba} (-13.55)
	10	2.47 ± 0.56^{a}	2.50 ± 0.75^{bc} (1.28)	1.92 ± 0.41^{b} (-22.08)	2.71 ± 0.55^{a} (9.86)
Boiled dried berries	1	2.44 ± 0.47^{a}	2.30 ± 0.49^{bc} (-5.73)	1.60 ± 0.18^{b} (-34.40)	1.98 ± 0.11^{bc} (-18.84)
	10	2.22 ± 0.38^{a}	2.77 ± 0.19^{b} (25.11)	2.60 ± 0.28^{ba} (17.22)	2.40 ± 0.26^{ba} (8.4)
Fresh berries	1	2.00 ± 0.17^{a}	1.73 ± 0.24^{bc} (-13.73)	1.51 ± 0.12^{b} (-24.46)	1.35 ± 0.11^{c} (-32.61)
	10	2.56 ± 0.22^{a}	2.66 ± 0.56^{b} (3.98)	2.53 ± 0.36^{ba} (-1.17)	2.66 ± 0.89^{ba} (3.85)
Boiled fresh berries	1	1.30 ± 0.08^{b}	1.18 ± 0.04^{c} (-8.74)	1.38 ± 0.02^{b} (6.78)	1.61 ± 0.13^{c} (24.08)
	10	2.16 ± 0.04^{a}	2.30 ± 0.03^{b} (6.41)	2.33 ± 0.03^{ba} (7.96)	2.44 ± 0.03^{ba} (13.21)

Percentages of protection against thermally induced pain by warm water are in parentheses.
$^{a-c}$ Values carrying superscripts indicate difference ($P<0.05$).

CHAPTER 5

Non specific cytotoxicity of *Solanum aculeastrum* berries on cancerous and non-cancerous cells.

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*This chapter has been submitted in this format and is under review with **African Journal of Pharmacy and Pharmacology**

Non specific cytotoxicity of *Solanum aculeastrum* berries on cancerous and non-cancerous cells

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Abstract

Ethnobotanical studies conducted on *Solanum aculeastrum* Dunal (Solanaceae), have shown that extracts of the berry are used for the treatment of breast cancer. This claim has been validated by scientific studies. As part of our on going research, we compared the cytotoxic effect of the cold aqueous extracts of the fresh, dried, and also the boiled extracts of the fresh and dried berries on human cancer cells and non-cancerous liver cells. The extracts obtained from the berries were studied against HeLa, MCF-7, HT-29, and U973 human cancer cells and non-cancerous Chang liver cells using the MTT assay. Annexin V-FITC/PI assay was conducted to confirm apoptosis. The present results demonstrated that all the extracts of *S. aculeastrum* are potent inhibitors of human breast, cervical, and colonic carcinoma cells. In terms of relative potency, the cold fresh berries appeared to be the most active of the extracts, especially at low concentrations. However, not only did the extracts inhibit the growth of the cancer cells, but they were also cytotoxic against non-cancerous cells (both the actively dividing and confluent Chang liver cells). The observed cytotoxic effect of the extracts was confirmed by the Annexin V-FITC/PI test, where the extracts caused more necrosis than apoptosis in the U973 cells. For the boiled extracts, necrotic ratio decreased (See Table 2).

The lack of differential effect of *S. aculeastrum* extracts on cancerous and non-cancerous cells implies that in addition to efficacy, safety considerations should govern possible therapeutic uses of the extracts of the berries of this plant.

Key words: Apoptosis, cytotoxicity, MTT assay, *Solanum aculeastrum*.

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1.0 Introduction

Biologically active products from plants have been sources of new therapeutic agents for cancer treatment (Hartwell, 1982). These products have served either as commercial drugs or as lead structures for the development of modified derivatives possessing enhanced activity (Cordell *et al.,* 1991). Research is ongoing to discover more natural sources of drugs for the treatment of cancer. The increase in the awareness and utilization of these drugs in the treatment of cancer has been due to the non-reliability and indefinitive cure by conventional medicines. One means of drug discovery has been the collection of information on the plants used in the treatment of cancer by different communities all over the world.

In Africa, herbal medicines are often used as treatment for cancer and for conditions often referred to as cancer such as warts, tumors, polyps, abscesses, calluses and hard swellings (Cragg and Newman, 2005). These herbal medicines are prepared either by drying, boiling or ashing of plant materials to increase their biological activity. Various reports exist on the increased biological properties resulting from steam processing of medicinal plants (Jung and Jin, 1996; Kim *et al.,* 1999; Yoo *et al.,* 2007). Therefore, in our study on *Solanum aculeastrum* Dunal (Solanaceae), a plant widely used in South Africa in the treatment of various diseases in humans and domestic animals (Hutchings *et al.,* 1996), the effect of boiling of the fresh and dried berries on cytotoxicity was investigated. The extract of the boiled berries of *S. aculeastrum* is used in the treatment of jigger wounds, gonorrhoea (Agnew and Agnew, 1994), rheumatism, and ringworm in cattle and horses (Watt and Breyer-Brandwijk, 1962). An ethnobotanical report by Koduru *et al.,* (2006b) showed that the aqueous extracts of the boiled berries of

S. aculeastrum are used in the treatment of breast cancer. Studies conducted by the same author reported that the cold aqueous extracts of the dried berries and leaves, and steroidal alkaloids isolated from *S. aculeastrum*, showed significant activity against HeLa, MCF-7, and HT-29 cancer cell lines (Koduru *et al.,* 2006a, 2007).

In this study, in addition to the investigation of the cytotoxic effects of the cold and boiled extracts, the selective cytotoxicity of the extracts against the MCF-7, HT-29, U973 cancer cell lines and non-cancerous Chang liver cells was determined.

2.0 Materials and methods

2.1 Plant Material

Fresh berries of *Solanum aculeastrum* were collected from Kayalethu village in the Eastern Cape province of South Africa in December 2008. The fruit was authenticated by Prof. D.S Grierson at the Department of Botany, University of Fort Hare and a voucher specimen (SA/Med 01) deposited at the Giffens herbarium.

2.2 Preparation of Extracts

The fresh berries collected were washed, cut into pieces and divided into two portions. The first portion was oven dried at 40°C , a part of which (400 g) was boiled in distilled water (BDB) and the other (300 g) soaked in cold water (DB) by shaking for 24 h on an orbital shaker. The second portion of the fresh berries was also divided into two parts, one part (1500 g) was soaked in cold water (FB) and the other (2000 g) boiled separately with distilled water (BFB). The extracts were filtered using a Buchner funnel and Whatman No 1 filter paper, then freeze dried (Vir Tis benchtop K, Vir Tis Company, Gardiner, NY) to give yields of 11.85 g (BDB), 13.16 g (DB) per 100 g of dry plant material, 2.03 g (BFB), 2.01 g (FB) per 100 g of fresh plant material.

2.3 Human Carcinoma cell lines and culture medium

HT-29 (colonic adenocarcinoma), HeLa (cervical carcinoma), MCF-7 (breast adenocarcinoma) and Chang liver cells (originally isolated in 1954 from a normal human liver by RS Chang from Harvard School of Public Health, Boston, MA) were cultured in 10 cm culture dishes in growth medium [antibiotic-free RPMI 1640 medium, (Sigma, Germany) containing 10% heat inactivated fetal bovine serum (Highveld Biological, South Africa), 25 mM HEPES and 2 mM glutamine] in a humidified 5% CO_2 incubator at 37°C. HT-29, HeLa, MCF-7 and Chang liver cells were obtained from the American Type Culture Collection (ATCC), USA and U937 cell lines from Highveld Biological, South Africa.

2.4 In vitro Cytotoxic Assay

The cells (200 µL aliquots) were seeded into 96-well culture plates (Nunclon, Roskilde, Denmark) at a density of 6000 cells/well to determine their viability. Cells were allowed to attach for 24 h in a humidified 5% CO_2 incubator at 37°C. The cold aqueous extracts of the fresh and dried and boiled aqueous extracts of the fresh and dried berries were solubilized in DMSO before further dilution with growth medium. The final DMSO concentration in culture wells never exceeded 0.5%. Cisplatin was used as a positive control at concentrations of 10 and 100 µM. Cells were exposed to the extracts and cisplatin for 48 h. Immediately following the 48 h incubation period, cell numbers were determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide) assay as previously described by Alley *et al.,* (1988) and Brauns *et al.,* (2004). The cells were incubated with 200 µL MTT (Sigma) (0.5 mg/mL in growth medium) for

3 h at 37°C. The formazan product was then dissolved in DMSO and plates were agitated on a shaker for 5 min, before the absorbance was read at 540 nm on multiwell scanning spectrophotometer (Multiskan MS, Labsystems). The values obtained were used to determine the percentage inhibition of cell growth caused by the extracts (Hagopian *et al.,* 1999; Huq *et al.,* 2004).

2.5 Annexin V-FITC/PI

U937 cells (1.0×10^6 cells/mL) were seeded in growth medium and 10 µM cisplatin and 15.6 µg/mL of DB, BDB, FB and BFB respectively were added to the test flasks. The cells were incubated for 24 h before the Annexin V-FITC Apoptosis detection kit (Beckman Coulter, Fullerton, CA, USA) was used to distinguish between apoptotic and necrotic cells. The reagents were prepared as per kit instructions. The cells were collected and centrifuged for 5 min at 500 × g at 4°C. The supernatant was discarded and the pellet re-suspended in ice-cold 1X binding buffer to make 2.0×10^6 cells/mL suspension. The tubes were kept on ice. To 100 µL of the cell suspension, 1 µL of Annexin V-FITC solution and 5 µL of dissolved propidium iodide (PI) were added and mixed gently. The tubes were incubated for 15 min on ice and in the dark before 400 µL of ice-cold 1X binding buffer was added. The samples were analyzed on a Beckman Coulter FC500 flow cytometer. A minimum of 10,000 events were acquired per sample.

2.6 Cell cycle analysis

The cell suspensions prepared for Annexin V-FITC/PI staining were also used for cell cycle analysis. Cells were fixed and stained using the Coulter DNA Prep Reagents kit

(Beckman Coulter). The cells were analyzed on a Beckman Coulter FC500 flow cytometer. A minimum of 10,000 events were acquired per sample.

2.7 Statistical analysis

Screening for cytotoxicity and log dose-dependent responses were performed in triplicate and quadruplicates respectively. The results were expressed as percentage growth inhibition of control. The treatment values were compared to control values using the two-sample student t-test. IC_{50} values for growth inhibition was derived from a nonlinear regression model (curve fit) based on sigmoidal dose response curve (variable) and computed using GraphPadPrism version 4 (GraphPad).

3.0 Results

3.1 In vitro cytotoxic assay

The relative cytotoxic activity of the cold extracts of the fresh and dried and the boiled aqueous extracts of fresh and dried berries of *Solanum aculeastrum* on human tumor cells was measured by means of MTT assay in the MCF-7, HT-29 and HeLa cancer cell lines. The growth inhibition of the extracts against the cancer and non-cancerous cell lines is depicted in Figure 1. All the extracts inhibited the cancer cell lines, with the best activity observed in the breast cancer (MCF-**7**) cell line. Table 1 represents the IC_{50} values of extracts on the cell lines. All the extracts showed cytotoxic effect on all the cancer cells with the cold fresh berries possessing the lowest IC_{50} values across the cancer cells.

3.2 Growth Inhibition of non-malignant Chang human liver (Chang)

To assess whether the extracts demonstrated toxicity in non-cancerous cells, Chang liver cells were plated at a density of 6000 cells/well and also to confluence. These cells were then cultured in the presence of varying concentrations of the extracts for 48 h. Table 1 shows that these extracts also inhibited the growth of these cells. Interestingly, the cold extract of the fresh berries also showed the lowest IC_{50} values in both the Chang and confluent Chang liver cells.

3.3 Annexin V-FITC/PI

To investigate the effect of FB, DB, BFB and BDB on the induction of apoptosis in U937 cells, Annexin V-FITC/PI staining and flow cytometric measurements were used to quantify the percentage of apoptosis in the total cell population. As shown in Table 2, after incubation with the extracts at a concentration of 15.6 µg/ml and cisplatin at 10 µM for 24 h, the percentage of necrotic cells were more than the early apoptotic cells in all the treatments. However, on boiling of the fresh and dried berries, the percentage of viable and early apoptotic cells increased while necrotic cells decreased.

4.0 Discussion

A number of the currently cytotoxic chemotherapeutic drugs in use as systemic therapy have been designed not only to specifically kill or inhibit cancer cells but any rapidly dividing cell (Lind, 2004). These results from the inability of the chemotherapeutic drugs to differentiate between normal and malignant cells, consequently causing damage to rapidly regenerating tissues such as bone marrow, hair, GIT cells, and resulting in side effects such as hair loss, gastro-intestinal disturbances,

myelosuppression etc. (Corrie, 2008). The problems associated with these drugs have led to the intensive search for naturally occurring products with anticancer potentials. This has led invariably to an increase in the use of traditional medicines for cancer treatments, even though only a few peer-reviewed reports are available regarding their efficacy, active principles, mode of action, side effects and possible adverse drug interactions (Cuzzolin *et al.,* 2006).

Solanum aculeastrum berries have been reported to be used in the treatment of breast cancer in the Eastern Cape Province of South Africa (Koduru *et al.,* 2006b), and previous studies have demonstrated that the extracts and chemical constituents of the berries possess cytotoxic activity against estrogen receptor positive human breast carcinoma cells (MCF-7), cervical (HeLa) and colonic cancer (HT-29) cells, by the induction of cell cycle arrest and apoptosis (Koduru *et al.,* 2006a; 2007). Therefore, as part of our on going research of this plant, we compared the cytotoxic effect of the cold extracts as well as the boiled extracts of the fresh and dried berries of *Solanum aculeastrum* on HeLa, MCF-7, HT-29 and Chang liver cells. Furthermore, we also determined whether these extracts possessed selective cytotoxicity against the investigated cancer cells.

The present results demonstrated that all the extracts of *Solanum aculeastrum* tested are potent inhibitors of human breast, cervical and colonic carcinoma cells. In terms of relative potency, the cold fresh berry extracts appeared to be the most active of the extracts, especially at low concentrations. However, not only did these extracts inhibit the growth of the cancer cells, but they were also cytotoxic against non-cancerous cells (both the actively dividing and confluent Chang liver cells). The lack of differential effect

of *S. aculeastrum* extracts on cancerous and non-cancerous cells implies that in addition to efficacy, safety considerations should govern possible therapeutic uses of these plant extracts (Lee *et al.,* 2004). The indiscriminate increase in the use of extracts of plants without recourse to their safety and adverse effects is worrisome because they are considered natural (Gesler, 1992). Several reports of side effects after administration of traditional medicines have been reported worldwide (Niggemann and Grüber, 2003; Stout *et al.,* 2003). A key question for the possible use of the *S. aculeastrum* extracts evaluated in this study in cancer treatment should take into consideration the ratio of the effective therapeutic to toxic dose. However, it is worth mentioning that solasodine and tomatidine, compounds found in *Solanum aculeastrum,* have been found to possess significant cytotoxic activity against various cancer cell lines (Cham, 1994; Lee *et al.,* 2004).

Annexin V-FITC/PI staining was used as a quantitative marker to estimate whether the extracts induced cell apoptosis. Apoptosis is a regulated cell death process characterized by morphological and biochemical features occurring at different stages. One of the earliest signs of an apoptotic cell is the loss of plasma membrane asymmetry (Martin *et al.,* 1995). In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment (Martin *et al.,* 1995). Detection of such membrane changes by Annexin V has been suggested as a suitable assay of early apoptotic cells. Annexin V is a 35 kDa, Ca^{2+}-dependent phospholipid binding protein that has a high affinity for PS, and binds to cells with exposed PS (Martin *et al.,* 1995). Annexin V FITC Staining is typically used in conjunction with a vital dye such as

propidium iodide (PI) or 7-Amino-Actinomycin (7-AAD) (Allen and Davies, 2007). This allows the identification of early apoptotic cells (PI negative, FITC Annexin V positive), viable cells (PI and Annexin V negative) and necrotic cells (PI and Annexin V positive) (Allen and Davies, 2007). Our data showed that the extracts induced necrosis more than apoptosis in the U937 cells after 24 h exposure to Annexin V-FITC/PI. The high percentage of necrosis observed with the Annexin kit in this experiment correlates with the high level of cytotoxicity observed with the confluent Chang cell. Although, several studies have shown that *Solanum* alkaloids are not destroyed by boiling, baking, frying, or drying at high temperature (Bushway and Ponnampalam, 1981; Jadhav *et al.,* 1997), it is worth noting that the cells treated with the boiled extracts had a lower percentage of necrosis compared to those treated with the fresh extracts.

These results clearly demonstrate that extracts of *Solanum aculeastrum* was neither selective in the inhibition of carcinoma nor actively dividing and un-dividing cells. Although compounds found in *S. aculeastrum* are potent inhibitors of human carcinoma cells, it is still imperative to ascertain that any potent inhibitor of cancer cells must possess low IC_{50} values when tested against actively dividing cells and a high IC_{50} values against confluent cells.

Acknowledgement

This research was supported by the National Research Foundation (NRF) of South Africa.

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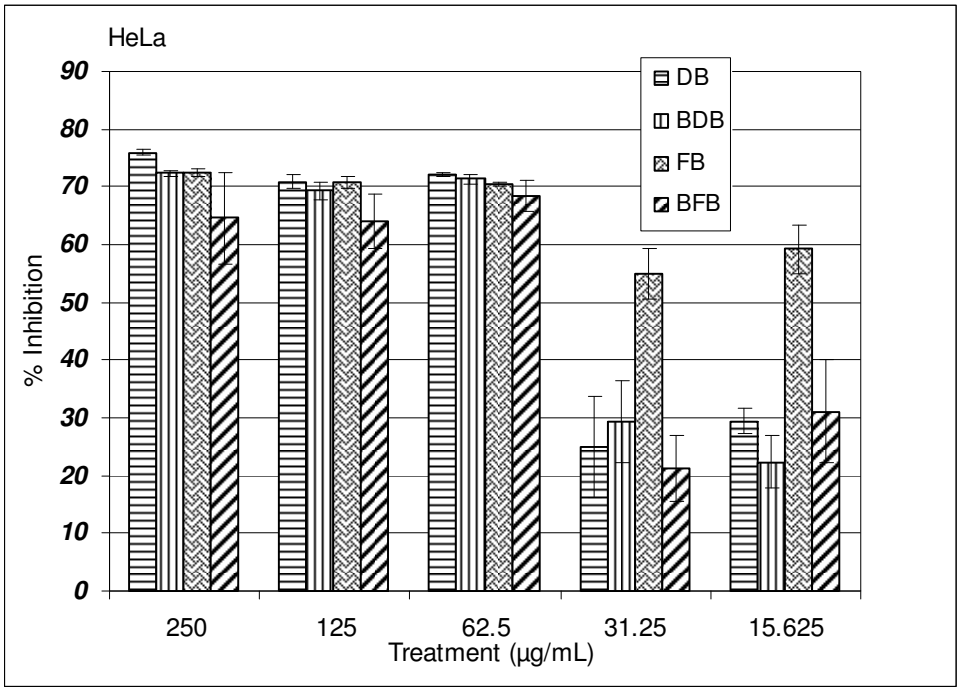
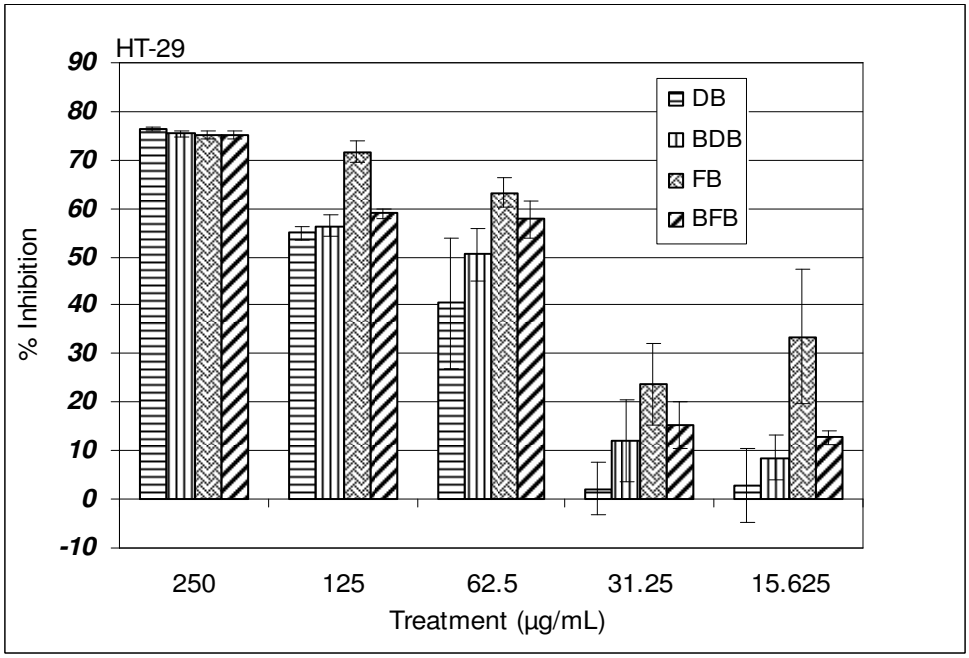
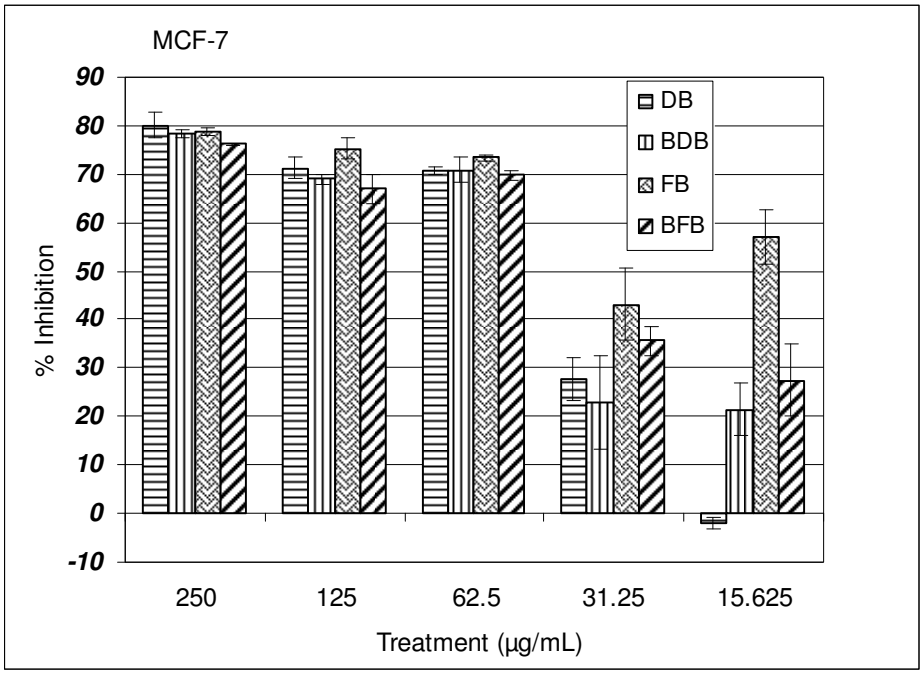

HeLa
90
80
70
60
50
40
30
20
10
0
% Inhibition
DB
BDB
FB
BFB
250
125
62.5
31.25
15.625
Treatment (µg/mL)


HT-29
90
80
70
60
50
40
30
20
10
0
-10
% Inhibition
DB
BDB
FB
BFB
250
125
62.5
31.25
15.625
Treatment (µg/mL)


MCF-7
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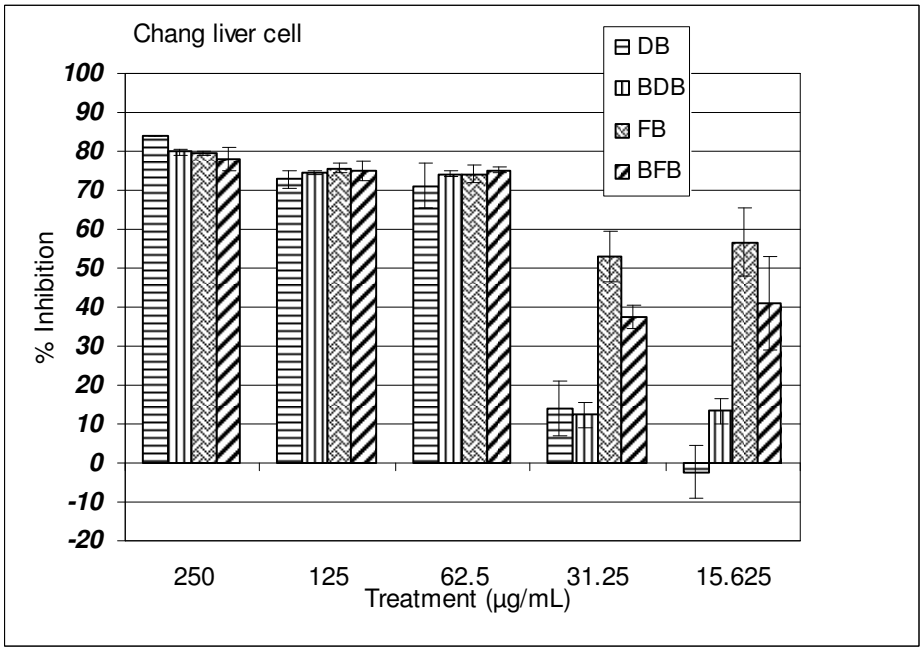
80
70
60
50
40
30
20
10
0
-10
% Inhibition
DB
BDB
FB
BFB
250
125
62.5
31.25
15.625
Treatment (µg/mL)


Chang liver cell
100
90
80
70
60
50
40
30
20
10
0
-10
-20
% Inhibition
DB
BDB
FB
BFB
250
125
62.5
31.25
15.625
Treatment (µg/mL)

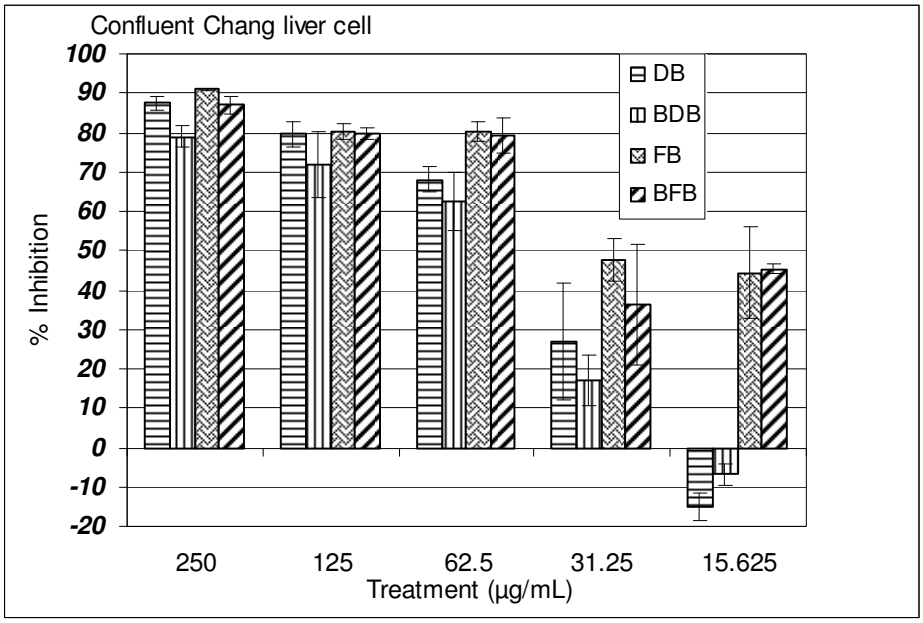


Figure 1: Comparative effect of boiling of the fresh and dried berries of *Solanum aculeastrum* on (a) HeLa; (b) HT-29; (c) MCF-7; (d) chang liver; (e) confluent chang liver cells. Mean ± SD (n = 4). Note: DB Dried berries; BDB Boiled dried berries; FB Fresh berries; BFB Boiled fresh berries

Table 1: IC_{50} values of the extracts of *Solanum aculeastrum* on some cell lines. Values in parentheses represent the selectivity index, calculated by dividing the IC50 for confluent Chang liver cells for a specific extract by the IC50 for the cancer cell line for the same extract.

Cell lines	FB	BFB	DB	BDB
		µg/ml		
HeLa	12.5 (2.6)	47.5 (0.84)	42.5 (1.12)	42.5 (1.12)
HT-29	50.0 (0.65)	52.5 (0.76)	100.0 (0.48)	55.0 (0.86)
MCF-7	12.5 (2.6)	42.0 (0.95)	45.5 (1.04)	47.5 (1.00)
Normal cells				
Chang liver (log phase)	12.5	40.0	47.5	47.5
Confluent Chang liver	32.5	40.0	45.0	50.0

Table 2: Apoptosis results from Annexin V-FITC/PI analysis using U937 leukemia cells

Treatment	Viable cells %	Early apoptotic %	Necrotic %
Control	67.5	2.6	2.0
Cisplatin (10 µM)	28.9	31.8	5.9
Fresh berries	5.4	2.1	42.2
Boiled fresh berries	25.5	7.7	20.7
Dried berries	3.1	3.2	28.8
Boiled dried berries	35.3	12.7	15.3

*Extract concentration: 15.6 µg/mL

*% DMSO: 0.5

CHAPTER 6

Toxicological evaluation of the aqueous extract of the berries of *Solanum aculeastrum* Dunal. berries in male Wistar rats.

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*This chapter has been accepted for publication in this format to **African Journal of Pharmacy and Pharmacology**

African Journal of Pharmacy and Pharmacology Vol. 4(1). pp. xxx-xxx, January, 2010
Available online http://www.academicjournals.org/ajpp
ISSN 1996-0816 © 2010 Academic Journals

Full Length Research Paper

Safety evaluation of aqueous extract of unripe berries of *Solanum aculeastrum* in male Wistar rats

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Accepted 17 December, 2009

The acute and sub-acute toxicity of aqueous extract of the fresh, unripe berries of *Solanum aculeastrum* was investigated in male Wistar rats. In the acute toxicity study, toxicity symptoms such as hypoactivity, respiratory distress and epistaxis which disappeared 72 h post treatment, were observed in all the extract treated animals. Except for the 125 mg/kg body weight of the extract, all the other dose levels (250, 500 and 1000 mg/kg body weight) produced mortality in the animals whose latency was inversely proportional to the doses. The extract produced no histopathological alterations in all the organs except the lungs where there was evidence of follicle formation and interstitial diseases following the administration of 125 and 250 mg/kg body weight of the extract. Again, with 500 and 1000 mg/kg body weight of the extract, the lungs became characterized with massive expansion of the bronchial lymphoid tissue (BALT), extension of lymphocytes and plasma cells through the mascularies into the submucosa and mucosa. In the sub-acute toxicity study however, the 50 and 75 mg/kg body weight of the extract significantly ($P < 0.05$) increased the body weight of the animals by 9.23 and 20.02% respectively. The extract decreased the weight of the liver whereas those of the lungs, spleen and the testes increased. All the dose levels also increased the concentrations of serum total protein, globulin, creatinine and MCV of the animals. Whereas the 50 and 75 mg/kg body weight of the extract increased the serum levels of albumin, urea, calcium, GGT, Hb and RBC, the 25 and 50 mg/kg body weight of the extract decreased the total and conjugated bilirubin. The 75 mg/kg body weight of the extract increased the levels of MCHC, WBC, Cl^-, total and conjugated bilirubin. Again, all the dose levels of the extract decreased the activities of serum ALP, ALT, inorganic phosphorus, MCH, platelets, lymphocytes, neutrophils, monocytes, eosinophils, LUC and basophils. The extract at 25 and 75 mg/kg body weight increased the RCDW and PCV levels respectively whereas the 75 mg/kg body weight of the extract reduced the RCDW. The extract at 25 mg/kg body weight decreased the serum AST activity, Hb, RBC, MCHC and WBC. The alterations in the haematological parameters, liver and kidney function indices as well as mortality observed in this study indicates that the aqueous extract of the fresh, unripe berries of *S. aculeastrum* is toxic and will adversely affect the normal functioning of the blood, liver and kidney of the animals. The follicular bronchitis observed in the lungs of the animals may be associated with immunodeficiency and hypersensitivity to the plant extract. Therefore, the extract is not completely safe as an oral remedy when repeatedly consumed on daily basis for 14 days at the doses investigated.

Key words: *Solanum aculeastrum*, safety, haematological parameters, functional indices, liver, kidney, histopathological alterations, oral remedy.

INTRODUCTION

Herbal remedies have been used in the management of various diseases from time immemorial. These remedies which are commonly self-medicated are more often with no proper dose regimen. Such indiscriminate use of medicinal plant simply because herbs are natural in origin, without recourse to safety and or adverse effects on biological system is worrisome.[1] Therefore, it has become imperative to assess the safety or otherwise, the toxicity of plants used for medicinal purposes in folklore

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medicine. One plant widely used in traditional medicine of South Africa without information on its effect on biological system is *Solanum aculeastrum* Dunal. This plant, commonly known as goat bitter apple (English), is widely distributed in southern Africa (Watt and Breyer-Brandwijk, 1962). It is a thorny, perennial plant that grows up to 2 - 3 m high with white flowers and lemon shaped berries that become greenish-yellow when ripe (Wanyonyi et al., 2002). The fruits of *S. aculeastrum* are used fresh, dried, boiled or ashed for the treatment of jigger wounds and gonorrhea, rheumatism as well as ringworm in cattle and horses (Agnew and Agnew, 1994; Watt and Breyer-Brandwijk, 1962). Ethnobotanical survey revealed that the berries are used in the treatment of breast cancer in the Eastern Cape Province of South Africa (Koduru et al., 2007a).

Preliminary pharmacological studies on the extracts of *S. aculeastrum* berries and leaves revealed that the plant parts possess antimicrobial, antitumor and antioxidant activities (Koduru et al., 2006 a, b, c; 2007b). The steroidal alkaloids in the berries have also been reported to be active against schistomiasis and cancer (Drews and Van Staden, 1995; Koduru et al., 2007b; Wanyonyi et al., 2002). *S. aculeastrum* berries have been used widely for the management of several diseases by the traditional healers of South Africa without recourse to its safety or toxicity risk (Koduru et al., 2007a). For example, several studies have also validated the use of this plant as an antimicrobial and anticarcinogen (Koduru et al., 2006 a, b, c; 2007b) without information on its toxic implication in the open scientific literature.

Again, study by Aboyade et al. (2009) have evaluated the toxicological effect of the repeated consumption of aqueous extract of the fresh berry, boiled fresh berry, oven-dried berry and boiled oven-dried berry of *S. aculeastrum* for 28 days at the doses of 1, 10 and 25 mg/kg body weight in male rats and concluded that the adverse effect on the normal functioning of the liver and kidney of the animals might be reduced by the drying and boiling treatments. Despite all these, information is still scanty on the toxic implications of the fresh, unripe berries of *S. aculeastrum* in animal model as used in the management of cancer by the traditional healers of South Africa. Therefore, this study was aimed at investigating the toxicological potentials of the aqueous extract of the fresh, unripe berries of this plant using male Wistar rats as model.

MATERIALS AND METHODS

Plant materials

Fresh, unripe berries of *S. aculeastrum* were collected from Kayalethu village in the Eastern Cape Province of South Africa. The fruit was authenticated by Prof. D.S Grierson of the Department of Botany, University of Fort Hare, South Africa and a voucher specimen (SA/Med 01) was deposited at the Giffen herbarium of the university.

Animals

Male rats (*Rattus norvegicus*) of Wistar strain weighing 187.29 ± 24.68 g were obtained from the Animal House of the Agricultural and Rural Development Research Institute (ARDRI), University of Fort Hare.

The animals were housed in clean aluminum cages placed in well ventilated house with optimum conditions (temperature 23 ± 1°C; photoperiod: 12 h natural light and 12 h dark; humidity: 45 - 50%). They were also allowed free access to food (Balanced Trusty Chunks (Pioneer Foods (Pty) Ltd, Huguenot, South Africa) and water. The cages were cleaned on daily basis. This study was carried out following approval from the ethical committee on the use and care of animals of the University of Fort Hare, Alice, South Africa.

Assay kits

The assay kits for creatinine, urea, calcium, phosphorus, chloride, magnesium, sodium, potassium, uric acid, albumin, bilirubin, alkaline phosphatase, gamma glutamyl transferase, alanine and aspartate aminotransferases were obtained from Roche Diagnostic GmbH, Mannheim, Germany. All other reagents used were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

Preparation of extract

The fresh berries (500 g) were extracted in 1000 ml of cold distilled water for 48 h on a mechanical shaker (Stuart Scientific Orbital Shaker SO1, United Kingdom).

The extract was filtered with a Buchner funnel and Whatman No 1 filter paper. This was later lyophilized using VirTis benchtop K freeze dryer (VirTis Company, Gardiner, NY) to yield 7.50 g which was reconstituted in distilled water to give the required doses used in this study.

Acute toxicity test

Thirty male rats were completely randomized into five groups (A - E) of six animals each and thereafter fasted (without food, but water) for 2 h before treatment.

The animals from group A (the control) received orally 0.5 ml of distilled water (the vehicle) with the aid of oropharyngeal cannula while those in groups B, C, D and E were treated like the control except they received equal volume of the extract containing 125, 250, 500 and 1000 mg/kg body weight respectively. Clinical symptoms of toxicity, mortality and its latency displayed by the animals were observed for an initial 2 h following the administration of the extract and continued throughout the 7 days treatment period.

Sub-acute toxicity test

In this experiment, twenty animals were grouped into four (A-D) consisting of five each. The animals from the control group received orally, 0.5 ml of distilled water with the aid of oropharyngeal cannula, once daily for 14 days, while those in groups B, C and D were treated like the control except they received equal volume containing 25, 50 and 75 mg/kg body weight respectively. The animals were sacrificed 24 h after their 14 daily doses. The changes in the body weight of the animals as well as the absolute weight of the liver, lungs, kidney, spleen, heart and testes were also determined.

Table 1. Acute toxicity of aqueous extract of unripe berries of *Solanum aculeastrum* in male Wistar rats n = 6.

Doses (mg/kg body weight)	D/T	Mortality (%)	Mortality latency (h)	Toxic symptoms
Control (distilled water)	0/6	0	0	No sign was observed.
125		0	0	Hypoactivity, respiratory distress.
250	1/6	0.17	72.00 ± 0.0	Hypoactivity, respiratory distress.
500	2/6	33.30	48.00 ± 4.50	Hypoactivity, respiratory distress, epistaxis.
1000	3/6	50.00	24.00 ± 2.60	Hypoactivity, respiratory distress, epistaxis.

D/T = dead / treated rats.
Mortality latency = time of death (in days) after the injection.

Preparation of serum

The procedure described by Yakubu et al. (2005) was used in the preparation of the serum. Briefly, under ether anaesthesia, the neck area of the rats was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to prevent contamination of the blood with interstitial fluid) were sharply cut with sterile scalpel blade and an aliquot (2 ml) of the blood was collected into sample bottles containing EDTA (BD Diagnostics, Pre-analytical Systems, Midrand, USA) for the haematological analysis. Another 5 ml of the blood was allowed to clot for 10 min at room temperature and then centrifuged at 1282 g x 5 min using Hermle Bench Top Centrifuge (Model Hermle, Z300, Hamburg, Germany). The sera were later aspirated with Pasteur pipettes into sample bottles and used within 12 h of preparation for the assay (Malomo 2000). The rats were thereafter quickly dissected in the cold and the organs (liver, kidneys, lungs, spleen, heart and testes) removed, freed of fat, blotted with clean tissue paper and weighed.

Determination of biochemical parameters

The serum was assayed for creatinine, urea, uric acid, aspartate (AST) and alanine amino transferases (ALT), alkaline phosphatase (ALP), potassium, sodium, calcium, phosphorus, chloride, magnesium, total bilirubin, total protein, albumin and gamma glutamyl transferase (GGT) using assay kits on Roche Modular Analyzer (model P800) Mannheim, Germany. The globulin concentration in the serum was computed using the expression of Tietz et al. (1994). The haematological parameters such as the red blood count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RCDW), large unstained cell (LUC), white blood cells (WBC), platelets, lymphocytes, neutrophils, monocytes, eosinophils and basophils were determined using the Horiba ABX Diagnostics - Pentra 80 (Montpellier, France).

Histopathological examination

The organs (liver, kidney, lungs, spleen, heart and testes) of the animals were fixed in 10% v/v formaldehyde, dehydrated through ascending grades of ethanol (70%, 90% and 95% v/v), cleaned in xylene and embedded in paraffin wax (melting point 56°C) (Krause, 2001). Tissue sections were prepared as described by Drury and Wallignton (1973) and stained with hematoxylin/eosin. The photomicrographs were taken at x400 with a Canon Power Shot G2 digital camera (Melville, NY).

Statistical analysis

Data were expressed as mean of five replicates ± SD except in the acute toxicity studies and were subjected to one way analysis of variance (ANOVA). Means were separated by the Duncan's multiple range test using SAS and complemented with Student's t-test. Values were considered statistically significant at $p < 0.05$.

RESULTS

Acute oral toxicity study

Toxicity symptoms such as hypoactivity, respiratory distress and epistaxis were observed in all the extract treated animals. The surviving animals were however free of these signs of intoxication, 72 h post treatment (Table 1). With the exception of the 125 mg/kg body weight of the extract, all the other dose levels (250, 500 and 1000 mg/kg body weight) produced mortality in the animals with latency that was inversely proportional to the doses (Table 1). The extract produced no histopathological alterations in all the organs except in the lungs. The cardiac architecture was with no evidence of inflammation, fibrosis or ischemia. The lobular architecture of the liver, seminiferous tubules of the testes, medullary and cortical architecture of the kidney and the spleen cells was preserved (photomicrographs not shown).

In contrast however, there was follicle formation and interstitial diseases in the lungs of animals treated with 125 and 250 mg/kg body weight of the extract (Plates 1a and b respectively). Again, at 500 and 1000 mg/kg body weight of the extract, the lungs became characterized with massive expansion of the bronchial lymphoid tissue (BALT) with extension of lymphocytes and plasma cells through the mascularies into the submucosa and mucosa (Plates 1c and d respectively). In addition, there was tracking of bronchitis into the terminal bronchioles. There was also marked narrowing of the bronchial lamina with acute pneumonic infiltrate in the alveoli (Plates 1a - d).

Subacute toxicity study

The repeated administration of 50 and 75 mg/kg body weight of the extract for seven days significantly ($P < 0.05$) increased the body weight of the animals by 9.23 and 20.02% respectively and this was sustained till the end of the 14 days experimental period. The absolute

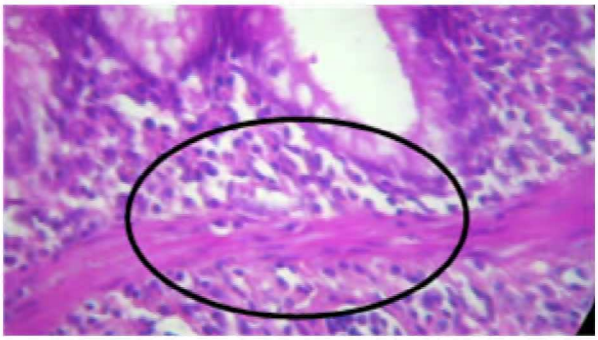

Plate 1a. Photomicrograph of male rat lung orally administered with 125 mg/kg body weight of aqueous extract of *S. aculeastrum* unripe berries for 7 days. The circled spot shows well preserved alveoli architecture with evidence of follicle formation (x400).

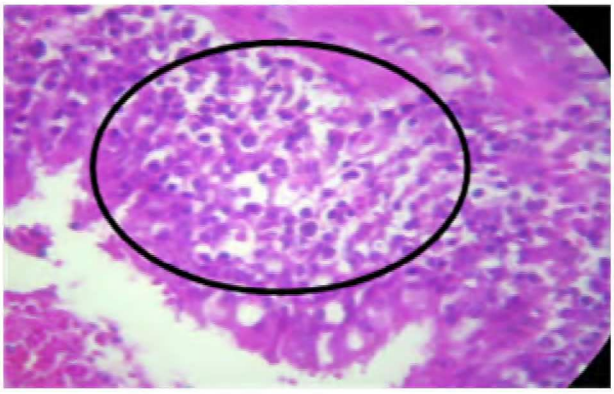

Plate 1b. Photomicrograph of male rat lung orally administered with 250 mg/kg body weight of aqueous extract of *S. aculeastrum* unripe berries for 7 days. The circled spot shows well preserved alveoli architecture with evidence of follicle formation (x400).

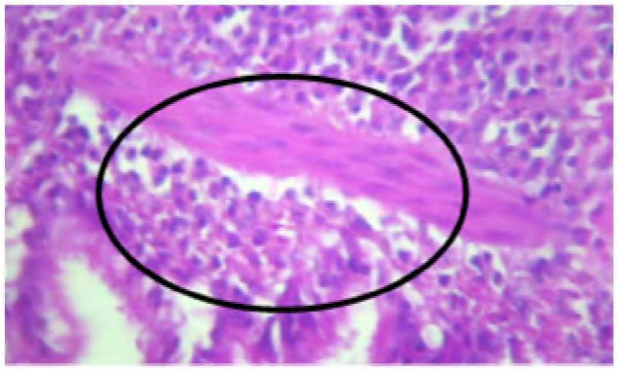

Plate 1c. Photomicrograph of male rat lung orally administered with 500 mg/kg body weight of aqueous extract of *S. aculeastrum* unripe berries for 7 days. The circled spot shows well preserved alveoli architecture with evidence of follicle formation, massive expansion of the bronchial associated lymphoid tissue (BALT) with extension of lymphocytes and plasma through the muscularis into the submucosa and mucosa. There is also acute pneumonic infiltrate in the alveoli as well as marked narrowing of the bronchial lamina (x400).

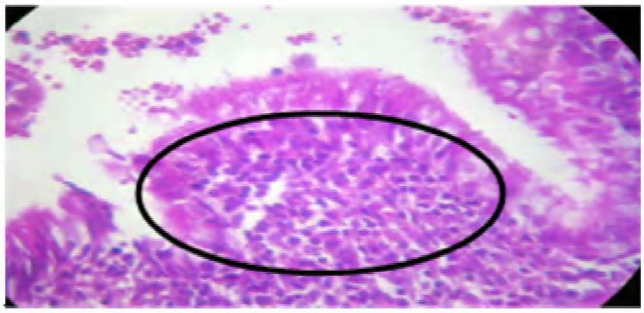

Plate 1d. Photomicrograph of male rat lung orally administered with 500 mg/kg body weight of aqueous extract of *S. aculeastrum* unripe berries for 7 days. The circled spot shows well preserved alveoli architecture with evidence of follicle formation, massive expansion of the bronchial associated lymphoid tissue (BALT) with extension of lymphocytes and plasma through the muscularis into the submucosa and mucosa. There is also acute pneumonic infiltrate in the alveoli as well as marked narrowing of the bronchial lamina (x400).

weight of the lungs, spleen and testes increased at all the dose levels of the extract whereas that of the liver decreased significantly. In contrast, the weight of the kidney and heart of the animals compared well with the control (Table 2).

The extract increased the concentration of serum total protein and globulin of the animals. Whereas the 50 and 75 mg/kg body weight of the extract decreased the serum albumin content, the 25 and 50 mg/kg body weight of the extract decreased the concentrations of total and conjugated bilirubin. In contrast, the 75 mg/kg body weight increased the levels of total and conjugated bilirubin (Table 3). Again, all the dose levels of the extract decreased the activities of serum ALP and ALT activity. The extract also produced dose specific responses in the activities of GGT and AST in the serum of the animals. For example, while there was decrease only in the activity of serum AST at 25 mg/kg body weight of the extract, the levels of GGT in the serum of the animals increased only at 50 and 75 mg/kg body weight of the extract (Table 3).

There was no significant alteration in the levels of sodium, potassium, magnesium and uric acid of the animals at all the doses investigated. While the concentration of creatinine in the serum of the animals was elevated at all the dose levels, that of inorganic phosphorus was reduced. The concentration of urea and calcium in the serum of the animals were also reduced at 50 and 75 mg/kg body weight of the extract, whereas, the level of chloride ions was elevated only at 75 mg/kg body

Table 2. Effect of aqueous extract of the unripe berries of Solanum aculeastrum on the weight of the body and absolute organs of male Wistar rats. n = 5, x ± SD.

Solanum aculeastrum (mg/kg body weight)				
Body weight of the animals (g)	Control	25	50	75
*Day 0	178.00 ± 10.12	178.03 ± 7.94	189.01 ± 6.80	223.87 ± 6.98
Day 7	191.00 ± 10.11	191.03 ± 10.12	208.63 ± 8.39	238.80 ± 3.73
Day 14	194.30 ± 28.37	194.33 ± 28.39	202.03 ± 22.21	206.80 ± 6.41
Organ weight (%)				
Liver	4.03 ± 0.09 ^{a}	3.52 ± 0.09 ^{b}	3.72 ± 0.07 ^{b}	3.97 ± 0.05 ^{b}
Lungs	0.83 ± 0.18 ^{a}	1.08 ± 0.21 ^{a}	1.14 ± 0.1 ^{b}	1.34 ± 0.07 ^{c}
Kidney	0.80 ± 0.05 ^{a}	0.76 ± 0.07 ^{a}	0.86 ± 0.18 ^{a}	0.81 ± 0.05 ^{a}
Spleen	0.27 ± 0.02 ^{a}	0.45 ± 0.06 ^{b}	0.44 ± 0.02 ^{b}	0.45 ± 0.09 ^{b}
Heart	0.41 ± 0.02 ^{a}	0.40 ± 0.03 ^{a}	0.42 ± 0.09 ^{a}	0.39 ± 0.01 ^{a}
Testes	1.03 ± 0.03 ^{a}	1.26 ± 0.07 ^{b}	1.46 ± 0.09 ^{c}	1.32 ± 0.02 ^{d}

Test values carrying superscripts different from their controls are significantly different ($p < 0.05$)
*Initial weight of the rats before treatment.

Table 3. Effect of aqueous extract of unripe berries of Solanum aculeastrum on some liver function parameters of male Wistar rats, n = 5, x ± SD.

Solanum aculeastrum (mg/kg body weight)				
	Control	25	50	75
Total Bilirubin (µmol/L)	8.67 ± 0.08^{a}	7.33 ± 0.08^{b}	7.00 ± 1.00^{b}	8.00 ± 1.00^{a}
Conjugated Bilirubin (µmol/L)	2.00 ± 0.00 ^{a}	1.33 ± 0.18^{b}	1.67 ± 0.18^{b}	3.00 ± 0.30^{c}
Total Protein (g/L)	56.67 ± 1.15^{a}	69.33 ± 2.08^{b}	70.00 ± 2.00^{b}	70.33 ± 3.06^{b}
Albumin (g/L)	17.33 ± 0.58^{a}	17.00 ± 1.00^{a}	15.00 ± 0.65^{b}	11.33 ± 1.15^{c}
Globulin (g/L)	39.33 ± 1.53^{a}	52.33 ± 3.06^{b}	55.00 ± 1.00^{b}	59.00 ± 2.00^{c}
Alkaline Phosphatase (U/L)	383.00 ± 1.14^{a}	261.00 ± 9.90^{b}	215.00 ± 8.49^{c}	244.50 ± 37.48^{b}
Gamma Glutamyl Transferase (U/L)	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	8.00 ± 0.61^{b}	5.50 ± 0.01^{c}
Alanine aminotransferase (U/L)	66.32 ± 2.08^{a}	55.33 ± 5.13^{b}	45.00 ± 3.61^{c}	37.67 ± 2.52^{d}
Aspartate aminotransferase (U/L)	158.50 ± 7.78^{a}	136.50 ± 9.19^{b}	166.50 ± 6.36^{a}	162.50 ± 3.54^{a}

Test values carrying superscripts different from the control for each parameter are significantly different ($p < 0.05$).

weight of the extract (Table 4).

All the doses of the extract increased the MCV of the animals. The MCH, platelets, lymphocytes, neutrophils, monocytes, eosinophils, LUC and basophils were reduced significantly reduced (Table 5). Whereas the levels of Hb and RBC decreased only at 25 mg/kg body weight of the extract, the 50 and 75 mg/kg body weight of the extract increased these haematological parameters. In addition, the levels of MCHC and WBC decreased at 25 and 50 mg/kg body weight of the extract, whereas, the 75 mg/kg body weight of the extract increased the blood indices. Again, the 25 and 75 mg/kg body weight of the extract increased the RCDW and PCV levels respectively whereas the RCDW was reduced only at 75 mg/kg body weight of the extract (Table 5).

DISCUSSION

Herbal remedies are used by about 80% of the population in the developing countries (Saggu et al., 2007). Despite this widespread use, few scientific studies are available that ascertained the safety of these traditional remedies (Saggu et al., 2007). Therefore, the need to provide information on the toxic implications of these remedies cannot be over emphasized. In this study, the aqueous extract of the unripe berries of *S. aculeastrum* was investigated for its toxic implications in male rats using clinical and biochemical indices such as mortality, haematological and functional parameters of the liver and kidney. The various biochemical indices evaluated in this study are useful parameters that can be employed in assessing the toxic potentials of botanicals. The observed clinical signs of toxicity such as hypoactivity, respiratory distress and epistaxis as well as the incidence of mortality at 250, 500 and 1000 mg/kg body weight suggest that the extract is toxic to the animals at these doses. Since post-mortem examination was not carried out, it was not possible to ascertain the actual cause of the death of the animals. However, it is possible that the oral administration of the extract might have affected the respiration of the animals. The epistaxis observed in the animals may be associated with platelet dysfunction (McGarry et al., 2005), as was revealed by the reduced platelet count in the present study. The increase in body weight of the animals treated

Table 4. Effect of aqueous extract of unripe berries of Solanum aculeastrum on some renal function parameters of male Wistar rats. n = 5, x ± SD.

Parameters	*Solanum aculeastrum* (mg/kg body weight)			
	Control	25	50	75
Sodium (mmol/l)	137.67 ± 0.58[a]	137.67 ± 0.58 [a]	137.67 ± 2.08 [a]	138.33 ± 0.58[a]
Potassium (mmol/l)	6.73 ± 0.32[a]	6.27 ± 0.55 [a]	6.77 ± 0.76[a]	8.07 ± 1.75[a]
Calcium (mmol/l)	2.51 ± 0.03[a]	2.52 ± 0.06 [a]	2.41 ± 0.10[a]	2.30 ± 0.10[b]
Magnesium (mmol/l)	1.15 ± 0.03[a]	1.16 ± 0.08[a]	1.16 ± 0.05[a]	1.20 ± 0.22[a]
Inorganic Phosphorus (mmol/l)	3.63 ± 0.15 [a]	3.07 ± 0.21[b]	3.10 ± 0.40 [b]	3.10 ± 0.13[b]
Chloride (mmol/l)	102.00 ± 1.00[a]	102.33 ± 0.58[a]	103.33 ± 1.15[a]	104.33 ± 1.15[b]
Urea (mmol/l)	7.07 ± 0.68[a]	7.13 ± 0.81[a]	6.90 ± 0.20[a]	5.77 ± 0.15[b]
Creatinine (mmol/l)	42.00 ± 2.00[a]	48.00 ± 3.46[b]	49.33 ± 4.04[b]	53.67 ± 7.50[b]
Uric acid (mmol/l)	0.09 ± 0.02 [a]	0.09 ± 0.01 [a]	0.10 ± 0.02 [a]	0.11 ± 0.01[a]

Test values carrying superscripts different from the control for each parameter are significantly different ($p < 0.05$).

Table 5. Effect of aqueous extract of unripe berries of *Solanum aculeastrum* on haematological parameters of male Wistar rats. n = 5, x ± SD.

	Solanum aculeastrum (mg/kg body weight)			
	Control	25	50	75
Haemoglobin (g/L)	15.53 ± 0.42a	14.63 ± 0.71b	16.63 ± 0.20c	16.20 ± 0.20c
Red blood cell (×1012/L)	8.51 ± 0.11a	8.26 ± 0.24a	8.73 ± 0.03b	8.88 ± 0.14b
Mean corpuscular haemoglobin (pg)	18.80 ± 0.46a	17.67 ± 0.31b	17.90 ± 0.17b	18.27 ± 0.15c
Mean corpuscular haemoglobin concentration (%)	31.57 ± 0.40a	29.93 ± 0.72b	30.93 ± 0.27b	36.67 ± 1.96c
Mean corpuscular volume (fl)	55.53 ± 1.94a	59.10 ± 0.99b	57.97 ± 0.63b	57.23 ± 0.36b
Cell distribution width (%)	12.40 ± 0.44a	13.70 ± 0.26b	13.37 ± 0.99a	11.20 ± 0.89c
White blood cell (×109 /L)	14.13 ± 1.46a	7.76 ± 1.23b	7.79 ± 2.22b	20.97 ± 2.87c
Lymphocytes (×109/L)	56.67 ± 2.63a	4.01 ± 0.54b	4.00 ± 1.21b	8.16 ± 1.94c
Neutrophils (×109/L)	5.43 ± 1.94a	0.45 ± 0.16b	0.53 ± 0.28b	1.05 ± 0.27c
Monocytes (×109/L)	28.17 ± 0.71a	2.06 ± 0.24b	2.82 ± 0.75b	8.90 ± 3.46c
Eosinophils (×109/L)	2.43 ± 0.25a	0.12 ± 0.09b	0.07 ± 0.04b	0.05 ± 0.01b
Basophils (×109/L)	0.43 ± 0.06a	0.08 ± 0.04b	0.13 ± 0.04b	0.18 ±0.03bc
Large Unstained Cell (×109/L)	6.93 ± 1.19a	1.00 ± 0.16b	1.00 ± 0.14b	1.33 ± 0.12b
Platelets (×109/L)	942.50 ± 2.12a	702.50 ± 15.96b	811.50 ± 4.95c	838.50±11.92c
Packed cell volume (L/L)	0.49 ± 0.02a	0.49 ± 0.02a	0.51 ± 0.02a	0.53 ± 0.01b

Test values carrying superscripts different from the control for each parameter are significantly different ($p < 0.05$)

with 50 and 75 mg/kg body weight of the extract for 14 days suggest that the normal growth in these animals was not impaired. This is in contrast to the least extract dosed group where no significant change in body weight was recorded. Therefore, it is unlikely that the extract will have adverse effect on the growth performance of the animals. This, however, contrast the loss of weight in the New Zealand rabbits, reported by Bello et al. (2005) following the oral administration of 750 mg/kg body weight of the aqueous extract of *S. melongena* fruit.

Organ weight is an index of swelling, atrophy or hypertrophy (Amresh et al., 2008). The increase in the weight of the spleen, lungs and testes following extract administration for 14 days may either imply hypertrophy or that growth of the organs is proportional to the growth of the animals. Again, the increase in the testes may be associated with anabolic effect of the extract arising from enhanced production of the secretory components of the testes (Yakubu et al., 2008), while that of the lungs may be due to hyperemia resulting from the ether anaesthesia. In addition, the reduction in the weight of the liver of the animals could possibly be due to cellular constriction. The absence of an effect on the weight of the heart and the kidney of the rats treated with the extract suggest that *S. aculaestrum* berry extract at the doses investigated did not cause organ swelling, atrophy or hypertrophy of these organs. This is an indication that the extract has selective effect on the weight of the organs investigated in this study.

However, since histopathological examination of these organs except the lungs showed lack of histoarchitectural alterations in the liver, kidney, spleen, heart and testes, therefore, the increase in the weight of the spleen and testes of the animals suggest that the growth of the organs was proportional to the growth of the animals. In contrast, the reduction in the weight of the liver notwithstanding the lack of histomorphorlogical changes the growth of the liver was not proportional to the growth of the animals. In addition, the follicular bronchitis observed in the lungs of the appeared to be a primary

process with secondary acute pneumonia. This follicular bronchitis may be due to immunodeficiency and hypersensitivity of the animals to the plant extract (Corrin, 2000). This may account for the respiratory distress and consequently, death observed in the extract treated animals.

Evaluation of serum proteins such as albumin and globulin is a good criterion for assessing the secretory ability/functional capacity of the liver (Yakubu et al., 2003). While the elevated levels of serum total protein and globulin at all the doses of the extract could possibly imply increase in the functional activity of the liver caused by the component of the extract, the non-definite pattern on the conjugated and total bilirubin as well as albumin suggest adaptation attempt by the animals to the effect of the extract. These alterations will adversely affect the normal function of the hepatocytes.

There are many enzymes found in the serum that did not originate from the extracellular fluid. During tissue damage, some of these biomolecules find their way into the serum probably by leakage through altered membrane permeability (Akanji and Yakubu, 2000). Serum enzyme measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue. The reduced levels of serum ALP and ALT at all the doses of the extract after 14 days suggest inactivation of the enzyme molecule *in situ* (Umezawa and Hooper, 1982) or inhibition of the enzyme activity at the cellular/molecular levels (Akanji et al., 1993; Karmen et al., 1965). The decrease in the activity of serum AST at 25 mg/kg body weight of the extract and the enhanced activity of GGT at 50 and 75 mg/kg body weight implies that the extract exhibited dose specific effect on these enzymes. The raised level of serum GGT at 50 and 75 mg/kg body weight might imply leakage of this enzyme from the tissues into the extracellular fluid.

Renal function indices such as serum electrolytes, urea, creatinine and uric acid can be used to evaluate the functional capacity of the nephrons of animals at the glomerular and tubular levels (Yakubu et al., 2003). The effect of the extract of *S. aculeastrum* berries was selective and dose specific on the kidney function indices investigated. Creatinine, urea and uric acids are major catabolic products of muscle, protein and purine metabolism respectively. Although, the levels of uric acid remained unaltered by the extract, the increase in the creatinine concentrations as well as decrease in urea further implies selective toxic effect of the extract. The elevated levels of creatinine at all the doses suggest glomerular dysfunction (Chawla, 1999).

The extract might have interfered with creatinine metabolism leading to increased synthesis or the tissue might have compromised all or part of its functional capacity of tubular excretion (Zilva et al., 1991), as renal disease which diminish the glomerular filtration rate lead to creatinine retention. It may also be an indication of adverse effect on the muscle biochemistry or inability of the kidney to excrete creatinine. Reduction in the urea level at 50 and 75 mg/kg body weight might suggest abnormality in the physiological excretion of urea caused by a non-renal factor which is the extract in this study. The non-significant effect of the extract on sodium, potassium and magnesium as well as the reduction in calcium levels coupled with the increase in chloride ions at specific doses further supported the selective effect of the extract on the biochemical indices of toxicity investigated in this study. In addition, the reduction in the level of phosphorus by the extract could be due to tubular dysfunction (Chawla, 1999). In this study, the assessment of haematological parameters was used as important biomarkers for evaluating the haematotoxic potential of the extract of *S. aculaestrum* berry. The reduction in WBC, although at 25 and 50 mg/kg body weight of the extract suggest that the constituents of the extract might have destroyed or impaired the production of these blood cells. It has been reported that granulocyte-macrophage colony stimulating factor, interleukins IL-2, IL-4 and IL-5 regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of these blood cells (Guyton and Hall, 2000; Ganong, 2001).

Therefore, the extract might have reduced the production of these regulatory factors or interfered with the sensitivity of the committed stem cells responsible for the production of these white blood cells (Adebayo et al., 2005). The elevated WBC at the highest dose may suggest enhanced production of these blood cells. The reduced platelet level at all the doses investigated might be an indication of platelet dysfunction (McGarry et al., 2005), resulting probably from adverse effect on the bone marrow.

Furthermore, the reduction in RBC and Hb levels only at the least dose (25 mg/kg body weight) may imply an attempt by the animals to adapt to the effect of the extract as the blood indices were increased at the higher doses investigated in this study. MCHC, MCH, MCV relate to individual red blood cells while haemoglobin, RBC, RCDW, LUC and PCV relate to the total population of red blood cells in the blood. The alterations in these parameters suggest localized, dose specific effect of the extract. Since lymphocytes, neutrophils, monocytes, eosinophils and basophils are related to the WBC, the decrease in WBC may as well account for the reduction in the levels of these factors and may adversely affect their normal functioning in the animals.

The biochemical data in the acute and sub acute studies present evidence of mortality as well as adverse effect on the absolute weight of some of the organs investigated, haematological parameters, liver and kidney function indices of male rats. Therefore, the aqueous extract of the unripe berries of *S. aculaestrum* is not safe as an oral remedy as it has the potentials of being nephrotoxic, hepatotoxic, haematotoxic and can also

result in the death of animals.

ACKNOWLEDGEMENT

This research was supported by the National Research Foundation of South Africa.

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Chapter 7

Studies on the toxicological effect of the aqueous extract of the fresh, dried and boiled berries of *Solanum aculeastrum* Dunal in male Wistar rats.

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*This chapter has been accepted for publication in this format to **Human and Experimental Toxicology.**

HET

Human and Experimental Toxicology
000(00) 1–11

DOI: 10.1177/0960327109354545
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Studies on the toxicological effect of the aqueous extract of the fresh, dried and boiled berries of *Solanum aculeastrum* Dunal in male Wistar rats

OM Aboyade, MT Yakubu, DS Grierson and AJ Afolayan

Abstract

The toxicological effect of the aqueous extract of fresh, dried and boiled berries of *Solanum aculeastrum* Dunal at 1, 10 and 25 mg/kg body weight was investigated in male Wistar rats for 28 days. The parameters used were the body weight of the animals and absolute weights of the organs, haematological parameters, renal and liver functional endpoints. The animals gained appreciable weight and showed no signs of clinical toxicity. The dried (DB), boiled dried (BDB), fresh (FB) and boiled fresh berry (BFB) extracts reduced ($p < .05$) the heart-, liver- and spleen-body weight ratio of the animals whereas that of the lung was not altered. The kidney and testes-body weight ratios were specifically altered by the different extract. All these were not accompanied by any histomorphological changes. The extracts did not alter ($p > .05$) the levels of RBC, Hb, PCV and albumin of the animals. The platelets were decreased by the DB and FB whereas BFB increased this parameter. The FB and BFB at all the doses also reduced the mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of the animals. With the exception of the FB where the creatinine and chloride levels decreased, other extracts did not alter the level of these kidney parameters. Only FB increased the levels of uric acid and urea. All the extract decreased the serum alanine aminotransferase (ALT) of the animal. The levels of total protein, globulin, total and conjugated bilirubin were not altered by DB and BDB whereas these indices were increased by FB and BFB. The DB and BDB increased the serum alkaline phosphatase (ALP) activity whereas FB decreased the activity of the enzyme. In contrast, DB and BDB decreased the serum aspartate aminotransferase (AST) activity of the animals whereas FB and BFB increased the activity of the enzyme. The FB and BFB also increased the levels of potassium, magnesium and phosphorus of the animals. Overall, the alterations in the biochemical parameters by the various extracts of *S. aculeastrum* berries at these doses indicated that the normal functioning of these organs may be adversely affected. However, drying and boiling might reduce the toxic effect of the berries.

Keywords

Solanum aculeastrum, solanaceae, hepatotoxic, haematotoxic, nephrotoxic

Introduction

The use of medicinal plants in the management of several ailments dates back to antiquity and is increasing empirically.[1] It has been estimated that more than half of the population of the world use herbal remedies.[2] In addition, a large and increasing number of people either use medicinal herbs directly or seek advice of traditional medical practitioners/physicians on the use of medicinal plants.[3] This has therefore necessitated the scientific scrutiny of medicinal plants for their safety in experimental animals. This study is a continuation of our screening of medicinal plants

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for safety in animal models. The plant, *Solanum aculeastrum* Dunal (Solanaceae), commonly known as goat bitter apple (English), is widely distributed in southern Africa.[4] It is a thorny perennial that grows up to 3 m in height, with white flowers and lemon-shaped berries that become greenish-yellow when ripe.[5]

Pharmacological studies on the crude extracts of the leaves and berries of this plant revealed that it possess antimicrobial, antitumor and antioxidant properties.[6-8] Similarly, steroidal alkaloids isolated from the berries showed activity against schistomiasis and cancer cells.[5,9,10] Despite the acclaimed uses of the fresh, dried, boiled and/or ashed berries of *S. aculeastrum* in the management of jigger wounds, gonorrhea, rheumatism, breast cancer,[11] ringworm in cattle and horses,[4,12] there is no report in the open scientific literature on the potential toxicological effects of the fresh, dried and boiled berries of this plant in animal models. This study was aimed at evaluating the toxic effects of the dried berry (DB), boiled dried berry (BDB), fresh berry (FB) and boiled fresh berry (BFB) of *S. aculeastrum* in animals using male Wistar rats as a model.

Materials and methods

Plant materials

Fresh berries of *S. aculeastrum*, collected in December, 2008, from Kayalethu village, Eastern Cape Province of South Africa, was authenticated by Prof DS Grierson of the Department of Botany, University of Fort Hare. A voucher specimen (SA/Med 01) was deposited at the Giffen's herbarium of the department.

Animals

Apparently, healthy, male rats (*Rattus norvegicus*) of Wistar strain weighing 187.29 ± 4.68 g were obtained from the Animal House of the Agricultural and Rural Development Research Institute (ARDRI), University of Fort Hare. The animals were housed in clean aluminum cages placed in well-ventilated house with optimum condition (temperature 23 ± 1°C; photoperiod: 12 hour natural light and 12 hour dark; humidity: 45%-50%). They were also allowed free access to food (Balanced Trusty Chunks, Pioneer Foods Pty Ltd., Huguenot, South Africa) and water free of contaminants.

Assay kits

The assay kits for creatinine, urea, uric acid, calcium, chloride, magnesium, sodium, potassium, phosphorus, albumin, globulin, total protein, bilirubin (total and conjugated), alkaline phosphatase, alanine and aspartate aminotransferases were products of Roche Diagnostic GmbH, Mannheim, Germany. All other reagents used were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

Preparation of extract

Fresh berries of the plant were washed in running tap water, blotted in tissue paper, sliced into pieces and divided into two portions. The first portion was oven-dried at 40°C until a constant weight was obtained. A part of this (400 g) was boiled for 20 min in 4 L of distilled water (BDB) and another (300 g) soaked in 3 L of distilled water (DB) and shaken for 24 hours on an orbital shaker (SO1 Stuart Scientific Orbital Shaker, Essex, UK). The second portion of the FB was again divided into two. The first part (1500 g) was soaked (FB) in 5 L of distilled water for 48 hours while the other (2000 g) portion was boiled (BFB) for 20 min in 5 L of distilled water. The extracts were filtered using a Buchner funnel and Whatman No 1 filter paper (Maidstone, UK), and then freeze-dried with a Vir Tis benchtop K (Vir Tis Company, Gardiner, NY) to give 47.40 g (BB), 39.48 g (DB), 30.15 g (FB) and 40.60 g (BFB). The yields were then reconstituted separately in distilled water to give the required doses (1, 10, 25 mg/kg body weight) used in this study. These doses and the 28 days exposure period in this study are in line with the way the traditional healers of Eastern Cape of South Africa use the plant extract in the management of cancer. The three dose levels employed in this study were those that were frequently claimed to be used in folklore medicine in the management of the disease.

Animal grouping and extract administration

The animals were assigned into 13 groups (A-M), each consisting of five rats. The extracts were administered orally once daily for 28 days using metal oropharyngeal cannula. Group A (control) received 0.5 mL of distilled water (the vehicle), while groups B to M were treated with the same volume of the extract preparation containing 1, 10 and 25 mg/kg body weight of the extracts, respectively. The animals were sacrificed 24 hours after their 28 daily doses. This study was carried out following approval from the ethical committee on the use and care of animals of the University of Fort Hare, South Africa.

Preparation of serum

The procedure described by Yakubu et al.[13] was used in the preparation of serum. Briefly, under ether anaesthesia, the neck area of the rats was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to prevent blood contamination by interstitial fluid) were sharply cut with sterile scapel blade and an aliquot (2 mL) of the blood was collected into sample bottles containing EDTA (BD Diagnostics, Preanalytical Systems, Midrand, USA) for the haematological analysis. Another 5 mL of the blood was allowed to clot for 10 min at room temperature and then centrifuged at 1282g × 5 min using Hermle Bench Top Centrifuge (Model Hermle, Z300, Hamburg, Germany). The sera were later aspirated with Pasteur pipettes into sample bottles and used within 12 hours of preparation for the assay. The rats were quickly dissected in the cold and the organs (liver, kidneys, lungs, spleen, heart and testes) removed, freed of fat, blotted with clean tissue paper and then weighed for the determination of organ body weight ratio using the expression of Yakubu et al.[14]

Determination of biochemical parameters

The serum was assayed for creatinine, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), potassium, sodium, calcium, chloride, phosphorus, total and conjugated bilirubin, total protein, albumin, globulin and uric acid using assay kits on Roche Modular Analyzer (model P800) Mannheim, Germany, as described by Tietz et al.[15] The haematological parameters: red blood count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet, white blood cells (WBC) and white blood cell differential count (basophils, eosinophils, neutrophils, monocytes and lymphocytes) were determined with the Horiba ABX Diagnostics-Pentra 80 (Montpellier, France). The analyzers were calibrated for the animal blood before being used for the study.

Histopathological examination

The organs (heart, liver, kidney, testes, lungs and spleen) were fixed in 10% v/v formaldehyde, dehydrated through ascending grades of ethanol (70%, 90% and 95% v/v), cleaned in xylene and embedded in paraffin wax (melting point 56°C).[16] Tissue sections were prepared according to the method described by Drury and Wallington[17] and stained with hematoxylin/eosin. The photomicrographs were taken at ×400 with a Canon (Melville, NY) Power Shot G2 digital camera.

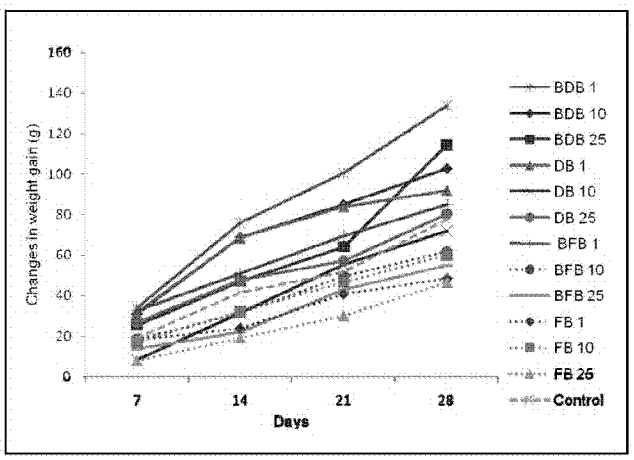

Figure 1. Changes in body weight gain over a period of 28 days for various doses of the aqueous extract of boiled dried berries (BDB), Dried berries (DB), boiled fresh berries (BFB) and fresh berries (FB) of *Solanum aculeastrum*.

Statistical analysis

Results were expressed as the mean ± SD of five replicates. Means were analyzed using a one-way analysis of variance (ANOVA) and this was complemented with Duncan Multiple Range Test to account for the significant differences among the means and the interaction between the variables. All statistical analyses were done using SAS. Values were considered statistically significant at $p < .05$.

Results

All the animals that were orally administered with 1, 10 and 25 mg/kg body weight of the extracts from *S. aculeastrum* for 28 days remained active throughout the experimental period. Although animals in all the groups gained weight, the least weight gain was observed in the rats administered with the 25 mg/kg body weight of FB. In addition, the weight gained by the animals was not dose related (Figure 1). In contrast, all the doses investigated for the DB, BDB, FB and BFB extract reduced the heart-, liver- and spleen-body weight ratios of the animals whereas that of the lung compared favourably with the control. Whereas the DB extract did not significantly ($p > .05$) alter the

Table 1. Organ-body weight ratio of rats administered with aqueous extracts of *Solanum aculeastrum* berry for 28 days ($n = 5$, $x \pm$ SD)

Treatments (mg/kg body weight)	Heart	Liver	Kidney	Testes	Lungs	Spleen
Control	0.38 ± 0.03^{a}	2.90 ± 0.98^{a}	0.69 ± 0.10^{a}	0.95 ± 0.12^{a}	0.97 ± 0.22^{a}	0.37 ± 0.19^{a}
Dried berries						
1	0.33 ± 0.01^{b}	2.13 ± 0.40^{b}	0.61 ± 0.03^{a}	0.80 ± 0.07^{b}	0.90 ± 0.04^{a}	0.27 ± 0.05^{b}
10	0.32 ± 0.02^{b}	2.28 ± 0.17^{c}	0.65 ± 0.01^{a}	0.74 ± 0.05^{b}	0.85 ± 0.17^{a}	0.25 ± 0.04^{b}
25	0.30 ± 0.03^{b}	2.25 ± 0.10^{c}	0.60 ± 0.05^{a}	0.74 ± 0.05^{b}	0.95 ± 0.15^{a}	0.22 ± 0.04^{b}
Boiled dried berries						
1	0.32 ± 0.01^{b}	2.35 ± 0.26^{d}	0.58 ± 0.08^{b}	0.78 ± 0.09^{b}	0.90 ± 0.09^{a}	0.28 ± 0.02^{b}
10	0.30 ± 0.04^{c}	2.28 ± 0.06^{c}	0.54 ± 0.02^{b}	0.74 ± 0.06^{b}	0.96 ± 0.16^{a}	0.24 ± 0.01^{b}
25	0.29 ± 0.01^{d}	2.27 ± 0.04^{c}	0.57 ± 0.04^{b}	0.76 ± 0.32^{b}	0.93 ± 0.12^{a}	0.22 ± 0.05^{b}
Fresh berries						
1	0.26 ± 0.18^{d}	2.60 ± 0.18^{e}	0.55 ± 0.05^{b}	1.19 ± 0.13^{c}	0.88 ± 0.12^{a}	0.23 ± 0.04^{b}
10	0.28 ± 0.02^{d}	2.74 ± 0.14^{f}	0.56 ± 0.02^{b}	1.17 ± 0.05^{c}	0.93 ± 0.20^{a}	0.22 ± 0.04^{b}
25	0.29 ± 0.02^{d}	2.80 ± 0.24^{f}	0.56 ± 0.01^{b}	1.14 ± 0.16^{c}	0.99 ± 0.08^{a}	0.27 ± 0.03^{b}
Boiled fresh berries						
1	0.27 ± 0.03^{d}	2.55 ± 0.40^{d}	0.58 ± 0.04^{b}	1.22 ± 0.17^{c}	0.89 ± 0.12^{a}	0.20 ± 0.02^{b}
10	0.28 ± 0.02^{d}	2.66 ± 0.30^{d}	0.56 ± 0.04^{b}	1.23 ± 0.33^{c}	0.91 ± 0.06^{a}	0.22 ± 0.05^{b}
25	0.27 ± 0.01^{d}	3.04 ± 0.34^{a}	0.56 ± 0.01^{b}	1.16 ± 0.02^{c}	0.94 ± 0.11^{a}	0.20 ± 0.02^{b}

[a-d] Test values carrying superscripts different from the control down the column for each organ are significantly different ($p < .05$) and those with the same superscript as the control are not significantly different ($p > .05$).

kidney-body weight ratio, the organ body weight ratio was reduced by the BDB, FB and BFB extract. Again, the DB and BDB extract reduced the testes-body weight ratio whereas the FB and BFB extracts increased it (Table 1). Compared with their respective controls, there was no evidence of distortion in the normal histomorphology of the organs investigated in this study (photomicrographs not shown). For example, the branching cardiac monocytes, coronary vessels as well as the endocardium and cardiac valves of the heart were normal, with no evidence of fibrosis, ischemia, inflammation or thrombosis. The pulmonary blood vessels, pleural surfaces, branchial lumina and alveolar architecture were also preserved in the lungs of the animals. Similarly, the epididymes, spermatocytes, seminiferous tubules as well as the amount of stroma were normal in the testes. The central veins, portal tract and capsule were also within normal histology in the liver of the animals. The medullary and cortical architecture as well as proximal and distal convoluted tubules with intervening loop of Henle were preserved. The cells of the spleen were also within normal histology.

The various extracts produced effects that were not dose-response related on the various biochemical indices of toxicity evaluated. For instance, all the extracts did not significantly alter the levels of RBC, Hb and PCV of the animals. While the extract of FB and BFB significantly reduced the levels of MCH and MCHC at all the doses, the DB and BDB of the plant did not alter the blood parameters. Whereas the extract of the FB of *S. aculeastrum* significantly decreased the levels of MCV of the animals, the blood index compared well with the control at all the doses following the administration of aqueous extract of DB, BDB and BFB. The platelets were decreased by all the doses of DB and FB whereas the level increased with boiled FB. The platelets were not significantly altered by the BDB extract. The RCDW was significantly increased only by the FB extract of *S. aculeastrum* (Table 2). Whereas there was no specific pattern of effect on the monocyte, eosinophils and basophils of the animals, the WBC, neutrophils, lymphocytes and LUC levels were reduced significantly (Table 3).

There was no significant difference in the levels of sodium and calcium of all the animals. With the exception of the FB extract that decreased the creatinine and chloride levels, all the other extract did not alter the level of these kidney parameters (Table 4). While the DB and BDB extract of *S. aculeastrum* did not affect the levels of potassium, magnesium and

Table 2. Effect of aqueous extract of *Solanum aculeastrum* berries on some haematological parameters of male Wistar rats ($n = 5$, $x \pm$ SD)

Treatments/doses (mg/kg b.w)	RBC ($\times 10^{12}$/L)	Hb (g/L)	PCV (L/L)	MCV (fl)	MCH (pg)	MCHC (%)	RCDW (%)	Platelet ($\times 10^{9}$/L)
Control	8.32 ± 0.24[a]	15.70 ± 0.21[a]	0.49 ± 0.03[a]	59.00 ± 1.34[a]	19.40 ± 0.49[a]	32.80 ± 0.07[a]	12.10 ± 0.07[a]	947.00 ± 33.80[a]
Dried berries								
1	8.30 ± 0.18[a]	15.85 ± 0.50[a]	0.51 ± 0.01[a]	59.85 ± 1.13[a]	19.90 ± 0.14[a]	32.85 ± 0.50[a]	12.05 ± 0.21[a]	861.00 ± 48.10[b]
10	8.36 ± 0.09[a]	15.95 ± 0.35[a]	0.49 ± 0.01[b]	59.80 ± 1.56[a]	19.50 ± 0.28[a]	32.70 ± 0.42[a]	12.10 ± 0.21[a]	844.00 ± 48.10[c]
25	8.35 ± 0.17[a]	15.65 ± 0.35[a]	0.48 ± 0.01[b]	59.90 ± 0.14[a]	19.45 ± 0.50[a]	32.80 ± 0.28[a]	12.05 ± 0.64[a]	854.00 ± 19.80[b]
Boiled dried berries								
1	8.48 ± 0.18[a]	15.75 ± 0.21[a]	0.51 ± 0.01[a]	60.00 ± 1.27[a]	19.45 ± 0.21[a]	32.75 ± 0.07[a]	12.10 ± 0.08[a]	947.00 ± 47.40[a]
10	8.37 ± 0.08[a]	15.80 ± 0.28[a]	0.48 ± 0.14[a]	59.97 ± 0.64[a]	19.45 ± 0.21[a]	32.65 ± 0.78[a]	12.15 ± 0.07[a]	947.00 ± 35.66[a]
25	8.36 ± 0.13[a]	15.75 ± 0.07[a]	0.49 ± 0.01[a]	59.95 ± 0.50[a]	19.55 ± 0.35[a]	32.80 ± 0.28[a]	12.00 ± 0.14[a]	956.00 ± 53.00[a]
Fresh berries								
1	8.47 ± 0.09[a]	15.80 ± 0.07[a]	0.50 ± 0.01[a]	57.60 ± 1.20[b]	17.70 ± 0.07[b]	30.70 ± 0.78[b]	14.80 ± 0.08[b]	803.00 ± 31.80[d]
10	8.39 ± 0.06[a]	15.90 ± 0.14[a]	0.49 ± 0.02[a]	56.50 ± 0.71[b]	17.20 ± 0.00[b]	30.40 ± 0.42[b]	14.90 ± 0.05[b]	612.00 ± 15.80[e]
25	8.34 ± 0.14[a]	15.90 ± 0.28[a]	0.50 ± 0.02[a]	54.20 ± 3.75[b]	16.90 ± 0.64[b]	29.20 ± 1.06[b]	15.1 ± 00.08[b]	764.00 ± 33.00[f]
Boiled fresh berries								
1	8.34 ± 0.04[a]	15.50 ± 0.57[a]	0.49 ± 0.04[a]	58.90 ± 0.07[a]	17.50 ± 0.35[b]	29.60 ± 0.57[b]	12.10 ± 0.15[a]	1175.00 ± 24.70[g]
10	8.32 ± 0.25[a]	15.80 ± 0.42[a]	0.50 ± 0.02[a]	58.40 ± 0.64[a]	17.70 ± 0.12[b]	28.95 ± 0.07[b]	12.05 ± 0.07[a]	1081.00 ± 29.90[g]
25	8.36 ± 0.02[a]	15.70 ± 0.28[a]	0.51 ± 0.01[a]	59.30 ± 0.71[a]	18.20 ± 0.07[c]	27.60 ± 0.57[c]	12.07 ± 0.07[a]	1128.00 ± 15.90[g]

RBC, red blood cell; Hb, haemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RCDW, red cell distribution width

[a-g] Test values carrying superscripts different from the control down the column for each haematological parameter are significantly different ($p < .05$) and those with the same superscript as the control are not significantly different ($p > .05$).

Table 3. Effect of aqueous extract of *Solanum aculeastrum* berries on white blood cells and the differentials of male Wistar rats ($n = 5$, $x \pm SD$)

Treatments/doses (mg/kg body weight)	WBC ($\times 10^9$/L)	Neutrophils (%)	Monocytes (%)	Lymphocytes (%)	LUC (%)	Eosinophils (%)	Basophils (%)
Control	14.57 ± 0.70^{a}	1.03 ± 0.26^{a}	2.80 ± 0.20^{a}	9.33 ± 0.89^{a}	1.30 ± 0.20^{a}	0.34 ± 0.05^{a}	0.09 ± 0.01^{a}
Dried berries							
1	9.87 ± 0.06^{b}	0.80 ± 0.13^{b}	2.58 ± 0.16^{b}	5.48 ± 0.01^{b}	0.65 ± 0.01^{b}	0.13 ± 0.04^{b}	0.10 ± 0.01^{a}
10	9.70 ± 0.10^{b}	0.72 ± 0.05^{c}	2.18 ± 0.03^{b}	5.43 ± 0.22^{b}	0.70 ± 0.21^{b}	0.24 ± 0.02^{c}	0.07 ± 0.02^{c}
25	9.26 ± 1.39^{b}	0.89 ± 0.23^{b}	1.77 ± 0.25^{c}	4.23 ± 0.83^{c}	0.85 ± 0.42^{c}	$0.18 \pm 0.06^{c,d}$	0.03 ± 0.00^{d}
Boiled dried berries							
1	9.15 ± 2.50^{b}	0.92 ± 0.48^{d}	3.64 ± 0.37^{d}	4.94 ± 0.39^{b}	0.95 ± 0.07^{d}	0.20 ± 0.01^{c}	0.10 ± 0.04^{a}
10	10.23 ± 0.25^{c}	0.74 ± 0.22^{c}	2.95 ± 0.22^{a}	6.18 ± 0.06^{d}	0.95 ± 0.07^{d}	0.12 ± 0.01^{b}	0.10 ± 0.01^{a}
25	10.38 ± 0.16^{c}	0.66 ± 0.03^{e}	2.37 ± 0.08^{b}	6.63 ± 0.35^{d}	0.85 ± 0.07^{c}	0.13 ± 0.02^{c}	0.05 ± 0.01^{c}
Fresh berries							
1	9.34 ± 0.39^{b}	0.94 ± 0.09^{d}	4.60 ± 0.06^{e}	6.23 ± 0.61^{d}	0.50 ± 0.14^{e}	0.42 ± 0.09^{e}	0.10 ± 0.01^{a}
10	9.20 ± 0.40^{b}	0.70 ± 0.04^{c}	2.80 ± 0.08^{a}	7.58 ± 1.20^{e}	0.40 ± 0.02^{e}	0.41 ± 0.11^{e}	0.09 ± 0.01^{a}
25	10.17 ± 0.57^{c}	0.51 ± 0.03^{e}	3.80 ± 0.06^{f}	7.66 ± 1.33^{e}	0.50 ± 0.14^{e}	0.24 ± 0.04^{c}	0.16 ± 0.06^{e}
Boiled fresh berries							
1	9.39 ± 0.05^{b}	0.84 ± 0.04^{b}	3.10 ± 0.17^{d}	4.50 ± 0.27^{c}	0.80 ± 0.01^{c}	0.22 ± 0.01^{c}	0.04 ± 0.01^{d}
10	10.98 ± 0.73^{d}	0.67 ± 0.17^{e}	3.90 ± 0.10^{f}	5.89 ± 0.62^{b}	0.85 ± 0.09^{c}	0.17 ± 0.03^{c}	0.06 ± 0.01^{c}
25	10.58 ± 0.65^{d}	0.61 ± 0.07^{e}	4.80 ± 0.20^{e}	4.33 ± 0.64^{c}	0.60 ± 0.04^{f}	0.42 ± 0.01^{e}	0.09 ± 0.02^{a}

WBC, white blood cell; LUC, large unstained cell.

[a-f] Test values carrying superscripts different from the control down the column for each haematological parameter are significantly different ($p < .05$) and those with the same superscript as the control are not significantly different ($p > .05$).

Table 4. Effect of *Solanum aculeastrum* berries on some renal function parameters of male Wistar rats ($n = 5$, $x \pm$ SD)

Treatments/doses (mg/kg body weight)	Sodium (mmol/L)	Potassium (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)	Calcium (mmol/L)	Magnesium (mmol/L)	Phosphorus (mmol/L)	Uric acid (mmol/L)	Chloride (mmol/L)
Control	140 ± 1.41[a]	5.15 ± 0.21[a]	5.70 ± 0.14[a]	51.50 ± 3.54[a]	2.42 ± 0.01[a]	0.90 ± 0.0[a]	2.55 ± 0.07[a]	0.07 ± 0.01[a]	99.5 ± 0.71[a]
Dried berries									
1	140 ± 1.41[a]	5.20 ± 0.14[a]	5.71 ± 0.14[a]	49.00 ± 2.83[a]	2.40 ± 0.02[a]	0.94 ± 0.03[a]	2.55 ± 0.07[a]	0.07 ± 0.03[a]	99.6 ± 1.41[a]
10	141 ± 0.71[a]	5.13 ± 0.04[a]	5.60 ± 0.71[a]	48.00 ± 1.41[a]	2.42 ± 0.05[a]	0.88 ± 0.08[a]	2.59 ± 0.01[a]	0.07 ± 0.00[a]	99.5 ± 0.71[a]
25	142 ± 0.71[a]	5.15 ± 0.07[a]	5.65 ± 0.21[a]	49.00 ± 5.66[a]	2.41 ± 0.01[a]	0.95 ± 0.01[a]	2.50 ± 0.00[a]	0.07 ± 0.01[a]	99.5 ± 0.71[a]
Boiled dried berries									
1	141 ± 0.71[a]	5.15 ± 0.11[a]	5.65 ± 0.35[a]	51.00 ± 2.83[a]	2.43 ± 0.01[a]	1.01 ± 0.02[a]	2.55 ± 0.08[a]	0.07 ± 0.01[a]	98.9 ± 0.83[a]
10	140 ± 1.41[a]	5.20 ± 0.07[a]	5.75 ± 0.19[a]	51.00 ± 1.41[a]	2.41 ± 0.05[a]	1.03 ± 0.02[a]	2.55 ± 0.06[a]	0.07 ± 0.01[a]	99.0 ± 0.71[a]
25	140 ± 1.41[a]	5.10 ± 0.18[a]	5.65 ± 0.21[a]	52.00 ± 1.41[a]	2.42 ± 0.02[a]	1.00 ± 0.11[a]	2.50 ± 0.04[a]	0.07 ± 0.01[a]	99.5 ± 0.71[a]
Fresh berries									
1	139 ± 1.71[a]	9.65 ± 0.35[b]	7.80 ± 0.14[b]	42.00 ± 3.66[b]	2.42 ± 0.04[a]	1.60 ± 0.00[b]	3.55 ± 0.50[b]	0.14 ± 0.01[b]	85.5 ± 0.21[b]
10	139 ± 1.54[a]	9.80 ± 0.20[b]	4.15 ± 0.29[c]	34.50 ± 4.95[c]	2.45 ± 0.01[a]	1.60 ± 0.10[b]	3.60 ± 0.28[b]	0.15 ± 0.08[b]	76.5 ± 0.71[c]
25	140 ± 0.30[a]	9.60 ± 0.57[b]	3.55 ± 0.07[d]	41.00 ± 0.11[b]	2.42 ± 0.03[a]	1.55 ± 0.10[b]	3.65 ± 0.35[c]	0.15 ± 0.02[b]	55.5 ± 0.71[d]
Boiled fresh berries									
1	140 ± 1.41[a]	9.40 ± 0.8b[b]	5.80 ± 0.28[a]	52.50 ± 10.6[a]	2.40 ± 0.02[a]	1.50 ± 0.10[b]	3.40 ± 0.14[d]	0.07 ± 0.00[a]	98.5 ± 2.21[a]
10	140 ± 1.41[a]	9.45 ± 0.78[b]	5.80 ± 0.27[a]	50.00 ± 4.95[a]	2.44 ± 0.04[a]	1.56 ± 0.10[b]	3.40 ± 0.28[d]	0.08 ± 0.01[a]	99.5 ± 1.21[a]
25	141 ± 0.71[a]	9.80 ± 0.28[b]	5.75 ± 0.07[a]	51.00 ± 2.83[a]	2.45 ± 0.01[a]	1.61 ± 0.10[b]	3.70 ± 0.14[c]	0.07 ± 0.02[a]	99.5 ± 0.71[a]

[a-d] Test values carrying superscripts different from the control down the column for each renal functional endpoint are significantly different ($p < .05$) and those with the same superscript as the control are not significantly different ($p > .05$).

phosphorus of the animals, the concentrations of these electrolytes increased with the FB and BFB extract. The FB extract increased the uric acid level at all the investigated doses. Also, the same extract (FB) increased the concentration of urea only at 1 mg/kg body weight, whereas this parameter was reduced at other dose levels (Table 4).

The various extract of *S. aculeastrum* berries did not significantly alter the levels of serum albumin in the animals whereas the activity of serum ALT was decreased (Table 5). The levels of total protein, globulin, total and conjugated bilirubin were not significantly altered by the DB and BDB extract while the FB and BFB extract of the plant increased the levels of all the molecules. In addition, the globulin content of the animals was not altered significantly by the BFB extract except in the 25 mg/kg body weight–treated animals where it was increased. The DB and BDB extract of the plant at all the doses investigated increased the activity of serum ALP whereas the FB extract decreased the enzyme activity. In addition, the serum ALP activity produced by the BFB extract was not different from the control group. Similarly, the serum AST activity decreased significantly at all the doses by the DB and BDB extract whereas the FB and BFB extract increased the activity of the enzyme in the serum of the animals (Table 5).

Discussion

The absence of clinical signs of toxicity in this study implied that the doses used did not accumulate sufficiently enough to manifest changes in the physical behaviour as well as cause the death of the animals. This may also explain the moderate activity of the animals when compared with the control group despite the alterations in their biochemical parameters.

A change in weight of the body and or the internal organs of animals is an important index of adverse side effect of a chemical compound including plant extract. Similarly, weight loss is a sensitive and simple index of toxicity.[18,19] The weight gained by the animals during the experimental period may be an indication that the extract did not hamper the growth of the animals.[20] Interestingly, the computed heart-, liver- and spleen-body weight were reduced whereas there was extract-specific alterations in those of the kidney and testes of the animals. Since the extract did not produce any histomorphological changes in these organs, the reduction in the heart, liver and spleen body weight ratios of the animals, therefore, suggests that the changes in the size and/or weight of these organs are not proportional to the growth of the animals. It is also possible that the reduced weight of the organs in this study may be the consequence of leakage of cytosolic enzymes into the extracellular fluid, the serum. The reduced level of circulating lymphocytes could also account for the reduction in the spleen–body weight ratio. In contrast, the increase in the computed testes–body weight ratio by the FB and BFB extract could be due to enhanced functional activity of the organ and this may result in increased secretory function of the testes.[14] Although secretory constituents of the testes such as the testosterone, protein, glycogen and sialic acid were not determined in this study, the alteration in the organ-body weight suggested adverse effect of the various extracts on the testes of the animals.

Assessment of haematological parameters is a useful index to determine the effects of foreign compounds including plant extracts on the blood constituents and its functions. Such analysis could have a higher predictive value for human toxicity, when the data are translated from animal studies.[21] Hb, RBC, PCV are associated with total population of red blood cells while MCH, MCHC, MCV and RCDW relates to individual red blood cells.[22] In this study, the individual red blood cells were adversely affected since levels of MCH, MCHC, MCV and RCDW were reduced by specific extract. We believe that drying, soaking and boiling might have reduced the phytotoxic component(s) in the plant berries. Similarly, the reduction in the level of platelets by the DB and FB extracts suggest impairment in the production of the blood cells. Such reduction in the platelet count might adversely affect normal coagulation, a process of blood clotting. Furthermore, it is also possible that the phytoconstitutents of the BFB extract might have promoted differentiation of the platelets that were hitherto absent in the BDB probably because of the drying treatment.

White blood cells and its differentials are useful indicators of the ability of an organism to eliminate infection. The decrease in the levels of WBC, neutrophils and lymphocytes may have adverse effect on the ability of the animals to fight infection, attack and destroy bacteria in the blood. It is also possible that the effector cells of the immune system might be adversely affected. Our findings in this study contrast that of Swenson and Reece,[23] who reported that toxic plants do not produce direct effect on WBC and its differentials. The non-specific pattern of effect by all

Table 5. Effect of *Solanum aculeastrum* berries on some liver functional endpoints of male Wistar rats ($n = 5$, $x \pm$ SD)

Treatments/doses (mg/kg body weight)	Total bilirubin (μmol/L)	Conj. bilirubin (μmol/L)	Total protein (g/L)	Albumin (g/L)	Globulin (U/L)	ALP (U/L)	ALT (U/L)	AST (U/L)
Control	5.50 ± 0.71[a]	1.50 ± 0.71[a]	64.50 ± 0.71[a]	17.00 ± 1.41[a]	49.50 ± 0.71[a]	348.00 ± 20.5[a]	54.50 ± 0.71[a]	169.00 ± 6.40[a]
Dried berries								
1	5.00 ± 0.41[a]	1.50 ± 0.71[a]	64.50 ± 0.12[a]	17.50 ± 0.71[a]	49.00 ± 0.83[a]	425.00 ± 35.4[b]	35.50 ± 0.71[b]	137.13 ± 4.95[b]
10	5.50 ± 0.71[a]	1.50 ± 0.71[a]	64.50 ± 0.54[a]	17.50 ± 0.71[a]	48.00 ± 2.24[a]	432.22 ± 11.3[b]	41.50 ± 0.71[c]	133.04 ± 7.70[b]
25	5.50 ± 0.71[a]	1.50 ± 0.71[a]	64.00 ± 0.83[a]	17.00 ± 0.00[a]	49.00 ± 0.24[a]	491.14 ± 20.5[c]	42.50 ± 0.71[c]	142.00 ± 0.00[b]
Boiled dried berries								
1	5.50 ± 0.71[a]	1.50 ± 0.71[a]	65.00 ± 0.41[a]	17.50 ± 0.71[a]	48.50 ± 2.12[a]	416.00 ± 4.24[b]	36.00 ± 2.83[b]	137.41 ±11.30[b]
10	5.50 ± 0.71[a]	1.50 ± 0.71[a]	65.50 ± 0.12[a]	17.50 ± 0.71[a]	49.00 ± 1.41[a]	424.16 ± 23.3[b]	44.00 ± 1.41[c]	130.00 ± 9.19[b]
25	5.00 ± 1.41[a]	1.50 ± 0.71[a]	65.00 ± 0.83[a]	17.50 ± 0.71[a]	49.50 ± 2.12[a]	482.00 ± 19.8[c]	36.50 ± 3.54[b]	143.22 ± 4.24[b]
Fresh berries								
1	12.00 ± 2.83[b]	5.50 ± 0.71[b]	71.00 ± 2.83[b]	16.50 ± 0.71[a]	56.50 ± 2.12[b]	241.00 ± 30.4[d]	25.00 ± 1.41[d]	215.81 ± 9.00[c]
10	16.50 ± 4.95[c]	6.00 ± 1.41[b]	71.50 ± 0.71[b]	16.00 ± 1.41[a]	58.50 ± 0.71[b]	228.20 ± 29.7[d]	28.00 ± 1.41[d]	214.05 ± 3.50[c]
25	14.50 ± 3.54[d]	6.00 ± 1.41[b]	71.00 ± 1.41[b]	17.50 ± 0.71[a]	61.40 ± 1.12[c]	212.17 ± 19.1[e]	21.00 ± 1.41[d]	220.13 ± 3.00[c]
Boiled fresh berries								
1	12.50 ± 0.71[b]	6.50 ± 0.71[b]	77.00 ± 1.41[b]	17.5 ± 0.71[a]	50.50 ± 0.71[a]	350.90 ± 15.71[a]	38.50 ± 0.71[b]	291.14 ± 5.70[d]
10	11.00 ± 2.83[b]	4.50 ± 0.12[c]	78.00 ± 4.24[b]	17.0 ± 1.41[a]	49.00 ± 0.83[a]	349.07 ± 20.51[a]	34.00 ± 3.4[b]	321.09 ± 7.61[e]
25	15.50 ± 2.12[c]	4.50 ± 0.71[c]	79.50 ± 0.71[b]	17.0 ± 0.0[a]	59.50 ± 0.71[b]	346.08 ± 23.19[a]	37.00 ± 2.38[b]	224.00 ± 7.11[c]

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

[a-e] Test values carrying superscripts different from the control down the column for each organ are significantly different ($p < .05$) and those with the same superscript as the control are not significantly different ($p > .05$).

the doses of the extracts on the monocyte, eosinophils and basophils may be attributed to physiological response to the effect of the foreign agent, the extract in this case.

Renal functional endpoints such as creatinine, urea, uric acid and serum electrolytes are indices that can be used to evaluate the normal functioning of the nephrons.[24] The reduction in serum creatinine, a major catabolic product of the muscle, may suggest that *S. aculeastrum* berries have the ability to boost the renal excretory system, thus increasing the elimination of these metabolic waste product. Similarly, the decrease in serum chloride ion content by the FB extract might imply adverse effect on the normal level of the ion and may affect chloride ion dependent process in the animals. Interestingly, we noted in this study that the levels of potassium, magnesium and phosphate were not altered by the DB extracts but by FB and BFB extracts. It is therefore possible that the oven-drying as well as soaking might have impact on the toxicity of the berries in the animals. Serum urea, a major catabolic product of purine nucleotide, which fluctuated at all the doses of the FB extract of *S. aculeastrum*, may be attributed to physiological responses to the effect of the extract. We believe that the FB extract is the most toxic since it showed the most remarkable adverse effect on the renal parameters.

Plasma proteins such as albumin are used for assessing the capacity of the liver, since most of them are synthesized in the hepatocytes[25] and they are important indicators of liver damage or biliary duct obstruction.[26] The alterations in the levels of total protein, globulin, total and conjugated bilirubin by only the FB and BFB extract of *S. aculeastrum* suggests selective adverse effect on the liver functional endpoints since the level of albumin was not altered by these extracts. This may affect the normal synthetic function of the liver with respect to these parameters. There are many enzymes found in the serum that did not actually originate from the extracellular fluid. During tissue damage, some of these enzymes find their way into the serum probably by leakage.[27] The increase in serum ALP following the administration of the DB and BDB extracts of *S. aculeastrum* suggests that these extracts might have disrupted the plasma membrane of the rats' organs since enzyme levels are only raised in the serum during damage to the plasma membrane. In contrast, however, the decrease in the serum ALP by the FB extract as well as similar decrease in the serum AST activities by the DB and BDB extracts of *S. aculeastrum* suggests inactivation or inhibition of the enzyme molecules by these extract. These will have consequential effects on the normal functioning of the enzymes in the animals.

We conclude that the alterations in the biochemical parameters by the various extract of *S. aculeastrum* berries at all the doses will have consequential effects on the normal functioning of these organs. Therefore, the various extracts of the berries of *S. aculeastrum* are toxicologically significant to the animals with that of FB extract producing the most profound effect. The repeated consumption of dried berry (DB), fresh berry (FB), boiled dried berry (BDB) and boiled fresh berry (BFB) extracts of *S. aculeastrum* for 28 days at the doses of 1, 10 and 25 mg/kg body weight (as claimed to be used in folklore medicine of South Africa for the management of cancer) may not be completely 'safe' as oral remedy. Oven-drying, soaking and boiling might reduce the toxic effect of the berries.

Acknowledgement

The authors are grateful to the National Research Foundation of South Africa for funding this research.

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