The Effects of Elytropappus rhinocerotis *Cass* and Pelargonium triste (L.) *L'Hérit* on Animal Health and Metabolism

By

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A thesis in partial fulfilment of the requirements for the degree of Magister Scientiae in the Department of Medical Biosciences, Faculty of Natural Sciences, at the University of the Western Cape.

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Dedication

To my Mom and Dad who always believed, encouraged and supported me through everything.

Acknowledgements

I would like to thank the following:

- My Mother, Father and two brothers Saeed and Abdullah for always being there and for all their encouragement.
- My supervisors, Prof. Q. Johnson and Frans Weitz.
- My friends and colleagues Natalie Vlotman, Cheryl Moses, Abigail Speelman and Andrea Bowie, who encouraged and supported me through good and bad times.
- Evan Springfield for his guidance.
- Pam Delport and Norman Coldrey for all their assistance.
- Ghakiem Singh, for always having faith in my capabilities and me.
- The National Research Foundation for financial support.
- Mr. L. Cyster (Department of Biodiversity and Conservation), for all his assistance, advice and support.

Declaration

I declare that "The Effects of Elytropappus rhinocerotis and Pelargonium triste on Animal Health and Metabolism" is my own work, only submitted to the University of the Western Cape, with all research resources used in this project duly acknowledged by means of complete references.

Nazeema Duarte

June 2003

Chapter 1: Introduction- The effects of Elytropappus rhinocerotis and Pelargonium triste on animal health and metabolism

Plants were once the source of all medicine used in the world and represent a sustainable source of new remedies (Swerdlow, 2000). Indeed, natural products and their derivatives represent more than 50% of all pharmaceutical drugs in clinical use today (van Wyk, van Oudtshoorn and Gericke, 2000).

There are four general types of herbal medicine. Asian, European, Indigenous and Neo-Western (DeSmet, 1995). In fact, indigenous medicinal systems are the most dynamic because of contact with other cultures (Elvin-Lewis, 2001). Traditional medicinal systems are the combination of both the physical knowledge and domain indigenous practices. Since much of this is based on spirituality, it may not have scientifically explicable reasoning (Posey, 2002). The informal oral traditions of the Khoi-San people, as well as that of the Nguni and Sotho speaking people have not yet been systemized. Seventy percent of South Africans still use traditional medicinal plants for ailments, and the majority of South Africans refer to practices of tradition where healthcare is concerned. Through trial and error, people have perfected the use of plants as food and medicine (Van Wyk and Viljoen, 1998).

There are numerous drugs that have been derived from natural products. Quinine, derived from the bark of Cinchona tree and Artemisinin from Artemisia annua is very effective in the treatment of Malaria (Phillipson, 1995). Malaria kills over one million people each year of which children under 5 years constitute 90% that live in Africa, south of the Sahara. Each year, there are 300 million clinical cases of malaria, which are 5 times as many as the combined cases of TB, AIDS and Leprosy (MRC, 2002).

Morphine and Codeine has been isolated from the latex of the opium poppy and is used as analgesics and as recreational drugs (Baskett and Hamilton, 2000). Natural products that act on the central nervous system include Caffeine, Codeine, Morphine, Reserpine (Phillipson, 2001) and Nicotine (Illback and Stalhandske, 2003).

Furthermore, Taxol is obtained from the bark of Taxus brevifolia and is active against cancer cells (Schreiber, 2000). In addition, Penicillium, Cephalosporium and Streptomycetes are all natural sources from which very important antibiotics are derived (Phillipson, 2001).

About 250 000 living plant species contain a much a greater diversity of bioactive compounds (Shu, 1998). Indigenous plant medicines have been used for reproductive health (Chaoudhary, Singh and Singh, 1991; Chaudhury, Chandrasekaran and Mishra, 2001; Al-Dissi, Al-Hajj and Salhab, 2001; Purohit, Satyanarayan and Shivalingappa, 2001; Amos, Binda, Gamaniel, Kapu, Kunle, Vongtau and Wambebe, 2000; Chaoudhary, Singh and Singh, 1991), and for combating bacteria (Afolayan and Grierson, 1999; Jager, McGraw and van Staden, 2001) viruses (Hay and Serkedjieva, 1998; Ivancheva and Serkedjieva, 1998) fungi (Dossaji, Kariba and Siboe, 2001) and parasites (Elvin-Lewis and Lewis, 1977), amongst others. Whilst secondary metabolites are sometimes responsible for the anti-infectiveness of a plant (Phillipson, 2001), many elements can contribute to overall health. Of these secondary compounds, Tannins, Carotenoids, essential oils, Lignin, Flavonoids and Alkaloids are the most common (Salisbury and Ross, 1992). In fact, it is estimated that 1/4 of prescription drugs contain at least one chemical that is originally identified and extracted from a plant (Reed, 2000).

Conventional medical systems have unfortunately marginalized the widespread use of medicinal plants, botanical therapeutics as well as phytochemical and phytonutrient supplements. The high cost of new and more effective medicines as well as the indiscriminate use of antibiotics and its consequences makes the search for less expensive alternative substances imperative (Eagles, Leng and Salie, 1996; Cruanes, Cruanes, Ferraro, Gutkind, Marino, Martino, Munoz, Penna and Vivot, 2001).

In South Africa, we are especially concerned about the impact of infectious organisms on human health. Our society is faced with the complexities of HIV/AIDS and secondary infections that steeply elevate morbidity and mortality. There are 42 million people living with AIDS/HIV worldwide. 38.6 million of these are adults and 3.2 million are children under the age of fifteen. In Sub-Saharan Africa there are 29.4 million people living with HIV/AIDS (Aids Epidemic Update, 2002).

A variety of medicinal plants are used against pathogenic microbes. Amongst these pathogens, Candida albicans, Staphyllococcus aureus, Mycobacterium smegmatis and Pseudomonas aeruginosa are especially problematic. Candida albicans is the cause of oral and vaginal thrush (Chan, Krieg and Pelczar (1986), which are common problems that are especially prevalent in HIV/AIDS compromised individuals. Staphyllococcus aureus is commonly found in spoilt foods and causes diarrhea. Diarrhea is especially a problem as it is the cause of 160-200 deaths per day of children under 5 years of age (MRC, 2001). Pseudomonas aeruginosa causes skin irritation and causes meat and food spoilage.

South Africa, specifically the Western Cape is reported to have the highest incidence of Tuberculosis in the world (Heap and Ramphele, 1991) and this condition further complex our challenges with HIV and Aids. South Africa is burdened by one of the worst tuberculosis epidemics in the world. The MRC estimated that the country had an estimated 180 507 cases (55% reported) in 1997, or 419 per 100 000 of the total population. Of these, 32,8% (73 679 cases) were probably infected with HIV (Fourie and Weyer, 1996).

Helichrysum italicum was tested against and showed positive results for 21 strains of S. aureus, 20 of which were hospital isolates from the Mediterranean region (Alonso, Basignano, Cannatelli, Crisafi, Germano and Nostro, 2001). Daferera, Donmez, Polissiou, Sokmen, Tepe and Unlu (2002) tested Achillea species from Turkey against Pseudomonas aeruginosa, Staphylococcus aureus, and Mycobacterium smegmatis, for which Pseudomonas aeruginosa was resistant to the oil of the species, while in Argentina they used Senecio graveolens against Staphylococcus aureus and Candida albicans, for which the essential oil was active against Candida albicans and inhibition of growth for Staphylococcus aureus (Agnese, Cabrera and Perez, 1999) and a study in Trinidad and Tobago, 29 species were tested against Staphylococcus aureus and Pseudomonas aeruginosa, the most susceptible organism was Staphylococcus aureus (Chariandy, Khambay, Phelps, Pollard and Seaforth, 1999) and India these same microbes was resistant to the Alstonia macrophylla (Bhadra, Chakraborty, Chattopadhyay, Kundu, Maiti, Mandal and Mandal, 2001).

Some plants used in Mexican traditional healing for the treatment of infectious diseases showed positive results for Staphylococcus aureus, Candida albicans and Pseudomonas aeruginosa (Losoya, Navarro, Rojas and Navarro, 1996).

What should be considered is the long term and short-term effect of taking alternative medicine and whether it is being taken in combination with other medication. Contrary to popular belief, herbal medicine can have adverse effects and should be tested and controlled (Elvin-Lewis, 2001). This is all a part of a larger screening of biological activities of plant extracts for anticancer, antiviral and anti- fertility drugs (Phillipson, 2001).

South Africa is a developing country that largely has rural areas and in these areas traditional medicines are used at the primary health care level (Jäger, McGraw and Van Staden, 2000). Compromised reproductive health and concomitant diseases pose significant challenges that threaten communities worldwide.

Herbal treatments have to be evaluated by clinical trials using currently accepted protocols. There is a great need to harness scientific and clinical research in order to investigate the quality, safety and efficacy of the medicinal plants (Phillipson, 2001).

Elytropappus rhinocerotis and Pelargonium triste have been used indigenously to combat a number of conditions that are caused by infective agents.

Elytropappus rhinocerotis from the Asteraceae family, is commonly called Renosterbossie is commonly used for influenza, indigestion, and lack of appetite, ulcers and stomach cancer. The powdered tops of the leaves are used for diarrhea in children (Breyer-Brandwyk and Watt, 1962; Green, 1991) and is used as an infusion of the young branches in brandy or wine (Van Wyk and Gericke, 2000). E. rhinocerotis has been found to have Cardio-glycosides, Tannin, Reducing Sugars, Tropane Alkaloids and Saponin (Duarte, 2001).

Pelargonium triste commonly known as Kaneeltjie belongs to the Geraniaceae family and is used as an infusion of the tubers to treat diarrhea and dysentery, which are caused by infectious organisms (Breyer- Brandwyk and Watt, 1962; Green, 1991). The active ingredients are tannins, which are used in Namaqualand to tan leather (van Wyk and Gericke, 2000).

This investigation is divided into two main parts, contained within chapters two and three, each focusing on Elytropappus rhinocerotis and Pelargonium triste respectively. These chapters are formatted to journal specifications and focuses on the individual plants.
To assess the effect of the medicinal plant, it was important to use identical methods in Chapter 2 and Chapter 3, in order to eliminate different methodology as a confounding factor.

In this investigation, the efficacy of Elytropappus rhinocerotis and Pelargonium triste was assessed against selected microbes and more especially the safety profiles of these extracts were evaluated in mice, with a focus on:

(a) metabolism parameters,

(b) haematological indices and

(c) tissue histology.

Consequently, this study aimed to first assess the antimicrobial value of these plant extracts, and more especially the effects of Elytropappus rhinocerotis and Pelargonium triste on animal health and metabolism.

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Chapter 2

The Effect of Elytropappus rhinocerotis on Animal Health and Metabolism

2.1. Abstract

Many South Africans use cultural traditions and practices in the management of their health. Elytropappus rhinocerotis is an important medicinal plant, which is widely distributed in regions of South Africa. The objective of this study was to evaluate the anti-microbial characteristics of Elytropappus rhinocerotis extracts and to assess its effects on animal and metabolism. Elemental analyses were done on the plant and soil samples to determine the concentrations of selected elements within the plant. The leaves were sectioned using freeze-microtomy. Elytropappus rhinocerotis was screened using a MeOH extract and the disc diffusion method against Staphylococcus aureus (ATCC29213), Mycobacterium smegmatis, Pseudomonas aeruginosa (ATCC27853) and Candida albicans (ATCC10231). The positive control for the bacteria was Ciprofloxicin and Amphotericin B for the fungal yeast. Elytropappus rhinocerotis aqueous extract was administered to female mice (NMRI strain), which were divided into two groups of 10 each. The mice were given 1g/100ml/kg/day of the plant extract for a period of 6 weeks. Various metabolic parameters were assessed over time and included the mice mass, stool production and selected elemental analyses, and water and food consumption. A variety of blood parameters and selected tissue samples were analysed after the animals were sacrificed. There was no significant difference between the plant and soil analyses. The trichomes are anti-herbivory or iridescent techniques of the plant. The sclereids around the vascular bundle are part of the desiccation prevention methods of the plant.The plant extracts had no effect on the microbial pathogens. Furthermore, there were no significant differences in metabolic parameters between the plant medicine and placebo group, except for the following: The mass of the Elytropappus rhinocerotis group was significantly higher ($P \leq 0.05$) at week two when compared to the placebo controls. On the other hand, the Elytropappus rhinocerotis group excreted less ($P \leq 0.05$) Cu at week 4 compared with the control group. The haematology indicated that the Elytropappus rhinocerotis group had elevated immune surveillance in that these animals had significantly higher ($P \leq 0.05$) levels of white blood cells, Neutrophils and Lymphocytes. The red blood cell parameters remained largely unaffected, except for mean cell haemoglobin concentration, which was higher ($P \leq 0.05$), and the mean cell volume, which was lower ($P \leq 0.05$) in the Elytropappus rhinocerotis group compared to the placebo. Moreover, there were no differences between the tissue architecture of both groups. This investigation has shown that Elytropappus rhinocerotis had no direct effect on microbial growth, but significantly improved immune cell numbers, with minimal physiologically important effects on red blood cell parameters. These outcomes compel us to further study Elytropappus rhinocerotis as a potential anti-infective indigenous phytotherapy that may exert its effects via immune enhancement, especially when we consider its favourable safety profile.

2.2. INTRODUCTION

Conventional medicinal systems have marginalized the widespread use of medicinal plants and have not taken advantage of the many potential therapeutic possibilities that may emanate from these natural materials. Compromised health and concomitant diseases pose significant challenges that threaten communities' worldwide (Elvin-Lewis, 2001).

The cost of medication has increased such that people are searching for safe effective and affordable medicines. People in the rural areas that are unable to get to clinics or hospitals; rely on medicinal plants for their primary health care (Eagles, Leng and Salie, 1996).

Elytropappus rhinocerotis is a candidate species with very distinct medicinal properties. E. rhinocerotis (Renosterbossie) belongs to the family Asteraceae and is commonly used for influenza, indigestion and lack of appetite, ulcers and stomach cancer (Breyer- Brandwyk and Watt, 1962). The powdered tops are used for diarrhea in children. It is used as an infusion of the young branches in brandy or wine (Van Wyk and Gericke, 2000).

The indiscriminate uses of antibiotics have resulted in many strains of microbes becoming drug resistant. Many herbal remedies have been used for its anti-microbial activities and therefore it would be useful to evaluate E. rhinocerotis for its potential antibiotic.

The aim of this study was to do an assessment of the anti-infective value of E. rhinocerotis. An assessment of the morphological and anatomical characteristics of the plant was done to determine differences and active sites within the tissue. Biologically active compounds and elements also have to be determined to see if they possess any anti-microbial activity.

In this investigation an in-vivo study was also conducted to determine the effects of Elytropappus rhinocerotis extracts on small mammal models, in terms of their effects on metabolism and health.

2.3. MATERIALS AND METHODS

2.3.1. Plant Description

Elytropappus rhinocerotis (Renosterbossie) (Asteraceae) is a thinly grey woolly, viscid shrub, which grows up to two metres in height. It has short whip like branches, leaves are scale like, adpressed and grayish green in colour. Flower heads are discoid, few at the tips of lateral branches, purple, mostly with three inconspicuous flowers. It flowers in February to April. It is found on dry sand stone slopes and flats. The species is widely distributed in the Western, Northern and Eastern Cape (Breyer- Brandwyk and Watt, 1962).

2.3.2. Field Work

A sample of Elytropappus rhinocerotis were collected at Houwhoek pass (Grid reference: 34°23'05S 19°20'11E; Altitude: 325m above sea level).

Shrubland vegetation type is common to the collection site. The substrate is stony with sandy soil, which indicates that the area is well drained. The plant grows in full exposure to the sun. The sample was found on flat land along a roadside rendering the vegetation disturbed. The local abundance is frequent.

2.3.3. Extraction Procedure

The plant material, Elytropappus rhinocerotis was collected on site and was placed in Methanol. The plant tissue was macerated after which more Methanol was added. Repeated Methanol extractions were completed to ensure most compounds were extracted. The extractions were filtered. The methanol was rotary-evaporated and the extract freeze-dried after which it was placed in the cooler at 5°C.

The rest of the plant material was cut into smaller pieces and oven dried at 37°C until completely dried. The dried material was ground, sieved and stored for Atomic Absorption Spectrophotometry.

2.3.4. Soil pH Determination

The soil pH was determined with an A PHM83 autocal pH meter.

2.3.5. Soil and Plant Material Analyses

Three soil samples were collected at the site where the plant specimen was collected. The soil and plant material were oven dried at 37°C. 4g of soil and 0.8g plant material was ground, sieved and weighed. The digestion system is an oxidizing system for organic material. This method from Chapman (1976) was used because it is a slow, less harmful oxidant, thus requiring a catalyst, which ensures that nitrogen is retained during the reaction. The advantages of this system are that Phosphorous, Nitrogen and other nutrients are not lost in the final solution. This digestion method is preferred as it prevents the sample from drying out (Chapman, 1976).

The digested samples were then diluted with distilled water to 100ml in a volumetric flask. The plant and soil digestions were made up to 100ml each. The mixtures were then further diluted- 15ml of original sample was diluted to 75ml with distilled water (Chapman, 1976). Phosphorous was determined from a method by Murphy and Riley (1962) with a Shimadzu UV 160A spectrometer.

2.3.6. Histology of Plant Tissue

Plant material was stored in FAA at the site of collection. Sectioning was done by using Leitz Watselar freeze- microtome using liquid carbon dioxide and Hamilton's freezing solution to freeze the sample. Sections were cut at 10-20 microns. Stains used were Alcian blue and Safrinin. Alcian blue stains cellulose, while Safrinin stains lignin and tannin. Photographs were taken with an Olympus photomicroscope. The description of all anatomical characteristics follows that of Fahn (1982) and Esau (1960).

2.3.7. Antimicrobial assessment

2.3.7.1. Plant material extraction method

The plant material was washed of excess soil with water and rewashed with distilled water. 100 grams of fresh Elytropappus rhinocerotis leaves and stems were extracted in methanol to ensure that most of the compounds were extracted. The extract was freeze-dried to remove water. E. rhinocerotis had a freeze-dried extract yield of approximately 14g. 2g of E. rhinocerotis extract were re-dissolved in 50ml methanol (2µg/ml).

2.3.7.2. Solution Concentrations

A stock solution of 2g/50ml was made. Five different concentrations were used ranging from 10µg/ml- 40µg/ml on the one end and 80µg/ml on the other. The disc diffusion method was used. Even though this method is not conclusive, it does give an indication as to whether the plant is active against these microbes or not. However, it does not specify which actives are responsible. 50µl of the various concentrations were pipetted onto sterile 9mm discs. The discs were placed in the incubator to evaporate the methanol. Once the discs were dry, it was placed on the agar plates, which were inoculated with the four microbes. The spread-plate method was used. Each extract was tested in triplicate.

Negative control discs contained 50µl of Methanol. The positive control for Staphylococcus aureus, Pseudomonas aeruginosa and Mycobacterium smegmatis was Ciprofloxicin and for Candida albicans it was Amphotericin. All plates were incubated for 24hrs, except Mycobacterium smegmatis, which was incubated for 48hrs. After the incubation period, inhibition zones were measured (mm) to estimate efficacy.

2.3.7.3. Microbes

Candida albicans (ATCC 10231) is a fungal yeast, which causes oral and vaginal thrush. Mycobacterium smegmatis (obtained from the former Tygerberg Medical School) was used instead of Mycobacterium tuberculosis since the latter is highly pathogenic and air-borne. M. smegmatis though, is equivalent in antibiotic sensitivity to M. tuberculosis. Pseudomonas aeruginosa (ATCC 27853) is an opportunistic pathogen, which can be isolated from wound, burn and urinary tract infections. Staphylococcus aureus (ATCC 29213) can cause boils, abscesses, wound infections, post-operative infections, Toxic Shock Syndrome (TSS), food poisoning in humans and Mastitis in cattle (Chan, Krieg and Pelczar, 1986). Nutrient agar was used for Pseudomonas aeruginosa, Candida albicans and Staphylococcus aureus. 7*H*11 agar was used for Mycobacterium smegmatis.

2.3.8. Animal studies

The room in which the animals were housed was light controlled in that the animals were exposed to 12 hours of light and 12 hours of darkness and temperature controlled at 21°C. The animals were kept in groups of five per cage. The cages were made of hard plastic and covered with a steel grid. It was 30cm long and 15cm wide. Food and water was available *ad libitum*.

Two groups of 10 mice (The NMRI strain, SA Vaccine Producer, Sandringham, JHB) were used. Group I was given an Elytropappus rhinocerotis infusion everyday for six weeks via gavage, whilst the control group was gavaged using distilled water.

2.3.8.1. Ethics

The UWC Ethics Committee approved this research project.

2.3.8.2. Herbal medicine

Elytropappus rhinocerotis leaves and stems were used to manufacture the infusion. 500ml of distilled water was boiled to which 5g of fresh plant material was added. The cooled infusion was filtered and refrigerated. This infusion corresponded to a solution of 1g/100ml/kg for the average 50kg human. The average weight of the mice was 25g each and they were gavaged with 0.5ml of the extract. To prevent contamination, fresh herbal extract was made every two weeks and kept in the refrigerator.

2.3.8.3. Metabolic analyses

Metabolic readings were taken at baseline (time 0), week 2, week 4 and week 6. Animals were individually placed in metabolic cages for 24hrs. 20g of food and 30ml water was placed in each cage after which the difference was taken as consumed over the 24hr period. Data collected included animal mass, water and food consumption. After the 24hr assessments, the animals were returned to their holding cages until the next evaluation. We were not in the position to collect enough uncontaminated urine samples for metabolic analyses and focused on quantifying stool as a significant indicator of elemental excretion.

Stool Analyses

The stool was collected over 24hrs,dried and ground to a powder for further analysis. The Atomic Absorption Spectrophotometer (Unicam Solaar M series), was used to measure, K, Na, Ca, Mg and Cu were analysed. The stool was analysed using the same method as for the plant and soil samples.

2.3.8.4. Haematology

Ether and chloroform was used to anaesthetise the animals. Terminal blood samples were taken from the left ventricle with a 2ml syringe and 36c needle. Blood samples were placed in 5ml EDTA vials. The blood was used to determine a number of fundamental haematology indices

We assessed red blood cell counts (RBC), white blood cell count (WBC), haemoglobin concentration, haematocrit value, platelet counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) using a microcell counter (CC-180A, Toa Medical Electronics, Hyogo, Japan). The analyses were completed at the Peninsula Technikon, Bellville.

2.3.8.5. Histology

The liver, adrenals, kidneys and ovaries were removed and fixed in Formal Saline.

Procedure

Fixing animal material causes life processes to stop within tissue with minimal structural disturbance and minimal distortion of the arrangement of the tissue. It must retain the undistorted structure and render material firm enough to withstand handling.

The lengths of time for fixing vary greatly depending on the bulk of tissue, the fluid used and the resistance of the material to penetration by reagents. (Carneiro, Junqueira and Long, 1971; Sass, 1958).

The dehydration process removes water from the fixed and hardened tissue. Dehydration has some washing action and makes the material firm, possibly hard and brittle as well. The process consists of treating the tissue with a series of solutions containing progressively increasing concentrations of alcohol (dehydrating agent) and a decreasing concentration of water. The alcohol (Ethanol) concentrations are diluted with distilled water. This takes approximately eight hours (Carneiro, et al, 1971; Sass, 1958).

Dehydration with dehydrant was approximately the same percentage of water as the fixing agent. The paraffin is the cleaning solvent. Paraffin solvents render tissues transparent, which takes a period of four hours. Xylene was used as rehydrating agent that takes four hours.

The paraffin matrix in which the tissues are embedded serves to support the tissue against the impact of the knife and to hold the parts in proper relation to each other after the sections have been cut (Carneiro, et al, 1971; Sass, 1958).

The tissue was then processed with a histokinette for 22 hours and embedded in wax. Tissues were cut with a microtome blade at 5 microns. Mayers Acid Alum Haematoxylin and Eosin were used. Haematoxylin and Eosin are useful to visualise different tissue components, but provide no insight into the chemical nature of tissue (Carneiro, et al, 1971). DPX Mountant was used to mount slides. Digital photographs were taken of the animal tissue with an Olympus Photomicroscope.

2.3.9. Statistics

Data was analysed using Microsoft Excel Stat version 5.1 (2000). Control and experimental animal groups were compared with one another and a minimum significance of $P \leq 0.05$ was determined for all metabolic parameters using the Mann-Whitney test. The Mann-Whitney's U is normalised and tested against the normal distribution.

2.4. RESULTS

2.4.1. Table1: Soil pH

Soil samples	1	2	3	Average SD
PH values	7.3	7.8	7.9	7.7 +/- 0.32

2.4.2. Soil and Plant Elemental Analysis

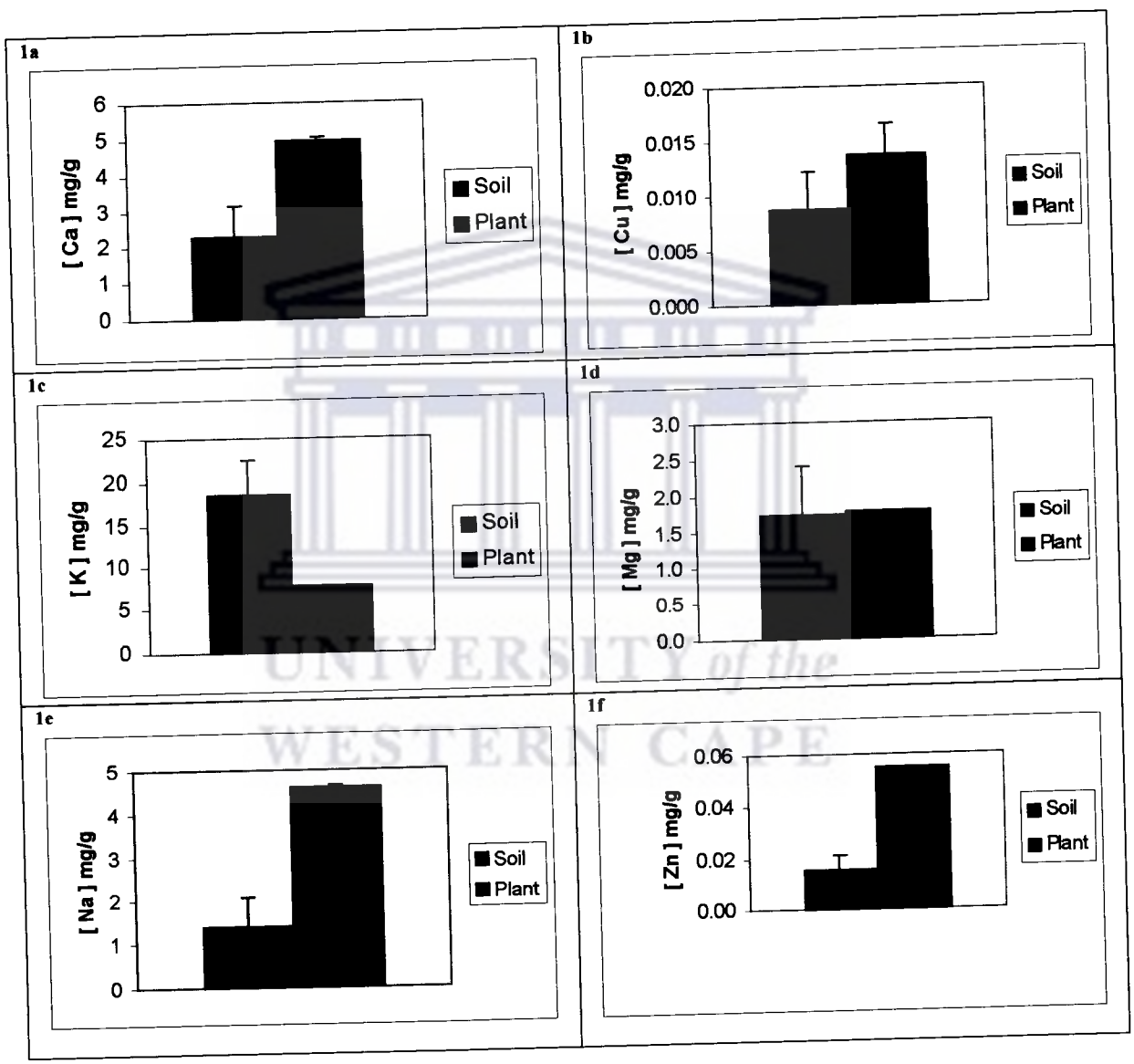


Fig 1a-f: The elemental levels of Elytropappus rhinocerotis leaves and stems and the soil in which the plant is found.

2.4.3. Plant Histology

The leaves of Elytropappus rhinocerotis (fig 2a) are small and are wrapped around the stem of the plant.

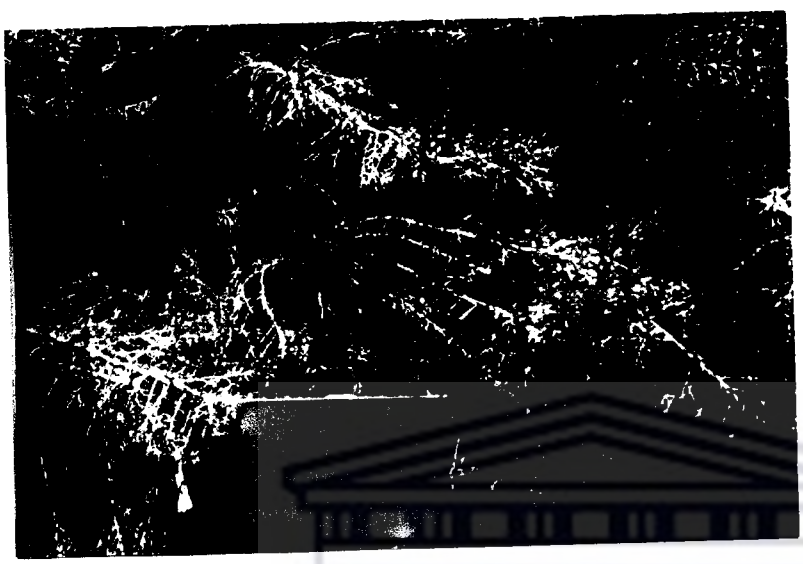


Figure 2a: Elytropappus rhinocerotis whole plant

Figure 2b: Elytropappus rhinocerotis *x/s*

2.4.4. Antimicrobial Assessments

Table 2: Growth inhibition (mm) of Elytropappus rhinocerotis against four different organisms.

	S. aur	P. aer	M. sme	C. alb
E. rhinocerotis				
10µg/ml	-	-	-	-
20µg/ml	-	-	-	-
30µg/ml	-	-	-	-
40µg/ml	-	-	-	-
80µg/ml	-	-	-	-
Methanol	-	-	-	-
Cip	4+	4+	4+	-
Amp	-	-	-	4+

Key:

S. aur - Staphylococcus aureus

P. aer - Pseudomonas aeruginosa

M. sme - Mycobacterium smegmatis

C. alb - Candida albicans

Cip - Ciprofoxicin

Amp - Amphotericin

-: no inhibition;

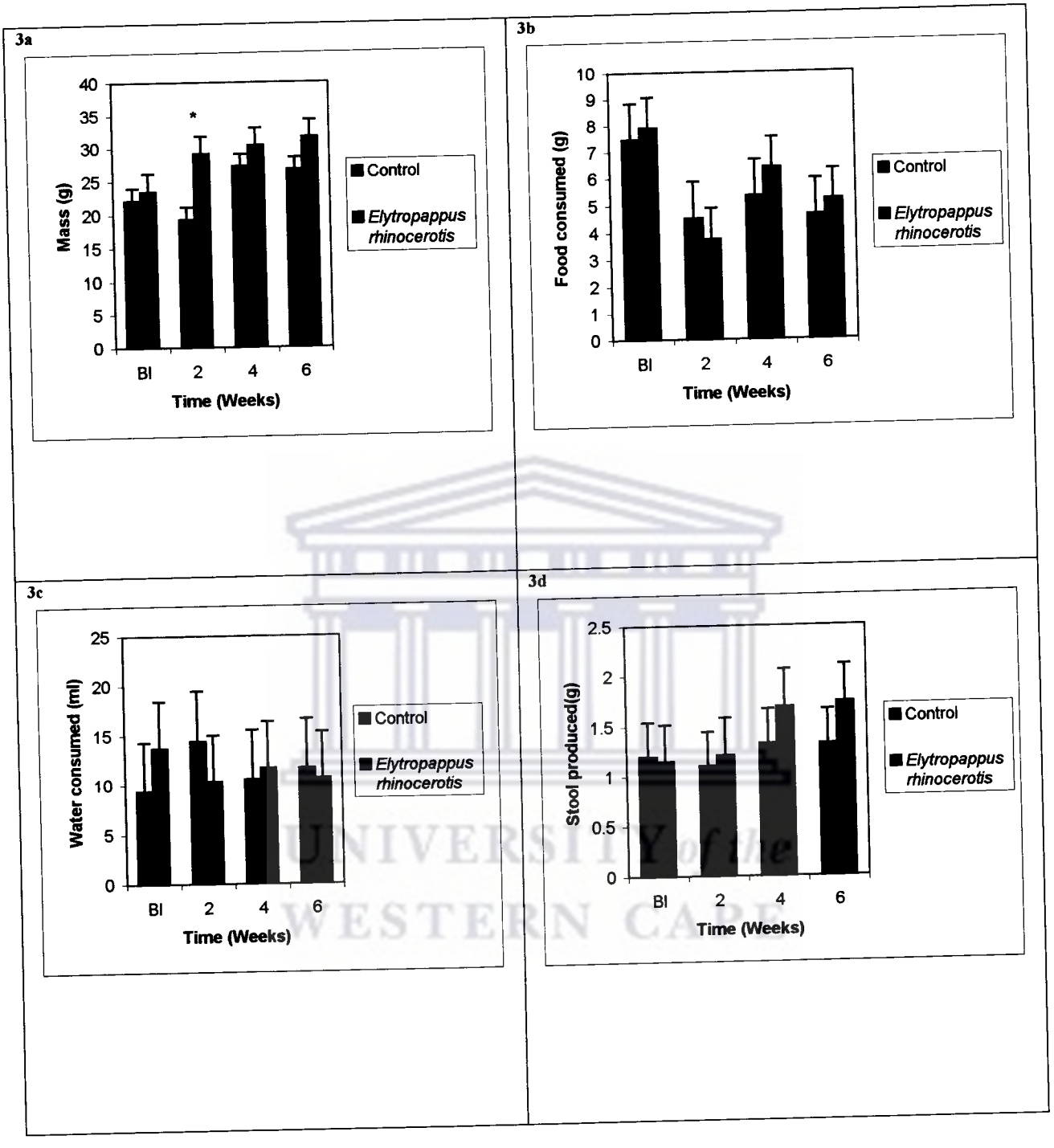
1+: 0-3mm;

2+: 3-6mm;

3+: 6-9mm;

4+: 9mm+

2.4.5. Metabolic Indices

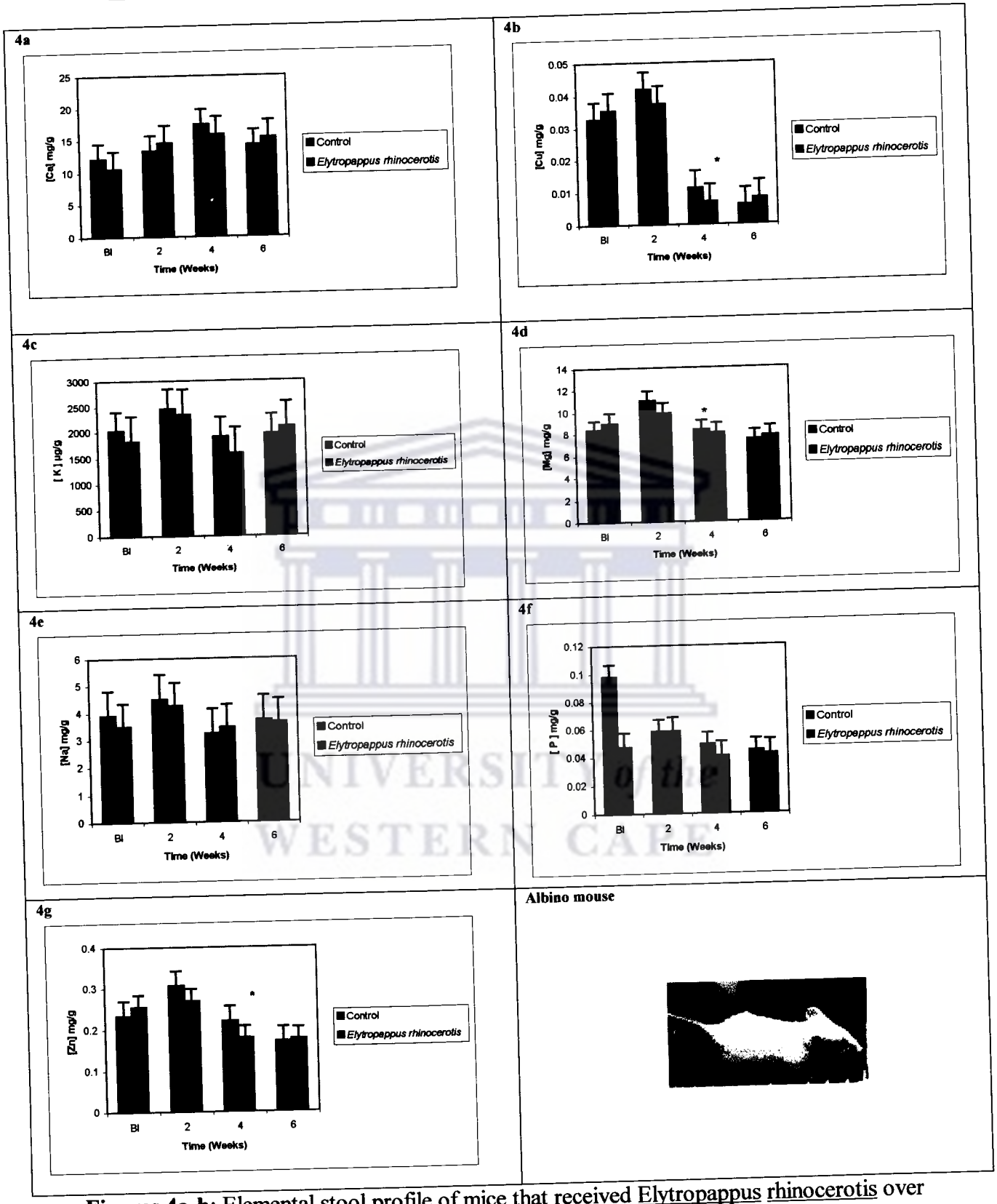


Figures 3a-f: Metabolic parameters of animals that received Elytropappus rhinocerotis extract compared to those who received a placebo.

Key: (*)= P≤0.05

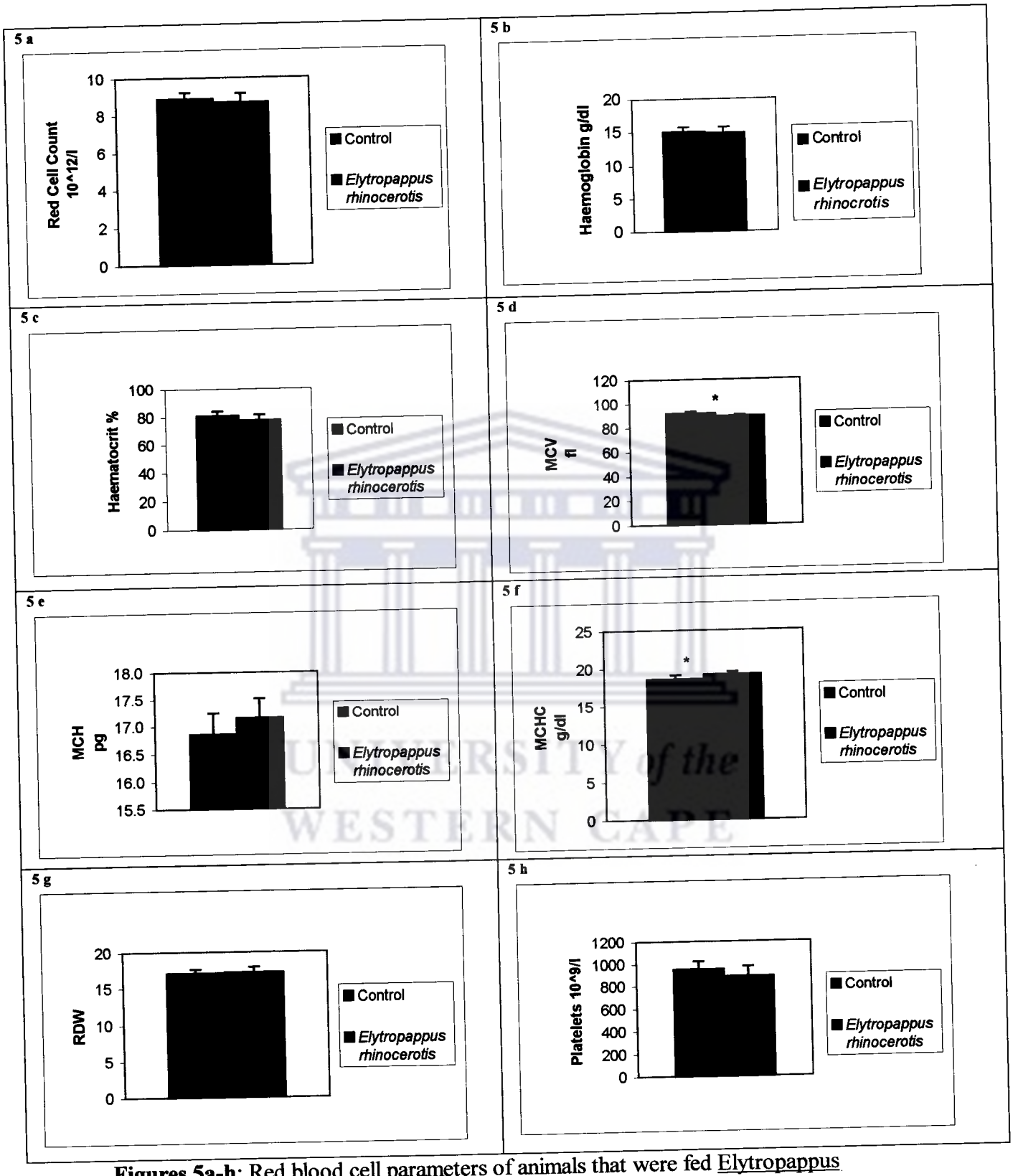
Bl- Baseline

2.4.6 Stool elemental profile



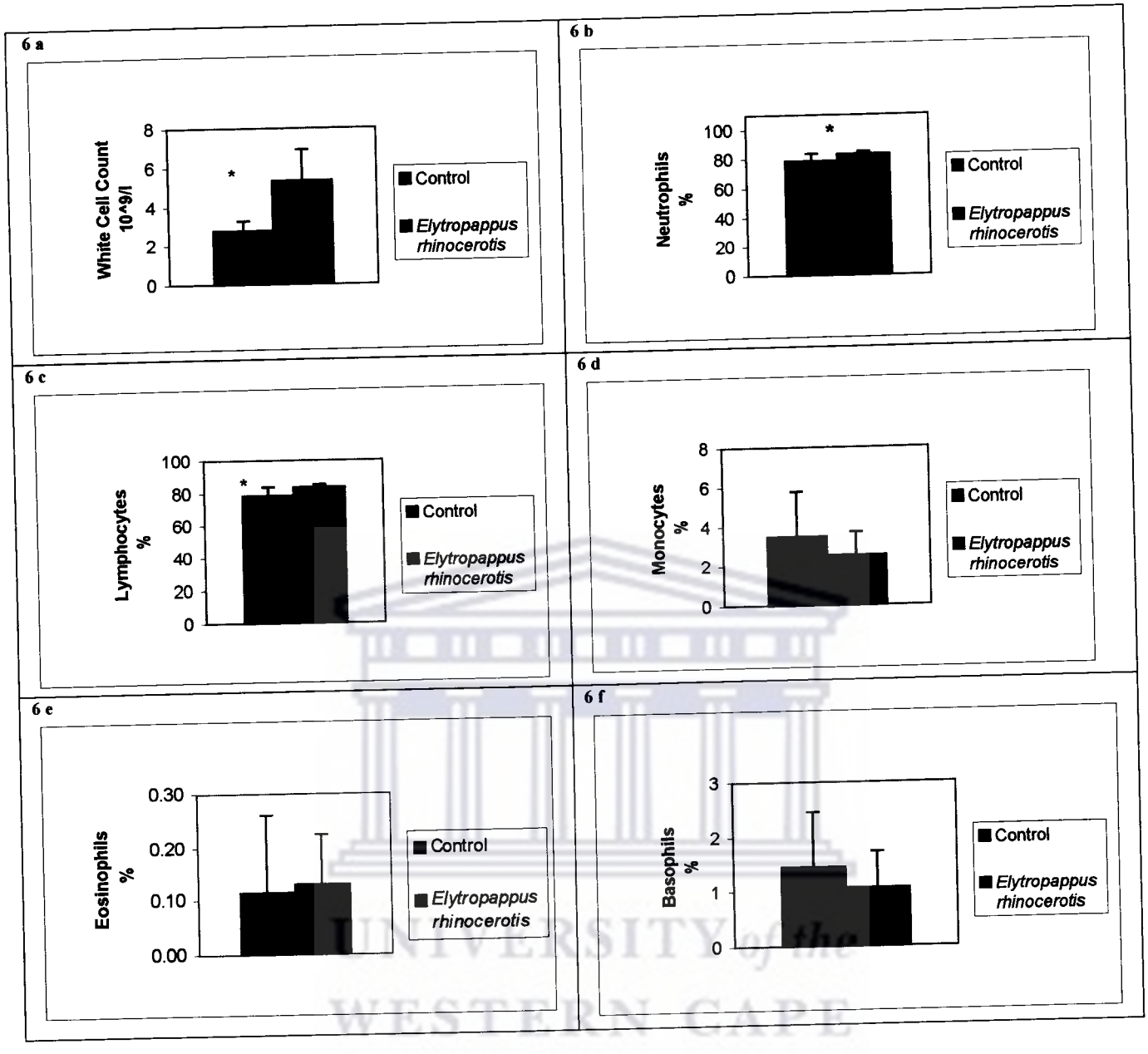
Figures 4a-h: Elemental stool profile of mice that received Elytropappus rhinocerotis over six weeks compared to those that received a placebo.
Key: (*)= P≤0.05
Bl- Baseline

2.4.7 Haematology



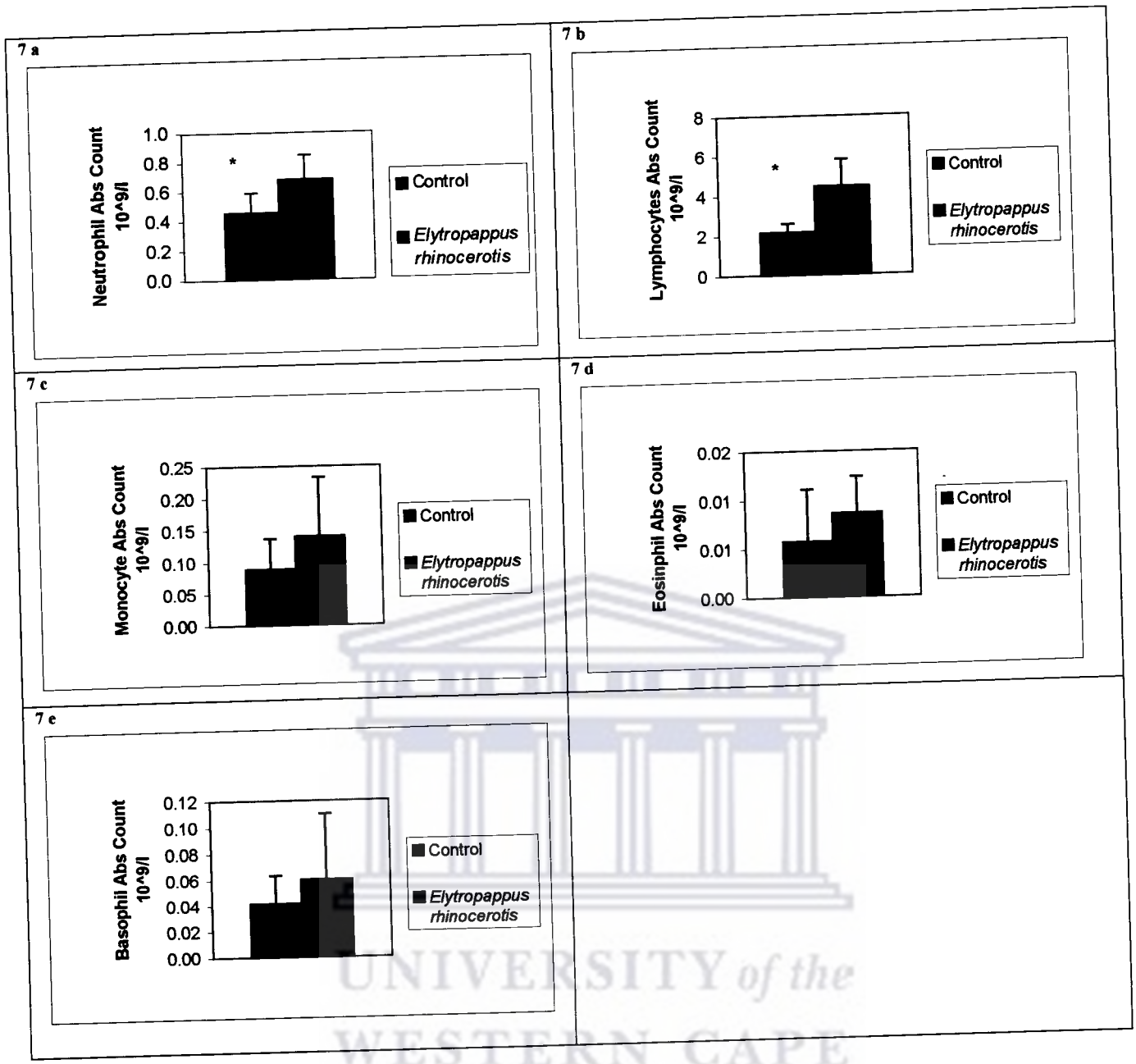
Figures 5a-h: Red blood cell parameters of animals that were fed Elytropappus rhinocerotis compared to those that received a placebo.

Key: (*)=P<0.05



Figures 6 a-f: White blood cell parameters of animals that received Elytropappus rhinocerotis compared to those that received a placebo

Key: (*)= P<0.05



Figures 7 a-e: White blood cell parameters of animals that were fed Elytropappus rhinocerotis compared to those that that received a placebo

Key: (*)= P<0.05

2.4.8 Animal Tissue Histology

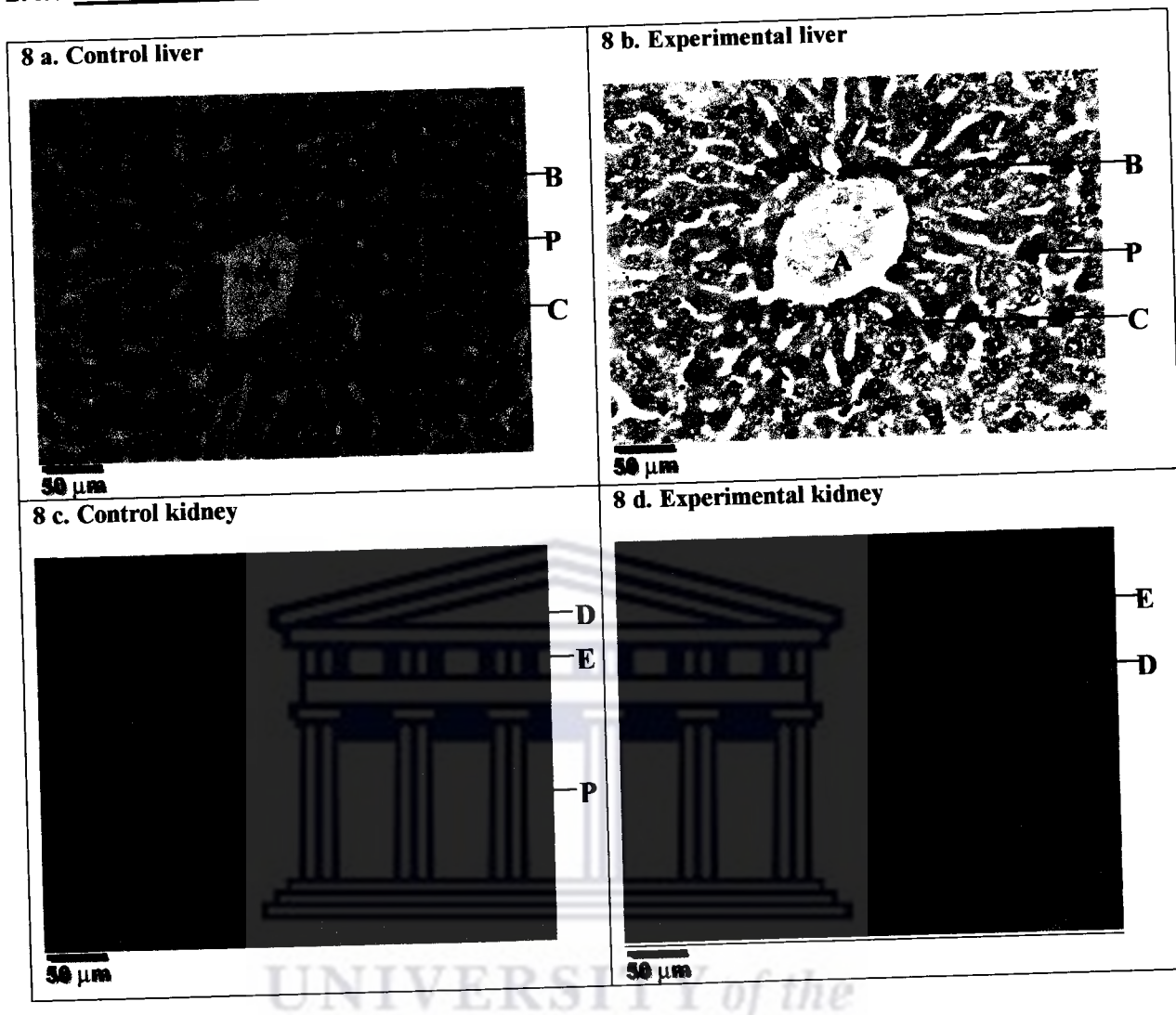


Figure 8a-d: The effects of Elytropappus rhinocerotis extracts (Experimental) and placebo (Control) on liver (a, b) and kidney (c, d) tissue.

A-Central Vein;

B- Sinusoid;

C- Liverplate;

D- Glomerular Space;

E- Glomerulus;

P- nucleus

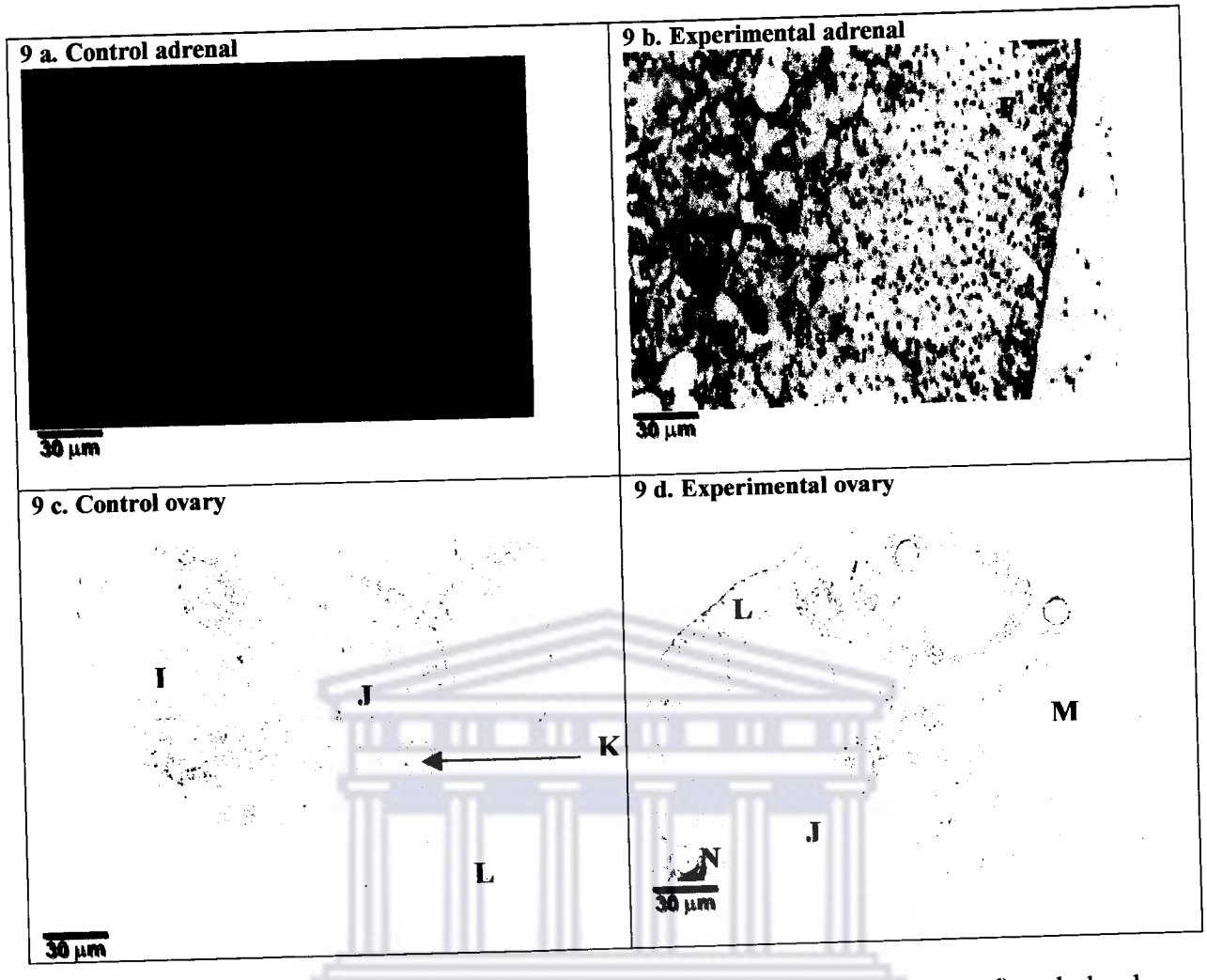


Figure 9a-d: The effects of Elytropappus rhinocerotis extracts (Experimental) and placebo (Control) on adrenal (a, b) and ovary (c, d).

F- Zona Glomerulosa;

G- Zona Fasciculata;

H- Zona Reticularis

I- Corpus Luteum;

J- Medulla;

K- Oocyte release;

L- Cortex;

M- Graafian follicle;

N- Primary Follicle.

2.5. Discussion

This investigation focused on the effects of Elytropappus rhinocerotis extracts on harmful microbes and their impact on animal health and metabolism.

Table 1 exhibits the pH (pH 7.7) from which it can be determined that Elytropappus rhinocerotis can grow on alkaline soil, which is the natural occurrence. $CaCO_3$ is a common soil constituent of high-level alkalinity soil. Ca can be 40-50% of soil content. The normal range for Magnesium is 0.003- 0.6% and Potassium is between 0.3- 2.5 %. Copper, which is a trace element, is less than 1µg/g-1 and Zinc is between 10- 300 µg-1. Na is 10-20 ml/l-1/ 10 000mg. The levels of these elements in the soil are within normal range. (Etherington, 1982) The soil and plant elemental analyses from figures 1a –f show no significant differences ($P > 0.05$). Therefore the plant is not absorbing unusual amounts of elements from the soil.

The soil is well drained and the plant is in full exposure to the sun. This would indicate that the plant has a tolerance for desiccation stress. Elytropappus rhinocerotis has ericoid leaves that are almost wrapped around the stem as scales. It has both trichomes (fig 2b), which are also known as hairs and glands (fig 2c), which are modified structures to cope with environmental stress as well as anti-herbivory characteristics. Glands usually rid the plant of secondary metabolites. Other structures signifying desiccation stresses are the papillae (fig 2c). The sclereids that surround the vascular tissue are meant to support the vascular bundle if the plant looses too much water so that the vascular bundle does not collapse. (Esau, 1960 and Fahn, 1982).

In Table 2, Elytropappus rhinocerotis showed no activity. A higher dosage could have made a difference when compared with the positive control (Ciprofloxicin). One of the uses of Elytropappus rhinocerotis, is that the powdered tops are used to treat diarrhea in small children. Staphyllococcus aureus causes foods to spoil and when eaten does in fact cause diarrhea for which E. rhinocerotis had no effect. However, there are a variety of factors that can cause diarrhea. Diarrhea can be a result of bacterial or viral enteritis, food and toxic poisoning or gastrointestinal allergy as well as parasitic infestations. (Jäger et al, 2000). Another contributing factor is that these were only methanol extracts for the purpose of screening, whilst plant extracts that are derived using hexane, chloroform and ethanol, may well have different effects.

The Asteracae family is very effective generally against Staphylococcus aureus. Nostro, Bisignano, Canatelli, Crisafi, Germano and Alonzo (2001) using DMSO and Rabe, Mullholland and van Staden (2002) isolated specific compounds and obtained positive results against Staphylococcus aureus. In a study done by Aburjai, Al-Abbadi, Al Khalil, Darwish and Mahafzah (2001), a combination of herbal extract using Methanol as solvent and various antibiotics against resistant and standard Pseudomonas aeruginosa, showed that the extract of Gundelia tournifortii (Compositae) negatively effects penicillin G and cephaloxin. Eagles, Leng and Salie (1996), using DMSO extraction method and Agnese, Cabrera and Perez (1999) showed anti-fungal properties of some species from the Compositae family against Candida albicans and Staphylococcus aureus. The essential oil of two Achillea species' essential oils has been used against Candida albicans, Mycobacterium smegmatis and Staphylococcus aureus for which it showed anti-microbial activity. The actives presumed to be responsible are camphor and their derivatives and eucalyptol (Űnlű, Dafarera, Donmez, Polissiou, Tepe and Sokmen, 2002).

This varying techniques extract different compounds from the crude extract and does affect the outcome of results.

The treated animals' weight (Fig 3a) increased significantly in comparison to the control in the second week of the experimental period. This a once off-off occurrence and may well relate to the animals requiring a longer acclimation period. The amount of food (Figure 3b) and water (figure 3c) consumed between the two groups were statistically the same. The latter outcome is also evident from the stool profile, which also chows no difference between the groups (Figure 3d).

The medicinal plant extract could also have affected mineral metabolism. From this study figures 4b (Cu) and d (Mg) and 4g (Zn) there is significant difference ($P \leq 0.05$) in week 4. Figures 4a (Ca), c (K), e (Na) and f (P) there is no significant difference ($P \geq 0.05$) between the two groups. When there are significant increases of these elements in the stool, it is of the control. There is no specific trend for when the elements are significant in a certain batch of stool. However, the plant may influence the elemental output in the stool, which means that the treated animals are discarding valuable elements however the treated animals do have a surplus of elements through the extracts compared to the control which received distilled water.

The haematology of the animals were monitored for differences. This investigation shows that for the red blood cell indices, MCV (Mean Cell Volume) (fig 5d) is significantly lower than the control. If the MCV is low it means the cell size is smaller (microcytic) than normal. This can be caused by severe blood loss, Lack of iron, vitamin B_{12}, or folic acid in the diet.

The MCHC (Mean Corpuscular Haemoglobin Concentration) (fig 5f) is however significantly higher ($P<0.05$) than the control. A high MCHC can be caused by a lack of Vitamin B_{12} or folic acid. The commonality seems to be the lack of vitamin B_{12} and folic acid (Nordenson, 1999).

The white blood cell count, Figure 6a, was significantly higher in the treated animals than the control ($P\leq 0.05$). Further more the Neutrophils (Fig 6b) and Lymphocytes (Fig 6c) compared to the control are significantly higher ($P\leq 0.05$). The Neutrophils absolute count (fig 7a) and Lymphocyte absolute count (fig 7b) were also significantly more ($P\leq 0.05$) than the control group. Neutrophils are amoeboid and phagocytises foreign material. There are two types of lymphocytes. T –cells, which attack cells that contain viruses and B -cells, which produce antibodies (Mader, 1996). The Neutrophils and Lymphocytes are key factors in anti-inflammatory effect; this could signify the anti-inflammatory and immunity enhancement effect of the plant (Amirghofran, 2000). However, all these indices are still within normal range (Harkness and Wagner, 1996)
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Tissue architecture is an index of health. Certain tissues more easily reflect toxicity because it is more sensitive to external influences. The description of anatomical features follows Amenta (1997) and Sternberg (1976). For the purpose of this study, liver, kidney, adrenal and ovarian tissue was assessed, as an index of the effect of Elytropappus rhinocerotis on cellular archtecture. Figures 8a (control) and b (experimental) reflects the tissue histology of the liver. The arrangement of the liver plate (C) and sinusoids (B) around the central vein (A) appear to be normal. At gross cellular level the cells appear normal. From figures 8c (control) and d (experimental) kidney, it shows that the gross cellular arrangement

appears to be normal, however the glomerular space (D) and tubules of figure 8d is slightly distended. This could be due to the fact that when the animals were sacrificed, it was early morning and they were just removed from the metabolic cages. Another possibility is that the treatment could act as a diuretic. This is inconclusive because the amount of urine was not monitored, but from personal observation, it appeared as though the treated animals excreted more urine.

Figures 9a (control) and 9b (control) reflect the tissue architecture of the adrenals, which are at 40x magnification. All the zones and cells at gross level are normal of both the control and treated group. The ovaries, Figure 9c (Control) and 9d (Experimental) are also normal. From the gross cellular level, the plant does not seem to negatively affect the tissue of the animals. It had no effect on the ovaries especially on the follicles.

This investigation has shown that Elytropappus rhinocerotis had no direct effect on microbial growth, but significantly improved immune cell numbers, with minimal physiologically important effects on red blood cell parameters. These outcomes compel us to further study Elytropappus rhinocerotis as a potential anti-infective indigenous phytotherapy that may exert its effects via immune enhancement, especially when we consider its favourable safety profile.

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Chapter 3

The Effect of Pelargonium triste on Animal Health and Metabolism

3.1. Abstract

The majority of South Africans refer to practices of tradition where healthcare is concerned. Pelargonium triste is a geophyte with a large woody tuber that is indigenously used to treat diarrhea and dysentery. The objective of this study was to evaluate the anti-microbial characteristics of Pelargonium triste extracts and to assess its effects on animal health and metabolism. Elemental analyses were done on the plant and soil samples to determine the concentrations of selected elements within the plant. The tubers were sectioned using slide-microtomy. Pelargonium triste was screened against Staphylococcus aureus (ATCC29213), Mycobacterium smegmatis, Pseudomonas aeruginosa (ATCC27853) and Candida albicans (ATCC10231) using the disc diffusion method. The positive control for the bacteria was Ciprofloxicin and Amphotericin B for the fungal yeast. Pelargonium triste extract was administered to female mice (NMRI strain), which were divided into two groups of 10 each. The mice were given 1g/100ml/kg/day of the plant extract for a period of 6 weeks. A variety of metabolic parameters were measured over time, and included the mice mass, stool excretion, water and food consumption. Terminal blood and selected tissue samples were taken for haematological and histological analyses. There was no significant difference between the plant and soil analyses. The tubers had starch and crystals throughout the tissue. The plant extracts showed effect against Mycobacterium smegmatis from 30µg/ml to 80µg/ml. Furthermore there was no significant differences in the metabolic parameters between the plant medicine and the placebo group except for the following: The food consumption of the Pelargonium triste group was significantly more ($P \leq 0.05$) when compared to the placebo controls. On the other hand, the placebo control group produced significantly more ($P \leq 0.05$) stool and on week six the Pelargonium triste group produced more stool ($P \leq 0.05$). The Pelargonium triste group excreted significantly more ($P \leq 0.05$) Ca in week six; the Placebo control group excreted significantly more ($P \leq 0.05$) P in week 2 and Zn in week two and week four. The haematology indicated that the Pelargonium triste group had elevated immune surveillance in that these animals had significantly higher ($P \leq 0.05$) levels of Neutrophils and Lymphocytes. The red blood cell parameters remained largely unaffected. Moreover, there were no differences between the tissue architecture of both groups. This investigation has shown that Pelargonium triste had an effect on microbial growth and improved immune cell numbers with no physiologically important effect on red blood cell parameters. These outcomes compel us to further study Pelargonium triste as a potential anti-infective indigenous phytotherapy, especially when we consider its favourable safety profile.

3.2. INTRODUCTION

In many parts of Africa, herbal medicines still plays a vital role in health care. Herbal remedies are less expensive than western medicines and it is this that affects the accessibility of medicines to disadvantaged communities. Generally herbal medicines do not only cure diseases and heal sicknesses; it boosts the health of people. (Cocks & Møller, 2002).

Many South Africans use cultural traditions and practices in the management of their health. People are searching for safe effective and affordable medicines. People in the remote where clinics and hospitals are sparsely located, take advantage of plant species in the areas to cure ailments and diseases (Eagles, Leng and Salie, 1996), (Ndubani and Hojer, 2001).

Pelargonium triste commonly known as kaneeltjie, is a candidate species with very distinct medicinal properties. P. triste belongs to the Geraniaceae family and is used as an infusion of the tubers is used to treat diarrhea and dysentery. The active ingredients in the tubers are tannins, which are used in Namaqualand to tan leather (van Wyk and Gericke, 2000; Breyer- Brandwyk and Watt, 1962).

Many strains of microbes become drug resistant because of the indiscriminate use of Antibiotics (Cruanes, Cruanes, Ferraro, Gutkind, Marino, Martino, Munoz, Penna and Vivot, 2001). Many herbal remedies have been used for its anti-microbial activities and therefore it would be useful to evaluate P. triste as a potential antibiotic.The aim of this study was to do an assessment of the anti-infective value of P. triste.

An assessment of the morphological and anatomical characteristics of the plant was done to determine differences and active sites within the tissue. Biologically active compounds and elements also have to be determined to see if they possess any anti-microbial activity.

In this study an in-vivo study was also conducted to determine the effects Pelargonium triste extracts had on small mammal models, in this case mice, in terms of their safety on metabolism and health. Hence, we looked at the general health of animals as a large number of indigenous medicines are anecdotally reported to affect health and metabolism.

3.3. MATERIALS AND METHODS

3.3.1. Plant Description

Pelargonium triste (Kaneeltjie) (Geraniaceae) is a geophyte with a large woody tuber. The leaves are prostate, 2 or 3 pinnatisect with linear segments that are softly hairy, to 30cm in diameter. Flowers are up to twenty on a stout peduncle, 15- 18mm in diameter. The flowers are pale yellow with dark maroon to black centers, clove scented at night; hypanthium's 25- 35mm long, which is much longer than the pedicel. Flowers between August and February. Found on sandy flats and slopes. (Breyer- Brandwyk and Watt, 1962).

3.3.2. Field Work

Samples of Pelargonium triste was collected in the Pauline Bohnen Nature Reserve, Stilbaai. The vegetation type of the collection site is coastal Fynbos. The substrate is soil and the soil type is sand. The moisture regime is well drained. The lithology is limestone. The sample, which was collected, was found on a gentle slope with a NW aspect. The area was disturbed, as there is a road along which the sample was collected. Mr. Frans Weitz from the Department of Biodiversity and Conservation at the University of the Western Cape verified the botanical material.

3.3.3. Extraction Procedure

The plant material, Pelargonium triste, was collected on site and was placed in Methanol. The plant tissue was macerated after which more Methanol was added. Repeated Methanol extractions were completed to ensure most compounds were extracted. The extractions were filtered. The methanol was rotary-evaporated and the extract freeze-dried after which it was placed in the cooler at 5°C.

The rest of the plant material was cut into smaller pieces and oven dried at 37°C until completely dried. The dried material was ground, sieved and stored for Atomic Absorption Spectrophotometry.

3.3.4. Soil pH Determination

The soil pH was determined with an A PHM83 autocal pH meter.

3.3.5. Soil and Plant Material Analyses

Three soil samples were collected at each sight where each plant specimen was taken. The soil and plant material were oven dried at 37°C. 4g of soil and 0.8g plant material was ground, sieved and weighed for analyses.

The digestion system is an oxidizing system for organic material. This method from Chapman (1976) was used because it is a slow, less harmful antioxidant, thus requiring a catalyst, which ensures that nitrogen is retained during the reaction.The advantages of this system are that Phosphorous, Nitrogen and other nutrients are not lost in the final solution. This digestion method is preferred as it prevents the sample from drying out (Chapman, 1976). The digested samples were then diluted with distilled water to 100ml in a volumetric flask. The plant and soil digestions were made up to 100ml each. The mixtures were then further diluted- 15ml of original sample was diluted to 75ml with distilled water. (Chapman, 1976). Phosphorous was determined from a method by Murphy and Riley (1962).

3.3.6. Histology of Plant Tissue

Plant material was stored in FAA at the site of collection. Sectioning was done by Anglio Scientific slide-microtome. Sections were cut at 10-20 microns. Stains used were Alcian blue and Safrinin. Alcian blue stains cellulose, while Safrinin stains lignin and tannin. Photos were taken with an Olympus photomicroscope. The description of all anatomical characteristics follows that of Fahn (1982) and Esau (1960).

3.3.7. Antimicrobial Work

3.3.7.1. Plant material Extraction method

The plant material was washed of excess soil with water and rewashed with distilled water. 200 grams of fresh Pelargonium triste tubers were extracted in methanol. The extract was freeze-dried to remove water. P. triste had a freeze-dried extract yield of approximately 20g. 2g of P. triste extract were re-dissolved in 50ml methanol (2μg/ml). P. triste was re-dissolved in 50%methanol.

Solution Concentrations

A stock solution of 2g/50ml was made. Five different concentrations were used, ranging from 10μg/ml- 40μg/ml and 80μg/ml. The disc diffusion method was used. Even though this method is not conclusive, it does give an indication as to whether the plant is active against these microbes or not. However, it does not specify which actives are responsible. 50μl of the various concentrations were pipetted onto sterile 9mm discs. The discs were placed in the incubator to evaporate the methanol. Once the discs were dry, it was placed on the agar plates, which were inoculated with the four microbes. The spread-plate method was used.

Each extract was tested in triplicate. Negative control discs contained 50µl of Methanol. The positive control for Staphylococcus aureus, Pseudomonas aeruginosa and Mycobacterium smegmatis was Ciprofloxicin and Amphotericin B was used for Candida albicans. All plates were incubated for 24hrs except Mycobacterium smegmatis, which was incubated for 48hrs. After the incubation period inhibition zones were measured (mm) to estimate the efficacy of the plant extract.

3.3.7.2. Microbes

Candida albicans (ATCC 10231) is fungal yeast, which causes oral and vaginal thrush. Mycobacterium smegmatis (obtained from the former Tygerberg Medical School) was used instead of Mycobacterium tuberculosis since the latter is highly pathogenic and air-borne. M. smegmatis however has a similar growth pattern and antibiotic sensitivity profile to M. tuberculosis. Pseudomonas aeruginosa (ATCC 27853) is an opportunistic pathogen, which can be isolated from wound, burn and urinary tract infections (Chan, Krieg, Pelczar and Pelczar, 1986).

Staphylococcus aureus (ATCC 29213) can cause boils, abscesses, wound infections, post-operative infections, Toxic Shock Syndrome (TSS), food poisoning in humans and Mastitis in cattle (Chan et al, 1986). Nutrient agar was used for Pseudomonas aeruginosa, Candida albicans and Staphylococcus aureus. Difco 7*H*11 agar was used for Mycobacterium smegmatis.

3.3.8. Animal Studies

The room in which the animals were housed was light controlled in that the animals were exposed to 12 hours of light and 12 hours of darkness.

The temperature in the housing facility was controlled at 21°C. The animals were kept in groups of five per cage. The cages were made of hard plastic and covered with a steel grid. It was 30cm long and 15cm wide. Food and water was available *ad libitum.*

Two groups of 10 mice (The NMRI strain, SA Vaccine Producer, Sandringham, JHB) were used. Group I was given a Pelargonium triste infusion everyday for six weeks via gavage, whilst the control group was gavaged using distilled water.

3.3.8.1. Ethics

The UWC Ethics Committee approved this research project.

3.3.8.2. Herbal medicine

Pelargonium triste tubers were used to manufacture the infusion. 500ml of distilled water was boiled to which 5g of fresh plant material were added. The cooled infusion was filtered and refrigerated. This infusion corresponded to a solution of 1g/100ml/kg for the average 50kg human. The average weight of the mice was 25g each and they were gavaged with 0.5ml extract. To prevent contamination; fresh herbal extract was made every two weeks and kept in the refrigerator at 0-5°C.

3.3.8.3. Metabolic Analyses

Metabolic readings were taken at baseline (time 0), week 2, week 4 and week 6. Animals were individually placed in metabolic cages for 24hrs when readings were taken for animal mass, water and food consumption. After the 24hr assessments, the animals were returned to their holding cages until the next evaluation.

We were not in the position to collect enough uncontaminated urine samples for metabolic analyses and focused on analysing stool produced as an indicator of elemental excretion.

Stool Analyses

The stool collected was dried to a constant weight and ground to a powder for further analysis using the Atomic Absorption Spectrophotometer for K, Na, Ca, Mg and Cu analysis. The digestion of stool followed the same protocol as that used for soil and plant material analyses.

3.3.8.4. Haematology

Ether and chloroform was used to anaesthetise the animals. Terminal blood samples were taken from the left ventricle with a 2ml syringe and 36c needle. Blood samples were placed in 5ml EDTA vials. The blood was used to determine a number of fundamental haematology indices.

We assessed red blood cell counts (RBC), white blood cell count (WBC), haemoglobin concentration, haematocrit value, platelet counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) using a microcell counter (CC-180A, Toa Medical Electronics, Hyogo, Japan). The analyses were completed at Peninsula Technikon, Bellville.

3.3.8.5. Histology

The liver, adrenals, kidneys and ovaries were removed and fixed in Formal Saline.

Procedure

Fixing animal material causes life processes to stop within tissue with minimal structural disturbance and minimal distortion of the arrangement of the tissue. It must retain the undistorted structure and render material firm enough to withstand handling. The length of time for fixing vary greatly depending on the bulk of tissue, the fluid used and the resistance of the material to penetration by reagents. (Carneiro, et al, 1971; Sass, 1958).

The dehydration process removes water from the fixed and hardened tissue. Dehydration has some washing action and makes the material firm, possibly hard and brittle as well. The process consists of treating the tissue with a series of solutions containing progressively increasing concentration of alcohol (dehydrating agent) and a decreased concentration of water. The alcohol (Ethanol) concentrations are diluted with distilled water. This takes approximately eight hours (Carneiro, et al, 1971;Sass, 1958).

Dehydration with dehydrant was approximately the same percentage of water as the fixing agent. The paraffin is the cleaning solvent. Paraffin solvents render tissues transparent, which takes a period of four hours. Xylene was used as rehydrating agent that takes four hours.

The paraffin matrix in which the tissues are embedded serves to support the tissue against the impact of the knife and to hold the parts in proper relation to each other after the sections have been cut (Carneiro, et al, 1971; Sass, 1958).

The tissue was then processed with a Shandon Elliot histokinette for 22 hours and embedded in wax. Tissues were cut with a microtome blade at 5 microns. Mayers Acid Alum Haematoxylin and Eosin were used. Haematoxylin and Eosin are useful to visualise different tissue components, but provide no insight into the chemical nature of tissue (Carneiro, et al, 1971). DPX Mountant was used to mount slides. Digital photographs were taken of the animal tissue with an Olympus Photomicroscope.

3.3.9. Statistics

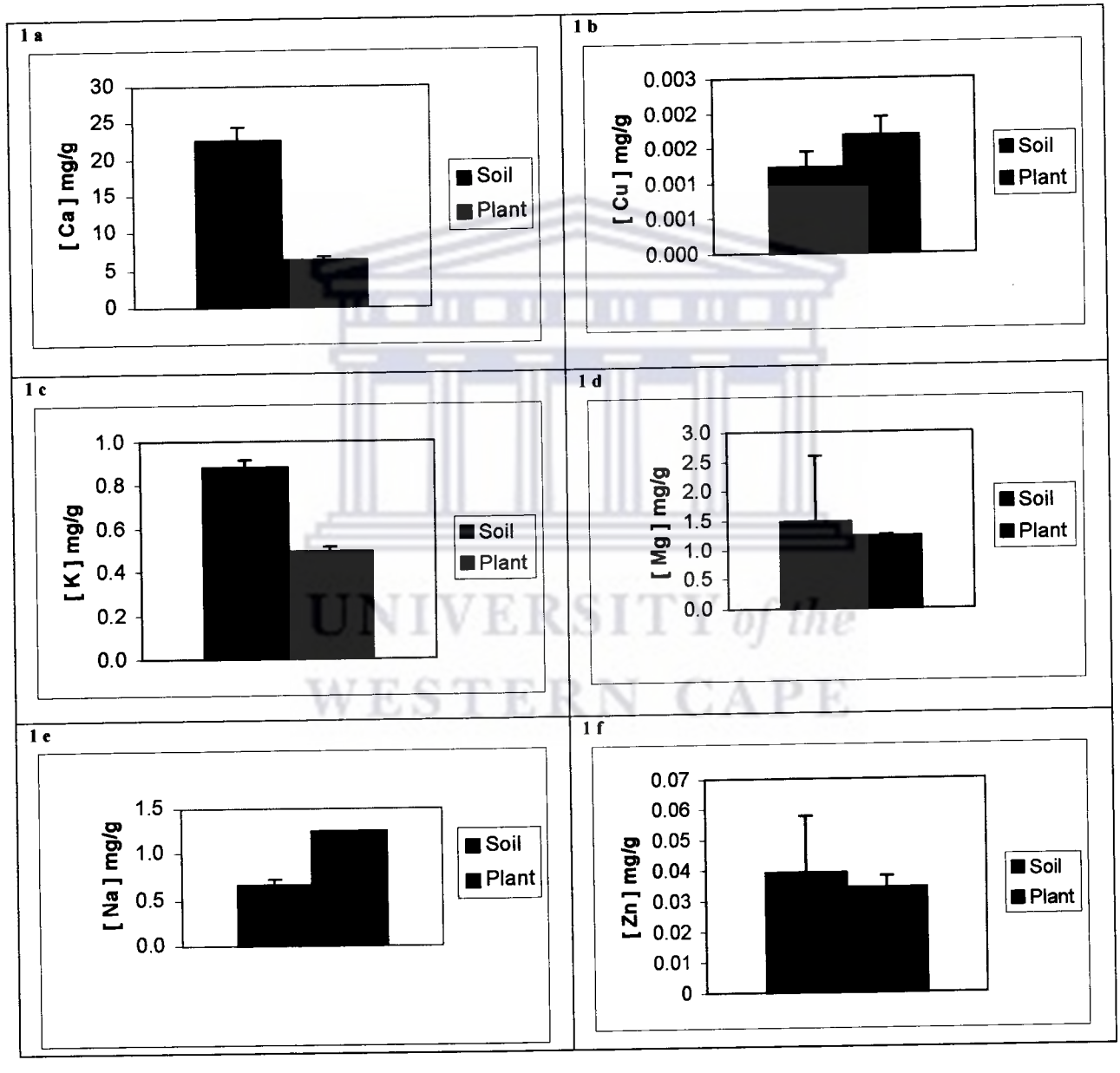
Data was analysed using Microsoft Excel Stat (2000). Control and experimental animal groups were compared with one another and a minimum significance of $P \leq 0.05$ was determined for all metabolic parameters using the Mann-Whitney test. The Man-Whitney is a two tailed non parametric test. The Mann-Whitney's U is normalised and tested against the normal distribution

3.4. RESULTS

3.4.1. Table1: Soil pH determination

Soil samples	1	2	3	Average SD
PH values	8.4	8.0	8.0	8.1 +/- 0.2

3.4.2. Soil and Plant Elemental Analysis



Figures 1 a –f: The elemental levels of Pelargonium triste extracts and the soil in which the plant is found.

3.4.3. Plant Histology

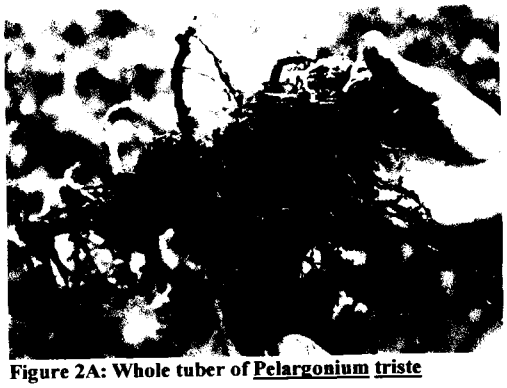


Figure 2A: Whole tuber of Pelargonium triste

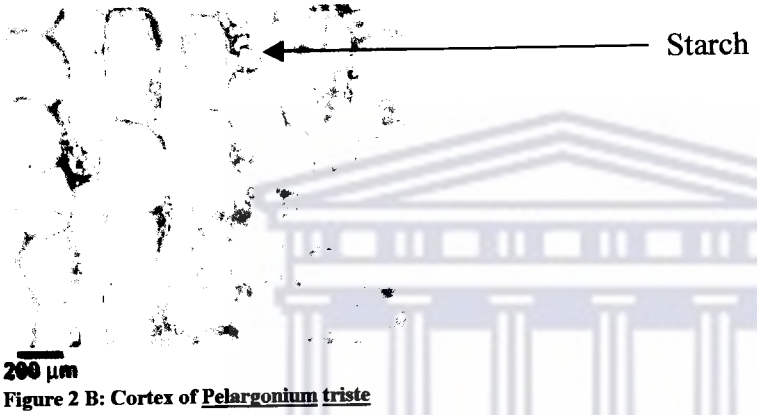


Figure 2 B: Cortex of Pelargonium triste

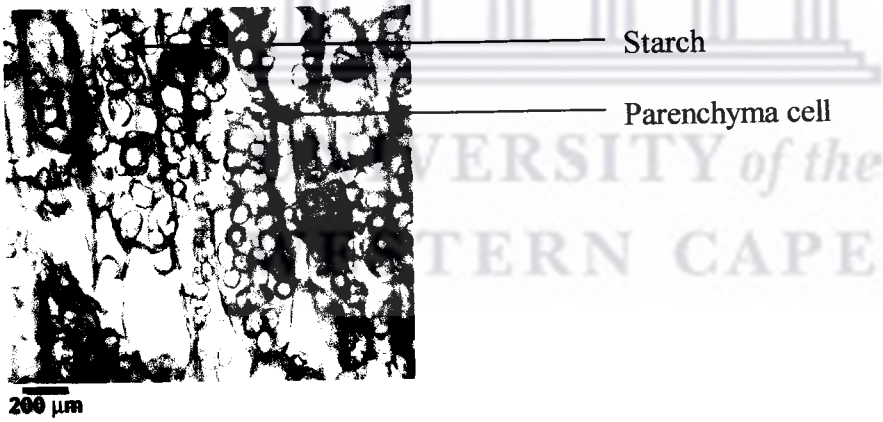


Figure 2 C: Pith of Pelargonium triste

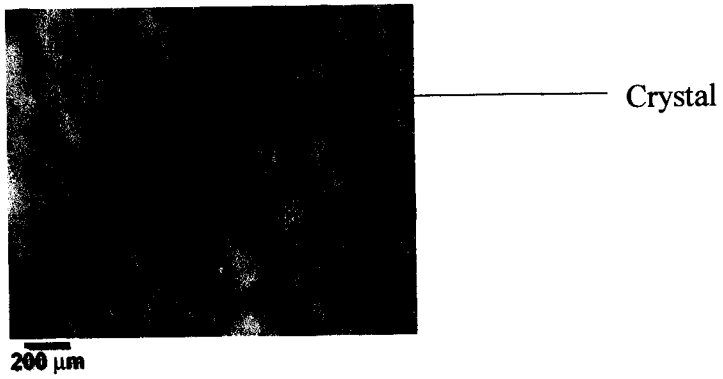


Figure 2 D: Crystal

3.4.4. Antimicrobial Results

Table 2: Growth inhibition(mm) of Pelargonium triste against four different organisms

	S.aur	P. aer	M. sme	C. alb
P. triste				
10µg/ml	-	-	-	-
20µg/ml	-	-	-	-
30µg/ml	-	-	1+	-
40µg/ml	-	-	1+	-
80µg/ml	-	-	2+	-
Methanol	-	-	-	-
Cip	4+	4+	4+	-
Amp	-	-	-	4+

Key:
S.aur - Staphylococcus aureus

P.aer - Pseudomonas aeruginosa

M.sme- Mycobacterium smegmatis

C.alb -Candida albicans

Cip - Ciprofoxicin

Amp - Amphotericin

-: no inhibition;

1+: 0-3mm;

2+: 3-6mm;

3+: 6-9mm;

4+: 9mm+

Antimicrobial Plates

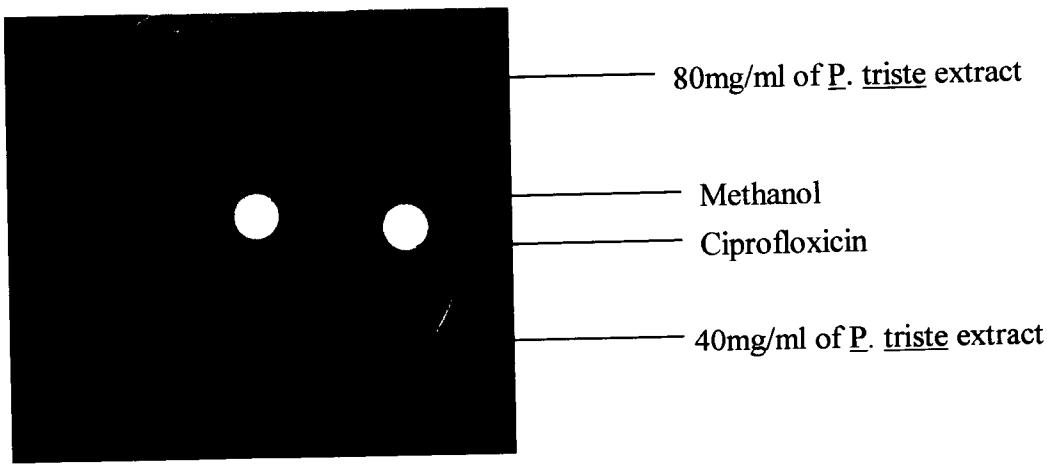


Figure 3a: Pelargonium triste extract on Mycobacterium smegmatis

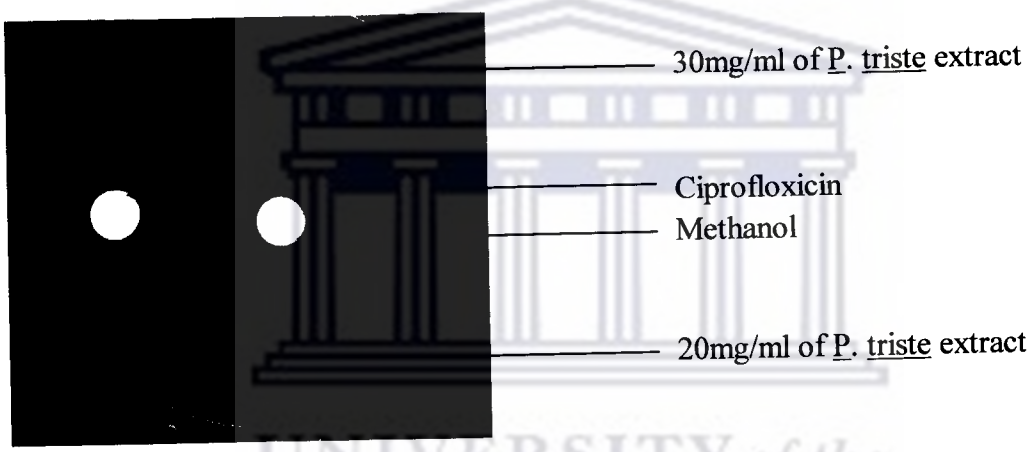


Figure 3b: Pelargonium triste extract on Mycobacterium smegmatis

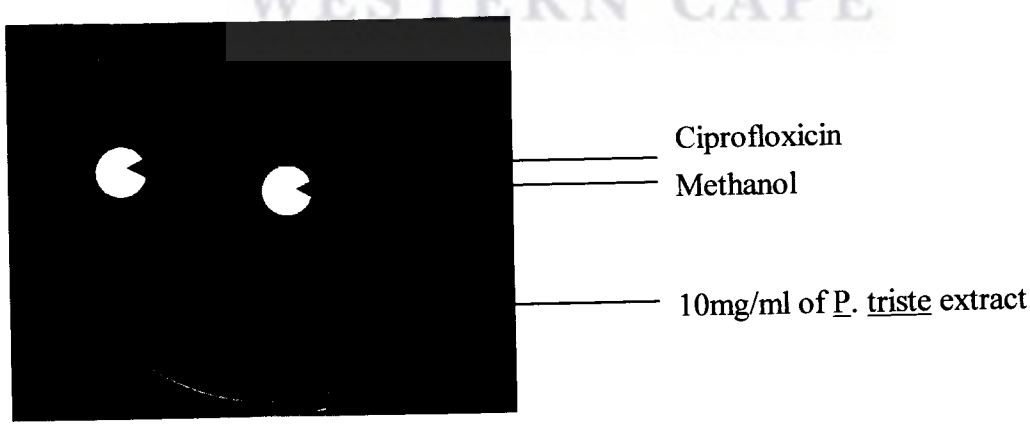
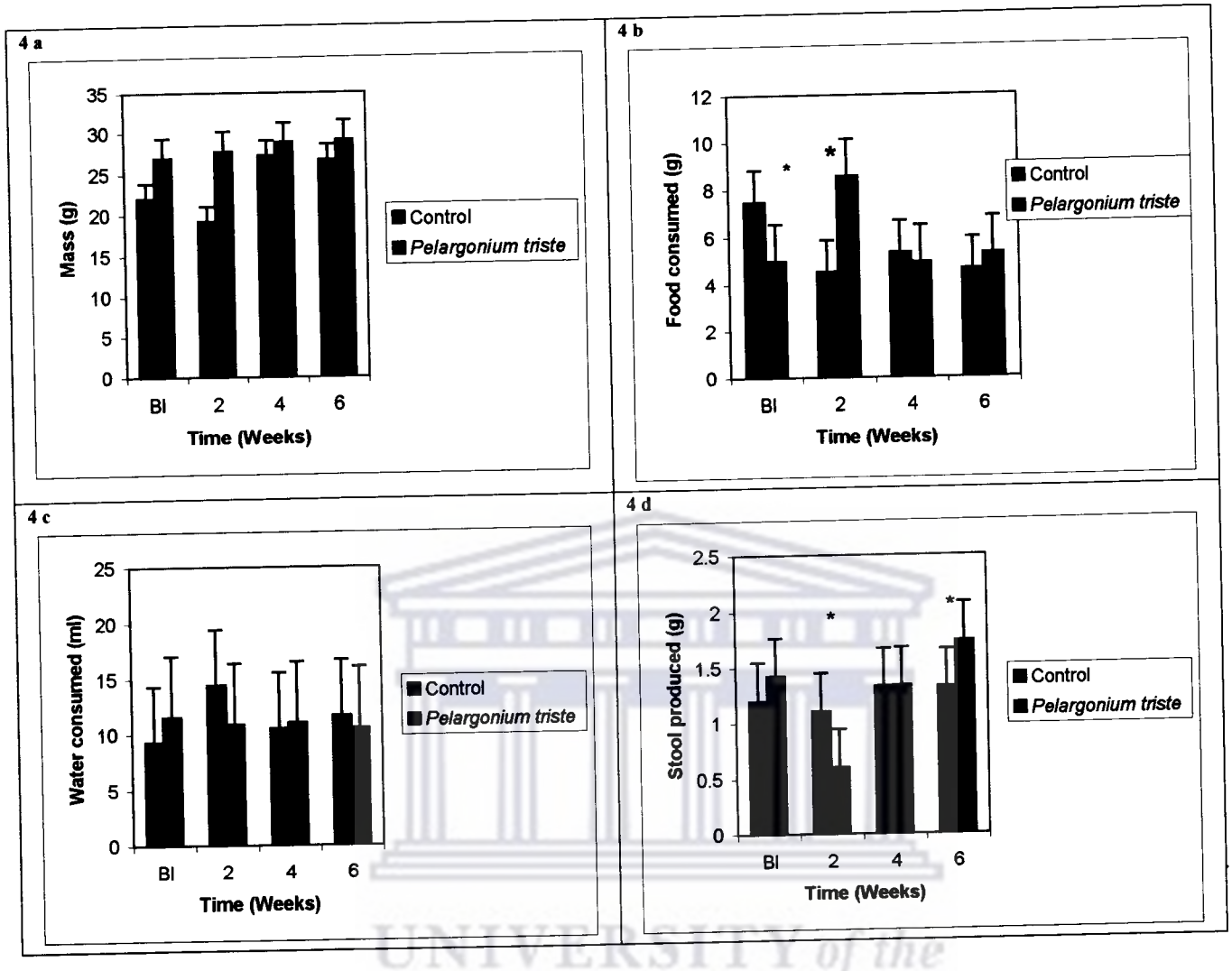


Figure 3c: Pelargonium triste extract on Mycobacterium smegmatis

3.4.5. Metabolic Indices

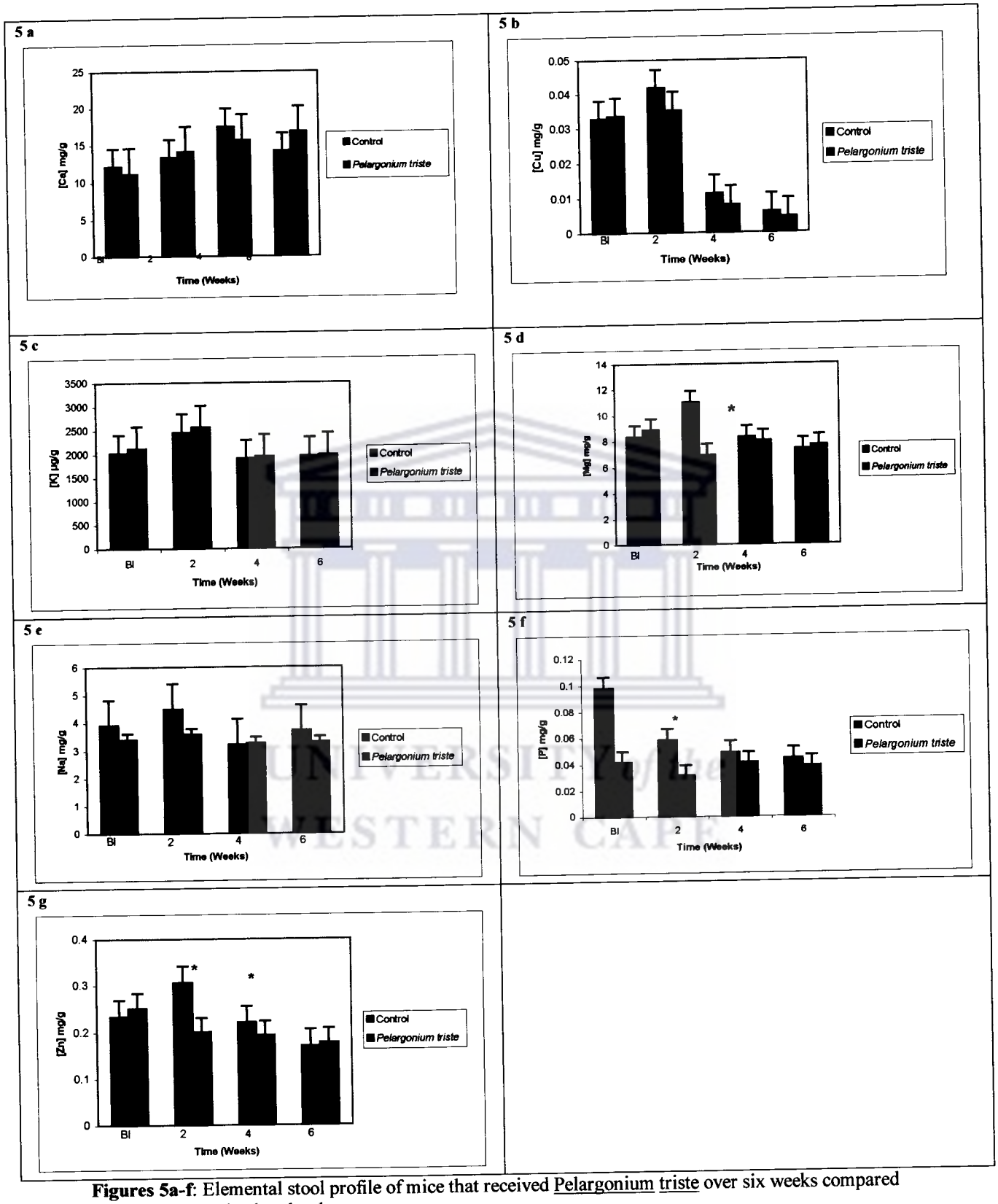


Figures 4a-d: Metabolic parameters of animals that received Pelargonium triste extract over six weeks compared to those that received a placebo.

Key: (*)= P≤0.05

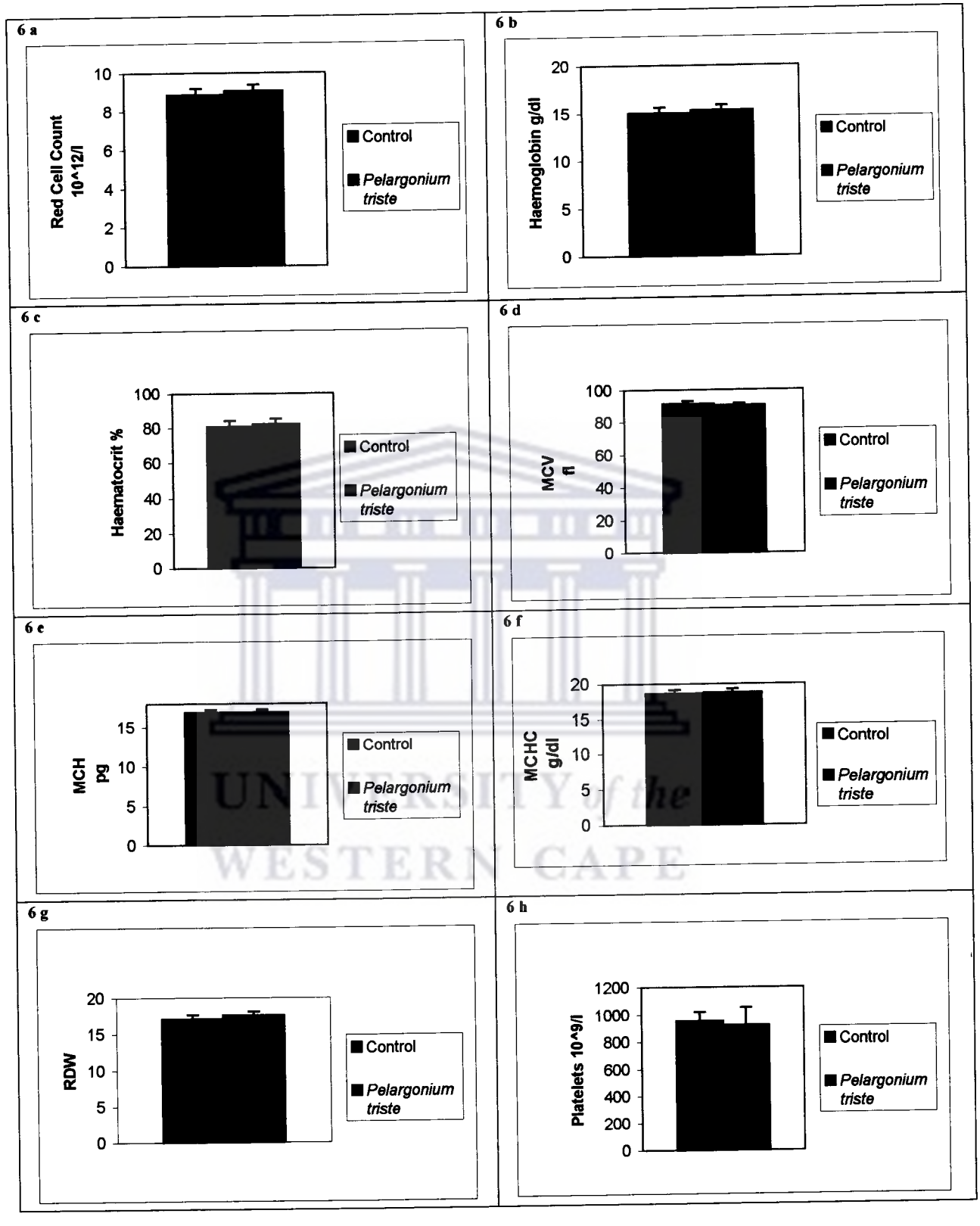
BL- Baseline

3.4.5.1. Stool Elemental Profile



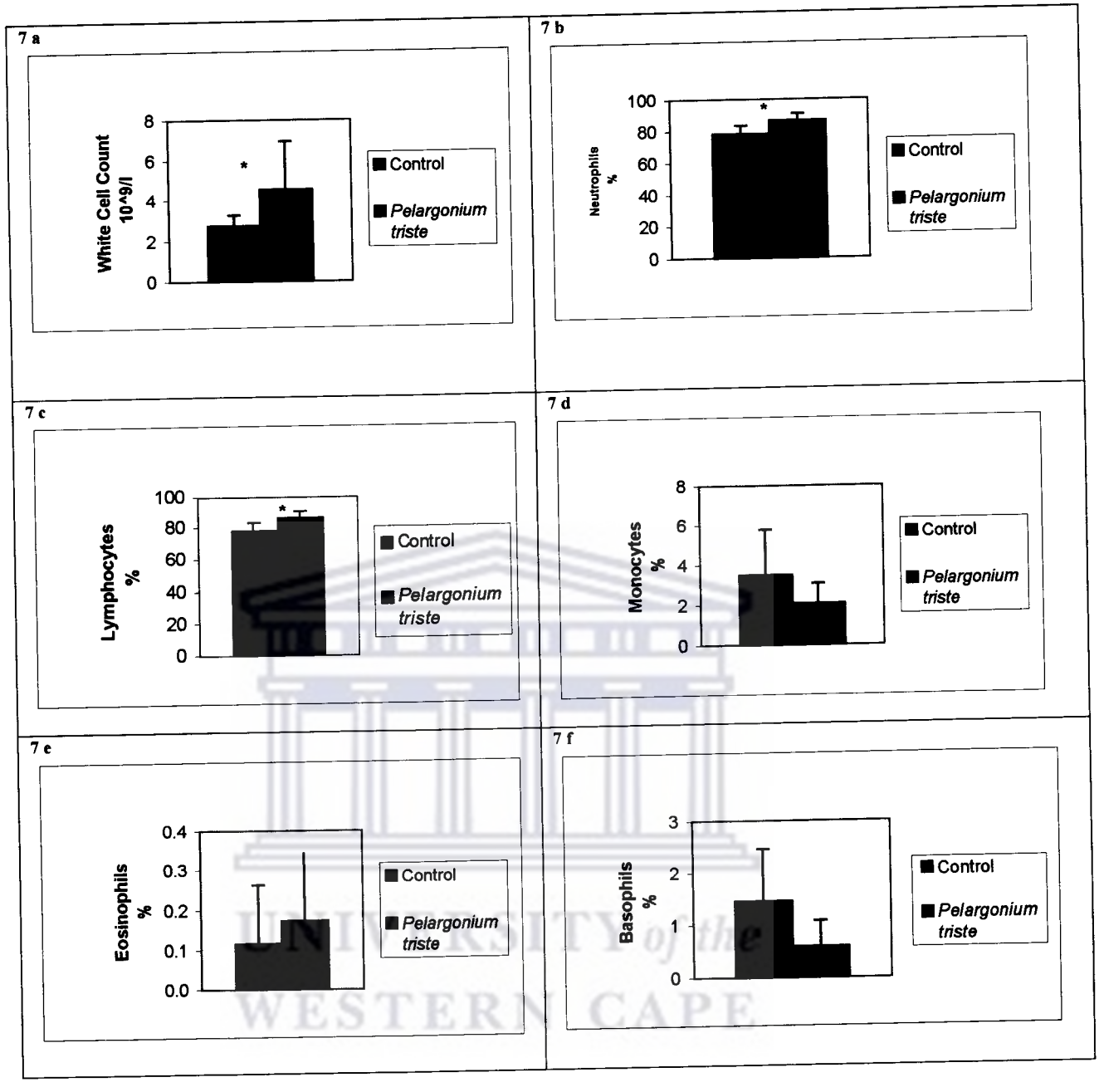
Figures 5a-f: Elemental stool profile of mice that received Pelargonium triste over six weeks compared to those that received a placebo
Key: (*)= P<0.05
Bl- Baseline

3.4.6. Haemotology



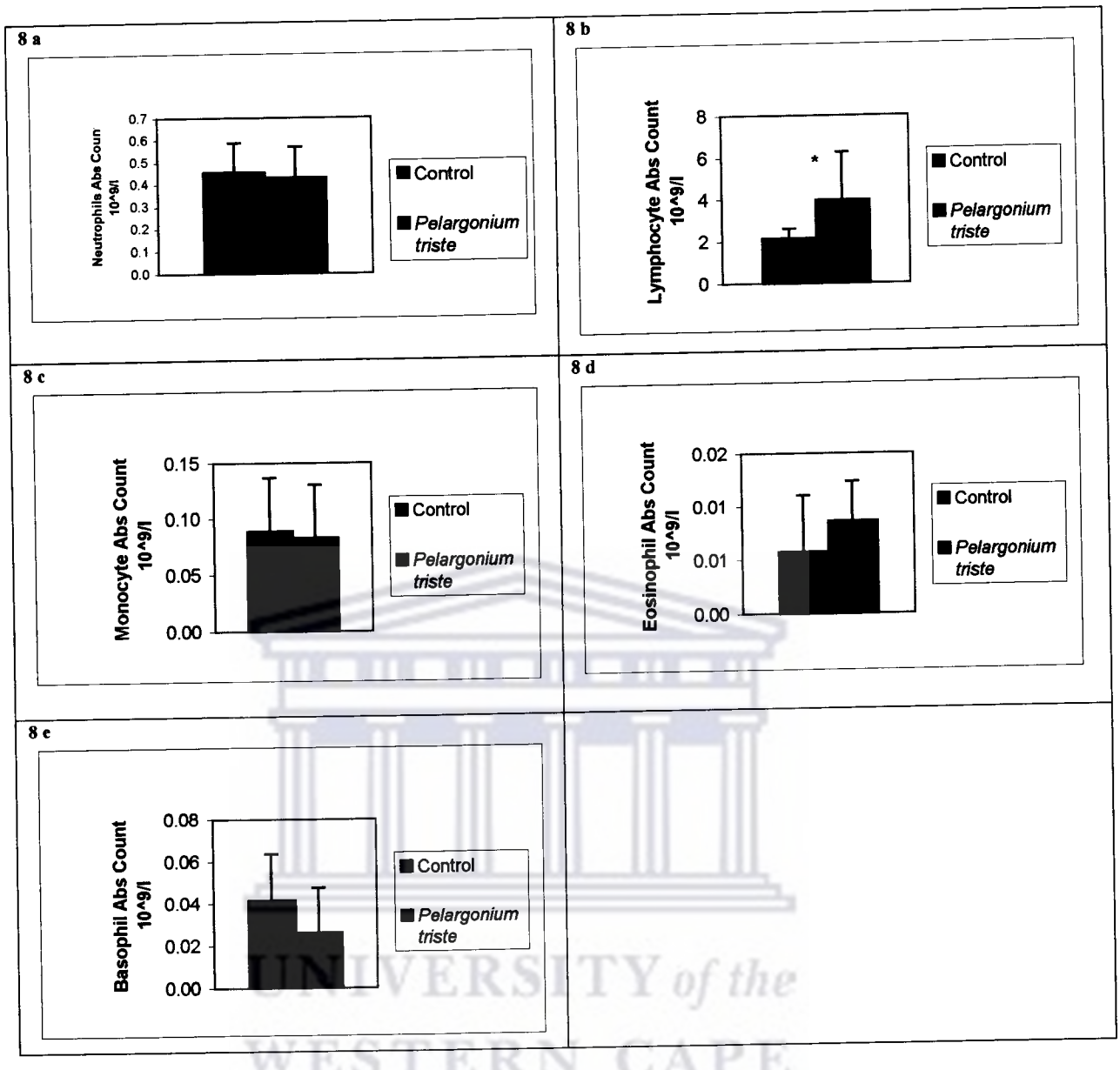
Figures 6 a-g: Red blood cell parameters of animals that were fed Pelargonium triste compared to those that received a placebo.

Key: (*)=P<0.05



Figures 7a-f: White blood cell parameters of animals that were fed Pelargonium triste compared with those that received a placebo

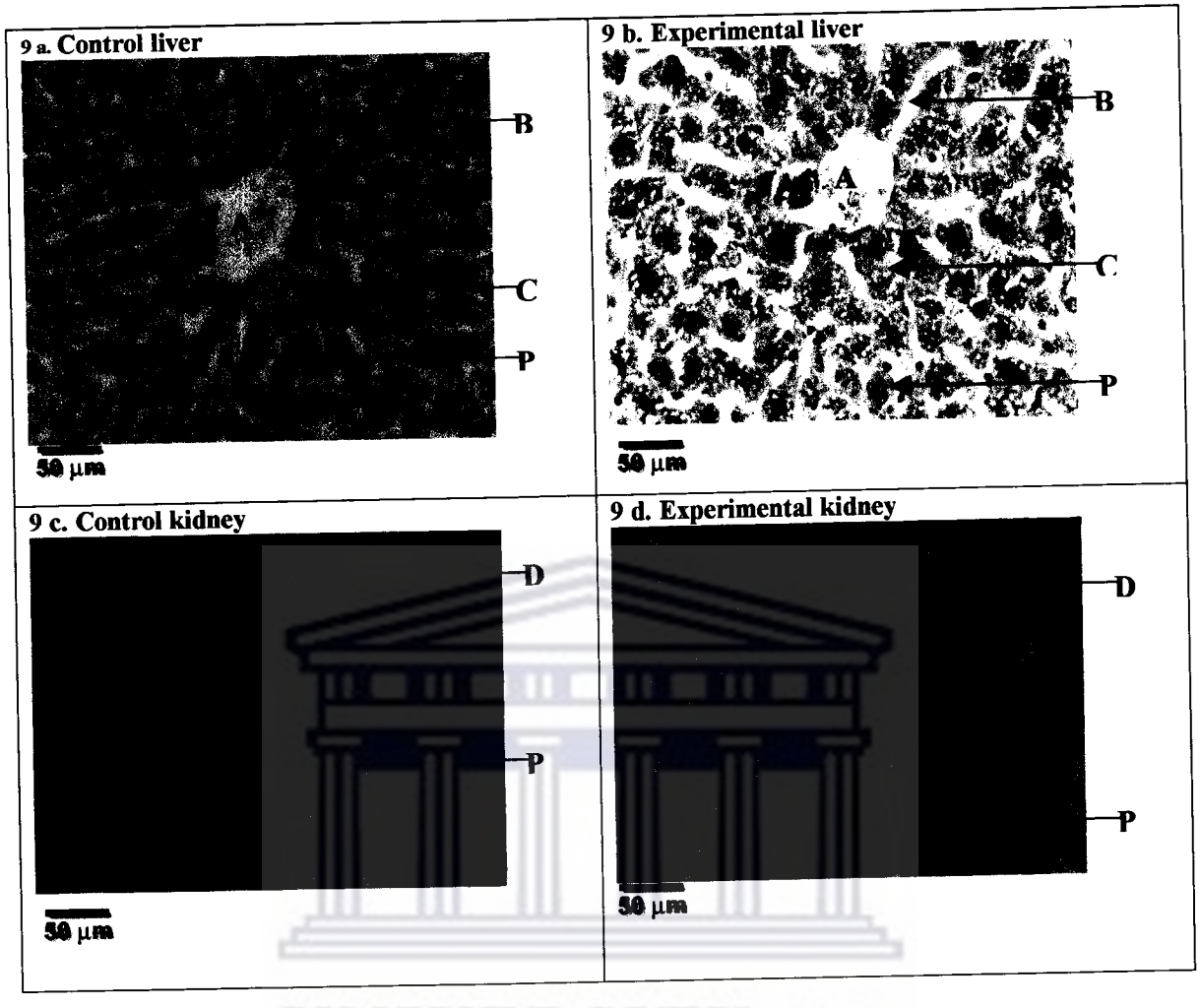
Key: (*)= P<0.05



Figures 8 a-e: White blood cell absolute levels of animals that were fed Pelargonium triste compared to those that received a placebo

Key: (*)= P<0.05

3.4.7. Animal Tissue Histology



Figures 9a-d: The effects of Pelargonium triste (Experimental) extracts and placebo (Control) infusionon liver (a, b) and kidney (c, d) tissue.

A-Central Vein;

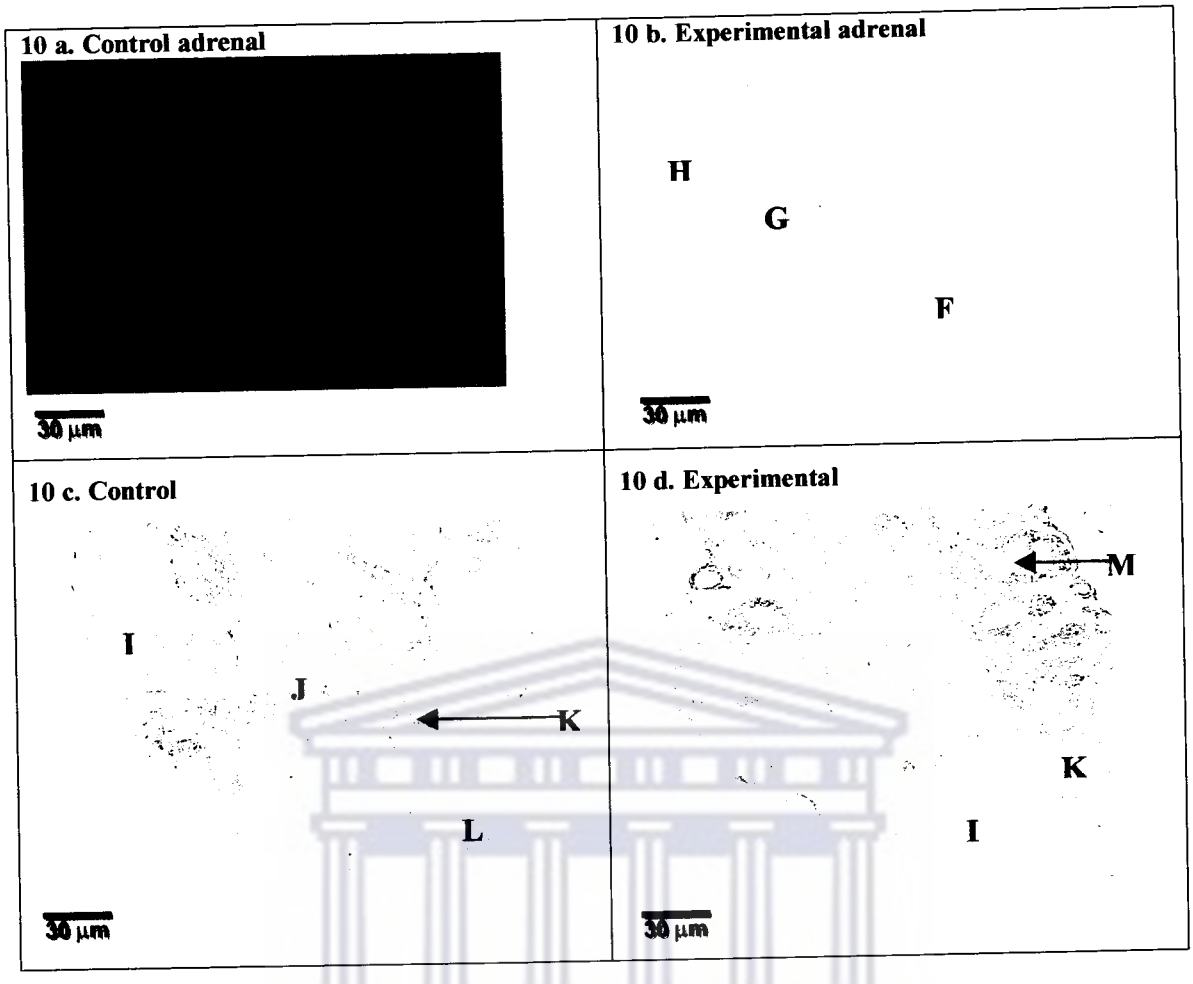
B- Sinusoid;

C- Liverplate

D- Glomerular Space;

E- Glomerulus;

P- Nucleus



Figures 10 a-d: The effects of Pelargonium triste extracts (Experimental) and Placebo (Conrol) on adrenals (a, b) and ovary (c, d).

F- Zona Glomerulosa;

G- Zona Fasciculata;

H- Zona Reticularis

I- Corpus Luteum;

J- Medulla;

K- Oocyte release;

L- Cortex;

M- Graafian follicle.

3.5. Discussion

This investigation focused on the effects of Pelargonium triste extracts on harmful microbes and their impact on animal health and metabolism.

It is known that soil pH (Table 1) plays a role in a plants capability to absorb elements. It is clear from the above mentioned table that Pelargonium triste soil- pH 7.5. $CaCO_3$ is a common soil constituent of high-level alkalinity soil. Ca can be 40-50% of soil content. The normal range for Magnesium is 0.003- 0.6% and Potassium is between 0.3-2.5 %. Copper, which is a trace element, is less than 1µg/g-1 and Zinc is between 10-300 µg-1. Na is 10-20 ml/l-1/ 10 000mg. The levels of these elements in the soil are within normal range (Etherington, 1982). In figures 1a-f the elemental profile of Pelargonium triste and the soil wherein it grows is compared. It is clear that the soil and plant elemental analyses show no significant differences ($P > 0.05$). Therefore the plant is not absorbing unusual amounts of elements from the soil.

The Pelargonium triste soil from Pauline Bohnen Nature reserve is alkaline. The plant is found in well-drained sandy soil and is in full exposure to the sun however the tubers are in the ground and protected from the elements. The tuber (Fig 2a) was used for sectioning as this is the part utilised. It is clear from the microanatomy that in the cortex (Fig 2b) and in the pith (Fig 2c) there is starch. The parenchyma cells in Fig 2c are long and well developed. The tubers have crystals throughout the tissue (Fig 2d). (Esau, 1960; Fahn, 1982).

The anti-infective value of Pelargonium triste is reflected in Table 2. From the data, it is clear that Pelargonium triste inhibits Mycobacterium smegmatis in a dose dependent (30mg/ml to 80mg/ml) fashion. Since this was only a screening of a crude extract, it is possible that it can be potentially safe, effective and affordable natural Tuberculosis therapeutic.

In Figure 4a, the mass of both groups of animals did not significantly ($P \geq 0.05$) differ over the length of the experiment. The food consumption patterns (Fig 4b) were no different over time except at week 2, where the Pelargonium triste group ate significantly more ($P \leq 0.05$) than the placebo group. This may well relate to the fact that the animals require a longer acclimation period, since this elevation in food consumed is a single event over the experimental period and it may have been that here is no competition in the metabolic cages but there may well be in the holding cages. Water consumption (Fig 4c) did not differ but stool excretion was lower at week 2 and higher at week 6 for the Pelargonium triste group compared to the placebo control. There is no specific trend when the metabolic indices are significant.

Mineral elements are thought to be important for human health. For the Elemental Analyses of the faeces, Figures 5b,c and 5e, show no significant differences. At individual metabolic reading there are significant differences in Figure 5 a (Ca) week 6, week 2, Figure 5f (P) and week 2 Graph 5g (Z) weeks 2 and 4. There is no specific trend for when the elements are significant but when it is the control has more significance, except in figure 5a (Ca), week 6. The control output of elements in the stool are more significant, this could mean that the treated animals are better able to utilize the elements for metabolism.

The blood indices were considered for differences. This study shows that white blood cell count (fig 7a) of the animals fed the aqueous extract of Pelargonium triste was significantly more ($P \leq 0.05$) in the treated animals than the control, specifically the Neutrophils (fig 7b) and Lymphocytes (fig 7c). T –cells attack cells that contain viruses and B -cells produce antibodies, these are two types of lymphocytes (Mader, 1996; Sell, 1987).

In the anti-inflammatory effect, Neutrophils and Lymphocytes are key factors and this could signify the anti-inflammatory effect of the plant (Amirghofran, 2000). However, the neutrophils and lymphocytes of the treated animals are still within normal range. The control's levels of neutrophils and lymphocytes are normal.

In this investigation the liver, kidney, adrenal and ovaries were assessed. The description of anatomical features follows Amenta (1997) and Sternberg (1976). Figures 9a is the Control liver and Figures 9b the Experimental liver at 200x magnification. The arrangement of the liver plate (C) and sinusoids (B) around the central vein (A) appear to be normal. At gross cellular level the cells appear normal. Figures 9c (control) and 9d (experimental) are of the kidney, the control and treated kidney respectively at 200x magnification. The gross cellular arrangement appears to be normal.

Figures 10a (control) and 10b (Experimental) are the adrenal. All the zones and cells at gross level are normal of both the control and treated group.

The ovaries, Figure 10c (Control) and figure 10d (Experimental) are also normal.

From the gross cellular level, the plant does not seem to negatively affect the tissue of the animals. It had no effect on the ovaries especially on the follicles. In conclusion, the animal tissue does not appear to be affected by the medicine.

This investigation has shown that Pelargonium triste had an effect on microbial growth and improved immune cell numbers with no physiologically important effect on red blood cell parameters. These outcomes compel us to further study Pelargonium triste as a potential anti-infective indigenous phytotherapy, especially when we consider its favourable safety profile.

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Chapter 4

Summary

Plants were once the source of all medicine in the world and still represent a sustainable source of new remedies. Natural products and their derivatives represent 50% of all drugs still used today. South Africa has a rich cultural diversity, which is reflected in the formal and informal medicinal systems that are practised in different parts of this country. Many people in rural South Africa still use traditional medicines for the management of their health. Through trial and error, people have perfected the use of plants as food and medicine. The indiscriminate use of antibiotics has also led to strains of microbes becoming resistant; this leads to the necessity of finding alternative means to cure illness and disease. However, the correct administration of medicine has been taken for granted. Morphologically similar but totally unrelated plants are often used to the detriment of the user.

Given that these indigenous medicinal plants have anecdotal anti-effective value, this study aimed to assess their potential as antimicrobial agents and to evaluate their impact on small mammal metabolism and health. These plants were selected as they are used as system cleansers, which are reported to improve health.

The anatomy of the plants was done to identify plants on a microscopic level, where any specialities in the tissues could help identification and possible evidence of secondary metabolites. Elytropappus rhinocerotis has pappilated epidermis, trichomes and glands that were the most outstanding features. Pelargonium triste had crystals throughout the tissue; this alludes to the possibility that this is the storage form of the secondary metabolites.

In vitro studies were done to show that the screening of plant extracts for anti-microbial activity has shown a potential as a new source of anti-infective agents. Elytropappus rhinocerotis showed no inhibition zones whereas Pelargonium triste showed activity against Mycobacterium smegmatis. These results were a screening and further testing could prove advantageous.

In vivo studies were done to determine whether the plant had any adverse effect on living organisms, mice being a representative of a human model. Toxicology testing had to be considered; therefore tests were performed on animals. The metabolic indices showed some significance but not beyond normal parameters. The animals were sacrificed, and blood samples were taken in order for the blood parameters to be analysed.

Considering that Elytropappus rhinocerotis had no effect on the microbes yet still acts as an immune-enhancer, does not cancel it out as an anti-infective because the mode of action is different. Pelargonium triste is however an anti-infective as well as an immune-enhancer. It acts directly and indirectly on microbes. These plants can possibly be used in the prevention of disease and illness instead of a curative agent.

Histological sections determined if there were any differences in the control and treated groups. Organs were removed and histological analyses were done. The liver, ovaries and adrenal do not show any abnormalities. These organs are particularly important because these organs are most sensitive to potential toxicity.

It is hoped that the results presented in this study will not only provide useful leads for the discovery of clinically useful drugs but also to encourage research on South African plants used in traditional medicine.