The effects of *Exomis microphylla* and *Atriplex lindleyi* on metabolism and health

By

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

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STATEMENT

I declare that " The effects of *Atriplex lindelyi* and *Exomis microphylla* on metabolism and health" 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.

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Cheryl Moses

December 2002

DEDICATION

To my Grandmother, my mother and aunt for all the support and love throughout my years of study.

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Chapter 1

Overview: The effects of *Exomis microphylla* and *Atriplex lindleyi* on metabolism and health

Plants have been used by people for centuries as a means of healing. Herbal medicines has been used in countries as an aid to health problems as well as to save ancient traditions.[1] Plants have been used by people for centuries as a means of healing. Information on the healing capabilities of plants have been recorded as far back as 5000 years to the Sumerians [2], and archeological records suggest even earlier use of medicinal plants. The historic bond between plants and human health began to unwind in 1897, when Friedrich Bayer and Co. introduced synthetic aspirin to the world. This was found to be safer that salicyclic acid which is an active ingredient of willow bark and was used as a remedy for aches and fevers.[3]

The seeds of the opium poppy *(Papaver somniferum)* and castor oil seed (*Ricinus communis*) were excavated from some ancient Egyptian tombs, which indicated their use in that part of Africa as far back as 1500 B.C. This indicates that man have been aware of the medicinal properties (and possibly the toxic effects) of some plants growing around them as long ago as 3 000 B.C.[4]

The status of traditional medicine generally, and the use of medicinal plants in particular, shows that whereas in some areas competition and confrontation with conventional health care is occurring, in others collaboration between the systems has occurred.

Traditional medicine can be seen as the total combination of knowledge and practice, whether explicable or not, used in diagnosing, preventing, or eliminating a physical, mental, or social disease and which may rely exclusively on past experience and observation handed down from generation to generation, verbally or in writing.[4]

There is a one in four chance that any bottle of prescription medicine at a pharmacy in the United States, Canada or Western Europe has its active ingredients derived from plants. In most cases these plant derived drugs were originally discovered through the study of traditional cures and folk knowledge of indigenous peoples i.e. the ethnobotanical approach.[5]

During the 1970's at least 25% of all drugs dispensed in the USA contained compounds derived from flowering plants[6], and an even greater proportion of phytochemicals were used as drugs worldwide.[7] At the dawn of the twenty-first century, 11% of the 252 drugs considered as basic and essential by the World Health Organization, were exclusively derived from flowering plants.[8]

At present people are researching the use of herbal medicine for the treatment of cardiovascular disorders[9-10], certain forms of cancer[11], AIDS[12], diabetes[13-17] inflammation[18--20], injuries and various infectious diseases more. Since pathogens are developing a greater resistance to antibiotics, significantly more attention has focused on extracts and biologically active compounds isolated from plant species used in herbal medicine.[21]

Westernization and urbanization of the African population of South Africa has caused in increase in the incidence in life style related illnesses such as hypertension and diabetes. A study was conducted in 1999 in South Africa on twenty plants used by traditional healers for the treatment of high blood pressure. The investigation was undertaken to determine the anti-hypertensive properties of the plants, utilizing the angiotensin converting enzyme assay and a 65% success rate was found.[22]

Over the years, diabetes has posed a huge concern. Research conducted in the last few decades have shown that many of the plants traditionally used in India have strong anti-diabetic properties. [23]

A number of plants have been studied which show potential as anti- diabetic medicines. Among these are the more commonly known *Aloe vera, Allium sativum and Eucalyptus globulus.* All of the plants used in the specific study showed varying degrees of hypoglycemic and anti-hyperglycemic activity.[24]

Sexually transmitted diseases (STDs) and acquired immunodeficiency syndrome (AIDS) are gaining significant importance at present due to rapid spread of the diseases, high cost of treatment, and the increased risk of transmission of other STDs and AIDS. Current therapies available for symptomatic treatment of STDs and AIDS are quite expensive and beyond the reach of ordinary individuals. A further complexing feature relates to the emergence of microbial resistance to current drug therapies. Due to this, many AIDS patients are now looking for alternative means of fighting STD's and AIDS. Although medicinal plants have been used in the past to treat infectious diseases, there has been no scientific evidence that it does or does not work. For this reason many researchers are now testing various plants that have been said to have medicinal value.[12]

A study was conducted in two islands in the Gulf of Guinea to investigate the antimalarial effects of medicinal plants traditionally used there by traditional healers. The study revealed that the traditional healers use several medicinal plants against fever and malaria that have strong antiparasitic activity in vitro.[25] Infectious diseases are a cause of great concern. As previously mentioned, bacterial strains build resistance to many of the drugs developed to cure these diseases and hence research has been undertaken into the use of medicinal herbs as potential therapeutics. In Russia, particularly Siberia, people use herbal medicines extensively to treat infectious diseases, inflammation and injuries.[26] A study conducted to determine the antimicrobial effect of 16 traditionally used medicinal plants against 5 species of microorganisms showed that of the 16 plants tested, 12 showed antimicrobial activity against one or more species of microorganisms.[26]

In another study conducted in Tanzania, it was found that most of the plants that were tested for antimicrobial activity was effective.[27] In addition to the methanolic, ethanolic and acetone extracts that were tested, water extracts were also tested. This was found to be just as effective, which demonstrates that the hot water decoctions of the species tested, used in traditional medicine in Tanzania, are effective in the treatment of bacterial infections.[27]

Whilst chapter one provides and overview of medicinal plants and human health, the essence of this study is written in the form of two papers that are separately presented in chapters two and three. Each paper focuses on an individual indigenous medicinal herb in an attempt to assess its:

a) Anatomical features
b) Mineral Element profile
c) Anti-infective value and
d) metabolic, haematological and histological effects

Anatomical studies were done in order to describe the components within the plant leaves and determine which may be responsible for storage of the chemical compounds responsible for the plants chemical effects. Mineral element assessments were completed to gain insight into the possible contribution of these indices to the health value of the herbs. Microbial studies were done to determine whether the plants posses any antimicrobial activity against selected bacterial and fungal strains. The effects of the plants on animal metabolism were also studied. This was done in order to determine whether the plants have any adverse effects on the metabolism and health of the animals. This included metabolic, hematological and histological evaluations.

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Chapter 2: The effects of *Atriplex lindleyi* on metabolism and health

2. Abstract

For many decades people have used plants for healing various ailments. Plant products have been used in herbal remedies especially for their anti-infective properties. The following study was undertaken to assess the anti-infective value of *Atriplex lindleyi* and to determine how the plant extract effects metabolism, and health of male rats. Plant anatomy was done to determine which components are present in the plant leaves and to assess which components might be responsible for the production or storage of the chemical products within the leaves that may contain the secondary chemical. The leaves were sectioned using a freeze microtome. Liquid CO_2 and Hamilton's freeze solution was used to freeze the plant leaves and sections were made ranging from 15-25 microns. Elemental analysis was also done on the plant material to determine the concentration of selected elements within the plants. The analyses were done using the Unicam Solaar M series atomic absorption spectrometer. Four microbes were used for the antimicrobial screening. Various concentrations of the plant extract was prepared and tested against *Staphylococcus aureus* (ATCC29213), *Pseudomonas aeruginosa* (ATCC27853), *Mycobacterium smegmatis* and *Candida albicans* (ATCC10231). Amphotericin B served as the positive control for *C. albicans* whilst Ciprofloxacin was used for the three bacterial strains. Screening was done using the disc diffusion method. Metabolic studies were done on 20 Wistar male albino rats. This was done to assess the metabolic, haematological and histological effects of the herbal extract on the animals.

The one component within the leaf that seemed to be most outstanding was the sclereids. Since sclereids have a protective function, it can be speculated that secondary compound storage and/or production might occur at this site, but further studies are required before absolute conclusions can be drawn in this regard. The difference in the elemental concentrations between the soil and plant samples was not statistically significant.

The antimicrobial screening showed no herb-induced inhibitory effects on the four selected microbes at either of the extract concentrations used. The metabolic studies showed a significant increase ($p \leq 0.05$) in urine excretion on days 15 and 45. This significance might indicate that the plant has diuretic properties. There is a significant decrease ($p \leq 0.05$) in the urine pH values between the control and experimental groups. The urine pH however, remained alkaline. The herbal extract caused a significant decrease ($p \leq 0.05$) in the Haemoglobin levels. There was a significant increase ($p \leq 0.05$) in both the mean cell volume (MCV) and Hematocrit percentage of the rats on the herbal extract. The mean cell haemoglobin count (MCHC) significantly decreased ($p \leq 0.05$) in the rats on the herbal extract. All white blood cell parameters remained ($p \geq 0.05$). Even though there was no herb-induced effect on the white cell parameters, the decrease in the red blood cell parameter Haemoglobin, may be reason for concern. Histological sections of the liver and testes showed no differences between the control and experimental rat groups. Whilst this medicinal herb showed no antimicrobial effects, it may possibly have diuretic value. Given its favourable safety profile, further studies on this medicinal plant should be pursued even though it had an input on certain blood biochemical indices, all of which though remained within physiologically normal limits.

2.1 Introduction

Plants have been used as herbal remedies for many decades. Original inhabitants such as the Khoi and San people have long since been using plants for medicinal purposes. Because of factors such as the high cost of conventional medicine, other local communities have also been making use of traditional medicine.[1] Traditional healing is a widespread practice in South Africa, and an estimated 80% of the black population consult with traditional healers.[2] A number of between 12 and 15 million South Africans are still dependant on indigenous herbal medicines from at least 700 indigenous plant species.[3]

The use of medicinal plants contributes a great deal to health care all over the world and especially in South Africa. A study was conducted in the Eastern Cape on the antimicrobial activity of selected plants on the treatment of wounds. The results justified the use of these plants for the treatment of wounds that had been contaminated through bacterial infection in the province.[4]
Atriplex lindleyi which is also known as the saltbush has not been extensively studied. Studies have however been done on other *Atriplex* species such as *Atriplex littoralis.*[5]

Women who ate the plant under famine conditions in China, developed oedema and then a pruritic bullous eruption of exposed skin. Men where rarely affected.[5] This disorder, known as atriplicism, has been interpreted as a photosensitisation[6] and attributed to ingestion of *Atriplex serrata* and *Amaranthus mangostanus* L. (fam. Amaranthaceae). It has beed recorded that species of both of these genera tend to grow on garbage dumps around human habitation.[7] *A. littoralis* was reported by Matignon (1897, 1900) to produce adverse effects.

Various Botanical supplements are frequently criticized for poorly proven efficacy and safety, lack of standardization and quality standards[8-9], and potential interactions with prescribed drugs.[10]

However, data from most of the randomized clinical trials performed with the top selling botanical supplements suggest that most are at least mildly effective against a specific indication.[11-12] In doing the following study we aim to scientifically substantiate the efficacy and safety of *Atriplex lindleyi.*

2.2 Distribution of *Atriplex lindleyi*

The family Chenopodiaceae consists of ±100 genera with ± 1500 species. It is cosmopolitan, but particularly common in semi-arid environments and in saline habitats. Few of the species have given rise to cultivars of agriculture and many occur as weeds of cultivation. Southern Africa has 14 genera of which two are exotic and 164 species have been identified.

Atriplex is one genus within the Chenopodiaceae family. These are perennial herbs, subshrubs or shrubs. They can be either monoecious or dioecious with some bisexual flowers. There is ± 250 species and is cosmopolitan. 19 species are found widespread in Southern Africa mainly in desert areas, saline habits and on waste ground in temperate and tropical regions. *Atriplex lin*dleyi, also known as *Blackiella inflata,* is a grey-mealy rounded annual, which grows up to 30 cm. The leaves are rhomboid and toothed. It can be found on dry stony flats and disturbed sites. In general the genus succeeds in full sun in any well-drained but not too fertile soil.[13] Most species in this genus tolerate saline and very alkaline soils.[13] No member of this genus contains any toxins, all have more or less edible leaves. However, if grown with artificial fertilizers, they may concentrate harmful amounts of nitrates in their leaves.

2.3 Ethics

This investigation received ethical approval from the University of the Western Cape.

2.4 Materials and Methods

2.4.1 Plant collection

Atriplex lindleyi was found 3 km north east of the turnoff to Herbertsdal in the Western Cape. The vegetation type is Renosterveld and it was found on stony rocky soil in gravel soil. The area was also well drained with full exposure to the sun. The plant was found growing towards the East at a gentle slope. Taxonomist Mr Frans Weitz verified the identity of the plants. Voucher specimens were deposited in the Herbarium of the Botany Department and the University of the Western Cape and a sample of the plant was also placed in FAA (Formaldehyde, Acetic acid and Alcohol) for later anatomical studies.

2.4.2 Plant Anatomy

The anatomy of all plants was completed by using a freeze microtome. A small section of the leaf which is used as a medicine, was cut out and then mounted on the freeze-microtome to be sectioned. Liquid CO_2 gas and Hamilton's freeze solution was used to freeze the plant sample and sections were made ranging from 15-25 microns. The sections were fixed on slides and stained with a safrinin red/alcian blue stain.

Each slide was viewed under an Olympus photomicroscope and the images were captured digitally.

2.4.3 Soil samples

Three soil samples were collected at the site where the plant was located. In the laboratory the soil was oven dried at 30 – 40 °C for a 48hr period. These samples were collected in order to determine elemental concentration within the soil. Soil pH was also determined using an A PHM83 Autocal pH-meter pH meter.

2.4.4 Phytochemistry

(a) Extraction

Chemical Extraction of the plant was done in the laboratory. The fresh plant material was left in methanol for ± three days. It was then filtered and the methanol was then evaporated by means of a rotary evaporator. Another methanol extraction was done, but the plant material was blended. This was repeated to make sure that all the compounds were extracted. This was then again evaporated using a rotary evaporator. The dried extract was then frozen overnight after which it was freeze dried. The freeze-dried extract was put in a 5ºC cold room.

The remaining plant material was oven dried at 30–40 ºC for a 72 hr period. The dried plant material was used for Atomic absorption spectrometry (AA).

2.4.5 Elemental analysis of *Atriplex lindleyi*

(a) Soil and Plant Digestion

Soil and plant material were prepared using acid digestion .[14] This type of digestion of an organic material is an oxidizing system and has the advantage that phosphorus and in some cases, even nitrogen can be determined on the final solution along with other nutrients.

Sulphuric acid is added to the digestion mixture to reduce the possibility of desiccation. The acid system used was Hydrogen Peroxide – Sulphuric Acid. This is suitable for nitrogen in addition to sodium, potassium, calcium, magnesium, iron, manganese and phosphorus.

(b) Procedure

To prepare the digestion mixture, 0.42 g Se and 14 g of $Li_2SO_4.H_2O$ were added to 350 ml H_2O_2. This was mixed well and then 420 ml of concentrated H_2SO_4 was carefully added to the mixing vessel which was surrounded by ice to keep the mixture cool. Four grams of dried soil was weighed into a glass tube (0.8 g dried ground plant material was used) and 8.8 ml of the digestion mixture was added.

This mixture was then digested at moderate heat setting of 200°C until the initial reaction subsided, after which the heat level was incrementally raised to 350°C. The digestion process proceeded until a clear, almost colorless solution was obtained. This solution was filtered and diluted with distilled H_2O to a volume of 100ml. A further five fold dilution was necessary in order to obtain a 1% H_2SO_4 solution. Furthermore, blank solutions were also prepared as reference samples, whilst a 200ml stock solution was also prepared.

(c) Dilutions of samples for Atomic Absorption Spectrometry

A 1% H_2SO_4 solution was made by using a 10 ml of 98% H_2SO_4 solution topping it up with water to a volume of 980 ml.

1000mg/l Merck Ca, Na, Mg, K, and Cu stock solutions were used to prepare the six standards used for the AA analyses. This was topped up with 1% H_2SO_4 in a volumetric flask in order to obtain a concentration of 100mg/l. These stock solutions were then used to prepare standards ranging from 0.05mg/l – 50mg/l. An air aceylene flame was used to determine all the elemental concentrations.

Each sample was diluted by using 15 ml of the sample and topping it to 75 ml with distilled water in order to obtain the 1% H_2SO_4 matrix

The samples were run through the Unicam Solaar M series atomic absorption spectrometer for elemental analysis using the flame mode. The following equation was used to determine the elemental concentrations:

$$\text{Total element \%} = \frac{C(mg.l^{-1}) \times sol.volume(ml) \times Dil.factor}{10^4 \times sampleweight(g)}$$

C =Concentration

Sol.vol = Solution volume

Dil.factor = Dilution factor

2.4.6 Antimicrobial screening of *Atriplex lindleyi*

The previously freeze-dried methanol extract was redissolved and various concentrations of the extract was prepared ranging from 10mg/l-80mg/l.

(a) Micro-organism and growth media.

Four microbes were used for antimicrobial screening. The extracts were tested against the micro-organisms *Staphylococcus aureus* (ATCC29213), *Pseudomonas aeruginosa* (ATCC27853), *Mycobacterium smegmatis* and *Candida albicans* (ATCC10231).All the micro-organisms were obtained from the Medical Biosciences Department at the University of the Western Cape. The *Mycobacterium smegmatis* was obtained from Tygerberg Hospital, in Tygerberg City.

(b) Disc diffusion test

Individual nutrient broths containing *Staphylococcus aureus, Pseudomonas aeruginosa, Mycobacterium smegmatis* and *Candida albicans* were streaked onto nutrient (Difco) and Mycobacteria 7H11 (Difco) agar plates respectively. The agar plates were streaked with sterilized swabs that were inserted into the suspensions. The plates were spread in three different directions to ensure an even-growing bacterial and fungal mat. Sterilized nine-mm filter paper discs were impregnated with 50µl of the methanol and aqueous plants extract in concentrations ranging from 10mg/l – 80mg/l. These were placed at equidistant spots on the inoculated agar plates. Methanol and water discs were used as controls. Amphotericin B (AMP) served as the positive control for *C. albicans* and Ciprofloxacin (Cip) was used for the three bacterial strains. The positive controls were supplied by the companies Bristol-Meyers Squibb and Bayer respectively. The plates were incubated at 37°C for 24 hours with the exception of *Mycobacterium smegmatis* that required a 48 hour growth period. All extracts were tested in triplicate. Inhibition zones were recorded at the end of the incubation period by measuring the growth–free zone between the discs and the bacteria and/or fungal yeast.

2.4.7 Animal studies

20 Wistar male albino rats were supplied by the Medical Research Council (MRC) and used in the experiment. These were divided into two groups of ten each. The rats were housed in pairs of two in plastic cages with mesh wire flooring and tops, in a temperature-controlled room. The cages were 35cmx35cm with a height of 23cm. One group was used as a control group receiving only distilled water as a substitute for the herbal extract, whilst the second group was medicated with an aqueous extract of *Atriplex lindleyi*.

(a) Medicine preparation

5 g of the dried plant material was boiled in 500ml of distilled water on a hotplate for approximately 15 minutes. This was left to cool down after which it was filtered and stored in a fridge until administration. The herbal medicine extract was prepared at a weekly interval.

(b) Metabolic Studies

Each of the ten rats per group were weighed individually. Exactly 4ml of either the *A. lindleyi* extract or the distilled H_2O was then administered to each individual rat by means of a specially made gavage needle. Each rat was then placed in a metabolic cage containing 40g food and 60ml distilled water for a 24 hour period. After this period the rats were again weighed and food consumption, water consumption, urine excretion, urine pH, and stool production was determined. The process was repeated every 15 days over a 45 day period. After the 45 day period the rats were terminated by using ether and chloroform as an anaesthetic. The study was conducted over a 45 day period at 15 day intervals. This arrangement would allow for the most appropriate assessment of the herbs safety within acceptable time limits.

(c) Hematology

At termination blood samples were taken from the left ventricle. Approximately 3ml of whole blood was collected in EDTA vials for biochemical analyses.

The following haematological parameters were assessed: red blood cell counts (RBC), white blood cell count (WBC), hemoglobin concentration, hematocrit value, platelet counts, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) using a microcell counter (CC-180A, Toa Medical Electronics, hyogo, Japan).

(d) Histology

The liver and testicles were removed from the rats. These were then fixed in a Bouins fixative. These organs were then processed and embedded in wax. The embedded organs were cut and slides were made for histological study.

2.5 Statistical Analyses

Data was statistically analyzed using Microsoft Excel Statistical Package, Version 2000. Due to the significant difference at the baseline for a number of parameters, corrections were made to data for the respective collections made over the experimental period, after which statistical analyses were applied. Control and experimental animal groups were compared with one another and a minimum value of $p \leq 0.05$ was accepted as significant. Significance for all metabolic data was determined by using the Mann-Whitney statistical test. Baseline corrections were not applied to blood data, which was directly compared. In this regard significant differences were also determined at a minimum level of $p \leq 0.05$ using the Mann-Whitney test.

2.6 Results

2.6.1 Plant Anatomy

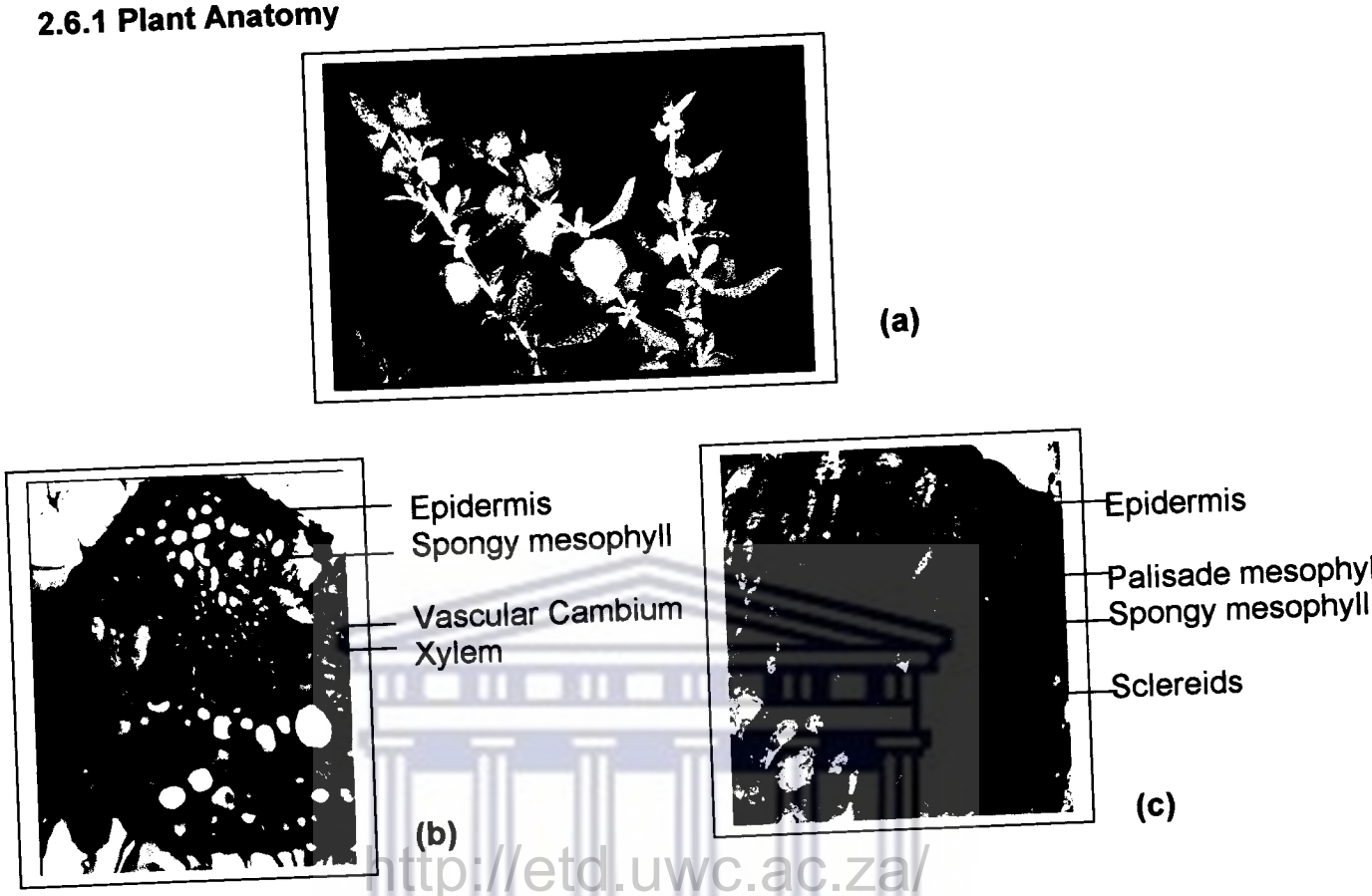


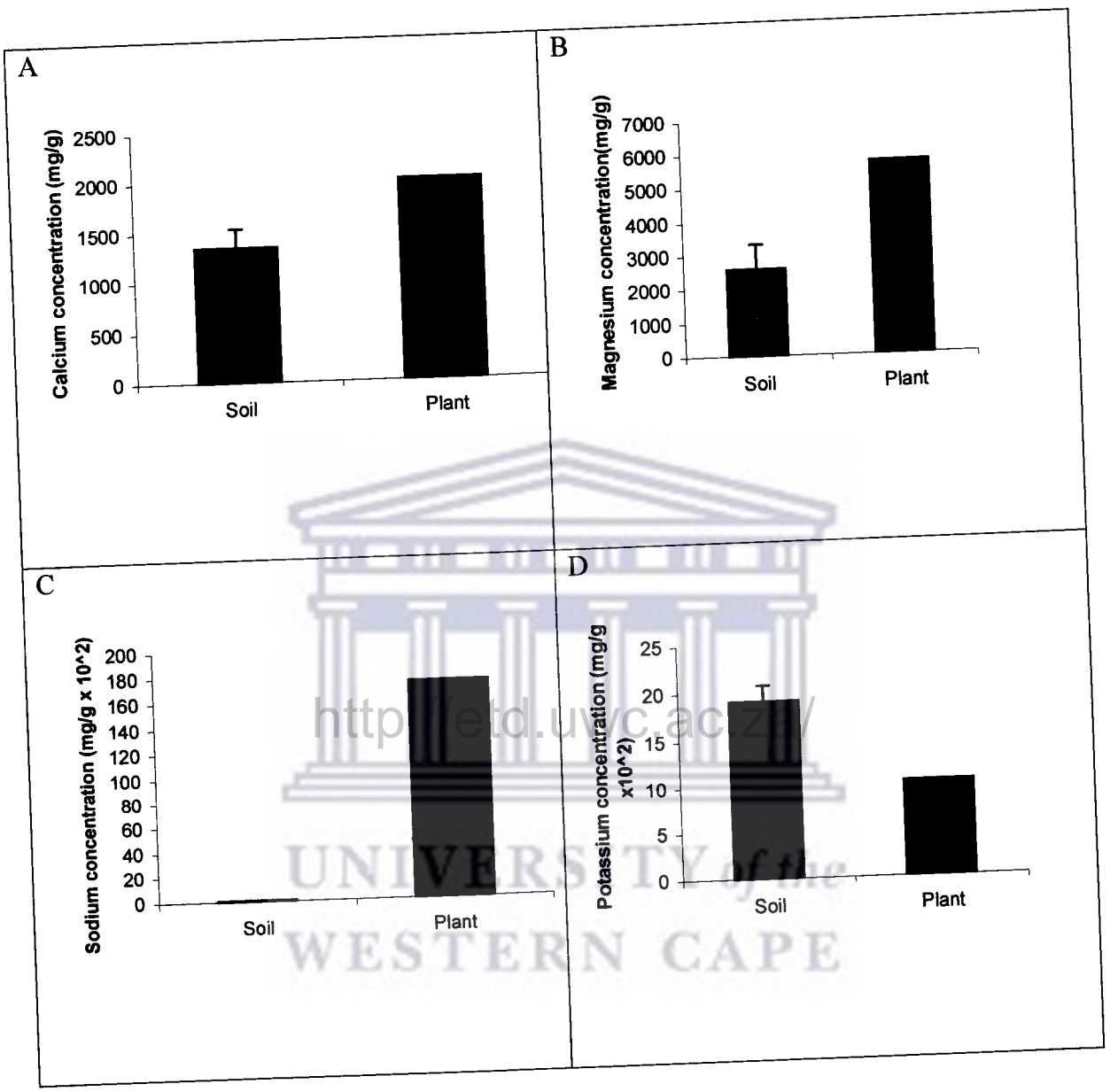
Fig. 1 The *Atriplex lindleyi* indigenous herb (a) whole leaf (b) leaf cross section[Mag 40x] and (c) leaf cross section [Mag 100x].

2.6.2 Soil pH

Table 1. pH of soil samples from *Atriplex lindleyi* showing Average and Standard Deviation(SD)

Soil samples	Site 1	Site 2	Site 3	Average and SD
pH value	8.22	8.90	8.05	8.39 ± 0.45

2.6.3. Elemental analysis



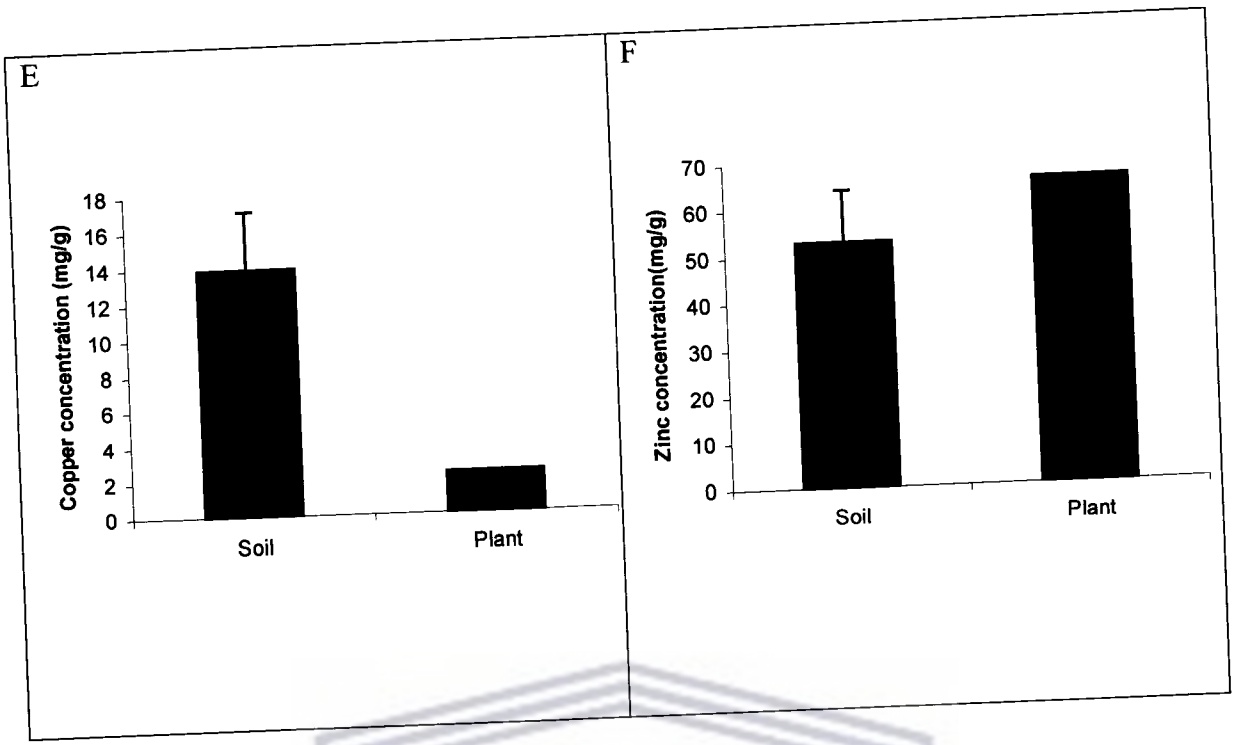


Fig. 2 A-F Elemental levels of *Atriplex lindleyi* (n=1)and the soil (n=3) in which it occurs.

2.6.4 Antimicrobial characteristics

Table 2 contains information that pertains to the effect of *Atriplex lindleyi* extracts on *S. aureus* , *P. aeruginosa, M. smegmatis* and *C.albicans*

Table 2: The effect of *Atriplex lindleyi* on growth inhibition (mm) of microbial pathogens at various concentrations (mg/ml)

		***Atriplex lindleyi* exctracts (mg/ml)**				
Micro-organism	Control	10	20	30	40	80
S. aureus	Cip(11mm)	-	-	-	-	-
P. aeruginosa	Cip(11mm)	-	-	-	-	-
M. smegmatis	Cip(12mm)	-	-	-	-	-
C.albicans	AMP(9mm)	-	-	-	-	-

2.6.5 Animal studies – Metabolic parameters

Metabolic parameters are represented by Fig 3 A – F.

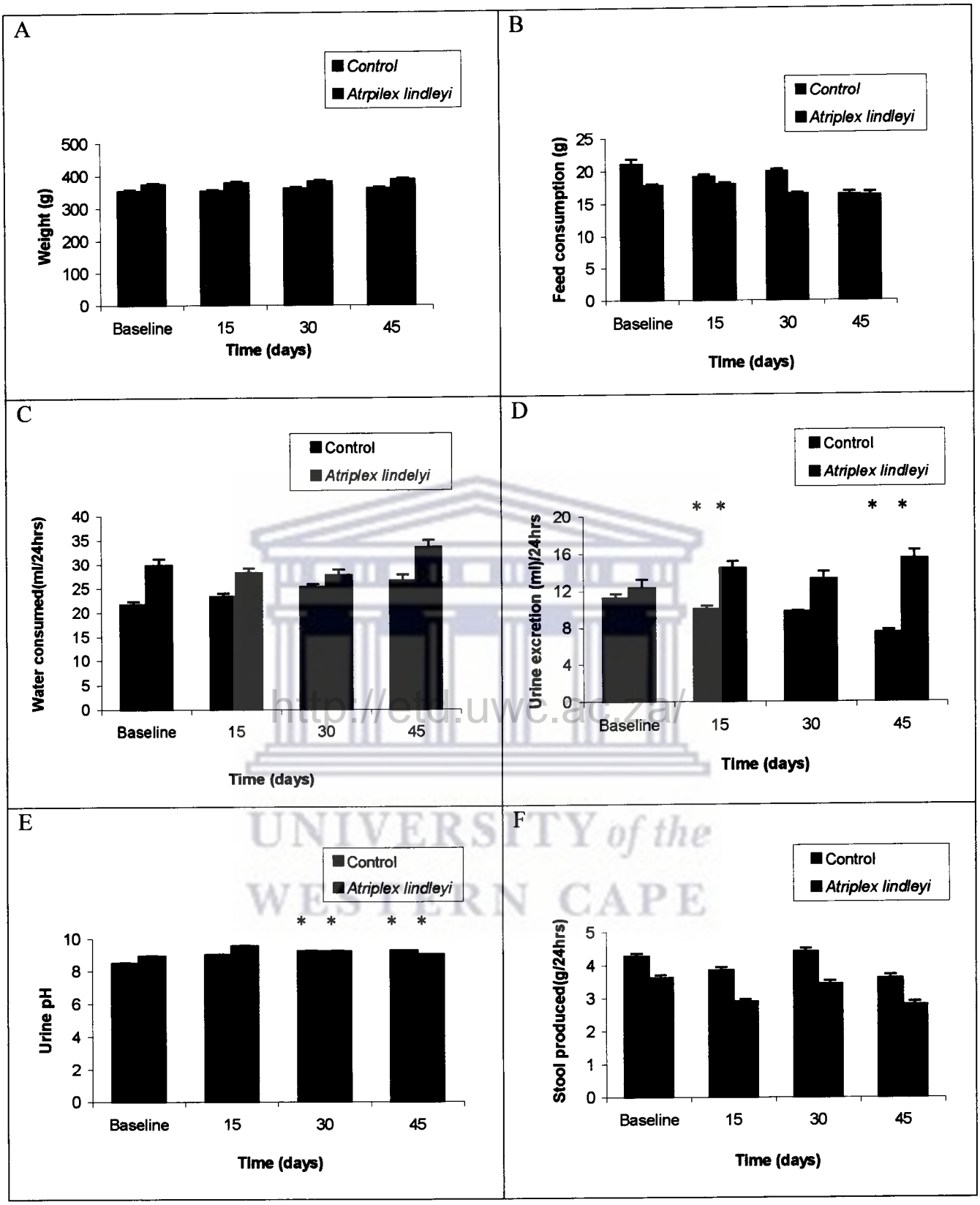
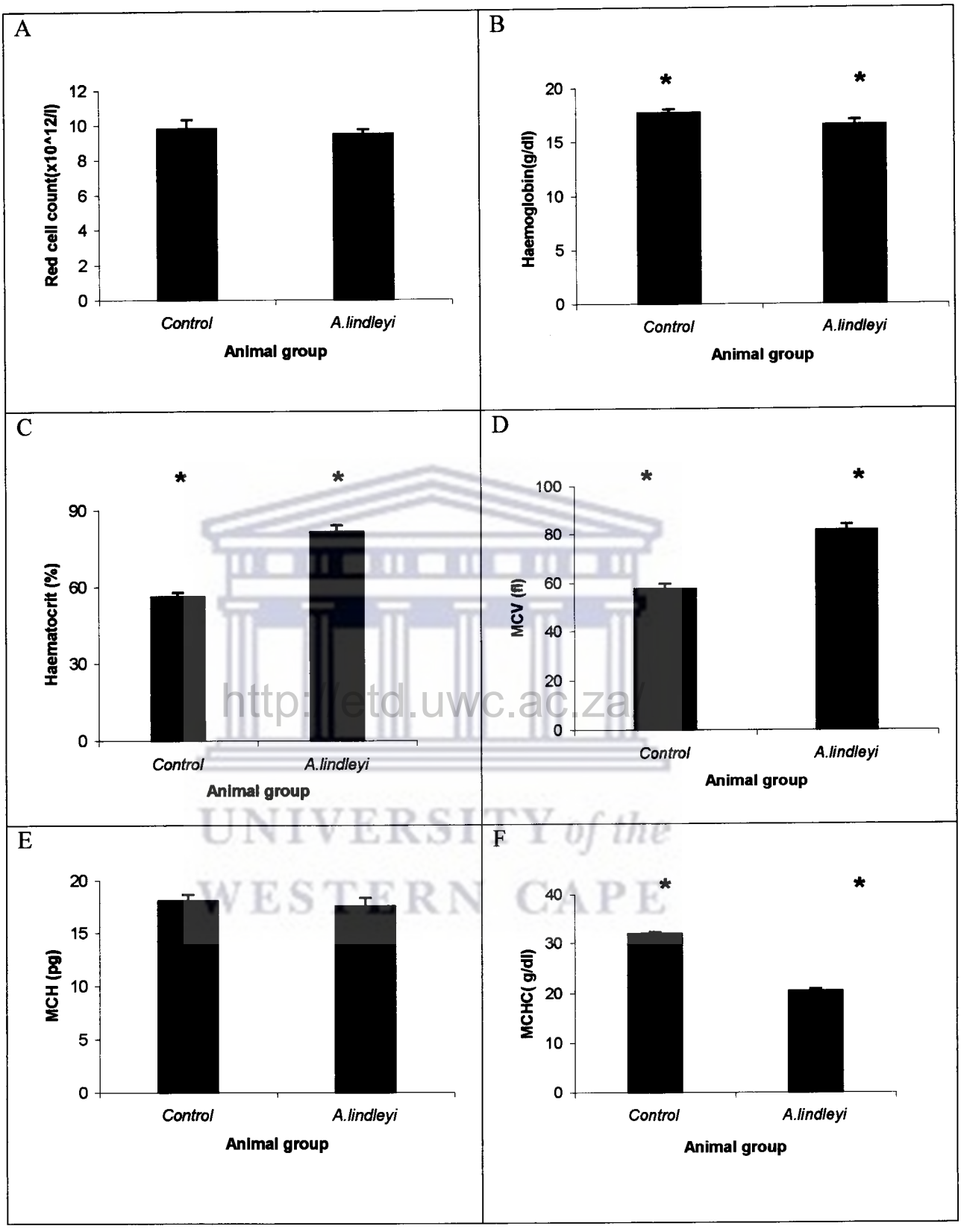


Fig 3A-F Metabolic parameters of male rats on *Atriplex lindleyi* monitored for 24 hrs over a 45 day period. * Significance between control and experimental group - $p \leq 0.05$ Panels with no stars have no significantly different data - $p \geq 0.05$. Baseline groups received no treatment.

2.6.6 Haematological indices.

Haematological indices are represented in Fig . 4 A - G and Fig. 5 A – L



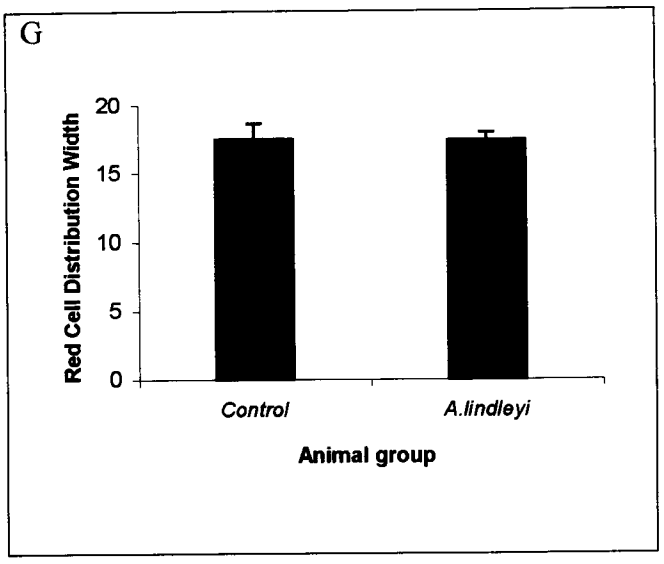
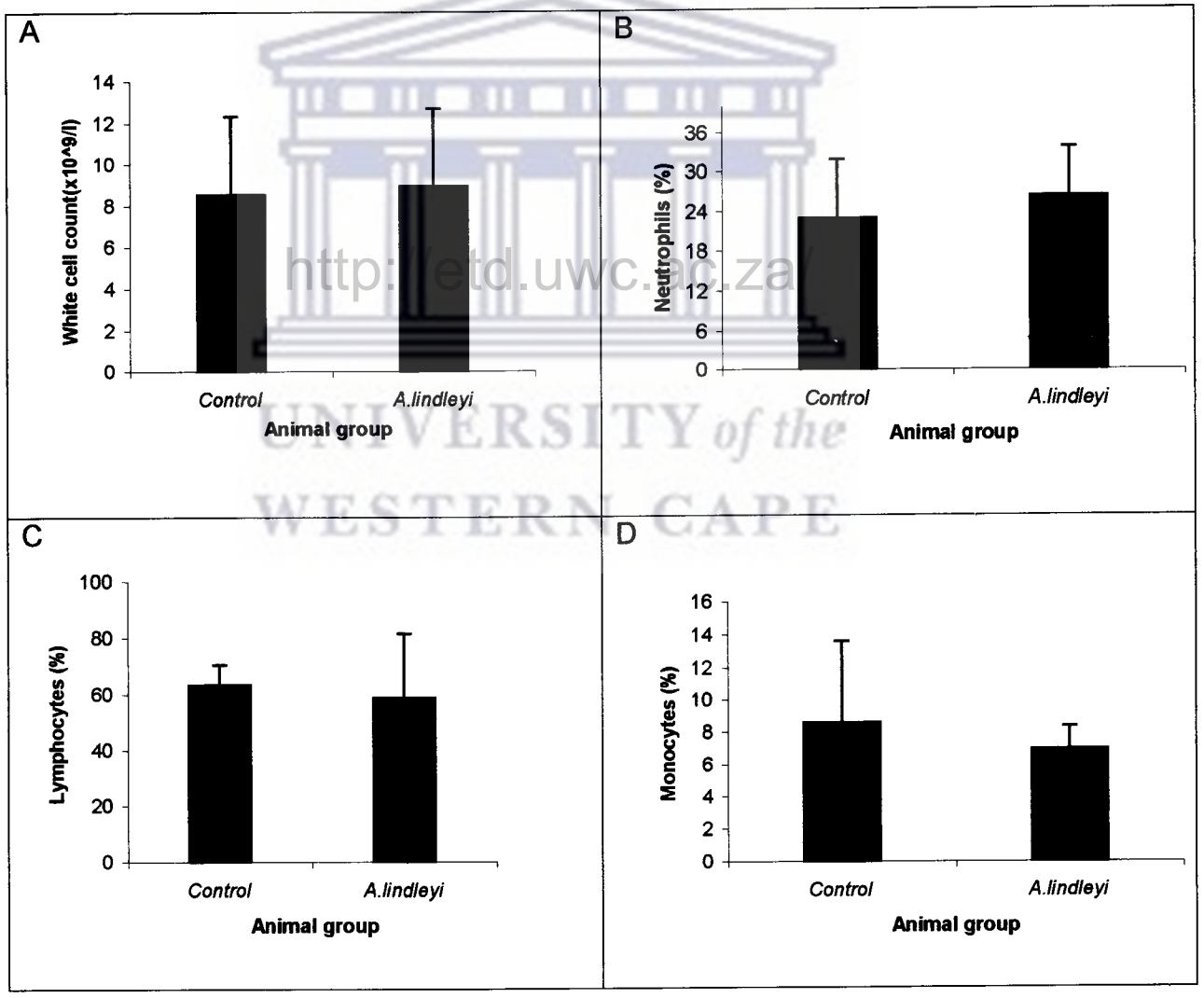
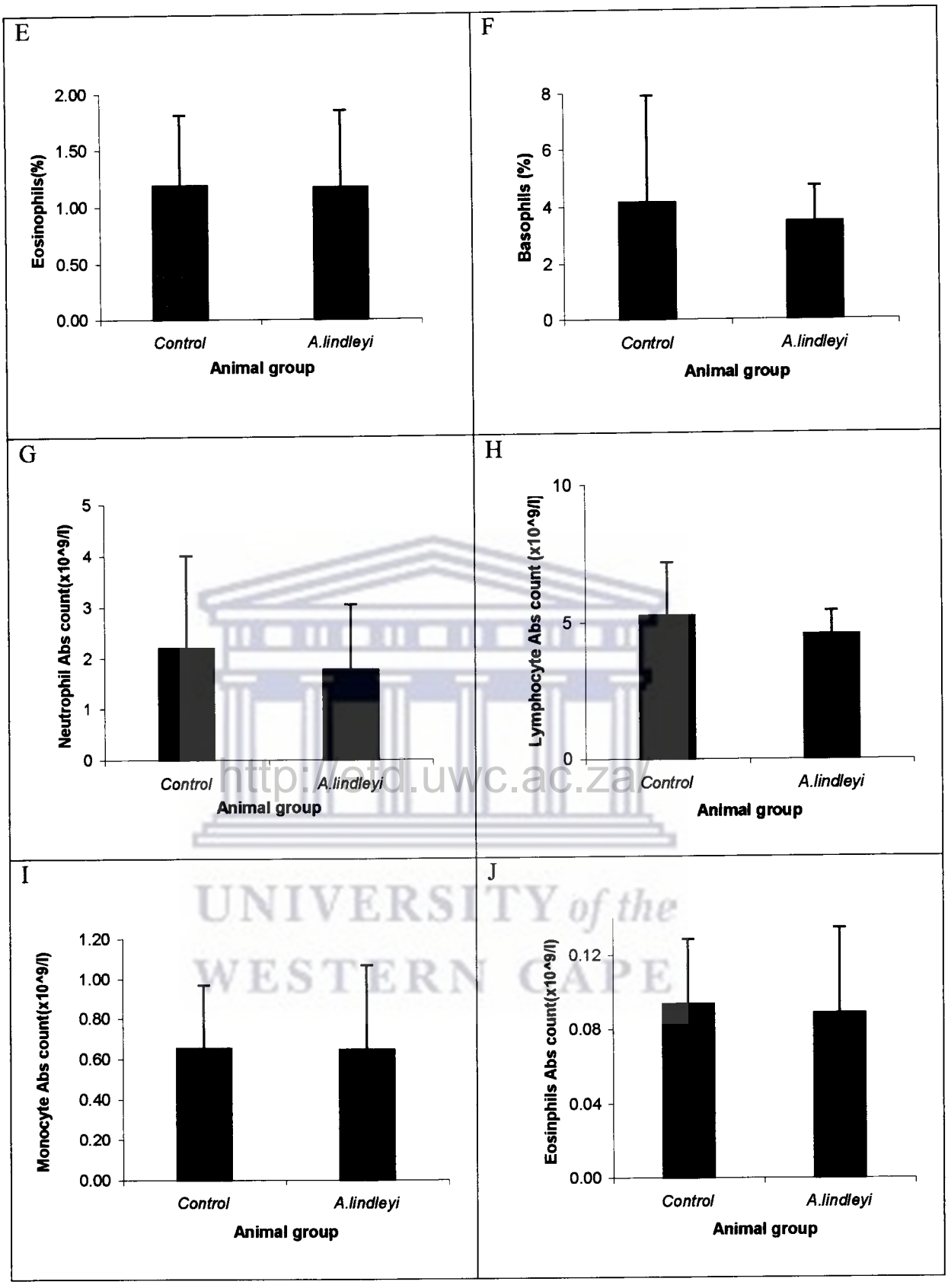


Fig 4A-G Red blood cell parameters of male rats on *Atriplex lindleyi* extracts over a 45 day period. * Level of significance – $p \leq 0.05$.
Panels with no stars, have no significantly different data - $p \geq 0.05$.





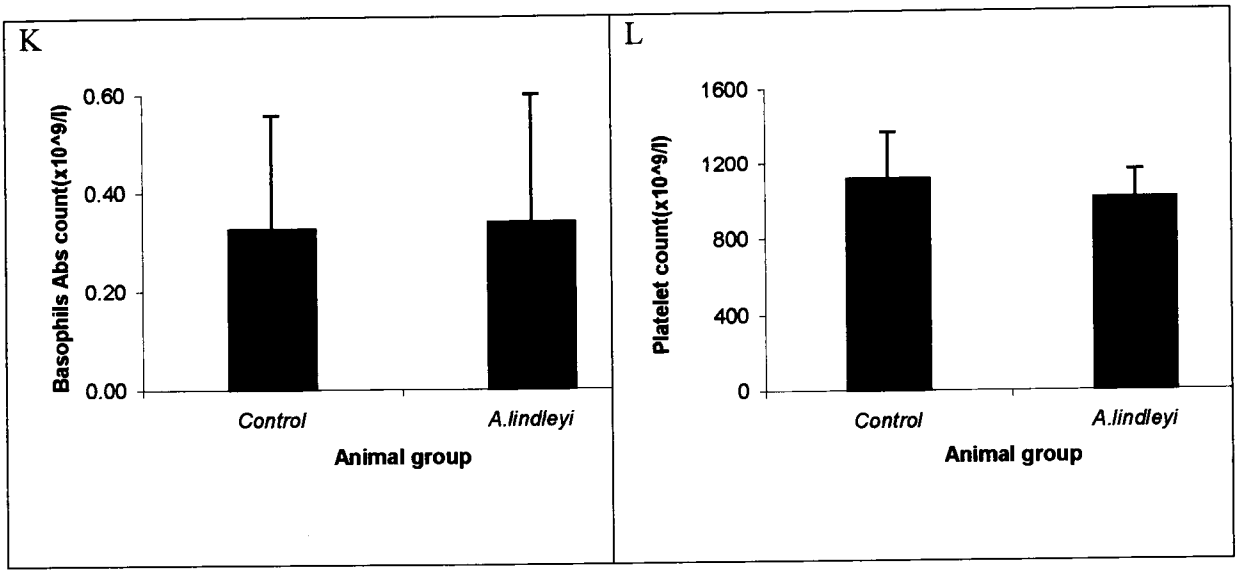


Fig5A-L White cell parameters of male rats on *Atriplex lindleyi* over a 45 day period Level of significance - $p \leq 0.05$. Panels with no stars, have no significantly different data - $p \geq 0.05$.

2.4.7 Animal Histology

Fig. 6 A – D reflects the macro-anatomy of the testes and liver, which are environments that are more sensitive to toxicological insult.

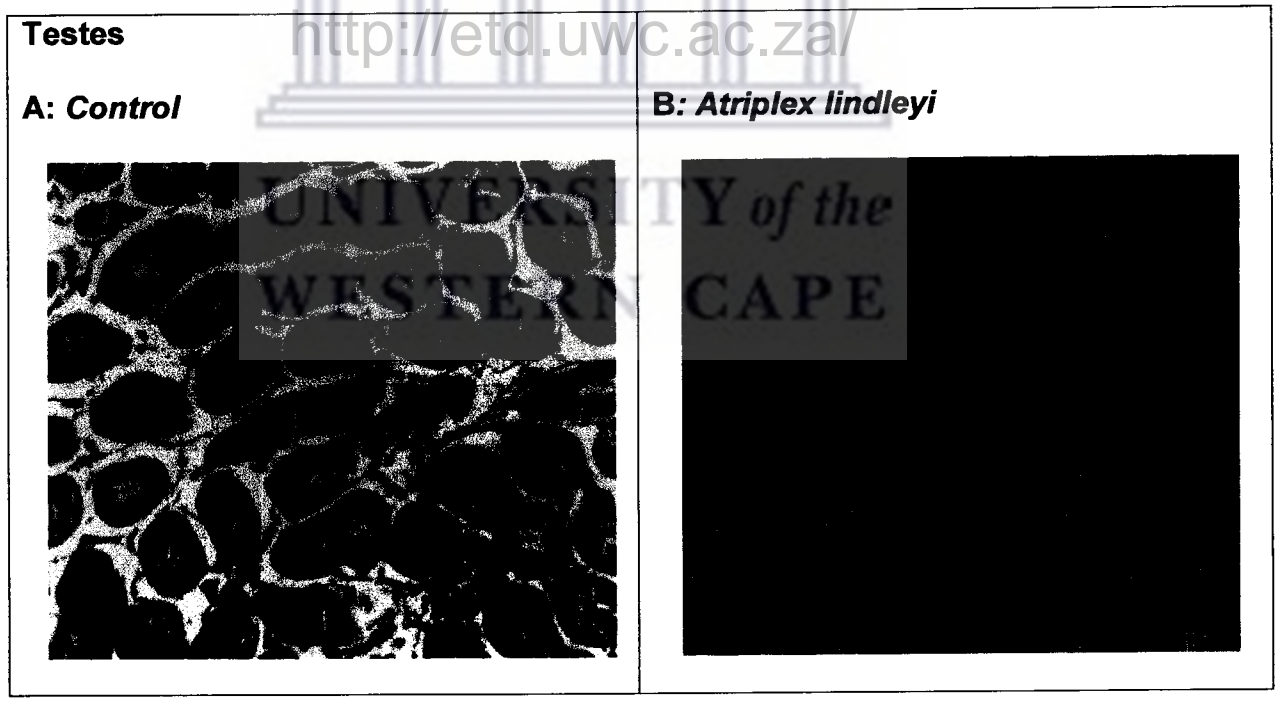


Fig 6. Cross sectional view of the testis of (A) the control group and (B) animals given *Atriplex lindleyi* extracts [Mag. 40x]

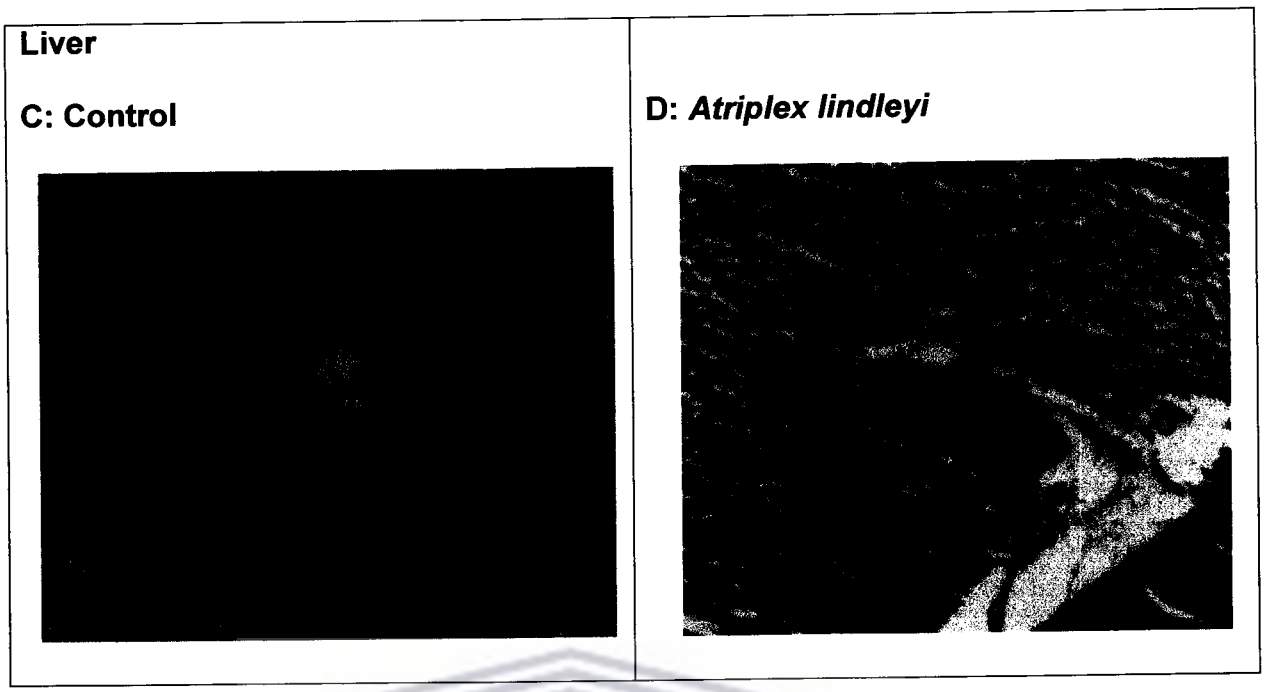


Fig 6. Cross sectional view of the liver of (C) control animals and (D) animals given *Atriplex lindleyi* extracts [Mag x200]

2.7 Discussion

Atriplex lindleyi can be classified as a C4 plant. These types of plants have different internal leaf structures and utilize carbon and energy efficiently at high temperatures. C4 plants are also more efficient in terms of water use.[15] The results from the plant anatomy slides show that the leaves of this plant contain a number of sclerieds. Sclereids are found in various different plant tissues and organs. They serve various protective, strengthening and other functions.[16] Sclereids called idioblasts can cause tissue to be coarse and gritty and less palatable to insects.[17-18] This could possibly mean that the sclereids in the leaves of this plant function as a protective mechanism against insects. However, this protective function gives no indication that these sclereids may have a potential role to play in the storage or production of secondary compounds that may provide the plant with its medicinal value. A more detailed study has to be done on the anatomy of this plant to determine the main organs for production and/ or storage of the secondary compound responsible for the medicinal vale of *Atriplex lindleyi.*

Soil pH influences many facets of crop production and soil chemistry, including availabilities of nutrients and toxic substances, activities and nature of microbial populations, solubility of heavy metals, and activities of certain pesticides. The effect of soil pH is great on the solubility of minerals or nutrients. Most minerals and nutrients are more soluble or available in acid soils than in neutral or slightly alkaline soils. The results in this study have shown that the soil in which *Atriplex lindleyi* grows is alkaline (Table 1). Extreme levels of acidity and alkalinity give rise to various toxicity problems due to the availability or inaccessibility of certain elements. The lime content of the soil is important because it is not only source of the major nutrient calcium but it has a very significant effect on the availability of other nutrients within the soil and also on the maintenance of many beneficial soil micro-organisms.

The determination of essential and toxic elements in medicinal plants are important for human health, obviously because these medicinal herbs are ingested in teas, elixirs, and even capsules.[19] The results portrayed in Fig. 2 A – F show that in general the elemental concentrations were higher in the soil that in the plant samples. These results were however not significant, since no statistical analyses were done. It would appear as if the sodium concentration in the plant samples were very high (Fig. 2 C). This may be because *Atriplex lindleyi* is a halophytic plant and absorbs high amounts of sodium from the soil. The copper concentration in the plants also appears to be higher than in the soil though not significantly (Fig. 2 E). In green leaves and important role played copper is in Superoxide Dismutase (SD), a copper and zinc containing enzyme in chloroplasts in higher land plants.[20] Further analysis should be done on the copper content in *Atriplex lindleyi* , since SD is a powerful antioxidant. If the copper content in *Atriplex lindleyi* is found to be significant it could mean that the plant exhibits powerful antioxidant value to perhaps help fight ageing in cells.

Table 2 shows the effect of the *Atriplex lindleyi* extract against the four microbes tested. The results have shown that *Atriplex lindleyi* had no effect on any of the microbes against which it was tested (Table 2). This might be because a higher concentration of the extract was not tested. Antimicrobial activity have been found in other Atriplex species.[21]

There is always a possibility that metabolism and health of an animal can be affected by what is ingested. The medicinal herb extract had no effect on animal weight (Fig 3 A). A similar trend was observed for food consumption, that did not differ ($p \geq 0.05$) between the groups (Fig 3B). The water consumption between the two groups also showed no difference ($p \geq 0.05$) (Fig 3 C).

The urine excretion for the experimental group increased significantly on during days 15 and 45 ($p \leq 0.05$). The control rat group showed a significant decrease in urine excretion ($p \leq 0.05$) (Fig 3D). The significant difference between urine excretion of the control group and the experimental group could be an indication that the plant acts as a diuretic. *Atriplex hortensis* is considered a diuretic, emetic, and emollient and has been suggested as a folk remedy for plethora and lung ailments.[22] The increased urine excretion could be an indication that *Atriplex lindleyi* also has diuretic effects.

From Fig 3 E it is clear that the urine of both experimental and control groups are alkaline throughout the study. Whilst there are significant ($p \leq 0.05$) differences in urine pH on days 15 and 45, the urine of the experimental group remained alkaline. The latter might well be connected to the alkalinity of the soil in which the plant occurs. Although it appeared as though the control group excreted more stool, no significant difference ($p \geq 0.05$) were found between stool productions of the two groups (Fig 3 F).

The red cell count is a measure of the total amount of erythrocytes in the blood sample. The red cells are the most numerous of the cellular elements and they carry oxygen to the lungs to the body tissue.[23]

The red cell count between the two rat groups (Fig 4 A) showed no significant difference ($p \geq 0.05$). However, haemoglobin, which is the protein-iron compound in the red blood cells that enables them to transport oxygen, was affected by the herbal extract. In fact there was a significant difference ($p \leq 0.05$) in the haemoglobin levels between the control and experimental rat groups, with the latter having lower levels of this compound (Fig 4 B). On the other hand, the haematocrit levels showed a significant difference ($p \leq 0.05$) with the rats on *A. lindleyi* having a higher haematocrit percentage compared with the control group (Fig 4C).

The rats on *A. lindleyi* had a significantly higher MCV count ($p \leq 0.05$) than the rats on the control group (Fig 4D). The MCH level showed no significant difference (Fig 4E).

The MCHC count was significantly higher for the control group than for the experimental group (Fig 4F). No significance ($p \geq 0.05$) was found for the RDW levels (Fig 4 G). It would appear from the red cell parameters, that the herbal extract had variable effects that depress haemoglobin indices. The latter could well impair oxygen transport, even though the herbal extract boosts the mean cell volume in animals that consume this herbal medicine.

The herbal extract did however not have any significant effects on the white blood cell parameters ($p \geq 0.05$) indicating that the medicine had no effect on the white blood cells (Fig5A-L). Studies done on various medicinal plants have shown that these herbal remedies help to boost the immune system.[24-25]

The safety of herbal extracts is always a matter of enquiry, as is the care with any other orthodox or alternative medicine. In this regard, any potential toxicity may well be more pronouncedly reflected in testicular and liver tissue, since these environments are particularly more sensitive to environmental insult. The macro histology of the testis (Fig 6A & B) and liver (Fig 6C & D) of the control and experimental group shows no significant differences in their macro-architecture. Studies done on the effects of medicinal plants have shown various effects on the reproductive systems in animals.[26] Varying results have also been found for studies done on the liver.[27-28]

The medicinal herbs showed no anitimicrobial activity, but the metabolic studies have shown that it might possible have diuretic value. Regardless of the herbs' input on certain blood biochemical indices, all of which remained within physiologically normal limits, its safety profile is fairly favourable, thus further studies should be pursued on this plant.

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Chapter 3: The effect of *Exomis microphylla* on metabolism and health.

3. Abstract

Through the years, various studies have been done on the effects of medicinal plants, with a strong focus on their anti-infective properties. The following study was undertaken to assess the anti-infective value of *Exomis microphylla* and to determine how the plant extract affects the metabolism, and health of male rats. Plant anatomy was done to determine which components are present in the plant leaves and to assess which components might be responsible for the production compounds. The leaves were sectioned using a freeze microtome. Liquid CO_2 and Hamilton's freeze solution was used to freeze the plant leaves and sections were made ranging from 15-25 microns. Elemental analysis was completed to determine the concentration of selected elements within the plants. The analyses were done using the Unicam Solaar M series atomic absorption spectrometer. Four microbes were used for the antimicrobial screening. Various concentrations of the plant extract was prepared and tested against *Staphylococcus aureus* (ATCC29213), *Pseudomonas aeruginosa* (ATCC27853), *Mycobacterium smegmatis* and *Candida albicans* (ATCC10231). Amphotericin B served as the positive control for *C. albicans* and Ciprofloxacin was used for the three bacterial strains. Screening was done using the disc diffusion method. Metabolic studies were done on 20 Wistar male albino rats. This was done to assess the metabolic, haematological and histological effects of the herbal extract on the animals. The leave anatomy did not show any structures that could be responsible for the production or storage of secondary compounds, which provides the plant with its medicinal value. The elemental concentration of the elements tested was seemingly higher in the plant than in the soil samples. This was however not statistically significant. The plant extract showed antimicrobial activity at 40μg/ml and 80μg/ml concentrations against *S. aureus*. The inhibition zones was however only between 1mm and 3mm. The metabolic analyses showed significant increase in urine excretion ($p \leq 0.05$) on days 30 and

45 for the rats on the herbal extract. Urine pH was also significantly higher ($p \leq 0.05$), and the stool production was significantly lower on day 45 ($p \leq 0.05$). The *Exomis microphylla* extract was responsible for a significant decrease ($p \leq 0.05$) in the haemoglobin levels of the rats receiving this extract. The haematocrit levels were significantly increased ($p \leq 0.05$) for the rats receiving the herbal extract, as was the mean cell volume ($p \leq 0.05$). MCHC levels decreased significantly for the rats on the herbal extract ($p \geq 0.05$). The white blood cell parameters remained unchanged showing no significant differences ($p \geq 0.05$). Histological sections done on the liver and testes showed no differences between the control and experimental rat groups.

The medicinal herb showed antimicrobial activity at 40μg/ml and 80μg/ml concentrations against *S. aureus* and may also have diuretic value. Since it has a favourable safety profile, further studies on this medicinal plant should be pursued even though it had an input on certain blood biochemical indices, all of which though remained within physiologically normal limits.

3.1 Introduction

Plants have been used as herbal remedies for many decades. Original inhabitants such as the Khoi and San people have long since been using plants for medicinal purposes. Because of factors such as the high cost of conventional medicine other local communities have also been making use of traditional medicine. [1] At least 12-15 million South Africans are still dependant on medicinal plants from at least ±700 indigenous plant species[2-3] and it has been reported that there are over 100 000 indigenous healers practicing in South Africa and using indigenous plants as their *materia medica.* There has been considerable growth in the medicinal plant industry in South Africa over the past few years. Large urban markets (e.g. Durban and Johannesburg) have developed for trade in traditionally used medicinal plants and products.

Because of the high demand for various popular medicinal plant species, traders are now reporting acute shortages and price increases. To date, several plant species, such as wild ginger (*Siphonochilus aethiopicus*) and the pepper-bark tree (*Warburgia salutaris*) have been exploited to such an extent that they are seldom found in unprotected areas.[4]

In recent years antibiotics have been used extensively and this has resulted in the emergence of numerous resistant bacterial strains. Researchers are now looking towards alternatives especially natural medicines to combat such problems. Data from most of the randomized clinical trials performed with the top selling botanical supplements suggest that most are at least mildly effective against a specific indication.[5-6] However, various Botanical supplements are frequently criticized for poorly proven efficacy and safety, lack of standardization and quality standards[7-8], and potential interactions with prescribed drugs.[9] Very little information is known about *Exomis microphylla,* thus in doing the following study we aim to scientifically substantiate the efficacy and safety of *Exomis microphylla.*

3.2 Distribution and anatomy of *Exomis microphylla*

The family Chenopodiaceae consists of ±100 genera with ± 1500 species. It is cosmopolitan, but particularly common in semi-arid environments and in saline habitats. Few of the species have given rise to cultivars of agriculture and many occur as weeds of cultivation. Southern Africa has 14 genera of which 2 are exotic and 164 species have been identified.

One of the Southern African genera is *Exomis.* These plants are sub shrubs with dichotomous branching and are usually ashy grey in colour with simple hairs. They are monoecious with few bisexual flowers.

This genus has only one species which is confined to dryish areas of Southern Africa, i.e. Namibia, Free State, Norhern Western and Eastern Cape. *Exomis microphylla* is a grey-mealy shrub that grows up to 1m. The leaves are ovate, oblong or sagittate. These plants are usually found on stony hillsides and are often coastal.

3.3 Ethics

This investigation received ethical approval from the University of the Western Cape.

3.4 Materials and Methods

3.4.1 Plant collection

The species *Exomis microphylla* was collected at the Pauline Bohnen Nature reserve, Stilbaai in the Western Cape. The species can be classified as coastal fynbos. It was found growing on lime stone in sandy loam. The area was well-drained and exposed to full sunlight. The aspect of growth was south and it was found on a gentle slope.

The man-made road alongside which it was found could influence its growth pattern. Taxonomist Dr Frans Weitz verified the identity of the plants. Voucher specimens were deposited in the Herbarium of the Botany Department and the University of the Western Cape and a sample of the plants was also placed in FAA (Formaldehyde, Acetic acid and Alcohol) for later anatomical studies.

3.4.2 Plant Anatomy

The anatomy of all plants was completed by using a freeze microtome. A small section of the leaf, which is used as a medicine, was cut out and then mounted on the freeze-microtome to be sectioned. Liquid CO_2 gas and Hamilton's freeze solution was used to freeze the plant sample and sections were made ranging from 15-25 microns. The sections were fixed on slides and stained with a safrinin red/alcian blue stain.

Each slide was viewed under an Olympus photomicroscope and the images were captured digitally.

3.4.3 Soil samples

Three soil samples were collected at the site where the plant was located. In the laboratory the soil was oven dried at 30 – 40 °C for a 48hr period.

These samples were collected in order to determine elemental concentration within the soil. Soil pH was also determined using an A PHM83 Autocal pH-meter pH meter.

3.4.4 Phytochemistry

Extraction

Chemical Extraction of the plant was done in the laboratory. The fresh plant material was left in methanol for ± three days. It was then filtered and the methanol was then evaporated by means of a rotary evaporator. Another methanol extraction was done, but the plant material was blended. This was repeated to make sure that all the compounds were extracted. This was then again evaporated using a rotary evaporator. The dried extract was then frozen overnight after which it was freeze dried. The freeze-dried extract was put in a 5°C cold room.The remaining plant material was oven dried at 30–40 °C for a 72 hr period. The dried plant material was used for Atomic absorption spectrometry (AA).

3.4.5 Elemental analysis of *Exomis microphylla*

Soil and Plant Digestion

Soil and plant material were prepared using acid digestion.[10] This type of digestion of an organic material is an oxidizing system and has the advantage that phosphorus and in some cases, even nitrogen can be determined on the final solution along with other nutrients. Sulphuric acid is added to the digestion mixture to reduce the possibility of desiccation. The acid system used was Hydrogen Peroxide – Sulphuric Acid. This is suitable for nitrogen in addition to sodium, potassium, calcium, magnesium, iron, manganese and phosphorus.

Procedure

To prepare the digestion mixture, 0.42 g Se and 14 g of $Li_2SO_4.H_2O$ were added to 350 ml H_2O_2. This was mixed well and then 420 ml of concentrated H_2SO_4 was carefully added to the mixing vessel, which was surrounded by ice to keep the mixture cool.

Four grams of dried soil was weighed into a glass tube (0.8 g dried ground plant material was used) and 8.8 ml of the digestion mixture was added.

This mixture was then digested at moderate heat setting of 200°C until the initial reaction subsided, after which the heat level was incrementally raised by 50°C every hour to a maximum of 350°C. The digestion process proceeded until a clear, almost colorless solution was obtained. This solution was filtered and diluted with distilled H_2O to a volume of 100ml. A further five fold dilution was necessary in order to obtain a 1% H_2SO_4 solution. Furthermore, blank solutions were also prepared as reference samples, whilst a 200ml stock solution was also prepared.

Dilutions of samples for Atomic Absorption Spectrometry

A 1% H_2SO_4 solution was made by using a 10 ml of 98% H_2SO_4 solution topping it up with water to a volume of 980 ml. A 1000mg/l Merck Ca, Na, Mg, K, and Cu stock solution was used to prepare the six standards used for the AA analyses. This was topped up with 1% H_2SO_4 in a volumetric flask in order to obtain a concentration of 100mg/l. These stock solutions were then used to prepare standards ranging from 0.05mg/l – 50mg/l. An air acetylene flame was used to determine all the elemental concentrations.

Each sample was diluted by using 15 ml of the sample and topping it to 75 ml with distilled water in order to obtain the 1% H_2SO_4 matrix. The samples were run through the Unicam Solaar M series atomic absorption spectrometer for elemental analysis using the flame mode. The following equation was used to determine the elemental concentrations:

$$\text{Total element \%} = \frac{C(mg.l^{-1}) \times sol.volume(ml) \times Dil.factor}{10^4 \times sampleweight(g)}$$

C =Concentration

Sol.vol = Solution volume

Dil.factor = Dilution factor

3.4.6 Antimicrobial screening of *Exomis microphylla*

The previously freeze-dried methanol extract was redissolved and various concentrations of the extract were prepared ranging from 10mg/l-80mg/l.

(a) Micro-organism and growth media.

Four microbes were used for antimicrobial screening. The extracts were tested against the micro-organisms *Staphylococcus aureus* (ATCC29213), *Pseudomonas aeruginosa* (ATCC27853), *Mycobacterium smegmatis* and *Candida albicans* (ATCC10231). All the micro-organisms were obtained from the Medical Biosciences Department at the University of the Western Cape. The *Mycobacterium smegmatis* was obtained from Tygerberg Hospital, in Tygerberg City.

(b) Disc diffusion test

Individual nutrient broths containing *Staphylococcus aureus, Pseudomonas aeruginosa, Mycobacterium smegmatis* and *Candida albicans* were streaked onto nutrient (Difco) and Mycobacteria 7H11 (Difco) agar plates respectively. The agar plates were streaked with sterilized swabs that were inserted into the suspensions. The plates were spread in three different directions to ensure an even-growing bacterial and fungal mat. Sterilized nine-mm filter paper discs were impregnated with 50µl of the methanol and aqueous plants extract in concentrations ranging from 10mg/l – 80mg/l. These were placed at equidistant spots on the inoculated agar plates. Methanol and water discs were used as controls. Amphotericin B (AMP) served as the positive control for *C. albicans* and Ciprofloxacin (Cip) was used for the three bacterial strains. The positive controls were supplied by the companies Bristol-Meyers Squibb and Bayer respectively. The plates were incubated at 37°C for 24 hours with the exception of *Mycobacterium smegmatis* that required a 48 hour growth period. All extracts were tested in triplicate. Inhibition zones were recorded at the end of the incubation period by measuring the growth–free zone between the discs and the bacteria and/or fungal yeast.

3.4.7 Animal studies

20 Wistar male albino rats were supplied by the Medical Research Council (MRC) and used in the experiment. These were divided into two groups of ten each. The rats were housed in pairs of two in plastic cages with mesh wire flooring and tops, in a temperature-controlled room. The cages were 35cmx35cm with a height of 23cm. One group was used as a control group receiving only distilled water as a substitute for the herbal extract, whilst the second group was medicated with an aqueous extract of *Exomis microphylla.*

(a) Medicine preparation

A 5 g bundle of oven dried plant material was boiled in 500ml of distilled water on a hotplate for approximately 15 minutes. This was left to cool down at room temperature after which it was filtered. The filtrate was then stored in a fridge until administration, whilst the residue was discarded. The herbal medicine extract was prepared at a weekly interval.

(b) Metabolic Studies

Each of the ten rats per group were weighed individually. Exactly 4ml of either the *E. microphylla* extract or the distilled H_2O a specific treatment was then administered to each individual rat by means of a specially made gavage needle. Each rat was then placed in a metabolic cage containing 40g food and 60ml distilled water for a 24 hour period. After this period the rats were again weighed and food consumption, water consumption, urine excretion, urine pH, and stool production was determined. The process was repeated every 15 days over a 45 day period. After the 45 day period the rats were terminated by using ether and chloroform as an anaesthetic.

(c) Hematology

At termination blood samples were taken from the left ventricle. Approximately 3ml of whole blood was collected in EDTA vials for biochemical analyses.

The following haematological parameters were assessed: red blood cell counts (RBC), white blood cell count (WBC), hemoglobin concentration, hematocrit value, platelet counts, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) using a microcell counter (CC-180A, Toa Medical Electronics, hyogo, Japan).

(d) Histology

The liver and testicles were removed from the rats. These were then fixed in a Bouins fixative. These organs were then processed and embedded in wax. The embedded organs were cut and slides were made for histological study.

3.5 Statistical Analyses

Data was statistically analyzed using Microsoft Excel Statistical Package, Version 2000. Due to the significant difference at the baseline for a number of parameters, corrections were made to data for the respective collections made over the experimental period, after which statistical analyses were applied. Control and experimental animal groups were compared with one another and a minimum value of $p \leq 0.05$ was accepted as significant. Significance for all metabolic data was determined by using the Mann-Whitney statistical test. Baseline corrections were not applied to blood data, which was directly compared. In this regard, significant differences were also determined at a minimum level of $p \leq 0.05$ using the Mann-Whitney test.

3.6 Results

3.6.1 Plant Anatomy

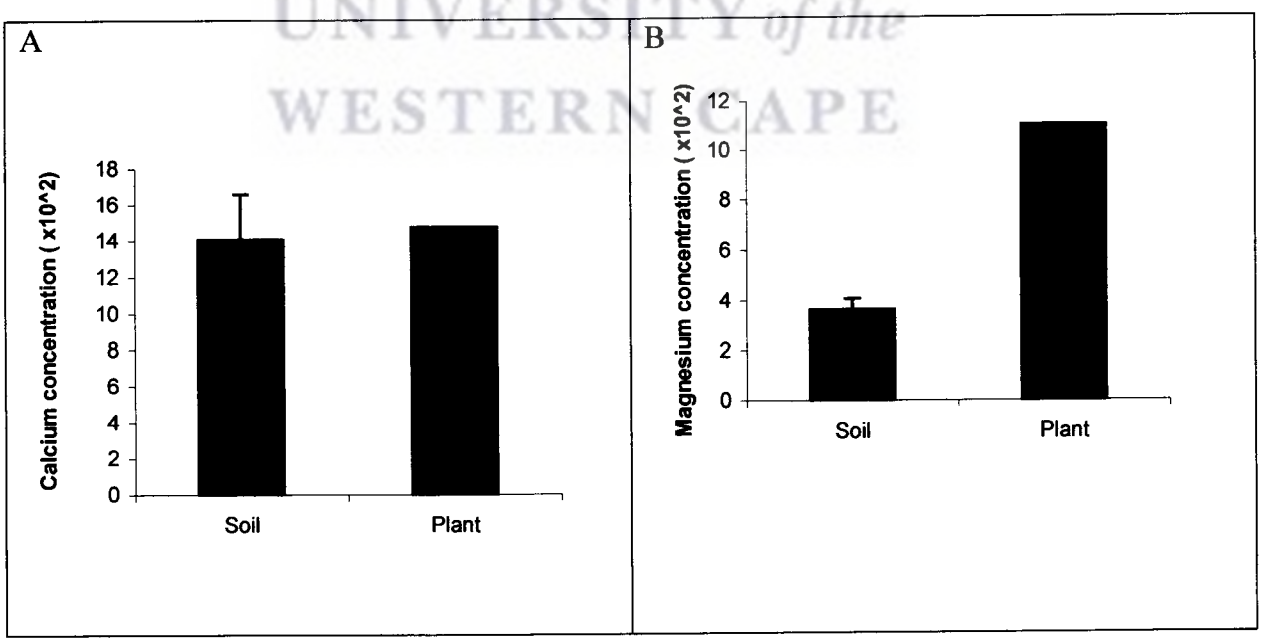
Fig 1. Cross section of the leaf of *Exomis microphylla* (Mag 40x)

3.6.2 Soil pH

Table 1. pH of soil samples from *Exomis microphylla* showing the average and standard deviation (SD) of the pH.

Soil samples	Site 1	Site 2	Site 3	Average and SD
pH value	8.17	8.24	8.03	8.15 ± 0.11

3.6.3 Elemental analysis



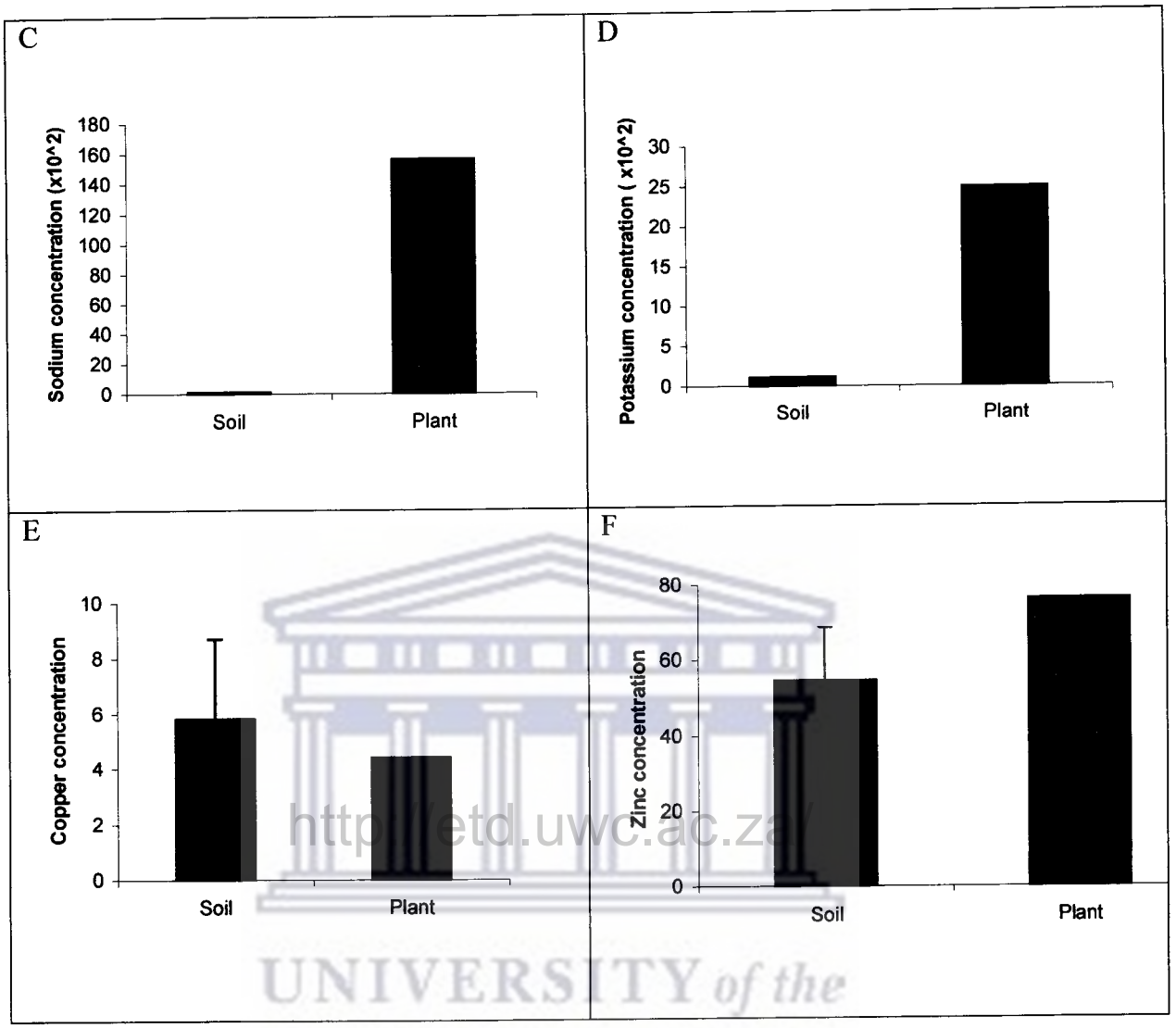


Fig. 2 A-F. Elemental levels of *Exomis microphylla* (n=1)and the soil (n=3) in which it occurs. All concentrations were measure in μg/g.

3.6.4 Antimicrobial characteristics

Table 2 contains information that pertains to the effect of *Exomis microphylla* extracts on *S. aureus* , *P. aeruginosa, M. smegmatis* and *C.albicans.*

Table 2: The effect of *Exomis microphylla* on growth inhibition (mm) of microbial pathogens at various concentrations (mg/ml)

		***Exomis microphylla* exctracts (mg/ml)**				
Micro-organism	Control	10	20	30	40	80
S. aureus	Cip(12mm)	-	-	-	2mm	3mm
P. aeruginosa	Cip(11mm)	-	-	-	-	-
M. smegmatis	Cip(12mm)	-	-	-	-	-
C.albicans	AMP(10mm)	-	-	-	-	-

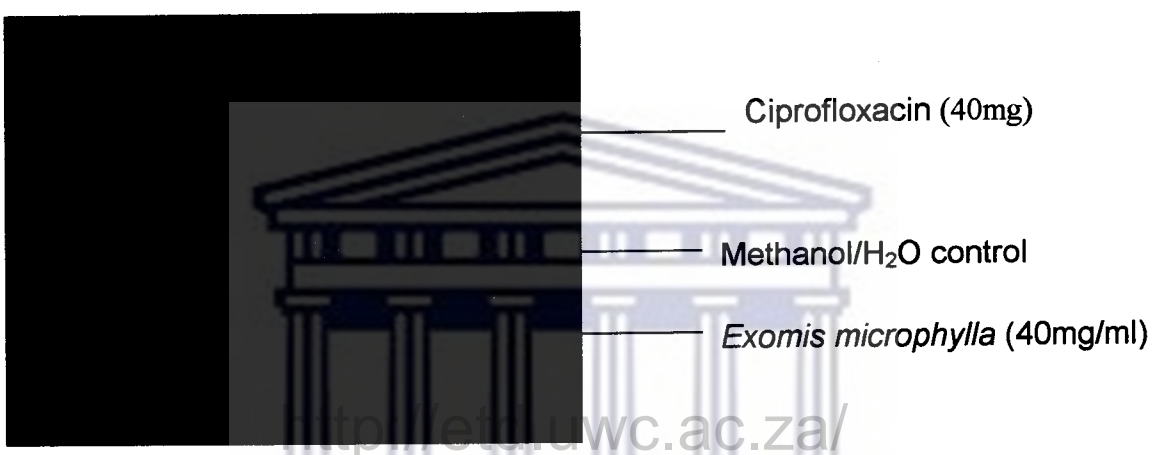


Fig 3a. *S. aureus* agar plate showing inhibition zones at a 40mg/ml concentration *E.microphylla*

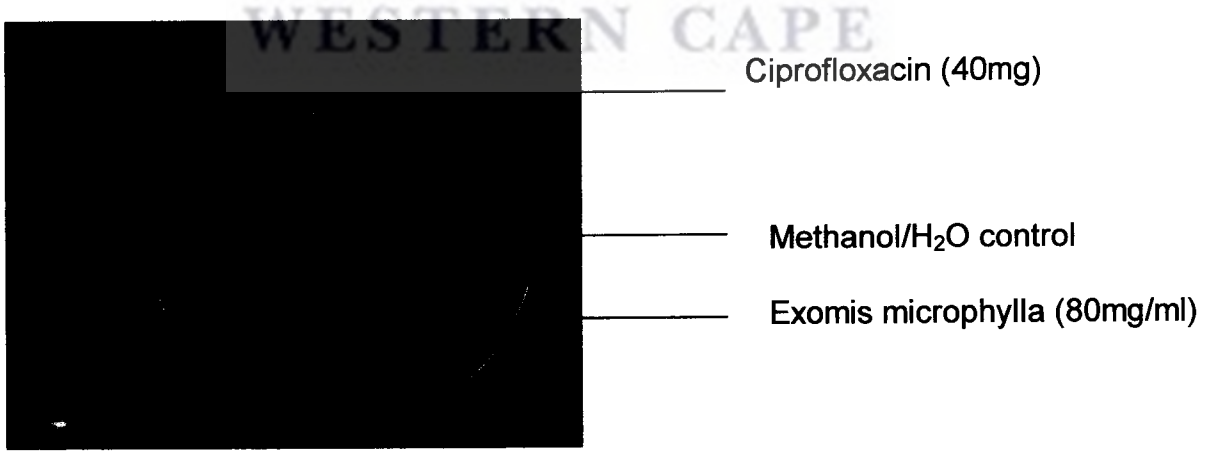


Fig 3b. *S. aureus* agar plate showing inhibition zones at 80mg/ml concentration *E.microphylla*

3.6.5 Animal studies – Metabolic parameters

Metabolic parameters are represented by Fig 4 A – F.

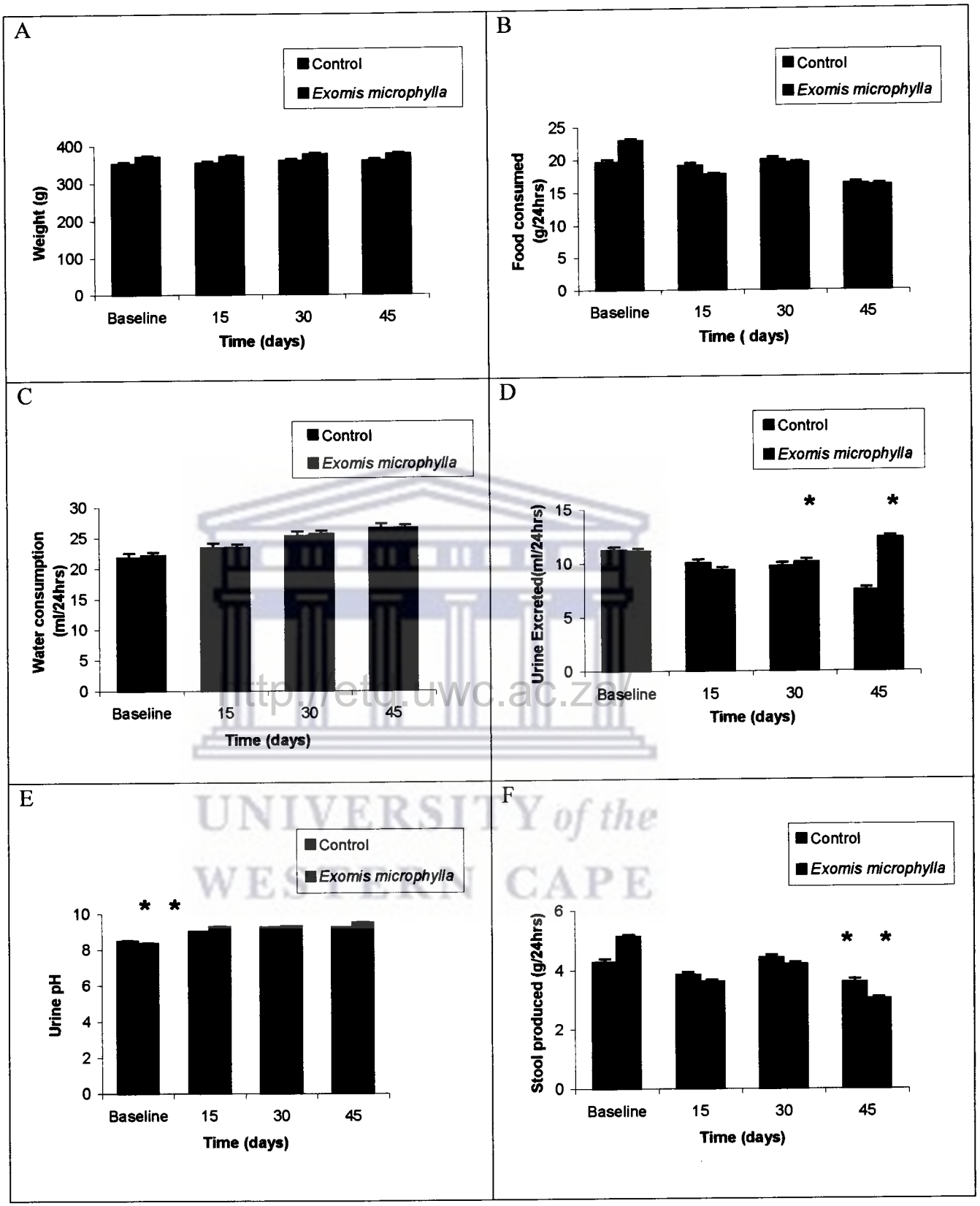
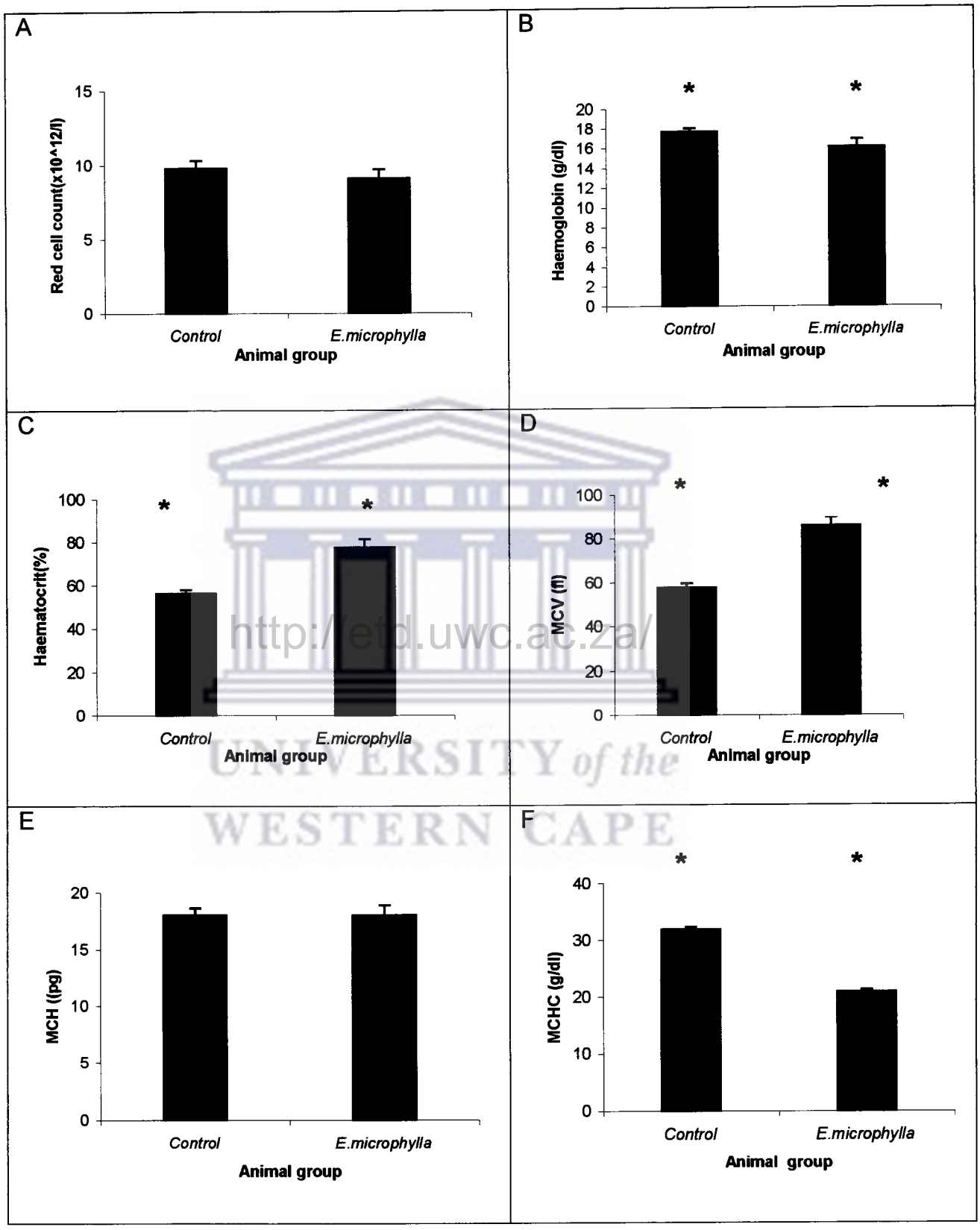


Fig 4A-F Metabolic parameters of male rats on *Exomis microphylla* monitored for 24 hrs over a 45 day period. * Significance between control and experimental group - $p \leq 0.05$
Panels with no stars have no significantly different data - $p \geq 0.05$

3.6.6 Haematological indices

Haematological indices are represented in Fig . 5 A - G and Fig. 6 A – L



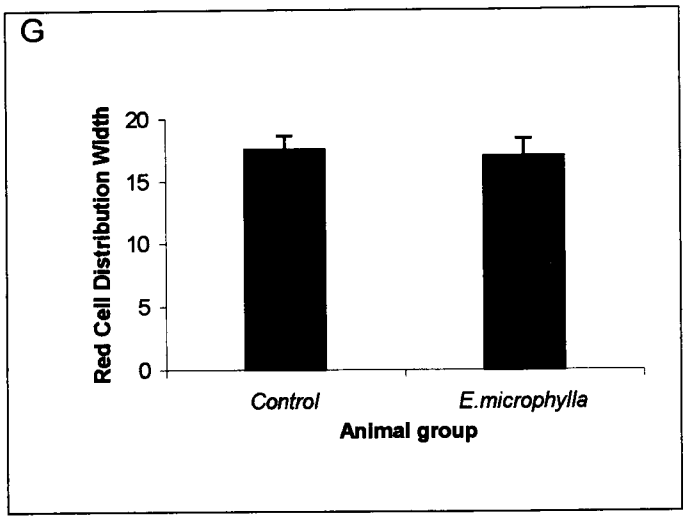
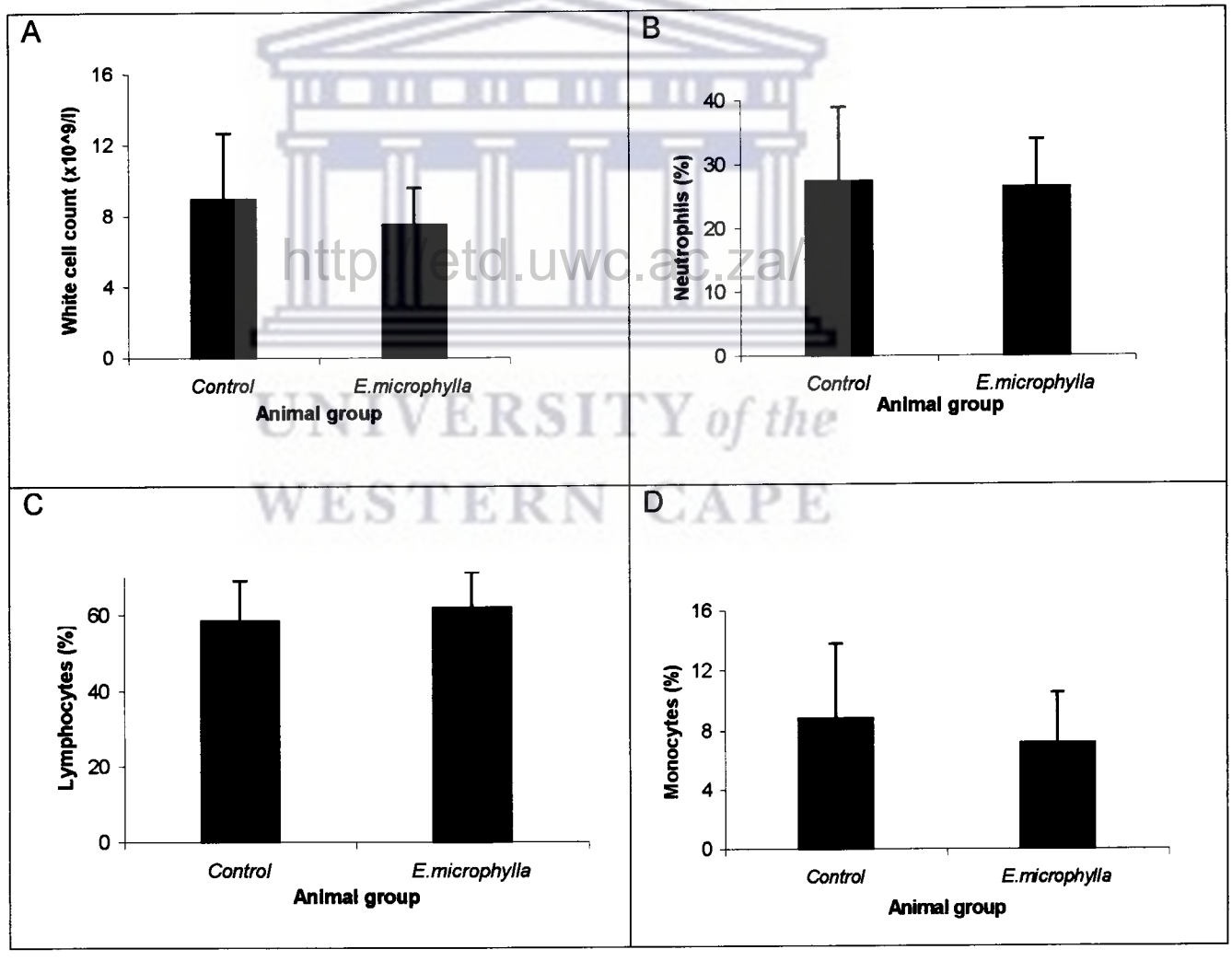
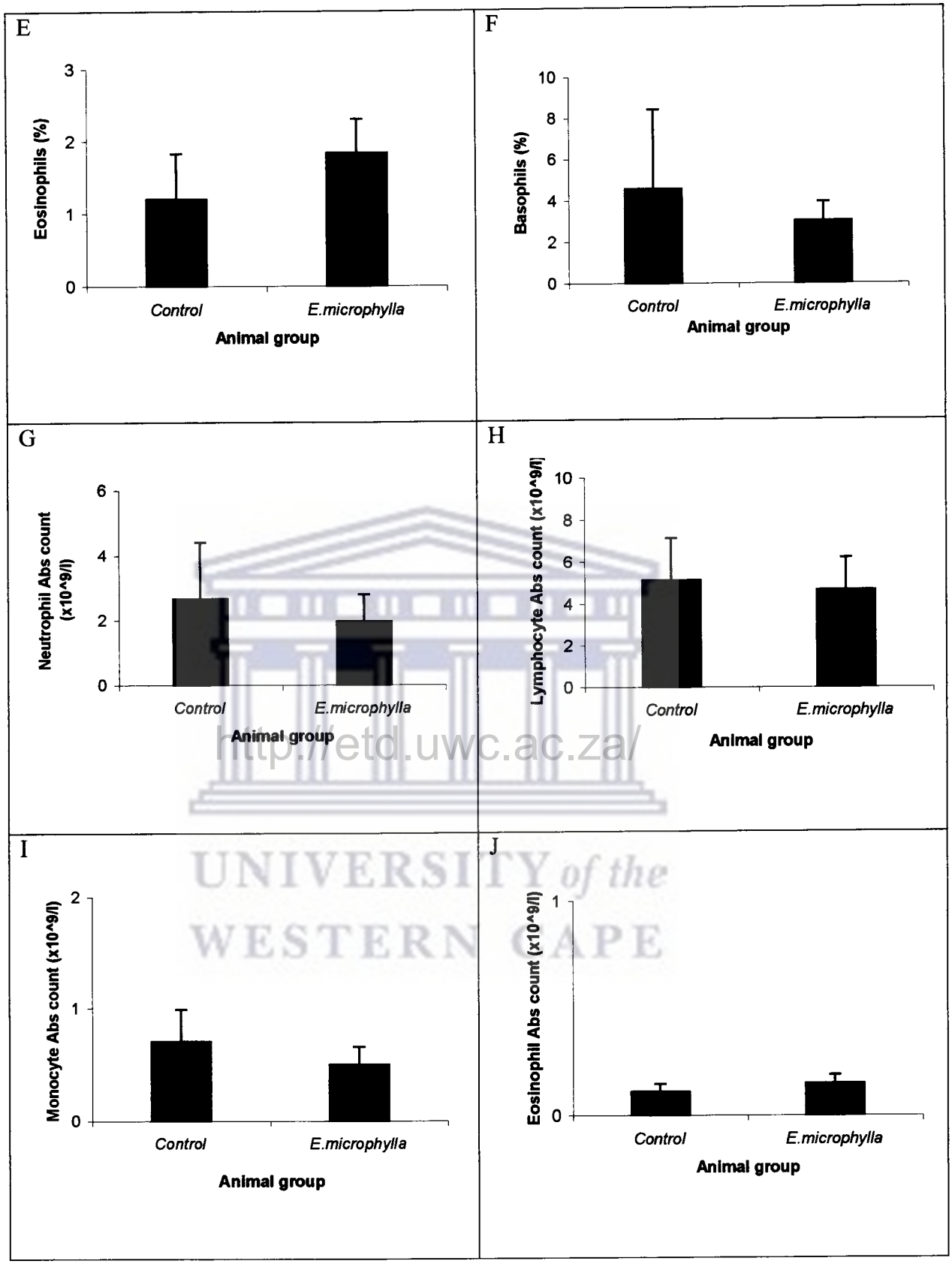


Fig 5A-G Red blood cell parameters of male rats on *Exomis microphylla* extracts over a 45 day period. * Level of significance – $p \leq 0.05$. Panels with no stars, have no significantly different data - $p \geq 0.05$.





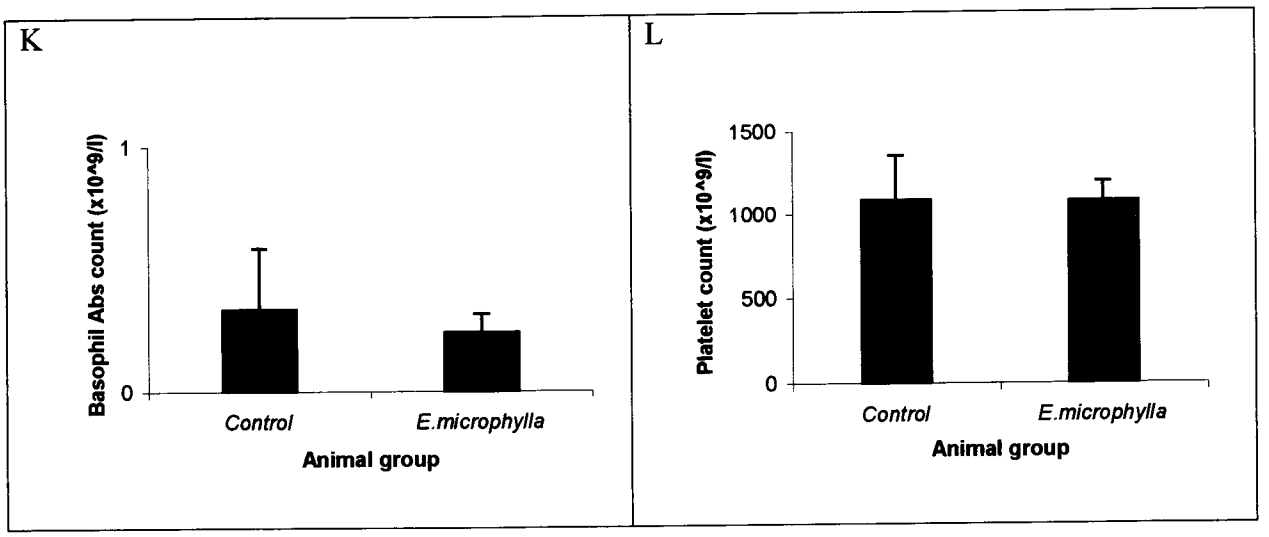


Fig 6 A-L White blood cell parameters of male rats on *Exomis microphylla* over a 45 day period. Level of significance - $p \leq 0.05$. Panels with no stars, have no significantly different data - $p \geq 0.05$.

3.6.7 Animal Histology

Fig. 7 A – D reflects the macro-anatomy of the testes and liver, which are environments that are more sensitive to toxicological insult.

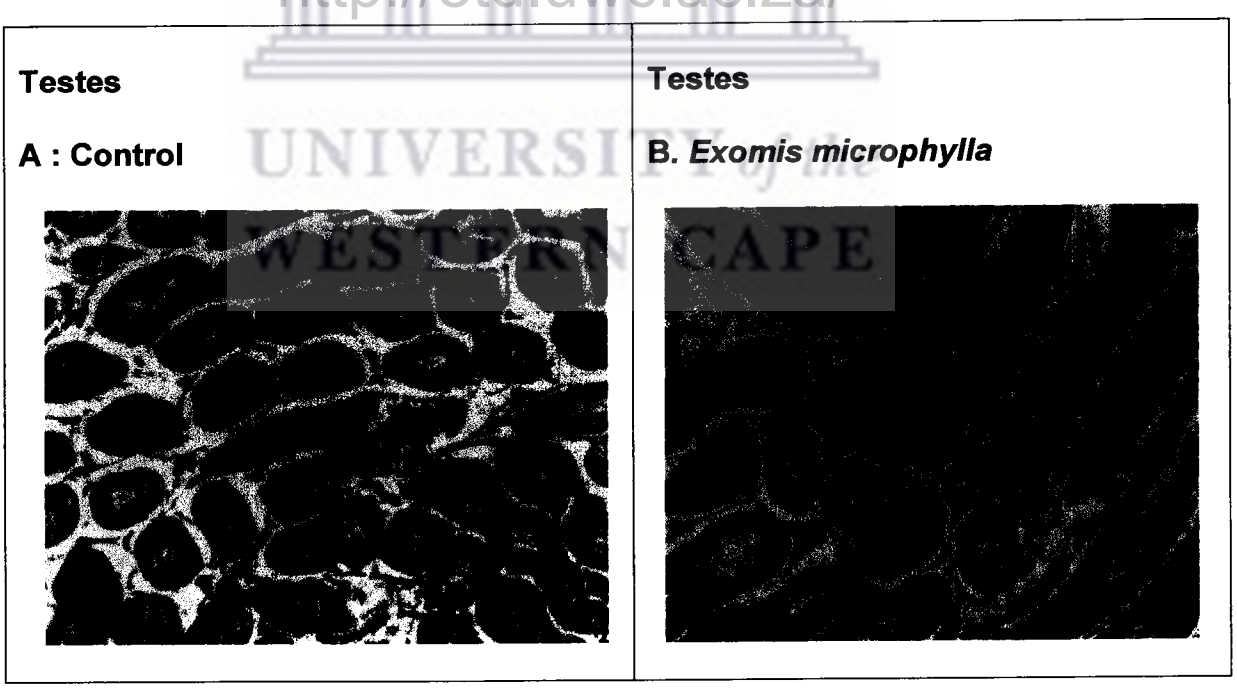


Fig 7. Cross sectional view of the testis of (A) the control group and (B) animals given *Exomis microphylla* extracts [Mag. 40x]

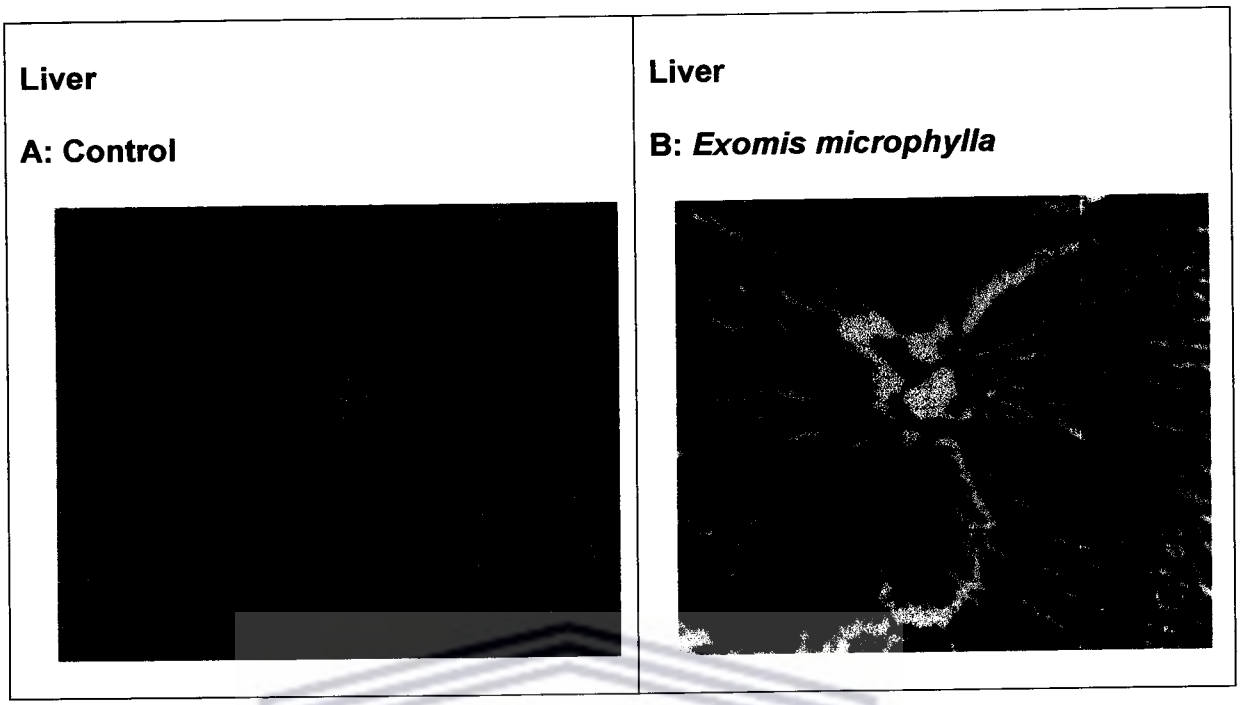


Fig 7. Cross sectional view of the liver of (C) control animals and (D) animals given *Exomis microphylla* extracts [Mag x200]

3.7 Discussion

The genus *Exomis* contains one sole species, which is found in Southern Africa. The species *Exomis microphylla has* not been well investigated. The anatomy of the plant has not shown any valuable information as far as the organs where the secondary compounds are produced or stored. Since very few studies have been done on this plant, more detailed analyses has to be done on the plants anatomy to determine where the production and/or storage of secondary compounds occur.

Soil pH is important to plants because (1) it influences the chemical form of many elements in the soil, and (2) it influences soil microbial processes. Some elements influenced by pH are essential nutrients for plants, so soil pH affects plant nutrition. Other elements are toxic when present in excessive amounts, and soil pH helps to determine how much is in solution at any one time.[11] Table 1 shows that the soil in which the plant occurs is alkaline.

Medicinal herbs are usually ingested in teas, elixirs, drops, and even capsules. Therefore it is important that the essential and toxic elements in these medicinal plants are determined because it is important for human health.[12] The results show a general trend where the elemental concentration in the plant is higher than in the soil (Fig. 2 A-F). It would seem that the sodium and potassium levels in the plant is particularly high (Fig 2C and D). Sodium is an essential element in the human body, needed for maintaining the correct balance of acidity and alkalinity and for regulating the volume of fluids. Evidence suggests that dietary potassium may play a role in decreasing blood pressure. Potassium is involved in nerve function, muscle control and blood pressure. Research suggests that the ratio of sodium to potassium in the diet may be more important than the specific amounts of sodium or potassium.[13] Statistics indicating the significance of these results are however lacking, therefore further studies will have to be done on the elemental concentrations in both the plant and soil in which it grows.

Over the years pathogens have become resistant to a number of antibiotics, therefore studies have been done on alternative medicines. Table 2 shows that *Exomis microphylla* had positive results at concentrations of 40 and 80 mg/g against the microbe *S. aureus*. The inhibition zones can be clearly seen on Fig.3 a and b. This result indicates that the herbal medicine *Exomis microphylla* could be used against *S.aureus*.

Any substances consumed by animals could possible have an effect, whether positive or negative, on metabolism and health. *Exomis microphylla* had no significant effect ($p \geq 0.05$) on the weight of the rats to which the extract was administered (Fig. 4 A). Similarly, there was no significant difference ($p \geq 0.05$) between the food consumption of the control rat group and the experimental rat group (Fig. 4 B). The water consumption between the two groups also showed no difference ($p \geq 0.05$) (Fig. 4 C). The urine excretion of the experimental rat group increased significantly ($p \leq 0.05$) on days 30 and 45 (Fig 4 D). Other Chenopodium species have been found to have diuretic effects.[14] And the increase in urine excretion, could be an indication that *Exomis microphylla* is also a diuretic. The diuretic effect could also be as a result of the high sodium content in the plant (Fig. 2 C). The pH levels for the urine were alkaline throughout (Fig 4 E). The fact that the plant grows in alkaline soil might be influential in this instance. The urine pH was significantly higher ($p \leq 0.05$) for the rats on the herbal extract at the baseline level. Stool production (Fig. 4 D) showed a significant decrease ($p \leq 0.05$) for the rats on the herbal extract on day 45. These outcomes may be indicative of the fact that this herbal extract could possibly help prevent acid-induced disease and diarrhea.

The red cells which are the most numerous of the cellular elements and carry oxygen to the lungs to the body tissue[15], showed no significance ($p \geq 0.05$) between the two animal groups (Fig 5 A). Haemoglobin, which is the protein-iron compound in the red blood cells that enables them to transport oxygen, was however affected by the herbal extract.

In fact, the herbal extract caused a significant decrease ($p \leq 0.05$) in the haemoglobin levels of the experimental rat group when compared with the control group (Fig. 5 B).

This decrease is reason for concern, because if the haemoglobin levels are low, it means that oxygen binding does not take place as it should and oxygen cannot be readily released to tissues that are low in oxygen concentration. A lack in haemoglobin also means that carbon dioxide is not transported away from tissues to the lungs where it is exhaled. A decrease in haemoglobin may very well lead to anemia. On the other hand, the hematocrit levels (Fig 5 C) and mean cell volume (Fig. 5 D) was significantly increased ($p \leq 0.05$) in the animals on the herbal extract. This indicates an increase in the portion of the blood that consists of red blood cells as well as an increase in the average volume of the red blood cells. The MCH level showed no significant difference (Fig. 5 E). The MCHC count was significantly higher for the control group than for the experimental group (Fig. 5 F) indicating an increase in the average concentration of haemoglobin in the blood. No significance ($p \geq 0.05$) was found for the RDW levels (Fig. 5 G). The results thus show that the herbal extract had variable effects that depress the haemoglobin indices, which might impair oxygen transport.

The white blood cell parameters showed no significant changes ($p \geq 0.05$) between the two animal groups. This indicates that the medicine had no effect on the white blood cells (Fig6A-L). Studies done on various medicinal plants have shown that these herbal remedies help to boost the immune system.[16-17]

As with any other orthodox or alternative medicine, the safety of herbal extracts is of great importance. For this reason, any potential toxicity may well be more pronouncedly reflected in testicular and liver tissue, since these environments are particularly more sensitive to environmental insult. The macro histology of the testis (Fig 7A & B) and liver (Fig 7C & D) of the control and experimental group shows no significant differences in their macro-architecture.

Studies done on the effects of medicinal plants have shown various effects on the reproductive systems in animals.[18] Certain herbal extracts can cause an increase in the weight of seminal vesicles and histological tests indicate a greater abundance of spermatozoa in the lumen of the seminiferous tubulus.[19] Varying results have also been found for studies done on the liver.[20-21]

In conclusion, it is clear that *Exomis microphylla* does have antimicrobial potential. The herbal extract also shows possible effects against acid-induced disease and diarrhea. Given its favourable profile, further studies on this medicinal plant should be pursued even though it had an input on certain blood biochemical indices, all of which though remained within physiologically normal limits.

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Summary

People have used plants for centuries as a means of healing. When Friedrich Bayer and co first introduced synthetic aspirin to the world in 1897, the bond between plants and human health began to unwind. Traditional medicine can be seen as the total combination of knowledge and practice, whether explicable or not, used in diagnosing, preventing, or eliminating a physical, mental, or social disease and which may rely exclusively on past experience and observation handed down from generation to generation, verbally or in writing.

At present people are researching the use of herbal medicine for the treatment of various ailments including cardiovascular disorders, diabetes, AIDS, and a number of infectious diseases. Since pathogens are developing a greater resistance to antibiotics, significantly more attention has focused on extracts and biologically active compounds isolated from plant species used in herbal medicine.

This study was conducted to determine the effects of two medicinal plants on health and metabolism. Chapter 2 assesses the anti-infective value of *Atriplex lindleyi* and determines how the plant extract affects metabolism, and health of male rats. *Atriplex lindleyi*, which is also known, as the saltbush has not been extensively studied. Studies have however been done on other *Atriplex* species such as *Atriplex littoralis.*

Atriplex lindleyi is found in alkaline soil. The results from the plant anatomy slides show that the leaves of this plant contain a number of sclerieds. Sclereids normally perform a protective function in plants. The sclereids in the leaves of this plant could possible function as a protective mechanism against insects. However, this protective function gives no indication that these sclereids may have a potential role to play in the storage or production of secondary compounds that may provide the plant with its medicinal value.

No statistical significance was found for the elemental analyses. Antimicrobial screening also showed no positive inhibition results. As far as the metabolism of the animals was concerned, effects were found on urine excretion and urine pH. The increase in urine excretion indicates that the plant might act as a diuretic, as does other Atriplex species. The pH of the rats was alkaline and this alkalinity might be directly linked to the fact that the plant is found in alkaline soil.

Significant results were also obtained for a number of red cell parameters. There were differences in the haemoglobin, haematocrit, MCV and MCHC indices. The decrease in haemoglobin could be a reason for concern since it could lead to anemia, due to the lack of oxygen that is bound to the red blood cells. The increase in haematocrit and MCV indicates an increase in the portion of the blood that consists of red blood cells as well as an increase in the average volume of the red blood cells. Furthermore, no histological differences were found between the testes and liver of the control and experimental animal groups. The integrity of the cellular architecture in these organs provides further evidence of the herbs' non-toxic nature.

Chapter 3 looked at the assessment of the anti-infective value of *Exomis microphylla* and determined how the plant extract affected metabolism, and health of male rats. The genus *Exomis* contains one sole species, which is found in southern Africa. The species *Exomis microphylla* has not been well investigated. The anatomy of the plant has not shown any valuable information regarding the organs where the secondary compounds may be produced or stored. As with *Atriplex lindleyi, Exomis microphylla* is also found in alkaline soil.

No statistically significant results were found for the elemental variables between the variable groups. Antimicrobial activity was found against the microbe *S. aureus* and inhibition zones of approximately 2 mm were found.

Metabolic parameters that varied significantly were urine excretion, urine pH and stool production. The differences occurred as a result of the herbal extract. This could mean that the herb can potentially be effective against acid-induced diseases and diarrhea. Significant results were obtained for a number of haematological indices.

There were differences in the haemoglobin, haematocrit MCV and MCHC indices. Again, no significance was found for the white cell parameters. The differences in these blood parameters are obviously as a result of the herbal extract. These results are closely related to those found for *Atriplex lindleyi.*

Histologically there were also no differences found in the testes and liver of the two animal groups. The integrity of the cellular architecture in these organs provides further evidence of the herbs' non-toxic nature.

In conclusion, *Atriplex lindleyi* showed no antimicrobial effects, but may possibly have diuretic value. *Exomis microphylla* has potential antimicrobial value, and could possibly also help prevent acid-induced diseases and diarrhea. Given the favourable safety profile of both these medicinal plants, further studies should be pursued even though it had an input on certain blood biochemical indices, all of which though remained within physiologically normal limits.