

Acute toxicity and 28-day oral administration of *euclea natalensis* extract in Swiss albino mice

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Euclea natalensis is a medicinal plant indigenous to South Africa that has been used for ages in the management of different diseases including pulmonary-related diseases. The present study was undertaken to investigate the toxicity effects of the medicinal plant in Swiss albino mice. Acute and 28-day repeated oral toxicity studies were performed following the Organization for Economic Cooperation and Development (OECD) test guidelines 423 and 407, respectively. For acute oral toxicity, *euclea natalensis* was administered at 500 mg/kg body weight repeatedly for seven days and the mice were observed for signs of toxicity for seven days. The gross pathological observation was performed at the end of the study. Haematology, clinical chemistry and histopathology examinations were performed for the acute study. No treatment-related deaths or toxic signs were observed with *euclea natalensis* in the acute toxicity experiments. For the 28-day oral toxicity study, the plant was administered at 500 mg/kg body weight, 250 mg/kg body weight and 125 mg/kg body weight. The animals were monitored and observed clinically every day after dosing, and body weight changes and feed consumption were monitored weekly. In the main study, no significant differences in body weight and feed consumption were observed between the controls and treated animals. Additionally, no gross pathological findings in relative organ weights and histopathological abnormalities were observed in either control mice or treated mice. Lesions observed were age-related and not dose-dependent. The blood results did not show any significant difference between the treated animals and the control animals. In conclusion, *euclea natalensis* has been found to be non-toxic and, therefore, the no observed adverse effects level (NOAEL) is 500 mg/kg body weight.

Keywords: medicinal plant, histopathology, toxicity, mice

Introduction

The use of traditional medicine has been of great importance since ancient times. This has been part of traditional practices within different societies worldwide.¹ In addition, a lot of work has been done on the ethnobotanical use of medicinal plants in South Africa, Uganda, Zimbabwe, Mexico and Asia. Well-documented records stipulate that many African and Mexican plants have been used in the management and treatment of various diseases such as tuberculosis and related symptoms, stomach problems, infertility and many more.²⁻⁵

South Africa is experiencing the burden of drug-resistance in patients suffering from tuberculosis; therefore, it is essential to research new anti-tuberculous agents which can be readily and simply produced from local sources.⁶ As stated by Balunas and Kinghorn,⁷ natural products continue to play the most significant role in drug discovery and development processes and plants are still recognised as useful resources of highly active anti-microbial metabolites. Having said so, Gittel⁸ together with the World Health Organization (WHO)⁹ emphasise screening programmes of medicinal plant remedies to validate the safe use of traditional medicines among societies, and to provide leads in the discovery

of new active principles. All these screening programmes have to be supported by toxicity studies.

Short-term studies (both maximum tolerable dose [MTD] and repeated dose – 28 days) were conducted to establish the MTD and the no observed adverse effect level (NOAEL) of the test compound of interest, to identify target organs, if any, and to decide on subsequent doses for future chronic studies in animals.¹⁰

Euclea natalensis (belonging to the family *Ebenaceae*) is a medicinal plant indigenous to South Africa and is mostly located along the coast of the country. It is also found in other African countries like Mozambique, Botswana and Zimbabwe. The genus *Euclea* is a small African genus of about 20 species. The name *Euclea* means 'of good report' or famous. As it is used traditionally by many African tribes, the medicinal plant bears many names. In English it is called 'natal ebony', in Afrikaans it is called 'swartbasboom', in Xhosa it is called 'Umkhasa' or 'Umtshekisan', in Zulu it is called 'Idungamzi' or 'Umzimane' and the Tsonga call it 'Umhlangu'la.¹¹ It is a shrub of approximately 4.1 m with a spreading crown in warm and well-watered conditions. It can spread to nearly 5–10 m with the trunk usually straight. The bark is dark grey, thin and finely cracked and branches are covered

with fine rusty hairs. Leaves are hard and leathery, dark green, shiny and pale underneath. Traditionally the twigs are used for toothbrushes, and the roots and bark are used as ingredients of a variety of traditional remedies to treat de-worming, stomach disorders, toothaches, headaches, chest complaints, urinary tract infections, venereal diseases, swellings, abnormal growths and skin leprosy.^{12,13}

The medicinal plant has been broadly investigated¹⁴ and it was discovered to have antimicrobial activity against many microorganisms including both drug-sensitive and drug-resistant strains of *Mycobacterium tuberculosis*. In that study, it was identified and documented that the root part of the plant contains a higher concentration of active compounds called naphthoquinones and triterpenes compared to the other parts. Hence, the root material was the substance of interest in this study. With more scientific revelations increasing over medicinal plants, not much is reported and documented on their safety and toxicity. Therefore, the present study is aimed at investigating the toxic effects of *euclea natalensis* root extract with repeated dose (seven and 28 days) toxicity tests on Swiss albino mice.

Materials and methods

In this toxicity investigation, two studies were conducted, namely seven days repeated oral toxicity study and 28 days repeated study. The acute study was conducted to establish the dosages to be used in the main 28-day study. The Organization for Economic Cooperation and Development (OECD) guides 423 and 407 were followed, respectively in these studies.

Collection and production of the extracts

The plant was collected from Xura River in Lusikisiki in the Eastern Cape province of South Africa. The collection was made possible with assistance from the National Biodiversity Institute of South Africa (NBISA). Lusikisiki is much nearer to the Walter Sisulu University (WSU) where the study was conducted and the exact location of the medicinal plant in Lusikisiki was obtained in the Kei Herbarium of the WSU. The specimen was authenticated in the Department of Botany at WSU, and voucher specimen number one was preserved in the Kei Herbarium. Roots were obtained and a week later these were chopped into smaller pieces and dried at 40 °C overnight.¹⁵ After drying, the roots were ground using a grinding apparatus.

The extract preparation

The extraction was carried out according to the procedure recommended by Draper¹⁶ and 100 g of the milled root was dissolved in 1 000 ml acetone. The mixture was left on the shaker for three days to allow for maximum extraction of the compounds at 25 °C at 180 r/min. The mixture was then sieved and filtered. The residue was washed twice with acetone to complete the extraction. The filtrate was then dried on a rota vapour machine at medium temperature ranging from 40–56 °C to obtain the crystallised black crude extract. The crude extract was then stored in a capped sterile plastic bottle at room temperature.

Animal selection and quantity

A total of 80 healthy adult Swiss albino mice were selected for both the studies. These animals were obtained from the breeding facility at the Orchid Chemicals & Pharmaceuticals Ltd., India (acute study) and the Shalom Laboratory Suppliers, South Africa (28-day study). The animals were 6–7 weeks old with an average weight of 25–30 g at the time of treatment. The mice were grouped into four groups of five per gender. They were selected in such a way that the mean body weight of all animals in a group fell between $\pm$ 20% of the mean body weight range to minimise biological variation. Female mice were nulliparous and non-pregnant.

Room sanitation

Before the animals could occupy the experimental rooms, the rooms were decontaminated. Room sterility was confirmed by performing a surface viable count. The floor of the experimental room was swept daily. All worktops and floors were mopped daily with a disinfectant (Suprasol LC 5%).

Animal welfare

All animals were handled similarly and with due regard for their welfare. Humane treatment and care of the animals complied with the Regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and South Africa, respectively (Protocol No 14-IAEC/02/TOX/2010).

All animals were allowed to acclimatise before treatment started.

Grouping

The animals were randomly allocated to and equally distributed into four groups: vehicle control G1 (0 mg/kg body weight), low dose G2 (125 mg/kg body weight), mid dose G3 (250 mg/kg body weight) and high dose G4 (500 mg/kg body weight). Each group comprised five males and five females.

Animal identification

Animals were given temporary numbers for identification during randomisation. After allocation of groups, permanent numbers were issued to all animals and each animal was given a tag bearing its number on the cage, with a unique colour for the specific group. (White tag for controls, pink tag for low dose, yellow tag for mid dose and red colour for high dose.)

Animal husbandry

Animals were housed individually in polypropylene mice cages (290 mm × 20 mm × 40 mm) using autoclaved paddy husk as bedding material. Each cage was fitted with a stainless steel top grill having provision to keep pellet feed and a polypropylene water bottle with stainless steel nozzle. Cages were placed on five-tier racks. Cages were changed once every three days and water bottles were changed once a week.

The experimental animals were fed ad libitum mice pellet feed (Nutrilab brand, Tetragon Chemie Pvt. Ltd, Bangalore) and had an unlimited supply of clean drinking water in polypropylene

Table I: Summary of group designations for 7-day repeated dose and 28-day toxicity studies

Group no	Treatment	Dose (mg/kg b.wt)	No of animals		Animal ID no	
			Male	Female	Male	Female
G1	Control	0	5	5	1–5	6–10
G2	Low dose	125	5	5	11–15	16–20
G3	Mid dose	250	5	5	21–25	26–30
G4	High dose	500	5	5	31–35	36–40

b.wt – body weight

bottles (capacity – 300 ml) filtered through an Aquaguard water filtration system. The quality of feed was checked, and the batch was accompanied by a certificate of analysis of the nutrient content. The quality of the water was monitored for any known chemical and microbial contaminants. There were no contaminants in the feed and water, which can affect the results of the experiment. Animals were maintained in a controlled environment with a temperature of 21–25 °C, humidity of 54–69%, light/dark cycle of ~12 hours and 14 fresh air changes per hour.

Study design

Four groups of Swiss albino mice comprising five males and five females per group were administered the test formulation at the dose levels of 0, 125, 250 and 500 mg/kg body weight through oral route for seven consecutive days. The same concentrations were maintained in the main study as well. The test substance was formulated in 20% dimethyl sulphur oxide (DMSO) and 80% of 0.25% carboxy methyl cellulose (CMC). The control groups received the vehicle alone.

Dose preparation and administration

The crude extract was repeatedly administered orally once daily using graduated syringe and stainless-steel intubation cannula for seven days and 28 days, respectively. The required quantity of the test substance was weighed using a calibrated analytical balance and was suspended in DMSO followed by CMC in the stipulated ratios. Owing to the thickness of the suspension, dose volume was maintained as 20 ml/kg body weight for all animals for both studies. The test suspension was prepared every day and was administered immediately. Individual dosage volume was calculated based on the initial body weight. The crude extract of *euclea natalensis* was suspended in DMSO/CMC to attain the required concentrations (i.e. 6.25, 12.5, and 25 mg/ml for the low, mid and high dose groups, respectively). A fresh suspension of *euclea natalensis* was prepared every morning prior to dosing. The dose volume administered to each mouse was calculated based on a constant factor of 20 ml/kg body weight throughout the study.

Repeated dose toxicity evaluation

Mortality

All the animals in the two studies were checked at least twice a day for mortality and signs of morbidity – in the morning after oral dosing and in the afternoon.

Clinical signs

Animals in the two studies were observed twice daily for all visible signs of reaction to the treatment such as skin and fur changes, eye and mucous membrane changes, changes to the respiratory, circulatory, autonomic and central nervous system, and somatomotor activity, behaviour pattern and general changes.

Body weight

The body weight of each individual animal was recorded prior to first dosing, and at day 4 and day 7 for the dose range finding study, and on days 7, 14, 21 and 28 (at necropsy) for the main study.

Feed consumption

Feed consumption was recorded cage wise for all animals at day 4 of commencement of exposure and at day 7. In the main study, feed consumption was recorded on weekly basis. The average daily feed consumption of an individual animal was calculated as follows:

Feed consumption = feed input – feed left over/No of mice in the cage × No of days

Clinical pathology observation

At the end of 7-day and 28-day periods the animals were fasted overnight. The following morning (prior to sacrifice, 7-day study), blood was collected from all the animals by puncturing the orbital sinus with heparinised capillary¹⁷ under mild isoflurane anaesthesia. Blood was collected into a 7.5 cm (length) capillary tube and the clotting time was recorded manually. Approximately 0.1 ml of blood was collected in vials containing Ethyldiminetetraacetic acid (EDTA) for haematology analysis.

The following haematological parameters were measured: total erythrocyte count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count and total differential leucocyte count (WBC).¹⁸ All parameters were determined using a haematological analyser (Sysmex KX 21). The procedure for analysis specified in the Sysmex's operational manual was followed.

From each animal, 0.5–0.8 ml of blood was collected in heparinised centrifuge tubes and then centrifuged using cooling centrifuge (Eppendorf Centrifuge 5804 R) at 5 000 revolutions per minute (RPM) for 10 minutes and plasma was separated.

Plasma biochemical parameters were analysed using a fully automated clinical chemistry analyser (ERBA XL 300, Transasia Biomedicals Ltd., Mumbai).

Gross examination and histopathology

All the animals were euthanised with a dose of isoflurane and subjected to a complete necropsy under the direct supervision of a veterinary pathologist at the end of treatment periods. The appearance of macroscopic abnormalities, if there were any, was recorded. The thoracic, abdominal and cranial cavities were then cut open and thorough examinations of the organs were carried out to detect changes or abnormalities, if any.

Organ weight

Absolute wet weights of the adrenals, brain, uterus, ovaries, testes, epididymides, heart, kidneys, liver, spleen and thymus were recorded immediately after dissection for all animals. The relative weights of these organs were calculated later.

The organs from the gastrointestinal system, the brain, renal system and adrenals, and both male and female reproductive systems of all the animals in the two studies were collected at necropsy and preserved in 10% buffered neutral formalin for processing and microscopic examination. All the preserved organs from the control and high-dose groups (G1 and G4) of the animals in both studies were processed following suitable histological techniques. Histopathology screening was carried out on all preserved organs and tissues of all the animals in control and high-dose groups. All carcasses were incinerated.

Statistical analysis of results

Raw data were processed statistically. The means and standard deviations were calculated with significance between the control and treated groups and all data were summarised in tabular form. All the parameters characterised by continuous data such as body weight, feed consumption, organ weight, relative organ weight, haematology and clinical chemistry were subjected to Bartlett's test to meet the homogeneity of variance. All homogenous data were analysed using analysis of variance (ANOVA) and data showing significance in their variances were subjected to Dunnett's t-test.

Significance was calculated at 1% as well as 5% levels, and indicated in the summary table as follows:

↑ – significantly higher than control ($p > 0.05$), ↑↑ – significantly higher than control ($p > 0.01$), ↓ – significantly lower than control ($p < 0.05$), ↓↓ – significantly lower than control ($p < 0.01$)

Results

No significant deviation of weights in the mean of all the treated groups in all genders was observed compared to the control group on day 7 and at terminal point for acute study. Similar observations were noted in the sub-acute study and no significant deviation was observed in all treated groups compared to the control group at week 1, week 2, week 3 and week 4 and at the termination of the study.

No significant deviation in feed consumption in the mean of the female treated groups was observed compared to the control group on day 4. The feed consumption started declining after day 4 in both the control group and the treated male animals at the lowest concentration (125 mg/kg body weight).

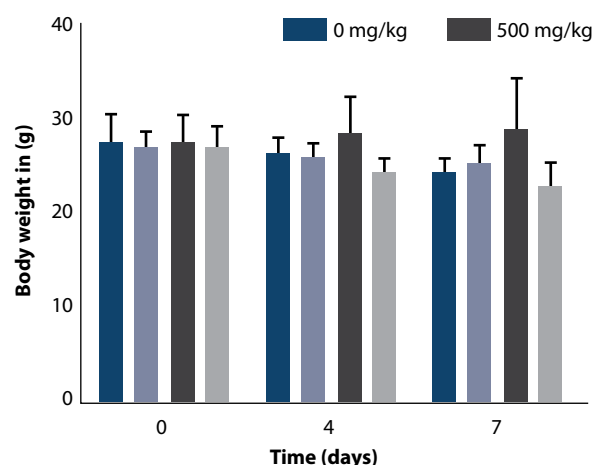


Figure 1: The body weight for male animals across all the concentrations at day 4 and day 7 of treatment with *euclea natalensis* root extract

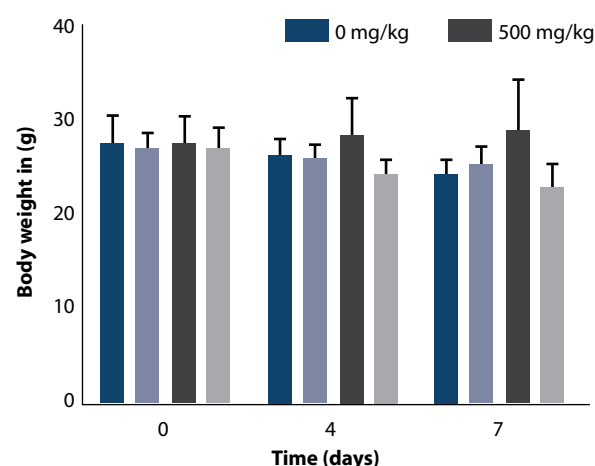


Figure 2: The body weight for female animals across all the concentrations at day 4 and day 7 of treatment with *euclea natalensis* root extract

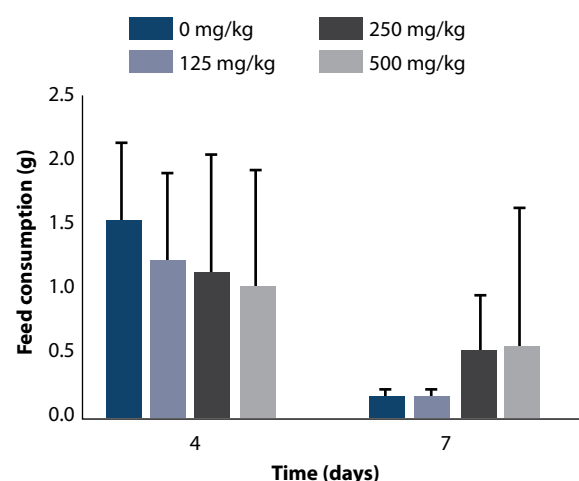


Figure 3: The feed consumption of male animals across all the concentrations at day 4 and day 7

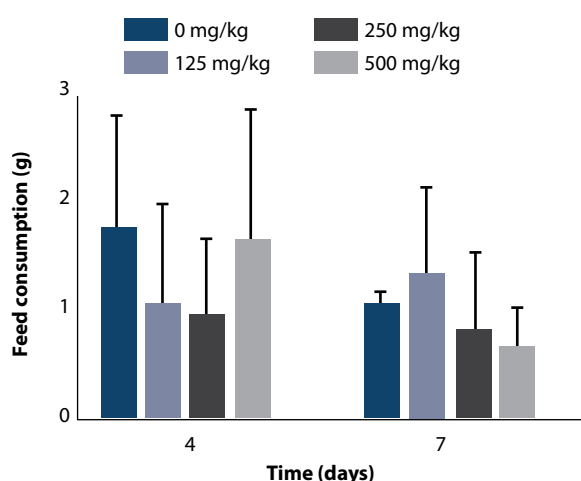


Figure 4: The feed consumption of female animals across all the concentrations at day 4 and day 7

No statistical significance was observed in all parameters in male treated animals, except for group G3 (250 mg/kg body weight)

which showed a decrease in total protein compared to the untreated control group at 1% significance level ($p \leq 0.01$). In the females, all treated animals showed a decrease in total cholesterol compared to the control group; although, statistically that is not significant. G4 (500 mg/kg body weight) showed an increase in phosphorus at 5% level compared to the control group. G2 (125 mg/kg body weight) showed a decrease in triglycerides at 5% level ($p \geq 0.05$) which was significant (Table II and III).

No statistically significant changes were noticed in any animals in all genders treated with *euclea natalensis* root extract compared to the control groups.

In this study, no significant changes in males were seen in any organ weight compared to the control group, except for the weight of testes in group G2 (250 mg/kg body weight) which was significantly higher than the control group at 1% level ($p \geq 0.01$) (Table VI).

Table II: Summary of biochemical results for male animals

Parameter	0 mg/kg b.wt	125 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt
GLU (mg/dl)	128.8 ± 34.9	126.7 ± 26.8	147.5 ± 67.4	111.7 ± 21.9
URE (mg/dl)	27.8 ± 8.0	26.4 ± 7.4	29.2 ± 1.3	31.0 ± 5.6
CRE (mg/dl)	0.40 ± 0.02	0.43 ± 0.04	0.45 ± 0.11	0.40 ± 0.06
T.PRO (g/dl)	7.0 ± 1.1	6.8 ± 0.8	↓5.7 ± 0.6	6.5 ± 0.6
ALB (g/dl)	3.8 ± 1.5	3.9 ± 1.1	2.3 ± 0.6.4	4.5 ± 1.3
CLO (mEq/l)	98.0 ± 4.1	101.1 ± 2.1	102.8 ± 2.3	96.9 ± 5.5
PHO (mg/dl)	12.3 ± 4.2	12.7 ± 0.6	11.9 ± 2.9	7.3 ± 1.3
CHOL (mg/dl)	143.8 ± 42.2	154.6 ± 24.4	118.6 ± 14.4	182.2 ± 25.4
TGL (mg/dl)	173.2 ± 41.3	181.6 ± 51.8	168.4 ± 61.2	194.0 ± 41.9
T.BIL (mg/dl)	2.3 ± 1.2	2.9 ± 0.6	2.4 ± 1.0	1.8 ± 0.9
GOT (IU/l)	149.7 ± 41.3	162.8 ± 49.6	133.1 ± 44.4	169.9 ± 17.5
GPT (IU/l)	103.9 ± 39.4	83.6 ± 14.9	97.4 ± 30.2	99.1 ± 19.0
ALP (IU/l)	118.2 ± 41.6	145.0 ± 32.4	135.8 ± 42.1	84.0 ± 20.9

↓ – significantly low at $p \leq 0.05$, ↓↓ – significantly low at $p \leq 0.01$, ↑ – significantly high at $p \geq 0.05$, ↑↑ – significantly high at $p \geq 0.01$

GLU – glucose, T.PRO – total protein, PHO – phosphorus, T.BIL – total bilirubin, URE – urea, ALB – albumen, CHOL – cholesterol, ALP – alkaline phosphatase, CRE – creatinine, CLO – chloride, TGL – triglycerides, GOT – glutamic-oxaloacetic transaminase, GPT – glutamic-pyruvic transaminase

Table III: Summary of biochemical results for female animals

Parameter	0 mg/kg b.wt	125 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt
GLU (mg/dl)	145.1 ± 12.9	127.3 ± 11.3	140.7 ± 11.9	138.8 ± 21.2
URE (mg/dl)	20.0 ± 2.1	18.2 ± 2.4	20.0 ± 1.4	21.8 ± 2.4
CRE (mg/dl)	0.38 ± 0.02	0.39 ± 0.03	0.40 ± 0.02	0.38 ± 0.03
T.PRO (g/dl)	5.9 ± 1.6	5.6 ± 0.4	6.2 ± 0.6	5.6 ± 0.5
ALB (g/dl)	3.4 ± 0.9	2.4 ± 0.3	3.2 ± 1.1	2.3 ± 0.2
CLO (mEq/l)	98.3 ± 1.5	98.9 ± 3.5	101.2 ± 3.5	100.5 ± 2.4
PHO (mg/dl)	8.9 ± 2.0	8.9 ± 2.0	↑12.0 ± 1.5	10.6 ± 1.4
CHOL (mg/dl)	146.6 ± 42.5	110.6 ± 9.3	121.4 ± 34.6	120.0 ± 14.1
TGL (mg/dl)	162.4 ± 21.9	↓131.2 ± 17.1	156.4 ± 68.8	169.2 ± 69.7
T.BIL (mg/dl)	1.5 ± 0.6	1.6 ± 0.6	1.5 ± 0.5	2.3 ± 1.2
GOT (IU/l)	146.8 ± 44.7	107.7 ± 33.3	163.6 ± 56.3	142.8 ± 85.8
GPT (IU/l)	60.4 ± 24.8	53.6 ± 16.8	64.9 ± 21.4	43.8 ± 6.8
ALP (IU/l)	166.6 ± 23.5	189.4 ± 34.3	146.8 ± 44.1	148.8 ± 31.4

↓ – significantly low at $p \leq 0.05$, ↓↓ – significantly low at $p \leq 0.01$, ↑ – significantly high at $p \geq 0.05$, ↑↑ – significantly high at $p \geq 0.01$

GLU – glucose, T.PRO – total protein, PHO – phosphorus, T.BIL – total bilirubin, URE – urea, ALB – albumen, CHOL – cholesterol, ALP – alkaline phosphatase, CRE – creatinine, CLO – chloride, TGL – triglycerides, GOT – glutamic-oxaloacetic transaminase, GPT – glutamic-pyruvic transaminase

Table IV: Summary of haematology results for male animals

Parameter	0 mg/kg b.wt	125 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt
RBC (106/ml)	9.1 ± 0.9	10.0 ± 0.7	9.4 ± 0.9	10.0 ± 0.7
Hgb (g/dl)	14.8 ± 0.4	15.1 ± 0.7	14.7 ± 0.8	15.2 ± 0.9
HCT (%)	49.7 ± 4.8	54.1 ± 2.8	51.8 ± 4.2	53.9 ± 4.4
MCV (fl)	55.0 ± 1.4	54.1 ± 2.8	55.3 ± 1.6	54.1 ± 1.6
MCH (pg)	16.5 ± 2.2	15.2 ± 0.9	15.7 ± 1.0	15.3 ± 0.8
MCHC (g/dl)	30.0 ± 3.9	27.9 ± 0.3	28.4 ± 1.2	28.3 ± 0.8
PLT X103/UI	1 359.6 ± 441.7	1 067.8 ± 374.9	1 333.2 ± 130.1	1 401.6 ± 249.1
CL.Time (Sec)	138.0 ± 12.6	135.0 ± 15.0	135.0 ± 15.0	129.0 ± 13.4
WBC (103/ml)	5.3 ± 0.9	5.2 ± 1.3	6.3 ± 1.5	5.8 ± 2.4
LYMP (%)	67.0 ± 11.4	69.5 ± 12.9	67.8 ± 8.9	63.6 ± 17.3
NEUTR (%)	28.4 ± 9.9	26.9 ± 11.9	28.3 ± 7.7	31.9 ± 15.8
MIX (%)	4.6 ± 1.9	3.6 ± 1.2	3.9 ± 1.3	4.5 ± 1.6

↓ – significantly low at $p \leq 0.05$, ↓↓ – significantly low at $p \leq 0.01$, ↑ – significantly high at $p \geq 0.05$, ↑↑ – significantly high at $p \geq 0.01$

RBC – red blood cells, MCH – mean cell haemoglobin, WBC – white blood cells, Hgb – haemoglobin, MCHC – mean cell haemoglobin concentration, MIX – other white cells, HCT – haematocrit, PLTS – platelets, LYMP – lymphocytes, MCV – mean cell volume, CL.Time – clotting time, NEUTR – neutrophils

Table V: Summary of haematology results for female animals

Parameter	0 mg/kg b.wt	125 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt
RBC (106/ml)	9.2 ± 0.7	9.4 ± 0.3	9.5 ± 0.3	9.6 ± 0.5
Hgb (g/dl)	14.6 ± 0.5	14.6 ± 0.6	14.9 ± 0.6	15.0 ± 0.4
HCT (%)	51.0 ± 2.0	51.6 ± 1.9	52.5 ± 3.2	52.4 ± 1.3
MCV (fl)	55.40 ± 2.7	55.10 ± 1.5	55.2 ± 2.0	54.6 ± 1.9
MCH (pg)	15.9 ± 0.8	15.6 ± 0.6	15.7 ± 0.3	15.7 ± 0.8
MCHC (g/dl)	28.6 ± 0.3	28.3 ± 0.5	28.4 ± 0.6	28.7 ± 0.5
PLT X103/UI	1 109.8 ± 131.09	1 015.6 ± 122.7	1 197.2 ± 172.9	1 131.8 ± 159.6
CL.Time (Sec)	138.0 ± 12.5	135.0 ± 10.6	147.0 ± 22.2	139.0 ± 10.8
WBC (103/ml)	4.6 ± 1.5	4.7 ± 1.9	6.4 ± 2.3	6.4 ± 2.3
LYMP (%)	80.8 ± 5.8	77.3 ± 8.3	68.5 ± 11.8	76.1 ± 14.0
NEUTR (%)	16.5 ± 4.9	19.9 ± 7.4	27.6 ± 9.9	20.3 ± 11.7
MIX (%)	2.7 ± 1.2	2.8 ± 0.9	3.9 ± 1.9	3.6 ± 2.3

↓ – significantly low at $p \leq 0.05$, ↓↓ – significantly low at $p \leq 0.01$, ↑ – significantly high at $p \geq 0.05$, ↑↑ – significantly high at $p \geq 0.01$

RBC – red blood cells, MCH – mean cell haemoglobin, WBC – white blood cells, Hgb – haemoglobin, MCHC – mean cell haemoglobin concentration, MIX – other white cells, HCT – haematocrit, PLTS – platelets, LYMP – lymphocytes, MCV – mean cell volume, CL.Time – clotting time, NEUTR – neutrophils

Table VI: Summary of absolute organ weight for male animals

Parameters	0 mg/kg b.wt	125 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt
Terminal body weight	24.1 ± 1.6	24.9 ± 1.6	28.5 ± 5.7	22.9 ± 2.3
Adrenals	0.012 ± 0.003	0.010 ± 0.003	0.012 ± 0.002	0.01 ± 0.004
Testes	0.1 ± 0.01	↑↑0.2 ± 0.01	0.18 ± 0.01	0.20 ± 0.02
Thymus	0.05 ± 0.02	0.05 ± 0.01	0.05 ± 0.02	0.04 ± 0.02
Epididymis	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01
Spleen	0.12 ± 0.02	0.12 ± 0.03	0.13 ± 0.02	0.12 ± 0.03
Heart	0.12 ± 0.01	0.16 ± 0.02	0.12 ± 0.03	0.15 ± 0.03
Brain	0.45 ± 0.02	0.47 ± 0.03	0.47 ± 0.03	0.45 ± 0.02
Kidneys	0.44 ± 0.06	0.45 ± 0.08	0.55 ± 0.17	0.40 ± 0.07
Liver	1.55 ± 0.25	1.54 ± 0.09	1.7 ± 0.29	1.39 ± 0.1

↑↑ – significantly high at $p \geq 0.01$, ↓↓ – significantly low at $p \leq 0.01$

No significant change in females was seen in any organs compared to the control group, except for the weight of the adrenal gland in the middle dose (250 mg/kg body weight) which was significantly higher than the control group ($p \geq 0.05$).

Absolute weight of the heart in female animals was significantly high in the low-dose group ($p \geq 0.01$). No significant changes were observed in weights in the rest of the organs (125, 250, 500 mg/kg body weight) (Table VII).

Table VII: Summary of absolute organ weight for female animals

Parameters	0 mg/kg b.wt	125 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt
Terminal body weight	22.6 ± 0.9	22.5 ± 3.9	23.6 ± 2.3	23.3 ± 1.6
Adrenals	0.01 ± 0.002	0.01 ± 0.01	0.07 ± 0.01	0.01 ± 0.004
Thymus	0.08 ± 0.05	0.07 ± 0.004	0.06 ± 0.02	0.07 ± 0.01
Uterus	0.19 ± 0.09	0.10 ± 0.08	0.95 ± 1.15	0.13 ± 0.03
Spleen	0.19 ± 0.11	0.15 ± 0.05	0.10 ± 0.04	0.16 ± 0.03
Heart	0.12 ± 0.01	0.13 ± 0.01	0.15 ± 0.040	0.12 ± 0.02
Brain	0.39 ± 0.08	0.459 ± 0.06	0.43 ± 0.05	0.46 ± 0.06
Kidneys	0.30 ± 0.05	0.39 ± 0.03	0.33 ± 0.06	0.33 ± 0.03
Ovaries	0.12 ± 0.13	0.02 ± 0.01	0.08 ± 0.03	0.05 ± 0.04

Table VIII: Summary of histopathology findings

Group	Animal no	Gender	Histopathological observation
Control (0 mg/kg)	SA45/1/2	Male	Heart: Degenerative cardiac myocytes
	SA/45/6/2	Female	Heart: Degenerative cardiac myocytes
	SA45/7/2	Female	Adrenal: Involution of the X-zone
Treated (500 mg/kg)	SA45/31/1	Male	Lung: Inflammation
	SA45/34/1	Male	Lung: Inflammation
	SA45/39/2	Female	Heart: Degenerative cardiac myocytes
	SA45/37/2	Female	Adrenal: Involution of the X-zone
	SA45/40/2	Female	Adrenal: Involution of the X-zone

Gross pathological findings

External findings and internal findings

Gross pathological observations did not reveal any abnormality either externally or internally in any of the animals belonging to both the control and treated groups at all concentrations.

Discussion

The history of traditional medicine and the use of traditional mixtures by different populations dates back 60 000 years ago¹⁹ and according to the WHO, approximately 80% of the world population is presently still relying on traditional medicines for primary healthcare.¹⁴ The use of traditional medicines has been very popular owing to availability, cultural practices and beliefs that these cause no side effects.²⁰ Medicinal plants and their products have been explored and investigated scientifically and have been proven to provide leads into discovery of new novel drugs for the treatment of acute and chronic diseases.²¹ Furthermore, the scientific discoveries of synthesising analogous metabolites from medicinal plant-derived compounds are gaining popularity.^{22,23}

South Africa is very rich in medicinal plants and approximately 2 062 species have been recorded and are used in the management of various conditions.²⁴ The massive discoveries on the beneficial effects of medicinal plants in South Africa and toxicity investigations primarily have been focusing on cell cultures and not on tissue level to completely explore any side effects. The reported cytotoxicity tests are conducted on cell cultures which are of short periods while animal models provide longer duration times for metabolism of the bio-active compounds identified in these. Hence, there is a need

to scientifically investigate and prove that medicinal plants are safe to use. Therefore, the aim of this study was to evaluate acute and sub-acute toxicity effects using the animal model for the ethanolic root extract of *euclea natalensis*, a medicinal plant that has shown in vitro antimicrobial activity on drug-sensitive and drug-resistant strains of *Mycobacterium tuberculosis*.²⁵ All animals in the MTD study survived the treatment period. In the 28-day repeated dose toxicity study, two males and one female animal belonging to the high-dose group died during the treatment period. There were no evident clinical observations during the study apart from poor feed consumption of the female animal. The deaths of these animals belonging to the high-dose group in the repeated dose study are attributed to the dose formulation resulting in ceasing of feeding in these animals.

No treatment-related changes were observed in any of the haematological and clinical biochemistry parameters in any of the animals treated at the dose levels of 125, 250 and 500 mg/kg body weight in both the MTD and the repeated dose studies.

Gross pathological findings could be directly related to specific clinical signs observed in animals which could ultimately contribute towards the cause of animal discomfort leading to death.²⁵ The increased weight of testes in the MTD study found in male animals belonging to group G2 (250 mg/kg) compared to the control group could probably be due to the proliferation of the germinating epithelium layer within the wall of the seminiferous tubules. The root extract at this dose probably influenced the proliferation process; other medicinal plants have been proven to have a similar effect on this layer in infertility investigations.^{26,27} However, similar changes were not observed in the high-dose group. Females from the control, mid- and high-dose groups showed a reduction in heart weight with

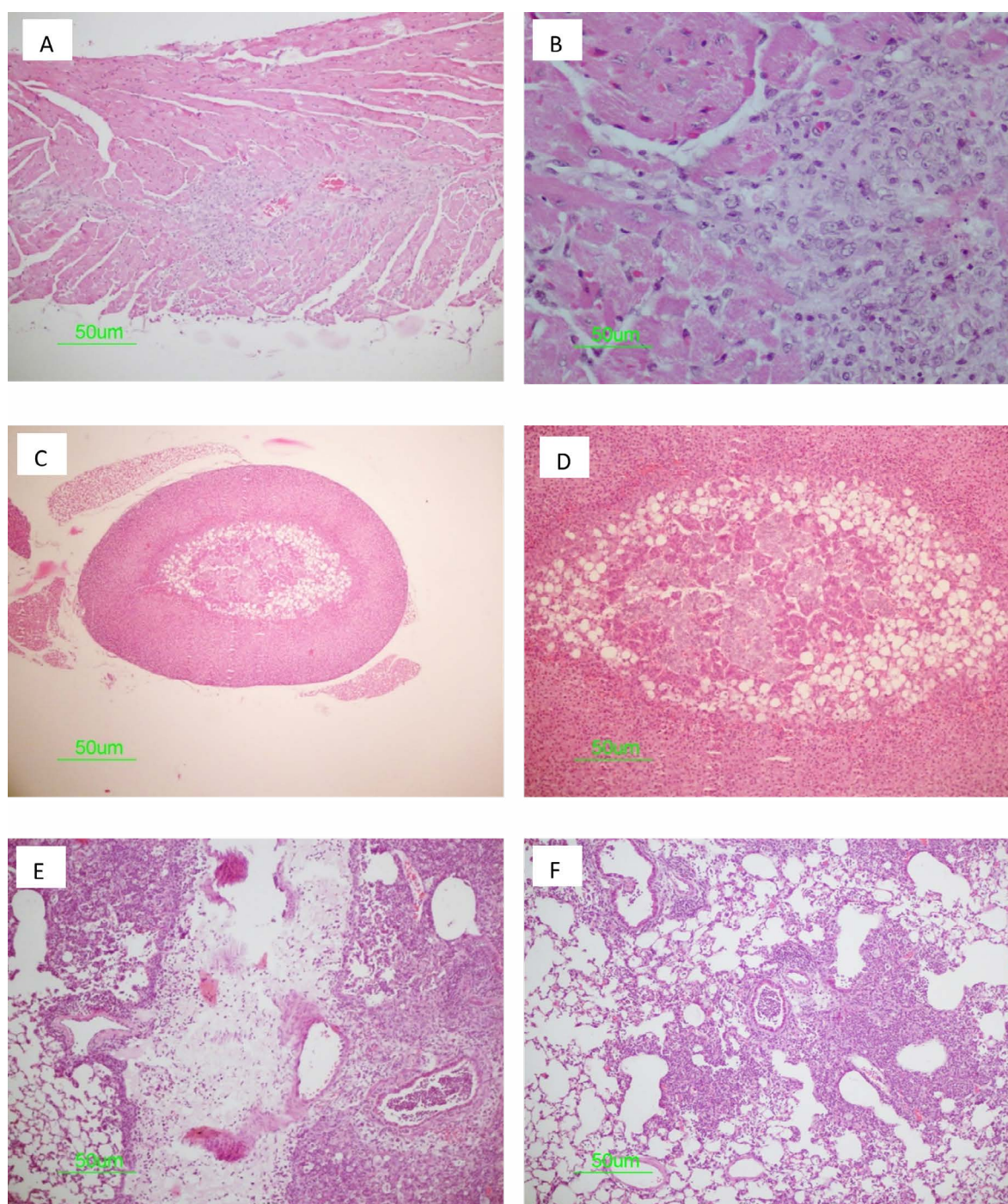


Figure 5:

A: Degenerated cardiac myocytes for the control mouse (male), stained with H&E, captured at 100x

B: Degenerated cardiac myocytes for the control mouse (male), stained with H&E, captured at 200x

C: Adrenal involution of X-zone, stained with H&E, for mouse treated with *euclea* at 500 mg/kg b.wt, captured at 100x

D: Adrenal involution of X-zone, stained with H&E, for mouse treated with *euclea* at 500 mg/kg b.wt, captured at 200x

E: Mononuclear infiltration of the lung airways (inflammation), stained with H&E, for mouse treated with *euclea* at 500 mg/kg b.wt, captured at 100x

F: Inflammation of the lung with dilatation of the alveoli for mouse treated with *euclea* at 500 mg/kg b.wt, stained with H&E

no statistical significance. This is evident in histopathological findings where degeneration of cardiac myocytes was noticed to a lesser extent. The degenerated muscle fibres were not dose dependent as the observation was seen in control animals, but rather these are spontaneous age-related lesions seen in mice.²⁸

The weights of adrenal gland in all treated female animals were significantly high at 1% level. This is a true demonstration which

is supported by studies which discovered that the growth and function of the adrenal gland is influenced by gender and age.²⁹ The question remains, however, why a similar observation is not seen in control animals.

The histopathological observation of the involution of the X-zone in the cortical region of the gland, in both control and treated animals, demonstrated lipid vacuolisation which demonstrated

lipid content. The question of why the biochemical results (cholesterol and triglycerides levels) were low compared to control animals remains the subject of further investigation. The opposite is seen in males where the cholesterol and triglyceride levels are higher than control animals (G2 and G4).

The histopathological findings in the repeated dose toxicity study demonstrated perivascular and peribronchial lymphoid aggregation in the lung as well as infiltration of the lung parenchyma by white blood cells in both acute and sub-acute studies. The alveolar sacs, alveolar ducts and alveoli of the control and treated animals are very much dilated and have lost normal structure. Inflammation suggests lung infection, and the dilated alveoli are trying to put in place a compensatory mechanism so that the lung can continue with normal function. Clinically this condition could lead to emphysema, a clinical condition that is characteristic of loss of elasticity due to the permanent destruction of parenchyma tissue structure.³⁰ Medial mineralisation of the brain and granulopoiesis hyperplasia of the liver was observed in both control and treated animals.

Conclusion

All lesions observed are spontaneous and age-related to laboratory animals and are not dose dependent. Based on these findings, the MTD of *euclea natalensis* root extract in Swiss albino mice is 500 mg/kg body weight. The NOAEL is 500 mg/kg body weight following oral dosing in Swiss albino mice for 28 consecutive days.

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Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

Ethical approval for this study was granted by the Regulation Committee for the Purpose of Control and Supervision of

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