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Toxicological and Phytochemical Evaluation of *Uvariodendron kirkii*

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Keywords
Uvariodendron kirkii, phytochemical screening, acute toxicity, subacute toxicity, LD_{50}, liver and kidney function.

Abbreviations
ALT – alanine aminotransferase
ANOVA – analysis of variance
AST – aspartate aminotransferase
BUN – blood urea and nitrogen
GHS- Globally Harmonized System of Classification and Labelling of Chemicals
H&E – haematoxylin and eosin
MCH – mean corpuscular haemoglobin
MCHC – mean corpuscular haemoglobin concentration
MCV – mean corpuscular volume
RBC – red blood cells
WBC – white blood cells

Abstract
Background: *Uvariodendron kirkii* is popularly used in Tana River County, Kenya for fertility regulation. This study is aimed at documenting its toxicity and phytochemical composition to validate its continuous usage in traditional medicine. Methods: Phytochemical screening was done to determine phytoconstituents of *U. kirkii* extracts. Acute toxicity was tested OECD test guideline 423. In sub-acute toxicity, three dose levels (62.5, 250, and 1000mg/Kg) were administered to mice for 28 days and observed as per OECD test guideline 407. Results: Phytochemical screening revealed the presences of tannins, terpenoids, alkaloids and saponins in the aqueous extract; and tannins, flavonoids and terpenoids in the organic extract. In acute toxicity, the LD_{50} was found to be >2000 mg/Kg for both extracts. In sub-acute toxicity, there were no adverse physical-clinical effects in all treatment groups. The aqueous extract caused a significant increase in thrombocyte counts, suggesting its usefulness in correcting thrombocytopenia and wound healing; and signs of hepatotoxicity indicated by a significant dose related increase in alanine aminotransferase levels and hepatocyte degeneration in mice that received 1000 mg/Kg of the extract. The organic bark extract produced signs of hepatotoxicity indicated by dose-related hepatocyte degeneration on histology; and signs of nephrotoxicity indicated by degeneration of renal tubules in the group that received 1000 mg/Kg of the extract. Conclusions: *U. kirkii* is safe when used orally for a short period. However, long-term administration may lead to nephrotoxicity and hepatotoxicity at high doses

therefore liver and kidney functions need to be monitored. Presence of phytochemical constituents suggest its usefulness in pharmaceutical research.

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1. Introduction

1.1 Background

Medicinal herbs have been used to manage various human ailments for many years. Traditional medicine (TM) is still popular to date, especially in developing countries. Eighty percent of the population in developing countries depends on traditional herbal medicine (Salatino et al., 2007). Recently, there has been a challenge in new drug discovery due to decrease in approval and increased costs, renewing interest in TM (Katiyar et al., 2012). Native medicine has gained recognition as a source of new knowledge on herbal and other remedies that traditional medicine practitioners (TMPs) have used for a long time; based on experience and ages of practical observation (Kokwaro, 2009). Lack of data on toxicity and dose response relationship are the main concerns in phytomedicine. There is therefore, need for well-designed clinical trials to prove their effectiveness and safety so as to gain public trust and conventionalize herbal remedies (Pal and Shukla, 2002). Due to rapid increase in population, fertility regulation finds great significance. Scientists have drawn attention towards plant products as fertility regulating agents due to perceived minimal side effects. WHO supports scientific exploration of traditional fertility regulating regimes. However, scattered evidence exists suggesting this area is suitable for novel drug development (Umadevi et al., 2013). A survey identified *Uvariodendron kirkii* to be among the most frequently used herbal contraceptives in Tana River County, Kenya (Kaingu, 2016). It is locally referred to as 'msaidizi' (Giryama) and is used as a fertility regulator. A decoction is made from boiling the root bark in water. One glass of this decoction is drunk daily for 30 days. Fresh decoction has to be prepared every 7 days (Kaingu et al., 2013). Despite its popularity and widespread use, its toxicity and phytochemical composition has not been intensively studied. This study therefore aims at documenting toxicological aspects and phytochemical constituents of *Uvariodendron kirkii* extracts.

1.2 Objectives of the research

The objective of the study was to provide scientific evidence to validate the continuous usage of *Uvariodendron kirkii* in TM by evaluating the toxicity and phytochemical composition of its root bark extracts.

1.3 Justification of the research

Modern contraceptives are highly inaccessible to women in developing countries, particularly in rural areas (Kaur et al., 2011). In addition, modern contraceptives are perceived to have adverse effects. This has caused lack of confidence in women and their partners causing them to forgo or discontinue family planning, or use methods in an irregular manner (Chebet et al., 2015; Chipeta et al., 2010). For these reasons, the use of herbal contraceptives is popular among women in developing countries. Determining the potency and toxicity of such plants may generate confidence and wider acceptance of herbal contraceptives. Information on toxicity is important to avoid toxic exposure and to avail remedies in case of toxicity. On the other hand, phytochemical screening is important in discovering the therapeutic importance of plants in pharmaceutical research. It was therefore justifiable to determine the phytochemical composition and toxicity profile of *Uvariodendron kirkii* to validate its traditional use.

2. Materials and Methods

This study was conducted on aqueous and organic extracts prepared from root barks that were collected from Tana River County, Kenya, Africa, which lies between latitudes 0° and 3° South, and longituted 38°30" East and 40°15" East. The botanical identity was authenticated by a taxonomist and a voucher specimen deposited

in the university herbarium at the School of Biological Sciences, University of Nairobi. The fresh root barks were cut into small pieces using a knife. They were then spread on a flat surface in a well-ventilated, rodent, insect and dust free room at room temperature and allowed to dry for two weeks. Once dry, the root barks were grounded into fine powder using a Cunningham grinder. The powder was stored in sachets within cool dry cupboards.

2.1 Preparation of extracts

2.1.1 Aqueous extract preparation

300g *Uvariodendron kirkii* of root bark powder was weighed using Lark digital weighing balance (LP502A, 500 G/0.01g). The powder was macerated in distilled water at a ratio of 1 to 6 (w/v) in a volumetric flask. The suspension was macerated for 48 hours at room temperatures with constant shaking. The suspension was filtered and the filtrate freeze dried for 5 days and the extract weighed to determine yield. The resultant extract was a light brown powder that readily dissolved in distilled water, which was used as the extract vehicle.

2.1.2 Organic extract preparation

Organic extraction was done using a mixture of dichloromethane (DCM): methanol at a ratio of 1:1 v/v (Harborne, 1998). Three hundred grams of *Uvariodendron kirkii* root bark powder was weighed using Lark digital weighing balance (LP502A, 500 G/0.01g). The powder was macerated in 800ml of the dichloromethane: methanol mixture and left to soak for 48 hours with constant shaking. The mixture was filtered using Whatman® number 1 filter paper. The filtrate was dried in vacuo using a rotary evaporator and finally completely dried in a sand bath set at 500C. The resultant extract was a dark-brown tar-like substance which formed a fine suspension in a mixture of dimethylsulfoxide (DMSO) and virgin olive oil at a ratio of 1:19(DMS0 : virgin olive oil) after warming.

2.2 Phytochemical screening

Qualitative methods were used to identify the phytochemical constituents of *Uvariodendron kirkii* root bark extracts. Test for tannins, flavonoids, anthraquinones, alkaloids, terpenoids, saponins and cardiac glycosides was done using methods described by Harborne (1998), Rukenya et al. (2015) and Trease and Evans (2009).

2.3 Acute oral toxicity of Uvariondendron kirkii extracts

The acute toxicity study protocol was conducted according to the OECD (2001) test guideline 423 with slight modification using nine healthy female rats weighing between 170-210g that were randomly assigned into three groups. Food was withheld overnight but water was provided ad libitum. The first group served as the negative control (untreated) group. The second and third groups received 2000 mg/Kg body weight of aqueous and DCM-methanolic *U. kirkii* root bark extracts respectively. These extracts were administered orally by gastric gavage. Food was withheld for a further 3-4 hours after extract administration. Clinical observations for signs were recorded for signs of toxicity and death. Special attention was given during the first four hours and thereafter daily for 14 days. The survival of these animals at the highest test dose dictated lack of need to lower the experimental dose. The rats were humanely euthanized using diethyl ether and necropsy performed.

2.4 Determination of sub-acute toxicity

2.4.1 Dosing and observation

The 28-day sub-acute toxicity study protocol was carried out as per the OECD (2008) guideline 407 using 40 female swiss albino mice weighing between 20-30 grams. The animals were randomly allocated into 8 groups of 5 each. The extracts were administered orally using intra-abdominal gavage daily for 28 days. Group 1, 2 and 3 received 62.5, 250 and 1000mg/Kg of *Uvariodendron kirkii* aqueous root bark extract respectively. Group 4, 5 and 6 received 62.5 mg/Kg, 250 mg/Kg and 1000mg/Kg of *Uvariodendron kirkii* DCM-methanol root bark extract respectively. There were two control groups (group 7 and 8) that received the extract vehicles (distilled water and 1:19(DMS0 : virgin olive oil), respectively) for a similar duration. The animals were observed daily for signs of toxicity and mortality and body weight changes were recorded weekly till the end of the study.

Feed and water consumption was calculated on a weekly basis.

2.4.2 Clinical test parameters

On day 28, the mice were fasted overnight and general anaesthesia was done with diethyl ether. Between 0.8 and 1.5 millilitres of blood was harvested from all animals through cardiac puncture from all animals. The collected blood was divided into two portions; one for haematolgical analysis, which was collected in Ethylenediaminetetraacetic acid (EDTA) containing vacutainer tubes, and the other for biochemical analysis, which was collected in plain (clot activator) vacutainer tubes. Blood for biochemical tests was centrifuged at 3000 revolutions per minute to obtain serum. The obtained serum was put in Eppendorf tubes and stored at -200C. Blood collected in EDTA tubes and serum in Eppendorf tubes were transported in a cool box containing ice packs and analyzed at the pathologist lancet laboratories. Haematological parameters determined included total erythrocyte count (RBC), total leucocyte count (WBC), platelet count, hematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Biochemical parameters evaluated included alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, blood urea and nitrogen (BUN) and creatinine. The ALT, AST and total protein levels were used to test the effects of *U. kirkii* root back extracts on liver function while the BUN and creatinine levels were used to test the effects of *U. kirkii* root back extracts on kidney (Mohan, 2010).

2.4.3 Pathological examination

At the end of the study, the animals were humanely euthanized using diethyl ether and subjected to post-mortem examination. Internal organs were examined for gross pathological changes. The liver and kidney were harvested; fixed in 10% formalin and processed for histopathological examination according to Kiernan (1981). The fixed tissue samples were trimmed and embedded in paraffin wax. The tissue blocks were sectioned at 5 µm using a microtone and then stained using haematoxylin and eosin (H&E) and observed under a light microscope (Leica DM 500®) under 40X, 100X and 400X objective lenses.

2.5 Data analysis

Test results were expressed as mean ± standard deviation of the mean and statistical significance was determined using one way analysis of variance (ANOVA) using IBM SPSS Statistics software version 21 and Tukey post hoc test. 95% level of significance ($P \leq 0.05$) was used in the analysis and P-value of <0.05 was considered statistically significant.

3. Results and Discussion

3.1 Phytochemical composition of Uvariodendron kirkii extracts

The phytochemical compounds detected on qualitative phytchemical screening are shown in table 1. The aqueous extract was found to have tannins, terpenoids, alkaloids, and saponins while the organic extract had tannins, terpenoids and flavanoids. Anthraquinones and cardiac glycosides were absent in both extract types. The presence of alkaloids, saponins and flavanoids justifies the traditional use of *U. kirkii* as a female anti-fertility drug. Alkaloids are thought to be responsible for anti-fertility properties of plants through various mechanisms which include blockage of ovulation, disruption of oestrus cycle, post-coital antifertility effect, abortive effects, endocrine disruption, anti-implantation activity and resorption of embryos. Saponins have arbotifacient, anti-zygotic and anti-implantation effects. Flavanoids have been shown to possess anti-zygotic, blastocytotoxic and anti-implantation properties (Kaingu, 2016). In addition to the reproductive effects, several other pharmacological activities can be deduced from the presence of phytochemicals in *U. kirkii* root bark extracts. Tannins have been associated with wound healing, anti-inflammatory, anti-oxidant, antimicrobial, cardioprotective, anti-diabetic and anti-obesity activities (Sieniawska and Baj, 2017). Terpenoids have been associated with anti-microbial, anti-cancer, antinociceptive, anti-spasmodic, hepatoprotective and anti-inflammatory activities (Ludwiczuk et al, 2017). Flavonoids possess antioxidant and free radical scavenging activity, hepatoprotective activity, antibacterial, anti-inflammatory, anti-cancer and antiviral activities. In this regard, flavonoids are

useful agents for coronary heart disease prevention, combating oxidative stress and growth regulatorion (Kumar and Pandey, 2013). Flavonoids have also been associated with anxiolytic and sedative effects (Aguirre-Hernández et al., 2016). Saponins have been shown to have cardioprotective, anti-diabetic, anti-cancer, immune stimulating effect (Yuan et al., 2010). They also have the ability to permeabilize the cell membrane (Thakur et al., 2011). Studies have associated alkaloids with anti-cholinesterase, anti-oxidant, anxiolytic, anti-inflammatory and anti-depressant properties in the treatment of symptoms and progression of certain diseases such as Alzheimers disease (Chaves et al, 2016). Others have suggested anti-microbial, anti-HIV and anti-parasitic activities (Patel et al., 2012). This study observed sedative and anxiolytic effects of *Uvariodendron kirkii* in the acute toxicity study. There is need for further investigation to quantify these metabolites and investigate the observed activities in acute toxicity study.

Table 1: Phytochemical composition of *U. kirkii* root bark extracts

Phytochemical compound	Aqueous extract	Organic extract
Tannins	+++	++
Alkaloids	++	-
Flavanoids	-	+++
Anthraquinones	-	-
Terpenoids	+++	+++
Saponins	+++	-
Cardiac glycosides	-	-

Key: +++ = strongly present; ++ = moderately present; + = mildly present; - = absent

3.2 Acute toxicity

The acute toxicity studies revealed that the LD_{50} of both the aqueous and DCM-methanolic extracts of *U. kirkii* root bark were above 2000mg/Kg. This corresponds with findings of similar studies on medicinal plants from the family Annonaceae which do not show acute toxic potential (Coria-Téllez et al., 2016; Olumese et al, 2016). Following the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), *U. kirkii* root bark extracts are classified as category 5, which are substances of low toxicity, and according to the WHO classification, it could be considered slightly hazardous (Category III). As shown in table 2, there were no adverse physical and behavioural changes in all mice that received both the aqueous and DCM-methanolic *U. kirkii* extracts at a dose of 2000mg/Kg body weight. However, during the initial phase of administration, rats treated with 2000mg/kg body weight of aqueous and DCM-methanolic U.kirkii root bark extract exhibited signs of sedation within the first two hours that normalized within thirty minutes. This may be attributed to presence of flavonoids, alkaloids, saponins and terpenoids in the plant extracts, which have been reported to cause anxiolytic and sedative effects observed in different plant extracts (Edewor-Kuponiyi, 2013; Aguirre-Hernández et al., 2016; Chaves et al., 2016). The results of weekly body weight changes in the different groups of rats are shown in table 3. The weight changes in both treated groups were not statistically significant as compared to the untreated group. Considering that the doses administered in the field and the effective dose of <800 mg/Kg (Kaingu, 2016) are much lower than the LD_{50} deduced from this study (>2000 mg/Kg), both the aqueous and DCM-methanolic extracts of *U. kirkii* may be considered safe for use.

3.3 Sub-acute toxicity

3.3.1 Physico-clinical changes

Oral administration of aqueous *U. kirkii* root bark extract for 28 days repeatedly did not lead to any physical and behavioural signs of toxicity even at the highest dose of 1000 mg/Kg body weight and did not significantly alter food (table 4) and water (table 5) consumption. There were no dose related significant changes in the live body weight (table 6). Sub-acute studies on the DCM-methanolic extract showed similar findings (table 7, 8 and 9).

Table 2: Effect of single dose of *U. kirkii* root bark extracts on physical and behavioural parameters

Observation	Control	Aqueous 2000 mg/Kg	Organic 2000mg/Kg
Respiratory changes	-	+	+
Circulatory changes	-	-	-
Skin and fur changes	-	-	-
Gripping strength	+	+	+
Sound response	+	+	+
Response to touch	+	+	+
Locomotion	+	+	+
Urination	+	+	+
Defaecation	+	+	+
Diarrhoea	-	-	-
Righting reflex	+	+	+
Lethargy	-	+	+
Sedation	-	+	+
Tremors	-	-	+
Convulsions	-	-	-
Mortality	-	-	-
Post-mortem changes	-	-	-

Key: +=present -=absent

Table 3: Effect of single dose of *U. kirkii* root bark extracts on weekly mean live weight variation

Extract Type and Dose	Initial Weight	Day 7 Weight	Day 14 Weight	P-value
Control – untreated	189.06 ± 4.64	206.94 ± 14.77	210.49 ± 5.29	-
Aqueous – 2000mg/Kg	209.94 ± 10.97	207.72 ± 15.11	213.3 ± 19.24	0.570
DCM:met – 2000mg/Kg	194.34 ± 18.24	206.65 ± 16.56	216.83 ± 11.73	0.878

Values are expressed as mean ± standard deviation Significant difference ($p \leq 0.05$)

Table 4: Effect of 28-day repeated dose administration of aqueous *U. kirkii* root bark extract on food consumption

DOSE	WEEK 1	WEEK 2	WEEK 3	WEEK 4	P-value
Control	4.98	4.99	4.51	4.73	-
62.5 mg/Kg	5.35	5.48	5.42	5.44	0.008
250 mg/Kg	4.87	4.40	5.21	4.87	0.996
1000 mg/Kg	4.84	4.64	4.62	4.47	0.730

Values are recorded in grams per mouse per day
Normal reference range – 3-6g per mouse per day (Derelanko,2018)

Table 5: Effect of 28-day repeated dose administration of aqueous *U. kirkii* root bark extract on water consumption

DOSE	WEEK 1	WEEK 2	WEEK 3	WEEK 4	P-value
Control	5.71	3.71	4.57	4.14	-
62.5 mg/Kg	5.71	5.71	5.29	5.00	0.271
250 mg/Kg	5.57	5.00	4.29	4.89	0.521
1000 mg/Kg	4.29	4.89	3.43	3.86	0.810

Values are recorded in average milliliters per mouse per day
Normal reference range – 3-7ml per mouse per day (Derelanko, 2018)

Table 6: Weekly mean live body weights of mice dosed with the aqueous *U. kirkii* root bark extract for 28 days

DOSE (mg/Kg)	INITIAL WEIGHT	WEEK 1	WEEK 2	WEEK 3	WEEK 4	P-value
Control	22.79±1.61	22.92±3.78	22.58±3.82	22.51±3.99	21.88±4.21	-
62.5	26.97±2.15	26.66±1.93	27.74±1.11	28.08±1.48	26.28±1.26	0.000
250	27.83±2.54	26.37±2.27	25.69±3.03	25.51±3.11	24.13±2.78	0.000
1000	23.58±2.39	22.26±2.51	21.75±3.01	21.59±2.97	21.7±3.16	0.919

Values are expressed as mean ± standard deviation
Significant difference ($p \leq 0.05$)

Table 7: Effect of 28-day repeated dose administration of DCM-methanolic *U. kirkii* root bark extract on food consumption

DOSE	WEEK 1	WEEK 2	WEEK 3	WEEK 4	P-value
Control	4.46	4.93	4.72	4.55	-
62.5 mg/Kg	4.54	4.66	4.68	4.57	0.997
250 mg/Kg	4.97	4.73	5.25	4.89	0.686
1000 mg/Kg	3.35	3.75	4.95	4.03	0.121

Values are recorded in grams per mouse per day
Normal reference range – 3-6g per mouse per day (Derelanko,2018)

Table 8: Effect of 28-day repeated dose administration of DCM-methanolic *U. kirkii* root bark extract on water consumption

DOSE	WEEK 1	WEEK 2	WEEK 3	WEEK 4	P-value
Control	3.57	4.29	4.14	3.71	-
62.5 mg/Kg	4.43	4.29	4.00	3.00	1.000
250 mg/Kg	4.29	4.14	3.86	4.29	0.895
1000 mg/Kg	3.00	3.71	4.00	3.29	0.536

Values are recorded in average milliliters per mouse per day
Normal reference range – 3-7ml per mouse per day (Derelanko, 2018)

Table 9: Weekly mean live body weights of mice dosed with the DCM-methanol *U. kirkii* root bark extract for 28 days

DOSE	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28	P-value
0 (control)	20.46±3.26	21.41±2.85	21.79±2.46	21.99±2.50	22.08±2.28	-
62.5mg/Kg bwt	26.96±3.67	25.52±4.27	26.01±4.30	26.29±4.24	25.08±4.01	0.000
250mg/Kg bwt	27.77±4.71	25.38±3.43	24.43±3.68	24.35±4.10	26.52±2.07	0.000
1000mg/Kg bwt	23.52±2.57	22.45±2.93	22.84±2.83	22.64±2.19	22.63±2.56	0.158

Values are expressed as mean ± standard deviation, Significant difference ($p \leq 0.05$)

3.3.2 Haematology

The effects of oral administration of various doses of aqueous and DCM-methanolic extracts of *U. kirkii* root barks on cumulative blood cell counts are shown in table 10 and table 11 respectively. Haematological analysis showed that all control and treated mice had lower mean thrombocyte counts than the normal reference range. This could be attributed to EDTA in the vacutainers used for blood collection which has been associated with pseudothrombocytopenia due to platelet aggregation (Fang et al., 2015). Significant elevation of thrombocyte counts was noted in all groups of mice that received aqueous extract of *U. kirkii* root bark compared to the control. This suggests that the aqueous extract could increase platelet counts in cases of thrombocytopenia in diseases such as Dengue fever and could be helpful in wound healing (Subenthirian et al, 2013; Atik et al, 2018). Other blood parameters were not affected in aqueous extract treatment groups compared to the control group. The mice that received sub-acute doses of DCM-methanolic *U. kirkii* root bark extract did not show significant dose-related changes in the cumulative blood counts.

Table 10: Effect of 28-day repeated dose administration of aqueous *U. kirkii* root bark extract on cumulative blood cell counts

Dose	RBC (10^6/uL)	WBC (10^3/uL)	Platelets (10^3/uL)
Control	9.89 ± 1.54	5.85± 1.72	264.67± 70.57
62.5	9.88 ± 0.56	5.13± 1.61	854.00± 153.64
250	10.40 ± 0.45	4.89± 2.14	936.20± 260.36
1000	10.99 ± 0.33	4.64± 0.57	674.40± 100.76
Referenced range	7 – 12	3 – 12	1000 – 1600

Values are expressed as mean ± standard deviation Normal referenced range (Derelanko, 2018)
Significant difference ($p \leq 0.05$)

Table 11: Effect of 28-day repeated dose administration of DCM-methanolic *U. kirkii* root bark extract on cumulative blood cell counts

Dose (mg/Kg)	RBC (10^6/uL)	WBC (10^3/uL)	Platelets (10^3/uL)
Control	10.30± 0.59	6.03± 1.75	626.33± 128.03
62.5mg/Kg	10.88± 0.51	16.00± 8.06	561.80± 135.27
250mg/Kg	10.01± 0.34	7.31± 3.00	450.00± 174.56
1000mg/Kg	10.37± 0.39	6.62± 0.53	756.40± 122.76
Referenced range	7 – 12	3 – 12	1000 – 1600

Values are expressed as mean ± standard deviation
Normal referenced range (Derelanko, 2018)
Significant difference ($p \leq 0.05$)

The effects of oral administration of various doses of aqueous and DCM-methanolic extracts of *U. kirkii* root barks on erythrocyte indices are shown in table 12 and table 13 respectively. There was a significant increase in the mean MCHC in the mice that received 1000mg/Kg aqueous *U. kirkii* root bark extract compared to the control. There were no other significant changes in mean erythrocyte indices in groups treated with the aqueous extract. This could however be considered an analyzer error as MCHC values have been seen to be prone to such errors (Bull et al., 2018). Significant dose-related differences were not detected in mice that received the DCM-methanolic *U. kirkii* root bark extract.

Table 12: Effect of 28-day repeated dose administration of aqueous *U. kirkii* root bark extract on erythrocyte indices

DOSE (mg/Kg)	PCV (%)	HEMOGLOBIN (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Control	49.40± 6.75	14.00± 2.29	50.07± 1.62	14.20± 0.78	28.30± 1.05
62.5	49.18± 23.00	14.98± 0.61	49.83± 1.96	15.20± 0.53	30.50± 1.72
250	49.22± 2.68	14.62± 0.73	47.14± 0.64	14.00± 0.25	29.66± 0.34▫
1000	49.50± 1.71	15.72± 0.46	45.08± 2.22	14.30± 0.34	31.80± 1.71
Reference range	40 – 54	13 – 17	43 – 54	13 – 18	31 – 34

Values are expressed as mean ± standard deviation
Normal referenced range (Derelanko, 2018)
Significant difference ($p \leq 0.05$)

Table 13: Effect of 28-day repeated dose administration of DCM-methanolic *U. kirkii* root bark extract on erythrocyte indices

DOSE	PCV (%)	HAEMOGLOBIN (g/dL)	MCV (fl)	MCH (pg)	MCHC (g/dL)
Control	48.67± 1.01	14.47± 0.45	49.30± 2.88	14.40± 0.56	29.10± 0.78▫
62.5	50.88± 1.79	15.44± 0.60	46.86± 3.40	14.20± 0.48	30.38± 1.94▫
250	46.50± 1.37	15.22± 0.29	46.52± 2.71	15.20± 0.62	32.76± 1.26
1000	49.26± 2.20	15.48± 0.62	47.98± 2.94	14.86± 0.75	31.44± 0.92
Reference range	40 – 54	13 – 17	43 – 54	13 – 18	31 – 34

Values are expressed as mean ± standard
Deviation, Normal referenced range (Deralanko, 2018)
Significant difference ($p \leq 0.05$)

3.3.3 Liver and kidney function

Serum analysis was focused on liver function tests and kidney function tests as they are the main organs of drug metabolism and excretion, respectively. Table 13 and table 14 show the effects of aqueous and DCM methanolic *U. kirkii* root bark extracts respectively. Dose-dependent differences attributed to the aqueous *U. kirkii* root bark extract were observed only in the ALT levels whereby the group that received 1000mg/Kg of the extract had a significantly elevated mean ALT level. This suggests that the aqueous extract has potential to cause liver damage at high doses. This finding is supported by histopathological changes in the liver which revealed varying degrees of hepatocyte degeneration and hepatocyte necrosis as shown in figures 1b and 1c. This could be attributed to presence of alkaloids that have been implicated in hepatocyte degeneration in previous studies (Benouadah et al., 2016). The histological structure of the liver from mice dosed with 62.5mg/Kg and 1000mg/Kg of the DCM-methanolic extract showed early signs of hepatic degeneration suggesting potential for liver damage on prolonged use as seen in figures 1d and 1e respectively. Though not statistically significant, creatinine levels were elevated in mice that received aqueous *U. kirkii* extract. Kidney damage could be implicated by the fact that histopathology showed degeneration of renal tubules in a dose-dependent manner shown in figures 2b and 2c. There were no significant differences in all biochemical parameters of mice that received the DCM-methanolic *U. kirkii* root bark extract indicating lack of physiological damage to the liver and kidney physiology. The histological structure of the kidney from mice dosed with 1000mg/Kg, as seen in figure 2d, revealed diffuse tubular degeneration suggesting kidney damage on prolonged use at high doses.

Table 14: Effect of 28-day repeated dose administration of aqueous *U. kirkii* root bark extract on biochemical parameters

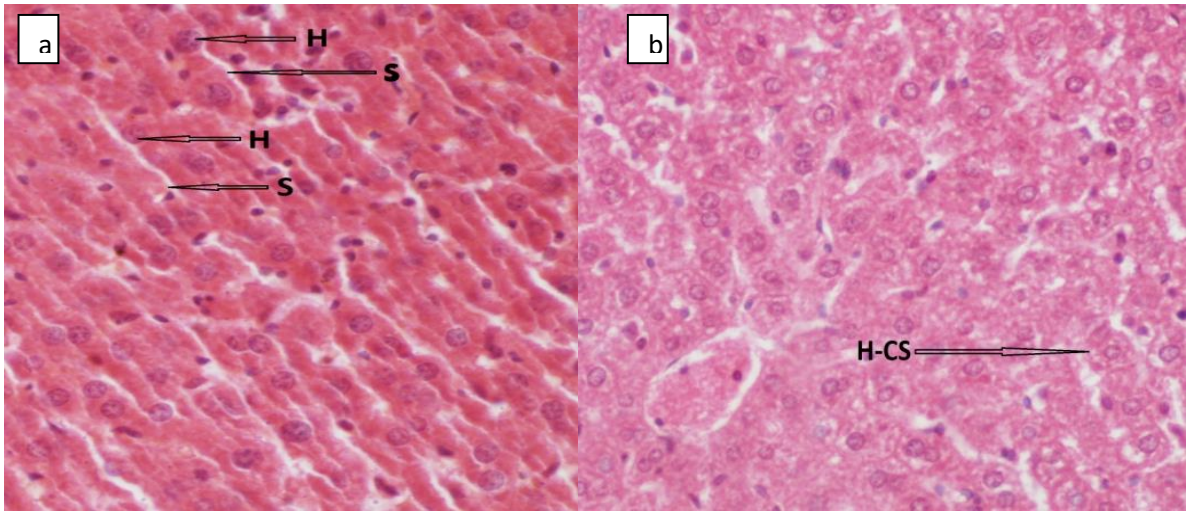
DOSE	ALT (IU/L)	AST (IU/L)	Total protein (g/L)	Urea	Creatinine
Control	62.02± 10.36	208.00± 36.51	66.37±▪ 22.25	8.45± 1.57	127.99± 50.42
62.5mg/Kg	70.07± 11.52	217.03± 66.44	71.82± 16.48	11.71± 2.14	145.53± 26.53
250mg/Kg	51.50± 17.42	211.13± 56.05	59.25± 14.92	8.67± 2.01	140.10± 30.25
1000mg/kg	b144.48± 53.61	233.86± 69.60	85.44± 14.22	16.86± 6.74	195.01± 77.73
Reference ranges	30 – 250	75 – 300	45 – 60	7.14 – 14.28	26.53 – 88.42

Values are expressed as mean ± standard deviation
Normal referenced range (Deralanko, 2018), Significant difference ($p \leq 0.05$)

Table 15: Effect of 28-day repeated dose administration of DCM-methanolic *U. kirkii* root bark extract on biochemical parameters

DOSE	ALT (IU/L)	AST (IU/L)	Total protein (g/L)	Urea	Creatinine
Control	121.45± 45.97	430.32± 81.11	89.57± 9.07	15.11± 3.95	196.45± 63.68
62.5mg/Kg	193.94± 75.29	90.11± 133.01	139.98± 62.40	18.20± 10.21	223.53± 128.18
250mg/Kg	152.07± 17.84	632.37± 315.92	40.15± 21.03	20.53± 3.27	267.77± 36.82
1000mg/kg	143.07± 41.85	543.43± 126.69	123.07± 39.60	16.00± 3.15	207.25± 41.97
Reference ranges	30 – 250	75 – 300	45 – 60	7.14 – 14.28	26.53 – 88.42

Values are expressed as mean ± standard deviation
Normal referenced range (Deralanko, 2018
Significant difference ($p \leq 0.05$)



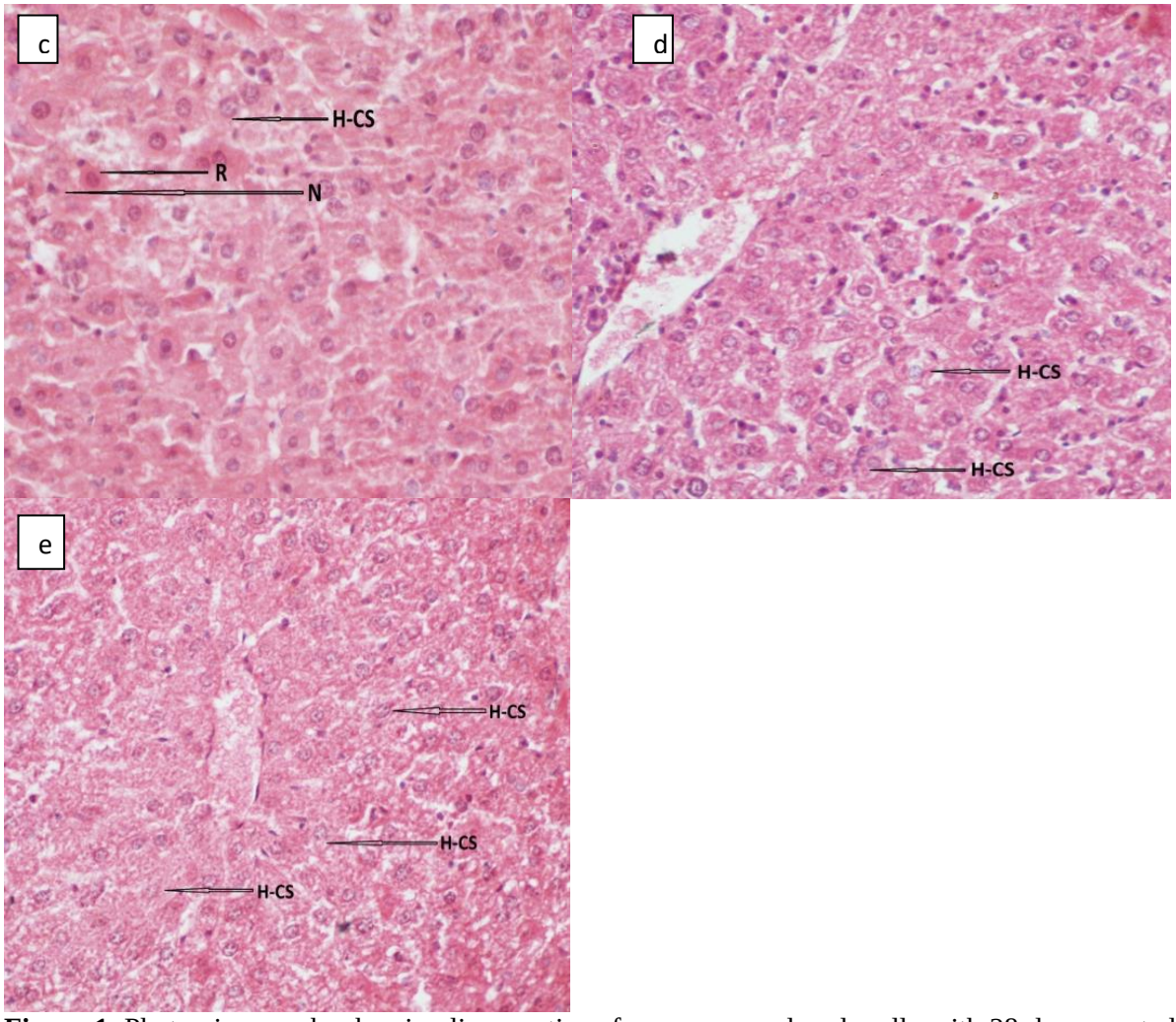


Figure 1: Photomicrographs showing liver sections from a mouse dosed orally with 28-day repeated doses *U. kirkii* root bark extracts, stained with H&E (400X magnification). **1** is a liver section from a control (normal) mouse, showing the normal parenchymal structure with evenly distributed hepatocytes separated by sinusoids. **2** is a liver section from a mouse dosed with 28-day repeated dose of 250mg/Kg body weight of aqueous *U. kirkii* root bark extract with multifocal hepatocyte cloudy swelling. **3** is a liver section from a mouse dosed with 28-day repeated dose of 1000mg/Kg body weight of aqueous *U. kirkii* root bark extract with diffuse cloudy swelling of hepatocytes and hepatocyte necrosis. **4** is a liver section from a mouse dosed with 28-day repeated dose of 62.5 mg/Kg body weight of DCM-methanolic *U. kirkii* root bark extract with focal hepatocyte degeneration. **5** is a liver section from a mouse dosed with 28-day repeated dose of 1000mg/Kg body weight of DCM-methanolic. *U. kirkii* root bark extract with diffuse hepatocyte degeneration. Key; H=hepatocyte, S=sinusoid, H-CS=hepatocyte with cloudy swelling, N= hepatocyte necrosis, R=regenerating hepatocyte.

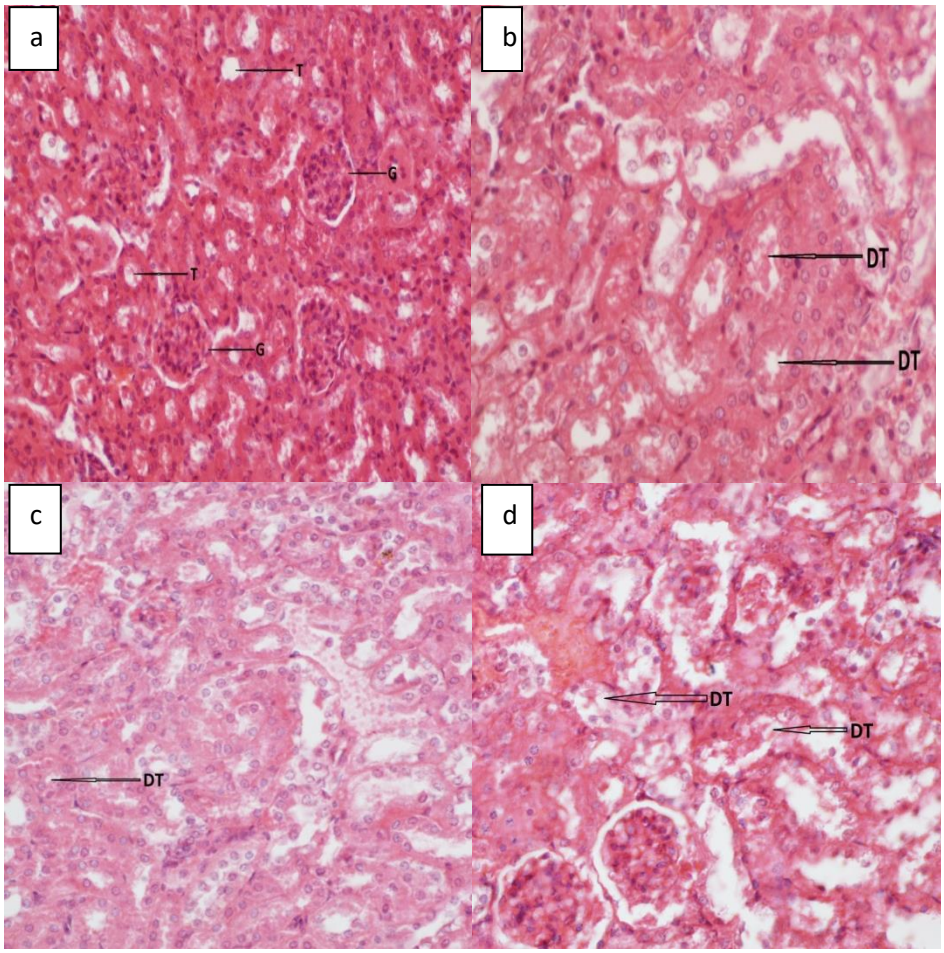


Figure 2: Photomicrographs showing a kidney sections from a mice dosed orally with 28-day repeated doses of *U. kirkii* extracts stained with H&E (400X magnification). **1** - kidney section of a control mouse with regular glomeruli and complete regular renal tubules. **2** - kidney section of a mouse dosed with 250mg/Kg aqueous *U. kirkii* extract with focal degeneration of the renal tubules. **3** - kidney section of a mouse dosed with 1000mg/Kg aqueous *U. kirkii* extract with focal degeneration of renal tubules. **4** - kidney section of a mouse dosed with 1000mg/Kg DCM-methanolic *U. kirkii* extract with diffuse tubular degeneration. Key; G=glomerulus, T=renal tubule, DT= Renal tubular degeneration

Conclusion

Uvariodendron kirkii root bark extracts can be considered safe when given orally for a short time. Long term use does not result in severe tissue destruction but liver and kidney function needs to be monitored especially for higher doses. The aqueous *U. kirkii* root bark extract showed a dose-related elevation of thrombocyte counts and could have a corrective effect in cases of thrombocytopenia and enhancement of wound healing. Various phytochemicals were found in *U. kirkii* root bark extracts, hence it could be considered useful in pharmaceutic discovery.

Research Highlights

1. Aqueous and DCM-methanolic extracts of *Uvariodendron kirkii* root bark have phytochemicals that are of medicinal value.
2. Acute oral toxicity studies did not cause mortality or adverse clinical signs.
3. Sub-acute toxicity studies did not cause adverse physico-clinical changes
4. 28-day repeated administration of higher doses caused signs of nephrotoxity and hepatotoxicity.

Research Limitations

This study is limited to qualitative phytochemical screening, acute and sub-acute toxicity study of *Uvariodendron kirkii* extracts. Quantification of phytochemical compounds and chronic toxicity studies have not be conducted.

Recommendations

Traditional use of *Uvariodendron kirkii* as a female contraceptive is safe. However, when used repeatedly for a long time at high doses, liver and kidney functions need to be monitored. Furthermore, chronic toxicity studies need to be conducted to establish its long term effect. Presence of phytochemical compounds supports continuation of its use in traditional medicine and its use in pharmaceutical research. Reseach needs to be conducted to quantify its phytochemical constituents.

Value to Global Audience

This study is of great value to those who advocate for the use of herbal remedies and development affordable alternative medicine for poor people.

Authors' contributions and competing interests

Kenana J.K. – performed the study and drafted the manuscript
Mbaria J.M. – was the main supervisor for the study and contributed in interpretation and discussion of results
Kaingu C.K. – was responsible for collection of plant materials used by the traditional healers, supervised the study and contributed in interpretation and discussion of results Okumu P.O. – contributed in histopathological analysis of liver and kidney sections the interpretation and discussion of results.
All the authors declare no competing interests.

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