Journal of Drug Metabolism and Toxicology

Musila et al., J Drug Metab Toxicol 2017, 8:1
DOI: 10.4172/2157-7609.1000222

Research Article Open Access

Acute and Sub-Chronic Oral Toxicity Study of Methanolic Extract of *Caesalpinia volkensii* (Harms)

Michael N Musila[1*], David N Ngai[1], Jane W Mbiri[2], Shardrack M Njagi[1], Wilton M Mbinda[3] and Mathew P Ngugi[1]

[1]*Department of Biochemistry and Biotechnology, Kenyatta University, Nairobi, Kenya*

[2]*Biochemistry and Biotechnology, School of Pure and Applied Sciences, South Eastern Kenya University, Kenya*

[3]*Department of Physical Sciences, School of Pure and Applied Sciences, Karatina University, Kenya*

[*]**Corresponding author:** Michael Ndile Musila, Department of Biochemistry and Biotechnology, Kenyatta University, P. O. Box 43844, 00100, Nairobi, Kenya, Tel: +254725270341; E-mail: 2mmusila@gmail.com

Received date: December 12, 2016; **Accepted date:** January 5, 2017; **Published date:** January 12, 2017

Abstract

Medicinal plant *Caesalpinia volkensii* is used to treat Malaria, conjunctivitis, retinoblastoma, and eyelid swelling as well as gonorrhoea and bilharzia. Animal toxicity information on this plant is limited. This medicinal plant was collected in Embu County in Kenya to evaluate its Acute and sub-chronic toxicity using Wistar rats. The rats were orally administered with different doses of the plants extracts. They were weighed on first day and after every 7 days during treatment with the extract. Signs of toxicity were also observed. After 28 days, the rats were sacrificed and blood samples taken for full hemogram, renal and liver function tests. Weight of internal organs was also recorded. Data was analysed using Minitab statistical software version 17. In acute toxicity studies, *C. volkensii* extracts did not result to death at extract dose of 2000 mg/kg body weight. In sub-chronic toxicity studies, there was no significance difference in body weight and organ weight among the groups treated with the plant extract ($p>0.05$). Treatment with extract dose of 1000 mg/kg body weight resulted in significant increase in total bilirubin and aspartate aminotransferase ($P<0.05$). In addition, 100 mg/kg body weight resulted in significant decrease in haemoglobin and haematocrit. Qualitative phytochemical screening confirmed the presence of various phytochemicals which have the ability to protect erythrocytes from oxidative damage as well as erythropoietin stimulatory activities. It was therefore concluded that methanolic leaf extract of *C. volkensii* is safe for acute oral administration. However, care should be exercised in sub-chronic exposure at 1000 mg/kg body weight and above to avoid liver injury.

Keywords: Bioassay; Blood Clotting; Anticoagulant; Haemoglobin; Mortality

Introduction

The ability of plants to synthesize varieties of chemical compounds gives them both physiological and pharmacological effects hence their popular use as treatment agents and food [1]. Moreover, medicinal plants use has been accompanied by great research interests, which provides scientists and policy maker accurate information to regulate their use [2].

There are several plants with diverse medicinal use in Kenya [3]. However, their safety evaluation cannot match the rate at which they are used. Therefore, there is need to bridge this gap. Only a handful of medicinal plants have undergone safety profiling. In addition, there are no elaborate regulatory bodies in place. This predisposes most of herbal users to toxicity [4].

Easy availability of herbal medicine has led to their increased use [5]. This has resulted to increased reports of their suspected toxicity and adverse events. Such unwanted reactions can be due to side effects; reactions occurring as a result of overdose, over duration, tolerance, dependence-addiction; hypersensitivity, allergic and idiosyncratic reactions; mid-term and long-term toxic effects. It is such reaction that necessitates toxicity evaluation.

Mechanisms of toxicity can present in several ways. One of them is on target whereby the toxicant binds to a targeted receptor with unintended high affinity resulting to untoward reaction. Off target is another mechanism whereby the toxicant binds to unintended receptor resulting to unintended reaction [6].

Nontoxic compounds can be metabolized to toxic end products in the body organs [7]. Idiosyncratic reactions are reactions which may result from medicinal plants use [8]. They have no apparent explanations for their occurrence and can only be approached from an individual perspective. They are related to the genetic uniqueness of an individual [9].

The toxicant or the toxicant metabolites can be excreted by the body and if not, the accumulated toxicant or its products of metabolism can react with DNA resulting to DNA adducts which are mutagenic and can lead to cancer [10]. On the other hand, proteins adduct formed may cause abnormal immune response that can lead to cellular damage [11]. In addition, toxic medicinal plants may impair the oxidative protective mechanisms leading to cell death through apoptosis or necrosis [12].

Caesalpinia volkensii (Harms) is a woody climber of length ranging between 1.8-4 m and contains bipinate leaves, which are paired 3 to 6. It has yellow flowers and petals of about 16 mm by 3.5-4.5 mm. The plant's fruits have seeds that are smooth, shiny and hard to crack [13, 14]. This plant is native to Ethiopia, Uganda, Tanzania and Kenya [15].

In Kenya, it grows in low land forest and is mainly cultivated in parts of central Rift Valley, Coast and Eastern regions [3].

In East Africa, *C. volkensii* is used to treat malaria [16] and to relieve abdominal upsets during pregnancy [17]. The plant is also used to treat conjunctivitis, retinoblastoma, and eyelid swelling as well as gonorrhoea and bilharzias [17]. The plant's seeds are used to treat stomach ulcers [17]. Various studies done on this plant have found that it to contain hypoglycaemic properties [5] anti-nociceptive properties [18] and anti-inflammatory activity [19].

Despite preliminary evidence of therapeutic benefits and traditional use of C. volkensii, the scientific validation and systematic safety evaluation of *C. volkensii* has not been comprehensively established so far. In this study, we endeavoured to evaluate the possible acute and sub-chronic toxicity of orally administrated methanolic extracts of *C. volkensii* on Wistar rat models.

Materials and Methods

Collection and preparation of plant materials

Fresh leaves of *C. volkensii* were collected from Embu County, Kenya, with the help of an herbalist and transported in polythene bags to Biochemistry and Biotechnology laboratories at Kenyatta University (KU) for further processing. The plant samples were then provided to a taxonomist at the National Museums of Kenya for botanical authentication and a specimen deposited at the University Herbarium for future reference. Plant leaves were shade dried at room temperature after washing under tap water to remove any debris. The leaves were then ground into fine homogenous powder using an electric mill and sieved through a mesh sieve.

Extraction

To obtain the extract, 500 g of plant fine powder sample material was soaked in one litre of methanol for 24 h. The extract was filtered using Whatman's filter paper No. 1 and the filtrate concentrated under reduced pressure using a rotary evaporator at 40°C. The concentrate was then put in an airtight container and stored at 4°C before use in bioassay studies.

Experimental design

Experimental animals: Adult male Wistar rats aged between 8-10 weeks old and weighing between 140-160 g were used to perform in vivo toxicity studies. The animal breeding colony was acquired and bred in the Animal Breeding and Experimentation Facility of the Department of Biochemistry and Biotechnology, Kenyatta University. These animals were maintained at 12/12 h light/dark cycle, 25°C ± 2°C temperatures and relative humidity of 60% ± 5%. They were fed on standard animal pellets diet and water ad libitum.

Four groups of five animals each were randomly selected, marked for identification and housed before beginning the experiment. The rats were then allowed to acclimatize for 5 days before beginning the experiment. Guidelines by Organization for Cooperation and Development (OECD) and ethics committee of the Kenyatta University on research on animal models were followed.

In vivo toxicity studies

Acute toxicity testing: Based on the information obtained from previous work [5] and information from structurally related compounds, a limit test at 2000 mg/kg body weight was conducted using five groups (5) of Wistar rats each group consisting of one (1) rat [20]. The animals were fasted overnight and their body weight taken. The fasted body weights were used to calculate individual doses of the test substance used for oral administration. The animals were sequentially dosed at 48 h intervals through oral gavages using bulb tipped gastric gavage needle. After oral administration of the plant extract, the animals were denied access to food for the initial 4 h only. For every animal dosed, signs of toxicity were observed for 30 min and periodically for 48 h before dosing the next animal. Each animal was observed for a maximum of 14 days. The results were recorded as 0: Survival and x: dead.

Sub-chronic toxicity testing: For sub-chronic toxicity testing, twenty (20) Wistar rats were used. They were divided into four (4) groups of five (5) male Wistar rats in each group. Group 1 was the control group, which was orally given 1% Dimethyl Sulfoxide (DMSO) daily, for 28 days. Wistar rats in group 2, 3 and 4 were orally administered with 100 mg/kg, 300 mg/kg, and 1000 mg/kg body weight respectively, of the plant extract dissolved in 1% DMSO daily for 28 days. The doses were calculated from the progression factor of 3.2, starting from 1000 mg/kg body weight downwards, which is the standard acceptable toxicity limit [21]. All the groups received standard pellet diet and water ad libitum.

Clinical signs, body weight changes and mortality: During the entire dosing period, the animals were observed daily for clinical toxicity signs and mortality. The rats were weighed prior to dosing, after every 7 days, and before sacrifice on the last day. Weight of each rat was recorded separately. After administration of the plant extract for twenty-eight days, the animals were anaesthetized in airtight dissection jar containing cotton soaked in chloroform. Each anaesthetized animal was laid on a dissecting board and a pair of scissors used to open up the animal by cutting through vertical mid-line from neck to peritoneum [21]. Brain, heart, liver, pancreas, spleen and kidneys were excised and weighed after washing them in normal saline. The organs were preserved in plastic containers containing 10% buffered formalin solution.

Blood samples collection: Blood samples were collected through cardiac puncture. After general anaesthesia with chloroform, a 22 gauge needle attached to a 3 ml syringe was inserted to the notch at the caudal aspect of the sternum and directed to the heart. The position was determined by palpating for the heartbeat. The plunger was pulled backwards gently in order to draw blood. The collected blood was divided into two portions; one for haematological analysis, which was collected in tubes containing anticoagulant Ethylenediaminetetraacetic Acid (EDTA) and the other for biochemical analysis, which was collected in tubes without anticoagulant. Blood for biochemical tests was kept for 1 hour at room temperature to allow adequate clotting. This was followed by centrifugation at 3000 revolutions per minute (rpm) for 10 min to obtain serum. The serum obtained was put in Eppendorf tubes and stored at -20°C awaiting biochemical analysis.

Haematological assays: Blood for haematological tests was collected through cardiac puncture. The blood from all experimental animals was collected in EDTA containing tubes for haematological assay. Parameters including white blood cells differential counts (WBC) and red blood cells (RBC) counts, red blood cells distribution width

(RDW), haemoglobin concentration (HB), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC), mean cell volume (MCV), platelets counts and platelet distribution width (PDW) were determined, using Cell-tac alpha automated haematology analyser (Nihon Kohden Corporation, Tokyo, Japan) according to the manufacturer's instructions.

Determination of biochemical parameters: The biochemical parameters determined included alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, proteins, albumin, urea, creatinine, and electrolytes. They were determined using automated BS-200 biochemistry auto analyser (Shenzhen Mindray Bio-medicals Co., Ltd, Hamburg, Germany) according to the manufacturer's prescribed protocol.

Statistical data analysis

Both qualitative and quantitative data was presented in tables and graphs. Data on weight, haematology tests, liver and kidney tests results was analysed using one-way analysis of variance (ANOVA) with Minitab statistical computer software v.17 (Minitab Inc., Pennsylvania, U.S.A). The data was also subjected to descriptive statistics and the results expressed as mean ± standard error of mean. Means were separated using Tukey's Honest Significant Difference test at a confidence level of 95% ($p \leq 0.05$).

Qualitative Phytochemical screening

Using standard procedures, qualitative screening for saponins, cardiac glycosides, sterols, phenolic compounds, terpenoids, alkaloids and flavonoids was done to determine their presence or absence using methods described by Kotake; Ayoola et al. [22,23].

Test for saponins: To 2 ml of the plant extract, five (5) drops of sodium bicarbonate solution were added. The mixture was shaken vigorously for about 15 s, and allowed to stand for 15-20 min. The test results were interpreted as negative if there was no frothing and positive if there was frothing after 15-20 min [22].

Test for alkaloids: For alkaloids test, 2 ml of 1 molar hydrochloric acid was added to 2 g of the plant extract. The mixture was stirred, boiled in water bath for 5 min and allowed to cool. Thereafter, the mixture was filtered using Whatman's filter paper No. 1 and two drops of Dragendorff's reagent added. A colour change to orange after addition of Dragendorff's reagent indicated presence of alkaloids [22].

Test for Terpenoids: To 0.5 g of plant extract, 2 ml of chloroform was added. This was followed by carefully layering 3 ml of concentrated sulphuric acid. A reddish brown coloration at the interface indicated the presence of terpenoids [23].

Test for flavonoids: To 2 ml of each plant extract, 1 ml of 1% sodium hydroxide was added. An intense golden yellow precipitate indicated positive results [22].

Test for cardiac glycosides: Cardiac glycosides presence was tested by dissolving 0.5 g of the extract in 2 ml glacial acetic acid containing 2 drops of 10% ferric chloride solution. One millilitre of concentrated sulphuric acid (H_2SO_4) was carefully added. Formation of either a violet, brown or greenish ring at the interphase was regarded as positive for deoxysugar characteristic of cardenolides [23].

Test for steroids: The presence of steroids in the extract was tested by dissolving 0.5 g of the extract in 2 ml of chloroform. Three millilitres of 2 M sulphuric acid was carefully layered. A reddish brown coloration at the interphase indicated the presence of steroids [22].

Test for phenolics: To screen for the presence of phenols in the extract, 1 ml of ferric chloride solution was added to 2 ml of the extract. Formation of blue to green colour indicated the presence of phenolics [22].

Results

Acute toxicity test

There was no mortality reported or any toxicity signs including autonomic effects (perspiration, defecation, inconsistence in urination, salivation and pilo-erection) in animals administered orally with of 2000 mg/kg body weight of methanolic leaf extracts of *C. volkensii*. In addition, there was no sign of central nervous system intoxication (gait, lethargy, drowsiness, restlessness, convulsions and coma) reported.

Sub-chronic toxicity testing

Effects of methanolic leaf extract of C. volkensii on body and organ weights in Wistar rats: The weekly change in body and organ weight at the end of treatment period is represented in Tables 1 and 2, respectively.

Treatment	Weekly weight of Wistar rats (g)					Δ Weight/Week
mg/kg.bw	Day 1	Day 7	Day 14	Day 21	Day 28	(g/Week)
Control	146.86 ± 3.23^a	156.60 ± 4.27^a	181.52 ± 5.56^a	200.02 ± 5.59^a	202.94 ± 6.12^a	14.02 ± 1.26^a
100	144.76 ± 4.13^a	167.20 ± 6.90^a	177.90 ± 4.64^a	187.14 ± 7.78^a	200.10 ± 7.88^a	13.84 ± 1.54^a
300	157.40 ± 2.68^a	178.80 ± 5.03^a	193.44 ± 5.49^a	201.24 ± 2.94^a	207.36 ± 6.24^a	12.49 ± 1.56^a
1000	143.90 ± 4.38^a	155.20 ± 8.09^a	172.84 ± 8.68^a	184.02 ± 8.72^a	190.54 ± 9.93^a	11.66 ± 1.73^a
Values are expressed as Mean ± SEM for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p>0.05 (Analysed by ANOVA followed by Tukey's post hoc test).						

Table 1: Effect of oral administration of methanolic leaf extract of *C. volkensii* on body weight in Wistar rats.

Treatment groups				
Organ	**Control**	**100 mg/kg.bw**	**300 mg/kg.bw**	**1000 mg/kg.bw**
Brain	1.95 ± 0.10^a	1.84 ± 0.11^a	1.88 ± 0.06^a	2.01 ± 0.07^a
Liver	9.72 ± 0.57^a	8.60 ± 0.64^a	11.08 ± 0.43^a	9.56 ± 0.92^a
Kidney	1.67 ± 0.06^a	1.57 ± 0.09^a	1.83 ± 0.78^a	1.64 ± 0.14^a
Spleen	0.92 ± 0.03^a	0.81 ± 0.11^a	0.90 ± 0.01^a	0.91 ± 0.07^a
Lungs	2.55 ± 0.20^a	2.37 ± 2.37^a	2.64 ± 0.15^a	2.78 ± 0.14^a
Heart	0.69 ± 0.02^a	0.61 ± 0.06^a	0.78 ± 0.03^a	0.71 ± 0.07^a

Values are expressed as Mean ± SEM for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p>0.05 (Analysed by ANOVA followed by Tukey's test).

Table 2: Effects of oral administration of methanolic leaf extract of *C. volkensii* on the organ weight in Wistar rats

There was no behavioural change or death noted at the end of the treatment period. In addition there was gradual gain in weekly body weight in all treatment groups. However, gain in body weight in all treatment groups was insignificant when compared to the control group (p>0.05; Table 1). The variation in the weight of body organs after administration of the three doses of the extract, were not statistically remarkable (p>0.05).

Effects of methanolic leaf extract of C. volkensii on liver and renal functions in Wistar rats: Oral administration of methanolic leaf extract of *C. volkensii* at 100 mg/kg, 300 mg/kg, and 1000 mg/kg body weight for 28 days resulted in varied effects on liver and renal function tests. For example, extract dose of 1000 mg/kg body weight caused significant increase in aspartate aminotransferase (AST) and total bilirubin compared to the control group (p<0.05; Table 3). This was not reflected in treatment groups administered with extract dose of 100 and 300 mg/kg body weight. Conversely, all the other liver and renal function tests (proteins, albumin, alanine aminotransferase, aspartate aminotransferase, direct bilirubin, creatinine, urea, uric acid, sodium and potassium) did not show any significant difference from the control group (p>0.05).

	Dose of *C. volkensii* (mg/kbw/day)			
Parameters	**Control**	**100**	**300**	**1000**
GLU (mmol)	10.12 ± 0.93^a	7.54 ± 1.06^a	9.54 ± 0.77^a	9.46 ± 1.20^a
T.PROT (g/l)	61.28 ± 0.90^a	69.2 ± 2.26^a	59.38 ± 1.86^a	65.76 ± 1.63^a
ALB (g/l)	29.08 ± 0.38^a	27.60 ± 1.98^a	29.28 ± 1.40^a	32.78 ± 0.77^a
ALT (IU/L)	139.2 ± 10.8^a	147.4 ± 10.1^a	108.84 ± 4.00^a	143.8 ± 11.3^a
AST (IU/L)	228.96 ± 5.88^b	229.7 ± 12.3^b	217.9 ± 14.4^b	285.77 ± 14.5^a
AST/ALT	1.69 ± 0.15^a	1.59 ± 0.13^a	2.09 ± 0.09^a	2.16 ± 0.28^a
D.BIL (µm)	0.47 ± 0.22^a	0.64 ± 0.64^a	0.40 ± 0.25^a	0.66 ± 0.30^a
T.BIL (µm)	1.850 ± 0.23^b	3.20 ± 1.02^{ab}	2.79 ± 0.59^{ab}	5.564 ± 0.72^a
CREAT (µm)	45.80 ± 2.35^a	47.54 ± 3.88^a	45.72 ± 5.59^a	44.36 ± 3.74^a
UREA (mmol)	10.34 ± 0.44^a	11.53 ± 0.64^a	10.35 ± 0.54^a	10.51 ± 0.75^a
UA (µm)	349.7 ± 44.90^a	415 ± 55.30^a	297.7 ± 19.30^a	432 ± 39.70^a
Na (mmol)	275.6 ± 10.4^a	238.34 ± 7.99^b	288.80 ± 7.51^a	299.04 ± 9.49^a
K (mmol)	8.68 ± 0.47^a	7.92 ± 0.92^a	6.60 ± 0.22^a	7.56 ± 0.39^a

Values are expressed as Mean ± SEM for five animals per group. Values with the same superscript across treatments are not significantly different from each other at (p>0.05). (Analysed by ANOVA followed by Tukey's test). GLU: Glucose; UA: Uric acid; CREAT: Creatinine; UREA: Urea; Na: Sodium; K: Potassium; T. prot: Total

protein; D. bil: Direct bilirubin; T. bil: Total bilirubin; ALB: Albumin; ALT: Alanine amintransferase; AST: Aspartate aminotransferase; mmol: Millimoles; IU/L: International units per litre; µm: Micromoles; g/l: Grams per litre.

Table 3: Effects of methanolic leaf extract of *C. volkensii* on liver and renal functions in Wistar rats.

Effects of methanolic leaf extract of C. volkensii on haematological profiles in Wistar rats: There was significant decrease in haemoglobin (HB) concentration and packed cell volume (PCV) in the group of animals treated with extract dose of 100 mg/kg body weight ($p<0.05$; Table 4). Contrary, extract dose of 300 mg/kg and 1000 mg/kg body weight did not alter HB and PCV significantly. Apart from HB and PCV, other red blood cells indices were insignificantly affected in all experimental doses ($p>0.05$).

	Dose of *C. volkensii* (mg/kbw/day)			
Parameters	**Control**	**100**	**300**	**1000**
RBC (1012/L)	6.33 ± 0.15^{a}	6.02 ± 0.23^{a}	6.53 ± 0.18^{a}	6.67 ± 0.22^{a}
Hb (g/dl)	14.7 ± 0.28^{a}	11.22 ± 1.69^{b}	14.56 ± 0.05^{ab}	15.38 ± 0.44^{a}
Hct (%)	42.8 ± 1.30^{a}	37.84 ± 1.71^{b}	43.94 ± 0.45^{a}	44.18 ± 0.80^{a}
MCV (fL)	67.68 ± 1.33^{a}	62.82 ± 0.80^{a}	67.56 ± 1.58^{a}	66.46 ± 1.46^{a}
MCH (9 pg/cell)	23.20 ± 0.40^{a}	21.94 ± 0.51^{a}	22.32 ± 0.58^{a}	23.04 ± 0.45^{a}
MCHC (g/dL)	34.36 ± 0.51^{a}	34.96 ± 0.61^{a}	33.10 ± 0.27^{a}	34.76 ± 0.77^{a}
RDW (%)	16.24 ± 0.34^{a}	15.82 ± 0.25^{a}	17.08 ± 0.42^{a}	16.28 ± 0.30^{a}
WBC (109/L)	11.66 ± 1.19^{a}	6.44 ± 0.77^{a}	10.46 ± 1.03^{a}	9.66 ± 1.78^{a}
Neutrophils (%)	22.80 ± 4.18^{a}	24.60 ± 4.03^{a}	29.80 ± 5.15^{a}	33.20 ± 2.13^{a}
Lymphocyte (%)	71.80 ± 4.22^{a}	69.20 ± 3.50^{a}	63.20 ± 4.76^{a}	60.20 ± 2.40^{a}
Monocytes (%)	3.00 ± 0.58^{a}	3.80 ± 0.97^{a}	3.60 ± 0.51^{a}	4.80 ± 0.58^{a}
Eosinophils (%)	2.40 ± 1.05^{a}	2.40 ± 1.29^{a}	3.40 ± 0.81^{a}	4.20 ± 1.53^{a}
Basophils (%)	0.40 ± 0.26^{a}	0.40 ± 0.40^{a}	0.20 ± 0.20^{a}	0.60 ± 0.40^{a}
Platelets(109/L)	513.8 ± 15.8^{a}	481.8 ± 15.4^{a}	527.8 ± 15.4^{a}	484.20 ± 8.32^{a}
PDW	14.46 ± 0.09^{a}	14.58 ± 0.20^{a}	20.08 ± 5.61^{a}	14.52 ± 0.07^{a}
PCT%	0.45 ± 0.02^{a}	0.53 ± 0.18^{a}	0.43 ± 0.05^{a}	0.35 ± 0.02^{a}

Values are expressed as Mean ± SEM for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p>0.05$ (Analysed by ANOVA followed by Tukey's post hoc test). RBC: Red blood cell, Hb: Haemoglobin, Hct: haematocrit, MCV: Mean cell volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, RDW: Red blood cells distribution width, WBC: White blood cells, PDW: Platelet distribution width, PCT: plateletcrit.

Table 4: Effects of methanolic leaf extract of *C. volkensii* on haematological profiles in Wistar rats.

Moreover, there was no significant difference observed in differential leukocytes counts in all the treatment groups when compared to the control group.

Qualitative phytochemical screening: Qualitative phytochemical screening of methanolic leaf extract of *C. volkensii* established the presence of saponins, alkaloids, terpenoids, flavanoids, and phenols. However, the extract lacked cardiac glycosides and steroids (Table 5).

Discussion

Classification of the safety level of a substance to both the environment and people can be achieved by conducting acute toxicity test [24]. Acute toxicity testing of a substance gives an array of possible clinical signs likely to occur. It gives a dose range that may be used in subsequent testing. Acute toxicity testing is also useful in estimation of therapeutic index [25]. When there is information from structurally related compounds that the test material may not be toxic, a limit test at 2000 mg/kg body weight can be conducted [21].

Survival of the Wistar rats following administration of methanolic leaf of *C. volkensii* extract at 2000 mg/kg body weight implies that the median lethal dose (LD50) of methanolic leaf extract of *C. volkensii* is beyond 2000 mg/kg body weight.

Any material with toxicity beyond 1000 mg/kg body weight may be considered to be of low toxicity and safe to use [26]. However, LD50 cannot be considered as a biological constant because it may be

affected by caging conditions, animal species and the diet among other things [26]. The findings on *C. volkensii* suggest that it is nontoxic and safe for oral use. However, acute toxicity testing cannot indicate cumulative toxic effects of a substance, therefore, necessitating sub-chronic and chronic testing, to evaluate the safety profile of phytomedicine [25].

Phytochemicals	*C. volkensii*
Saponins	+
Alkaloids	+
Cardiac glycosides	-
Terpenoids	+
Flavonoids	+
Phenols	+
Steroids	-
The sign (+) denotes a positive phytochemical test while (-) denotes a negative phytochemical test.	

Table 5: Qualitative phytochemical composition of methanolic leaf extract of *C. volkensii.*

Caesalpinia volkensii caused insignificant increase of albumin and proteins at extract dose of 1000 mg/kg body weight. This suggests that the plant extract may be having protein synthesis stimulatory effects when given at high doses.

Many medicinal plants are known to have an effect on glucose metabolism [27]. In this study, methanolic leaf extract *C. volkensii* did not cause either hypoglycemia or hyperglycemia. However Murugi et al. found that *C. volkensii* has hypoglycaemic effects on alloxan induced diabetic mice [5]. The effect of the extracts may have not been palpable in this study because diabetes was not induced in the rat models.

Bilirubin presents in two forms; conjugated bilirubin and unconjugated bilirubin. Unconjugated bilirubin is protein bound, insoluble, toxic and cannot be excreted through the kidney, whereas conjugated bilirubin is water soluble and can be excreted in urine [28]. The amount of conjugated bilirubin in healthy individuals is very minimal and an increase implies liver disease. This is not always the case with unconjugated bilirubin [29].

Cesalpinia volkensii administered at extract dose of 100 and 300 mg/kg body weight did not result in any significance increase of both direct and indirect bilirubin. This shows that, the extract at this dose, neither resulted in hemolysis of red blood cells nor affected uptake and conjugation of bilirubin. However, at the extract dose of 1000 mg/kg body weight, direct bilirubin was elevated insignificantly, indicating that, high concentrations of the extract beyond 1000 mg/kg body weight may cause hemolysis of red blood cells or injury to the liver cells. A similar study done by Murugi et al. with aqueous extracts of *C. volkensii* at 450 mg/kg body weight, resulted to mild inflammation of the liver [5].

At extract dose of 1000 mg/kg body weight, indirect bilirubin was significantly raised signifying that methanolic leaf extract of *C. volkensii* given at high dose may affect bilirubin metabolism. Surprisingly, alanine aminotransferase (ALT) values did not increase significantly. Only the value of aspartate aminotransferase (AST) and indirect bilirubin increased significantly. Bilirubin, being a breakdown product of heme containing proteins, is tightly bound to albumin and transported to the liver for conjugation through glucuronidation and sulphation. An increase in indirect bilirubin (≥90%) is indicative of acute haemolysis of red blood cells or Gilbert syndrome [29].

Some drugs that affect the uptake and conjugation of bilirubin have been implicated in increasing unconjugated bilirubin [30]. Such drugs inhibit hepatic uptake of bilirubin by interfering with Z proteins. They include bunamiodyl and flavispidic acid [31,32]. Novobiocin is also known to compete with bilirubin for conjugation [28]. Increase in indirect bilirubin could have resulted from the extract of *C. volkensii* interfering with bilirubin metabolism in a similar manner. Haemolysis of red blood cells, which can lead to increase in indirect bilirubin, was ruled out following insignificant rise in HB at extract dose of 1000 mg/kg body weight. In addition, since both AST and indirect bilirubin rose significantly, liver toxicity cannot be ruled out.

Phytochemicals from medicinal plants affects food absorption from the gut in addition to various metabolic and catabolic processes taking place in the body, leading to weight changes in experimental animals. Some of the mechanisms by which phytochemicals act include blocking pancreatic lipase and alpha amylase, interfering with uptake of nutrients in the gut, while others act through combination of central and peripheral mechanisms [33]. Such effect of plant extracts results in weight changes, thus acting as an indicator of toxicity in animal models.

In the sub-chronic studies, there was no significance difference in body weight between the control group of animals and those that received plant extract. This comparable gain in weight shows that the extract did not have major toxic effects. In addition, the liver, kidneys, spleen, lungs, brain and the heart did not show any significant differences in weight. Nevertheless, these insignificant increase and decrease in body weight could have been as a result of variation in size of internal organs [34], but not as result of toxicity induced by methanolic leaf extract of *C. volkensii.*

The source of red blood cell, white blood cells and the platelets is the pluripotent stem cells in the bone marrow [35]. Toxicants including drugs like chlorophenical can depress the bone marrow and affect its activity [36]. Similarly, phytochemicals from plants may also affect the bone marrow thus interfering with production of cells [37]. Various enzymatic activities are also involved in the synthesis of blood

components in the bone marrow [38]. This suggests that any substance which affects these activities in the bone marrow adversely compromises the synthesis of blood cells. The effect of *C. volkensii* on red blood cell count at extract dose of 100 mg/kg body weight was unfavourably insignificant.

In addition, this extract dose lowered haemoglobin and haematocrit levels significantly suggesting that 100 mg/kg body weight dose could be the optimal concentration of methanolic leaf extract of *C. volkensii* which may cause plausible effect on the red blood cells indices. Some hematoxicants have been implicated in causing anaemia and some phytochemicals have been found to have effects on hematocrit [39]. Saponins have been found to be cytolytic and can result in anaemia. Therefore, low red blood cell indices including haematocrit and haemoglobin may be attributed to the presence of saponins [24]. However, as the extract dose increased, the red blood cells indices (HB, RBC and HCT) increased. This is an indication that the plant extract may be containing phytochemicals which may induce erythropoiesis when administered at high doses. Platelets are cellular fragments essential in homeostasis, which, during injury, forms platelet plug to prevent vascular leakage [40]. Methanolic leaf extract of *C. volkensii* did not show any thrombotic activity.

When tissue damage occurs, ALT and AST are released in the blood in levels proportional to the degree of tissue damage [41]. The ratio of AST/ALT can be used to determine whether it is the liver or other tissue that is/are is damaged though its application is of limited value [29]. There was no significant rise of ALT, giving an impression that hepatocytes were preserved from injury.

However, this is contradicted by the fact that there was significant rise in AST and total bilirubin in the group administered with the *C. volkensii* extract, at the dose of 1000 mg/kg body weight. The liver is possibly the culprit since both AST and total bilirubin rose significantly. Insignificant rise in ALT could have been impacted by Vitamin B6 deficiency resulting from phytochemical effects [21]. In addition, rise in AST may have resulted from the cytolytic nature of saponins present in the plant extract, which may have caused cellular damage [42].

Methanolic leaf extract of *C. volkensii* at the dose of 100 mg/kg body weight significantly resulted in hyponatremia. Hyponatremia is a condition whereby sodium level in the blood is lower than normal. Low plasma osmolarity inhibits the release of anti-duretic hormone leading to excretion of dilute urine in order to correct this condition. However, some drugs such as antipsychotic drugs, cyclophosphamide and vincristine sulfate can interfere with this process leading to hyponatremia [43].

Dilutional hyponatremia is the most common form and sets in when the water intake exceeds water output through the kidney. Furthermore, inappropriate secretion of antiduretic hormone deprives the kidney ability to excrete excess water. This therefore, may leads to hyponatremia. Excess plasma volume caused by fluid retention during hyponatremia can result to low hematocrit as witnessed in the results [44]. Similar results were obtained by Abubakar and Sule on aqueous extract of *Cassia occidentalis* [45]. In their study, the extracts altered electrolytes in Rat models in a dose dependent manner.

Liamis et al. highlighted the common causes of hyponatremia to include thirst stimulation, excess antiduretic hormone (ADH), inappropriate ADH stimulation and production, action of nonsteroidal anti-inflammatory drugs and polydipsia [43]. Through any of these processes, the phytochemicals in the plant extract may have precipitated hyponatremia. Often when the kidney is directly involved, a number of other electrolytes are altered. Since potassium level was not altered significantly, the hyponatremia was associated with either inappropriate secretion of antiduretic hormone, non-steroidal anti-inflammatory mechanism or polydipsia induced by the methanolic leaf extracts of *C. volkensii*.

Serum urea, creatinine, uric acid and electrolytes are markers of kidney injury [46,47]. There was no significant difference in levels of creatinine, urea, uric acid and potassium among the experimental groups, resulting from administration of methanolic leaf extract of *C. volkensii*. Despite change in sodium levels in non-dose dependent manner, the presence of kidney problem however, cannot be based only on deviation of a single parameter without consideration of others. All the other indicators of kidney injury in experimental groups of animals were not significantly different from the control group of animals hence kidney injury was ruled out.

The presence of terpenoids, saponins, alkaloids, flavonoids and phenols in the plant extracts was associated with various changes noted in the experimental animals. Alkaloids have been documented to have both toxic and therapeutic effects [48]. In addition, saponins cause hemolysis of red blood cells [24]. Tannins too have been associated with hemolysis in blood and necrosis in the liver and some terpenoids lead to cancer through formation of protein adducts [21].

Conclusion

Methanoic leaf extract of *C. volkensii* is not likely to produce any severe toxic effects. Its median lethal dose (LD50) of greater than 2000 mg/kg body weight justifies its safety. However, prolonged oral administration at high dose may cause observable changes in biochemical parameters, in particular total bilirubin and AST. Therefore, we suggest prolonged exposure to be avoided. Further study on histopathology, chronic in vivo toxicity and quantitative phytochemical screening is needed to reveal extensive effects of *C. volkensii* in the body and determine active compounds responsible for the observable changes.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgement

We acknowledge the Department of Biochemistry and Biotechnology Kenyatta University and Kwale Hospital for allowing us to use their facilities in conducting this study.

References

1. Briskin DP (2000) Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. Plant Physiol 124: 507-514.
2. Kpomah ED, Arhoghro EM, Uwakwe AA (2012) Biochemical and histopathological changes in Wistar rats following chronic administration of Diherbal mixture of Zanthoxylum leprieurii and Piper guineense. JNSR 2: 22-28.
3. Kareru PG, Kenji GM, Gachanja AN, Keriko JM, Mungai G (2007) Traditional medicines among the Embu and Mbeere people of Kenya. AJTCAM 4: 75-86.
4. Kigen GK, Ronoh HK, Kipkore WK, Rotich JK (2013) Current trends of traditional herbal medicine practice in Kenya: A review. AJPT 2: 32-37.

5. Murugi NJ, Piero NM, Mwiti KC, Ngeranwa JJN, Njagi ENM, et al. (2012) Hypoglycemic effects of Caesalpinia volkensii on alloxan-induced diabetic mice. AJPCR 5: 69-74.
6. Taniguchi CM, Armstrong SR, Green LC, Golan DE, Tashjian AH (2008) Drug toxicity (2nd edn.) Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy. Lippincott Williams and Wilkins, Philadelphia, USA, pp: 63-74.
7. Guengerich FP (2001) Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity. Chem Res Toxicol 14: 611-650.
8. Shaw D, Graeme L, Pierre D, Elizabeth W, Kelvin C (2012) Pharmacovigilance of herbal medicine. J Ethnopharmacol 140: 513-518.
9. Grattagliano I, Bonfrate L, Diogo CV, Wang HH, Wang DQ, et al. (2009) Biochemical mechanisms in drug-induced liver injury: certainties and doubts. World J Gastroenterol 15: 4865-4876.
10. Romanov Victor, Terry C, Whyard Wayne C, Waltzer Arthur, Grollman P, et al. (2015) Aristolochic acid-induced apoptosis and G2 cell cycle arrest depends on ROS generation and MAP kinases activation. Arch Toxicol 89: 47-56.
11. Ju C, Uetrecht JP (2002) Mechanism of idiosyncratic drug reactions: reactive metabolites formation, protein binding and the regulation of the immune system. Curr Drug Metab 3: 367-377.
12. Sinha K, Das J, Pal PB, Sil PC (2013) Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis. Arch Toxicol 87: 1157-1180.
13. Agnew AD (1974) Upland Kenya wild flowers. East Africa Natural History Society, Nairobi, Kenya.
14. Evans CWT, Evans (2002) Pharmacognosy (14th edn.). W.B. Saunders Company Ltd. pp: 419-421.
15. Ngomo JN (2013) Investigation of phytochemical and antimalarial activity of Caesalpinia volkensii harms seeds (PhD Thesis), University of Nairobi, Nairobi, Kenya. pp: 17-19.
16. Kuria KAM, De Coster S, Muriuki G, Masengo W, Kibwage I, et al. (2001) Antimalarial activity of Ajuga remota Benth (Labiatae) and Caesalpinia volkensii Harms (Caesalpiniaceae): in vitro confirmation of ethnopharmacological use. J Ethnopharmacol 74: 141-148.
17. Mworia JK (2016) Antinociceptive Activities of Acetone Leaves Extracts of Carissa Spinarum and Caesalpinia Volkensii in Mice. PHD Thesis, Kenyatta University, Nairobi, Kenya, p: 20.
18. Maina MB, Maina GS, Muriithi NJ, Kiambi MJ, Umar A, et al. (2015) Antinociceptive Properties of Dichloromethane: Methanolic Leaf Extracts of Caesalpinia volkensii and Maytenus obscura in Animal Models. J Pain Relief 4: 1-6.
19. Mwangi BM, Gitahi SM, Njagi JM, Mworia JK, Aliyu U, et al. (2015) Anti-inflammatory Properties of Dichloromethane: Methanolic Leaf Extracts of Caesalpinia Volkensii and Maytenus Obscura in Animal Models. IJCPR 8: 100-104.
20. OECD (2001) Acute Oral Toxicity-Up and Down Procedure. OECD Guidelines for Testing of Chemicals 425: 1-26.
21. Osano KO, Nyamai DW, Ogola PE, Ouko RO, Arika WM, et al. (2016). Evaluation of In Vivo Toxicity of Dichloromethane: Methanolic Leaf Extracts of Prosopis juliflora in Female Wistar Albino Rats. JDMT 7: 1-11.
22. Kotake, C. K. (2000). Practical pharmacolognosy. Vallabh prakashan, New Delhi. India 4: 107-111.
23. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, et al. (2008) Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop J Pharm Res 7: 1019-24.
24. Mugisha MK, Ndukui JG, Namutembi A, Waako P, Karlson AKB, et al. (2014) Acute and Sub-Acute Toxicity of Ethanolic Leaf Extracts of Rumex abyssinica Jacq. (Polygonaceae) and Mentha spicata L.(Lamiaceae). Pharmacol Ther 5: 309-318.
25. Aniagu SO, Nwinyi FC, Akumka DD, Ajoku GA, Dzarma S, et al. (2005) Toxicity studies in rats fed nature cure bitters. Afr J Biotechnol 4: 72-78.
26. Patrick-Iwuanyanwu KC, Amadi U, Charles IA, Ayalogu EO (2012) Evaluation of acute and sub-chronic oral toxicity study of baker cleansers bitters a polyherbal drug on experimental rats. EXCLI 11: 632-640.
27. Deng R (2012) A review of the hypoglycemic effects of five commonly used herbal food supplements. Recent Pat Food Nutr Agric 4: 50-60.
28. Fevery J (2008) Bilirubin in clinical practice: a review. Liver Int 28: 592-605.
29. Murali MR, Carey WD (2000) Liver test Interpretation-Approach to the patient with liver disease: A Guide to Commonly Used Liver Tests. Cleveland Clinic, Cleveland, USA.
30. Cui Y, König J, Leier I, Buchholz U, Keppler D (2001) Hepatic uptake of bilirubin and its conjugates by the human organic anion transporter SLC21A6. J Biol Chem 276: 9626-9630.
31. Billing BH, Black M (1971) The action of drugs on bilirubin metabolism in man. Ann N Y Acad Sci 179: 403-410.
32. Bock KW, Elder GH, Israels LG, Marcks GS, De Matteis F, et al. (2012) Heme and hemoproteins (Vol. 44). Springer Science and Business Media, New York, USA, pp: 26-28.
33. Tucci SA (2010) Phytochemicals in the control of human appetite and body weight. Pharm J 3: 748-763.
34. Chunlaratthanaphorn S, Lertprasertsuke N, Ngamjariyawat USATA, Suwanlikhid N, Jaijoy K (2007) Acute and subchronic toxicity study of the water extract from dried fruits of Piper nigrum L. in rats. Health 29: 109-124.
35. Wilson A, Trumpp A (2006) Bone-marrow haematopoietic-stem-cell niches. Nat Rev Immunol 6: 93-106.
36. Kar M, Ghosh A (2002) Postgraduate clinic. JIACM 3: 29-34.
37. Mohd Yousef Alshibly N (2014) Effect of Artemisia absinthium L. on Genotoxicity on Mice Bone Marrow Cells. World Appl Sci J 30: 770-777.
38. Bain BJ (2014) Blood cells: a practical guide. John Wiley and Sons, Oxford, UK, pp: 416-440.
39. Donkor K, Okine LN, Abotsi WK, Woode E (2014) Acute and Sub-Chronic Toxicity Studies of aqueous extract of root bark of Cassia sieberiana DC in Rodents. JAPS 4: 84-89.
40. Schwer HD, Lecine P, Tiwari S, Italiano JE, Hartwig JH, et al. (2001) A lineage-restricted and divergent β-tubulin isoform is essential for the biogenesis, structure and function of blood platelets. Curr Biol 11: 579-586.
41. Huang XJ, Choi YK, Im HS, Yarimaga O, Yoon E, et al. (2006) Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. Sensors 6: 756-782.
42. De-Paula Barbosa A (2014) Saponins as immunoadjuvant agent: A review. Afr J Pharm Pharmacol 8: 1049-1057.
43. Liamis G, Milionis H, Elisaf M (2008) A review of drug-induced hyponatremia. Am J Kidney Dis 52: 144-153.
44. Gross P, Reimann D, Henschkowski J, Damian M (2001) Treatment of severe hyponatremia: conventional and novel aspects. J Am Soc Nephrol 12: 10-14.
45. Abubakar S, Sule M (2010) Effect of oral administration of aqueous extract of Cassia occidentalis l. seeds on serum electrolytes concentration in rats. BAJOPAS 3: 183-187.
46. Muhammad S, Hassan LG, Dangoggo SM, Hassan SW, Umar KJ, et al. (2011) Acute and subchronic toxicity studies of kernel extract of Sclerocarya birrea in rats. Sci J 6: 11-14.
47. Burtis CA, Ashwood ER, Bruns DE (2012) Tietz Fundamentals of Clinical Chemistry (6th edn.). Elsevier Health Sciences, Phillaephia, USA, pp: 363-368.
48. Hoffman F, Manning M (2002) Herbal medicine and botanical medical fads. Haworth Press, New York, USA, pp: 29-43.