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Safety evaluation and antihyperglycaemic effect of root extract of *Maerua decumbens* (Brongn.) DeWolf in Wistar rats

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ABSTRACT

Local communities living in rural regions of Kenya use the plant, *Maerua decumbens* (Brongn.) DeWolf for various purposes including water purification (roots), as food (roots and fruits) and medicinal (various parts of the plant) for treatment of several ailments including diabetes mellitus. However, there is no scientific evidence on the safety and antidiabetic claims of *Maerua decumbens* roots despite its continued use. Therefore, this study aimed at evaluating oral acute and sub-acute toxicity of methanolic root extract of *Maerua decumbens* (400 and 800 mg/kg.b.w/day) in Wistar rats and also the antihyperglycaemic activity of the orally-administered extract in streptozotocin (STZ) induced diabetic rats. Phytochemicals were qualitatively analysed using standard procedures while the fingerprint chromatograph of the extract was revealed by high performance liquid chromatography (HPLC). Diabetes was induced in the experimental groups by single intraperitoneal injection of STZ at 50 mg/kg.b.w. Animal grouping was then done as follows; Groups 1: Normal control; 2: Normal on extract (400 mg/kg.b.w/day) 3; diabetic untreated control; 4, 5 & 6; diabetic treated with 100 and 400 mg/kg.b.w/day of root extract and metformin at 100 mg/kg.b.w/day, respectively. Effects of extract on fasting blood glucose (FBG) and body weights were monitored weekly in the 21 days anti-diabetic study. Also, liver malondialdehyde (MDA) levels were determined at the end of the treatment. For sub-acute toxicity studies, the extract did not affect organ weights of liver, kidney, heart and spleen and there were no pathological changes in the liver and kidney. Also, the blood biochemical and haematological parameters did not show any changes in all dosages although there were significant alterations in red blood cell' indices but the values were within the published normal reference ranges for the species. Thus the extract was found to be relatively safe after oral sub-acute treatment in rats. On the other hand, the phytochemical-rich extract as also exhibited by the UV spectra of the compounds on diabetic rats showed a marginal increase in body weight, significant decreases in FBG and liver MDA levels versus untreated diabetic rats ($p < 0.05$). Therefore, *Maerua decumbens* root extract has great potential for use as a safe alternative medicine for the management of diabetes mellitus due to its antihyperglycaemic effects and reduction in hepatic lipid peroxidation.

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Introduction

Diabetes mellitus (DM) describes a metabolic disorder of multiple aetiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from chronic and/or relative insulin insufficiency [1]. DM has caused significant morbidity and mortality due to microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (heart attack, stroke and peripheral vascular) complications [2]. The global prevalence of DM was estimated at 451 million (age 18–99 years) in 2017 worldwide and these figures are expected to increase to 693 million by 2045 [3]. It is also projected that the largest increase in DM prevalence will occur in developing countries, mainly in Asia and Africa attributable to the rapid rise in sedentary life styles, urbanization, nutrition transitions and ageing [4,5]. Hyperglycaemia has been implicated in the onset and progression of DM and causation of severe complications through various mechanisms including induction of oxidative stress, decreased nitric oxide bioavailability, glucose autoxidation and non-enzymatic protein glycation [6,7].

DM is treated using insulin or by oral hypoglycaemic agents such as alpha–glucosidase inhibitors, sulfonylureas, biguanides, and thiazolidinediones [8]. These conventional anti-diabetic drugs have serious adverse effects besides being costly [9]. There is therefore need to explore other treatment and management strategies of DM [10]. Mankind has long been using herbs for the treatment of various ailments since they are less costly, better tolerated, more safe, less side effects, widely available, and ecofriendly in nature [11]. Many plants contain phytochemicals with medicinal potential such as glycosides, alkaloids; terpenoids, flavonoids amongst others that have been reported to have antioxidant and antidiabetic activities [12]. For instance, local communities living in rural regions of Kenya use the plant, *Maerua decumbens* (Brongn.) DeWolf for various purposes including water purification (roots), as food (fruits) and medicinal (various parts of the plant) for treatment of several ailments [13]. Noteworthy is the use of the roots of *Maerua decumbens* for the treatment of Diabetes mellitus (DM) by the Keiyo community in Elgeyo Marakwet County of Kenya [14]. *Maerua decumbens* (Brongn.) DeWolf which belongs to Capparaceae family is a small shrub growing in arid and semi-arid habitats from Somalia, Ethiopia, Sudan to South Africa and in Kenya where it is widespread in Northern Baringo, Southern Turkana, Mandera and Kitui [13]. In Kenya, *Maerua decumbens* is known by different names amongst the different tribes as follows; Keiyo: Chepyetabei; Marakwet: Chepiliowo; Tugen: Yubuluswa; Kamba: Munatha; Pokot: Chepuluswo; Somali: Abarmogo; Luo: Amoyo amongst others [13,14].To the best of our knowledge, there are no studies on *in vivo* toxicity of *Maerua decumbens* root extract which have been described in literature despite its continued use. Therefore, in the present investigation, we aimed to evaluate the acute and sub-acute oral toxicity of *Maerua decumbens* methanolic root extract in rats in order to increase the confidence in their safety to humans to treat various ailments. Additionally, this study also sought to evaluate for the first time the antihyperglycaemic effect of *Maerua decumbens* methanolic root extract in streptozotocin (STZ) induced diabetic Wistar male albino rats to scientifically determine whether the plant roots may be efficacious in the treatment of DM as claimed. Qualitative phytochemical screening and high performance liquid chromatography (HPLC) fingerprinting of the root extract of *Maerua decumbens* was also performed.

Materials and methods

Materials

(STZ) was obtained from Wako Pure Chemical Industries Ltd, Osaka, Japan. Thiobarbituric acid (TBA) was obtained from BDH Chemicals Ltd, Poole, England. 1, 1, 3, 3-Tetramethoxypropane (TMP) was obtained from Sigma Aldrich, St. Louis, Missouri, USA. Metformin (Glucophage) was obtained from Lipha Pharma Ltd, UK. Any other chemicals used were of analytical grade.

Collection and identification of plant

Maerua decumbens (Brongn.) DeWolf (Capparaceae) roots were collected in its natural habitat from EMCEA village, Elgeyo Marakwet County, Kenya (0°26′20.6″N 35°36′54.9″E) in January 2017. The plant material was identified and authenticated by a qualified taxonomist, Mr. Denis Onyango at University of Eldoret and a voucher number M.U.H/MD/0021/17 was assigned and the plant specimen kept in the herbarium of Department of Biological Sciences at University of Eldoret. The name of the plant was checked on http://www.theplantlist.org/.

Preparation of crude root extract

The roots of *Maerua decumbens* were first washed to remove any debris. The dead bark was then peeled, chopped into small pieces, shade dried and ground into homogenous powder using an electric mill (Disk Mill FFc-23, China). The extract was obtained from the root powder of *Maerua decumbens* by a 72-hour cold maceration using methanol (1:5 w/v) with regular stirring. The mixture was filtered using Whatman filter paper number one and the combined extracts were then concentrated under reduced pressure at 50 °C using a Rotary Evaporator (Rotavapor type EL 30, model AG CH-9230, Germany). The brick red syrup like crude extract was further dried in an oven at 40 °C for two days to remove remaining

methanol. The percentage yield of the methanol extract was 15%. The concentrate was then put in an airtight container and stored at 4 °C until use.

Qualitative phytochemical screening and HPLC fingerprinting

The methanolic root extract of *Maerua decumbens* was subjected to qualitative phytochemical tests to identify classes of phytochemical constituents present using standard methods [15,16]. For HPLC fingerprinting analysis, the crude methanolic root extract was passed through a silica gel column using methanol and ethylacetate mixture (1:1) and then concentrated under reduced pressure on a rotary evaporator. The extract was then filtered through a 0.45 µm micro-filter and then subjected to HPLC at Jomo Kenyatta University of Agriculture & Technology (JKUAT), Kenya. Acetonitrile together with 40 mM potassium dihydrogen phosphate buffer at pH 7.8 was used as the mobile phase on a gradient elution program with a flow rate of 0.8 mL/min within 60 min and injection volume of 20 µL. A C-18 column of 250 mm × 4.6 mm, 5 µm was used for separation of the extract using PDA Shimadzu M20A HPLC (Kyoto, Japan) and the diode array detector was used and set to scan from 190–800 nm.

Ethical considerations

Animal experimentation and protocols were approved by the Research Ethics Committee of University of Eastern Africa, Baraton, Kenya (Reference; REC: UEAB/9/3/2017). The acute and sub-acute oral toxicity study was performed according to the Organization for Economic Co-operation and Development (OECD) Test Guideline 423 (Acute Oral toxicity–Acute Toxic Class Method) [17] and 407 (Repeated Dose 28-Day Oral Toxicity Study in Rodents) [18] respectively. The research was also conducted in compliance with the ARRIVE guidelines and in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Animals

Wistar albino rats *(Rattus norvergicus)* aged between 6–8 weeks were purchased from Zoology Department, Chiromo Campus, University of Nairobi, Nairobi, Kenya. The rats were maintained at 22–25 °C with 12 h light and 12 h dark cycles, and 50–55% humidity conditions. The rats were allowed to acclimatize for one week before experimentation. The rats were fed with standard rodent chow pellet (Unga Farmcare, East Africa Limited, Nakuru, Kenya) and allowed free access to drinking water.

Acute oral toxicity study

Six female rats were randomly divided into 2 groups of 3 rats each. 2000 mg/kg body weight of root extract was dissolved in normal saline which readily dissolved into a solution. Each animal in Group 1 received 1000 µL of extract solution administered orally. Group 2 were orally treated with vehicle (normal saline at 1000 µL) to establish a comparative negative control group according to the OECD test guideline 423; Acute Oral toxicity–Acute Toxic Class Method [17]. All animals were observed at least once during the first 30 min in the first 24 h with great consideration given for the first 4 h following vehicle or root extract administration and then once a day for 14 days. These observations were done to check the onset of clinical or toxicological symptoms. All observations included changes in skin and fur, eyes and mucous membranes and behavioural changes. In addition, consideration was given for observations of convulsions, tremors, diarrhoea, salivation, lethargy, sleep, coma and mortality. Food and water were provided *ad libitum* after extract administration. The fasting body weights of animals (after overnight fast) were recorded shortly before the administration of the tested substance and at the end of each week.

Sub-acute oral toxicity study

Rats were divided into three groups of 10 rats each (5 males and 5 females) and a satellite group (3 males and 3 females). Group 1 received vehicle (normal saline at 500 µL) by oral gavage and served as control. Groups 2 and 3 received oral doses of root extract at 400 and 800 mg/kg body weight, respectively. The satellite group was included to observe for any withdrawal symptoms, reversibility, persistence or delayed occurrence of toxic effects of the test material and was given the highest oral dose of root extract at 800 mg/kg body weight. The extract was dissolved in normal saline and the volumes orally administered for all treatments were at 500 µL. The animals were handled as per the OECD test guideline 407; Repeated Dose 28-Day Oral Toxicity Study in Rodents [18,19] and oral administration was performed once daily at 10.00 am by oral gavage for 28 days. The satellite group was scheduled for follow-up observations for the next 14 days without root extract administration. During the entire dosing period, the animals were observed daily for clinical signs of toxicity on behavioural changes, morbidity and mortality. The rats were weighed (after overnight fast) prior to dosing, after every 7 days, and before sacrifice on 29th day.

At the end of the study (day 29), animals were euthanized and blood samples collected via cardiac puncture under mild chloroform anaesthesia. Whole blood was collected in EDTA vacutainers for haematological assay while blood for serum preparation was collected in plain tubes. Serum was obtained after centrifugation at 3000 × *g* for 10 min and kept at −20 °C awaiting biochemical analyses. A full haemogram was performed using ADVIA 2120i haematology autoanalyzer (Siemens Healthcare GmbH, Erlangen, Germany) while the biochemical parameters, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), total protein, and albumin as liver function indices were determined in serum using COBAS INTEGRA 400 plus auto-analyser (Roche Diagnostics, Mannheim, Germany). The kidney function indices in serum, urea and creatinine, were also determined in a similar way as the liver function indices.

Vital organs (liver, kidney, heart and spleen) were removed through a midline incision in the rat's abdomen. The organs were cleaned of adhering fat and blotted with clean tissue paper, and then weighed using analytical balance (AUW220 Shimadzu Corporation, Tokyo, Japan). The relative organ's weight (ROW) were calculated and recorded in proportion to the body weight according to the following equation:

$$Relative\ organ\ weight\ (ROW) = \frac{Absolute\ organ\ weight}{Body\ weight\ on\ day\ of\ sacrifice} \times 100$$

Sections of the liver and kidney tissues were also fixed in 10% formalin for use in histological analysis. For histological processing of the tissues, the fixed tissues were firstly placed in STP 120 automatic tissue processor. 5µm-thick paraffin sections were obtained using a microtome (SLEE medical model GmbH, Mainz, Germany) and stained with haematoxylin and eosin [20]. Specimens were then examined for histopathological changes under a light microscope at 40X (model CX21FSI, Olympus Corporation, Tokyo, Japan) and photomicrographs taken using Redmi Note 4 model 2,016,102 phone from Xiaomi Communication Company Limited, Beijing, China.

Induction of diabetes

Diabetes was induced in overnight fasted rats by single intraperitoneal injection of streptozotocin (STZ) dissolved in cold 0.1 M sodium citrate buffer of pH 4.5 at the dose of 50 mg/kg body weight. 5 rats for normal control group and 5 normal rats for administration of the extract only were all injected intraperitoneally with 0.2 ml of vehicle (0.1 M sodium citrate buffer). After the injections, the animals were allowed to drink 5% glucose solution overnight to overcome hypoglycaemic shock with free access to rodent chow. The fasting blood glucose levels were measured using glucometer (Wellion CALLA Light, Med Trust, Ottendorf – Okrilla, Germany) after 5 days in whole blood drawn from the tail vein amongst the animals injected with STZ. Rats with glucose levels above 13.9 mmol/L were considered as diabetic and used for further experimentation.

Experimental design for antidiabetic study

The formula for calculation of sample size for comparison of two groups that was used was according to Charan & Kantharia [21]. The Wistar male albino rats were divided into 6 groups consisting of 5 rats each as follows:

Group 1: Normal control rats received normal saline.
Group 2: Normal rats received *Maerua decumbens* root extract at 400 mg/kg.b.w.
Group 3: Diabetic control untreated rats received normal saline.
Group 4: Diabetic rats received *Maerua decumbens* root extract at 100 mg/kg.b.w.
Group 5: Diabetic rats received *Maerua decumbens* root extract at 400 mg/kg.b.w.
Group 6: Diabetic rats received standard antidiabetic drug metformin at 100 mg/kg.b.w.

Animal treatment in the antidiabetic study

The root extracts and metformin were all dissolved in normal saline and were orally administered with animals receiving 500 uL each including controls that received vehicle only (normal saline) accordingly as per experimental design (described in section 2.10) daily for 21 days according to Cheng et al. [22]. The basis for the administration of the standard drug, metformin is its novel mechanism for plasma glucose-lowering action in STZ induced diabetic rats. Fasting body weights for each rat was taken on day 0, 7, 14 & 21 and dosing adjusted weekly as per the weekly body weights recorded. Fasting blood glucose levels were also determined on day 0, 7, 14 & 21. Rats had free access to food and drinking water except when measurements of fasting body weights and fasting blood glucose were being assessed whereby the rats were fasted overnight but with water *ad libitum*. On the 21st day, the rats were weighed, fasting blood glucose from tail vein were determined then the rats were euthanized under mild chloroform to minimize stress and pain during sacrificing. All the rats were then dissected to collect the liver for determination of malondialdehyde levels. Liver sections were excised and immediately washed in ice cold 0.9% normal saline and stored at –20 °C until analyses.

Determination of liver malondialdehyde levels

Levels of malondialdehyde (MDA), an end product of lipid peroxidation were determined in liver tissues, as described by Alam et al. [23,24]. Briefly, liver tissues initially frozen at –20 °C were defrosted. One gram of the liver tissue in 9 ml of 1.15%

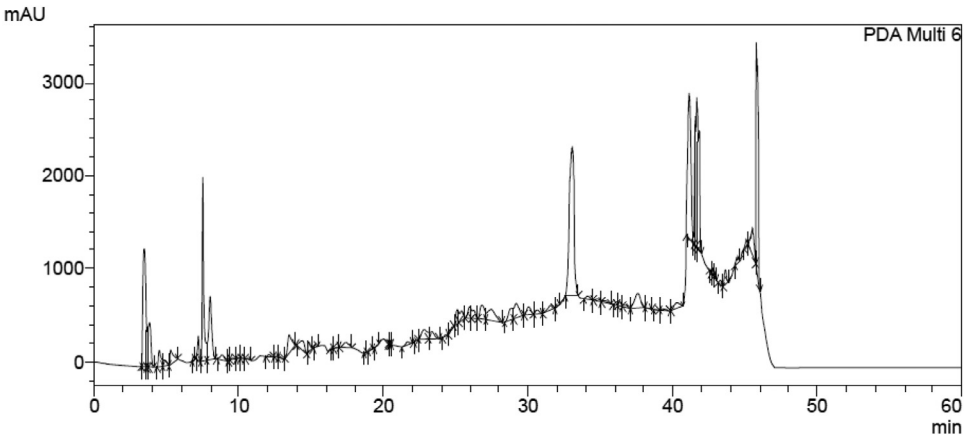

Fig. 1. HPLC fingerprint of *Maerua decumbens* root extract at 190–800 nm.

of cold potassium chloride (KCl) was homogenized with mortar and pestle then centrifuged at 2000 rpm for 10 min. The resultant supernatant (0.1 ml) was then mixed with 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid and 1.5 ml of 8% thiobarbituric acid (TBA). The volume of the mixture was made to 4 ml with distilled water and then heated at 95 °C after adding boiling chips on a water bath for 60 min. After incubation the tubes were cooled to room temperature and final volume made to 5 ml in each tube. 5 ml of butanol: pyridine (15:1) mixture was added and the contents vortexed thoroughly for 2 min. After centrifugation at 4000 rpm for 10 min, the upper organic layer was taken and its optical density measured using a spectrophotometer (Spectronic 21D, Milton Roy Limited, Pont-Saint-Pierre, France) at 532 nm. The MDA levels were obtained from a standard calibration curve generated using hydrolysed 1,1,3,3-tetramethoxypropane (TMP). The reference blank was 0.1 ml of 1.15% KCl and was treated in the same way as the samples and standards by addition of the same reagents. The level of MDA in samples was expressed in nM.

Statistical analysis

Quantitative data was expressed as mean ± standard error of the mean (SEM). Data was analysed by Student's *t*-test and one-way analysis of variance (ANOVA). The value with $p < 0.05$ was considered to be statistically significant.

Results

Phytochemical analysis and HPLC fingerprinting of Maerua decumbens root extract

The qualitative phytochemical tests of methanolic root extract showed the presence of alkaloids, glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids, while anthraquinones were not detected. On the other hand, the HPLC fingerprint of the methanolic root extract (**Fig. 1**) showed the presence of several compounds and the major peaks at 190–800 nm were at the retention time (minutes) of 7.49, 33.05, 33.43, 41.13, 41.57, 41.66, 41.83 and 45.78.

Clinical observations and body weight changes in acute oral toxicity of Maerua decumbens root extract in rats

It was observed that the methanolic extract of the roots of *Maerua decumbens* at 2000 mg/kg.b.w did not induce changes in the behaviour of female rats during the first 30 min and for a period of up to 4 h after oral administration. All the animals rubbed their mouth and nose with their front pawns and against the walls of the cage soon after dosing. All these symptoms disappeared completely after 30 min post dosing. The extract did not cause diarrhoea but the droppings in some test animals were watery and not well formed like pellets. There was no death during acute oral toxicity testing of *Maerua decumbens* root extract at 2000 mg/kg.b.w. All extracts treated animals showed a stable increase in body weights during the 14 days (**Table 1**). Therefore, the approximate acute lethal dose (LD_{50}) of *M. decumbens* extract in female rats was estimated to be higher than 2000 mg/kg.b.w in female rats.

Clinical observations, body weight changes and relative organ weights in sub-acute oral toxicity of Maerua decumbens root extract in rats

Daily oral administration of the animals at 400 mg/kg.b.w and 800 mg/kg.b.w dose of *M. decumbens* root extract for 28 days did not induce any obvious symptoms of toxicity in rats of both sexes, including the highest dose tested of

Table 1
Body weight changes and relative organ weights of rats in acute and sub-acute oral toxicity study with root extract of *Maerua decumbens*.

Acute oral toxicity				
	Sex		Control	2000 mg/kg.b.w
% body weight change on 14th day from day 0	F ($n = 3$)		10.5	16.0
Sub-acute oral toxicity				
	Sex	Control	400 mg/kg.b.w	800 mg/kg.b.w
% body weight change on 28th day from day 0	M ($n = 5$)	38.1	106.9	88.9
	F ($n = 5$)	28.2	85.6	63.1

Relative organs weights (%) of rats in sub-acute oral toxicity

Organ	Sex	Control	400 mg/kg.b.w	800 mg/kg.b.w
Liver	M ($n = 5$)	3.60 ± 0.28	4.13 ± 0.25	4.34 ± 0.16
	F ($n = 5$)	4.54 ± 0.32	4.07 ± 0.13	4.73 ± 0.27
Kidney	M ($n = 5$)	0.81 ± 0.03	0.84 ± 0.01[a]	0.83 ± 0.02
	F ($n = 5$)	0.79 ± 0.04	0.78 ± 0.04	0.85 ± 0.01[a]
Heart	M ($n = 5$)	0.43 ± 0.01	0.39 ± 0.00[a]	0.42 ± 0.01
	F ($n = 5$)	0.45 ± 0.00	0.39 ± 0.02[a]	0.45 ± 0.02
Spleen	M ($n = 5$)	0.38 ± 0.01	0.55 ± 0.03[a]	0.50 ± 0.04[a]
	F ($n = 5$)	0.45 ± 0.00	0.53 ± 0.03[a]	0.41 ± 0.04[a]

Values are expressed as mean ± SEM. M, male; F, female. [a] $p < 0.05$ values are significantly different compared with normal control.

800 mg/kg.b.w. Transient clinical signs that were most pronounced after dosing and that lasted for about 30 min included raised fur and rubbing at the oral cavity indicating irritation at any time treatments were administered. No deaths or additional signs of toxicity were found in any groups throughout the experimental period. Physical observation of the treated rats throughout the study indicated that none of them showed signs of toxicity in their skin, fur, eyes, mucus membrane, or behavioural changes, diarrhoea, tremors, salivation, sleep, and coma. The satellite group was observed daily for the next 14 days beyond day 28 days of daily treatment with 800 mg/kg.b.w of root extract and the rats in this group did not show any delayed behavioural signs of toxicity. The percent change in body weights between day 0 and day 28 were such that there was steady increase in body weights in all the extract treated groups (**Table 1**). The relative organ weights of organs recorded at necropsy in the treatment groups did not show a significant difference compared to the control in the liver at doses of 400 and 800 mg/kg.b.w of extract in both genders. There seem to have been incidental and dose unrelated significant decreases and/or increases of relative organ weights of kidney (increase in low dose male and high dose female), heart (reduced in low dose male and female) and spleen (increased in low and high dose male and low dose female, and reduced in high dose female) in extract administered treated rats versus respective controls (**Table 1**).

Effect of Maerua decumbens root extract on haematological and serum biochemical parameters in sub-acute toxicity study

The haematological parameters measured (neutrophils, lymphocytes, monocytes, basophils, and platelets count) in treated rats showed no significant difference from their respective controls. However, there were a few alterations that appear not to be dose dependant and gender specific such as significant variations in certain parameters as white blood cells, eosinophils, haemoglobin, haematocrit, RDW, HDW, MCHC, MCV, MCH, and MPV ($p < 0.05$) as shown below in **Table 2**. The *M. decumbens* methanolic root extract had no statistically significant difference ($p < 0.05$) on the liver function parameters that is alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in all dosage groups in male and female rats compared to their respective normal control groups at $p < 0.05$. There was no significant change in albumin in all treatment groups compared to respective normal controls. Total protein in both female (400 and 800 mg/kg.b.w) and male (800 mg/kg.b.w) group had significant reduction compared to respective controls (**Table 2**). In the kidney function parameters; urea, was significantly reduced in male and female groups at 800 mg/kg.b.w dose (30.77% and 25.96%) respectively compared to corresponding normal controls, while creatinine had significant reduction in female at 800 mg/kg.b.w dose (18.90%) compared to its respective control at $p < 0.05$ (**Table 2**).

Effect of Maerua decumbens root extract on rat's liver and kidney histology in sub-acute toxicity study

Light microscopic examination of sections of liver and kidney of control and *M. decumbens* extract administered groups showed absence of any gross and microscopic lesions (**Fig. 2**). Histopathological section of liver in representative control group and *M. decumbens* extract treated male and female groups at a dose of 400 and 800 mg/kg.b.w for 28 days showed normal liver architecture (**Fig. 2**). The portal triads and central veins in liver tissues were found to be normal in both control and extract administered rats (**Fig. 2**). There was no evidence of toxic signs observed in the liver tissues as there were no

Table 2
Effect of *Maerua decumbens* root extract on haematological and serum biochemical parameters of rats in oral sub-acute toxicity study.

Parameter	Sex	Treatment groups Control	400 mg/kg.b.w	800 mg/kg.b.w
Haemoglobin (g/dL)	M ($n = 5$)	15.78 ± 0.58	14.66 ± 0.34[a]	14.08 ± 0.90
	F ($n = 5$)	15.02 ± 0.26	13.18 ± 0.59[a]	13.70 ± 0.29[a]
Red blood cells (10^{-6}/μL)	M ($n = 5$)	8.67 ± 0.30	7.83 ± 0.24[a]	7.13 ± 0.47[a]
	f ($n = 5$)	8.39 ± 0.27	6.79 ± 0.28[a]	6.91 ± 0.28[a]
haematocrit (%)	m ($n = 5$)	49.10 ± 2.06	47.56 ± 1.26	43.52 ± 2.45
	F ($n = 5$)	47.60 ± 1.13	41.40 ± 1.81[a]	41.78 ± 1.23[a]
MCV (fL)	M ($n = 5$)	56.58 ± 0.51	60.74 ± 0.66[a]	61.36 ± 2.14[a]
	F ($n = 5$)	56.84 ± 1.05	60.94 ± 0.75[a]	60.68 ± 1.81[a]
MCH (pg)	M ($n = 5$)	18.20 ± 0.38	18.74 ± 0.24	19.76 ± 0.36[a]
	F ($n = 5$)	17.96 ± 0.40	19.40 ± 0.25[a]	19.92 ± 0.54[a]
MCHC (g/dL)	M ($n = 5$)	32.16 ± 0.57	30.84 ± 0.16[a]	32.26 ± 0.71
	F ($n = 5$)	31.58 ± 0.37	31.84 ± 0.67	32.86 ± 0.52[a]
White blood cells (10^{-3}/μL)	M ($n = 5$)	3.05 ± 0.97	5.45 ± 0.85	6.98 ± 0.45[a]
	F ($n = 5$)	4.82 ± 0.98	5.86 ± 0.79	4.42 ± 1.17
Neutrophils (%)	M ($n = 5$)	10.03 ± 2.97	12.96 ± 3.17	10.30 ± 4.72
	F ($n = 5$)	16.58 ± 5.78	11.30 ± 3.40	10.23 ± 1.12
Lymphocytes (%)	M ($n = 5$)	34.23 ± 1.94	46.80 ± 8.05	48.36 ± 8.56
	F ($n = 5$)	45.98 ± 2.71	55.26 ± 4.70	54.92 ± 7.17
Monocytes (%)	M ($n = 5$)	25.65 ± 3.23	26.54 ± 4.41	17.10 ± 1.50
	F ($n = 5$)	30.60 ± 7.20	20.98 ± 2.15	24.08 ± 1.94
Eosinophils (%)	M ($n = 5$)	1.05 ± 0.29	2.88 ± 0.21[a]	0.65 ± 0.23
	F ($n = 5$)	4.50 ± 1.62	1.72 ± 0.63	0.60 ± 0.17[a]
Basophils (%)	M ($n = 5$)	0.84 ± 0.14	0.66 ± 0.25	1.22 ± 0.23
	F ($n = 5$)	0.68 ± 0.22	0.60 ± 0.10	1.30 ± 0.59
Platelets (10^{-3}/μL)	M ($n = 5$)	351.67 ± 28.66	332.33 ± 20.85	354.40 ± 26.79
	F ($n = 5$)	423.25 ± 59.23	306.75 ± 33.92	347.00 ± 43.97
RDW (%)	M ($n = 5$)	13.92 ± 0.21	12.78 ± 0.26	14.30 ± 0.71[a]
	F ($n = 5$)	14.18 ± 1.06	13.70 ± 0.56	13.68 ± 0.20
MPV (fL)	M ($n = 5$)	8.68 ± 0.19	9.44 ± 0.21[a]	9.38 ± 0.20[a]
	F ($n = 5$)	8.84 ± 0.19	9.38 ± 0.10[a]	9.34 ± 0.18[a]
ALP (U/L)	M ($n = 5$)	182.08 ± 13.64	179.43 ± 8.56	223.03 ± 15.61
	F ($n = 5$)	109.10 ± 22.17	93.17 ± 13.51	177.20 ± 17.94
AST (U/L)	M ($n = 5$)	119.18 ± 11.65	162.45 ± 5.51	154.84 ± 11.84
	F ($n = 5$)	144.72 ± 10.09	163.97 ± 6.21	159.25 ± 15.60
ALT (U/L)	M ($n = 5$)	64.76 ± 12.12	80.16 ± 2.15	99.62 ± 5.66
	F ($n = 5$)	84.96 ± 15.57	81.80 ± 5.29	92.36 ± 11.26
Total protein (g/L)	M ($n = 5$)	68.54 ± 1.28	64.78 ± 0.76	63.06 ± 1.35[a]
	F ($n = 5$)	72.22 ± 1.92	66.00 ± 1.38[a]	63.60 ± 1.74[a]
Albumin (g/L)	M ($n = 5$)	33.57 ± 1.28	36.11 ± 0.69	35.12 ± 0.74
	F ($n = 5$)	37.26 ± 1.14	37.12 ± 0.64	35.44 ± 2.30
Urea (mmol/L)	M ($n = 5$)	10.40 ± 1.06	9.63 ± 0.30	7.21 ± 0.27[a]
	F ($n = 5$)	9.90 ± 0.77	9.28 ± 0.64	7.33 ± 0.58[a]
Creatinine (mmol/L)	M ($n = 5$)	33.20 ± 3.12	27.80 ± 1.16	31.40 ± 2.77
	F ($n = 5$)	32.80 ± 2.03	31.50 ± 1.58	26.60 ± 0.93[a]

Values are expressed as mean ± SEM. Means followed by superscript "[a]" across rows are significantly different at $p < 0.05$ as compared to the corresponding control. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width; MPV, mean platelet volume.

inflammations in all the treatment groups (**Fig. 2**). Kidney histopathology sections for normal control group and extract treated groups at doses of 400 and 800 mg/kg.b.w also showed normal architecture (**Fig. 2**).

Effect of root extract of Maerua decumbens on body weight of rats in anti-diabetic study

In order to monitor the effect of methanolic root extract of *Maerua decumbens* on body weight in diabetic rats, the fasting body weight of each animal was recorded on 0, 7th, 14th and 21st day of treatment. There was a relatively higher increase in body weight observed in normal control rats versus normal rats treated with the methanolic root extract (28.73% and 35.23% respectively) after 21 days of treatment compared to day 0 (**Table 3**). There was however weight decrease (−15.32%) in diabetic control group after 21 days from day 0. On the other hand, marginal increases in body weights were observed in diabetic animals treated with the standard drug metformin (2.1%), plant extract; 100 mg/kg.b.w (2.0%) and 400 mg/kg.b.w (3.7%) after 21 days of treatment (**Table 3**).

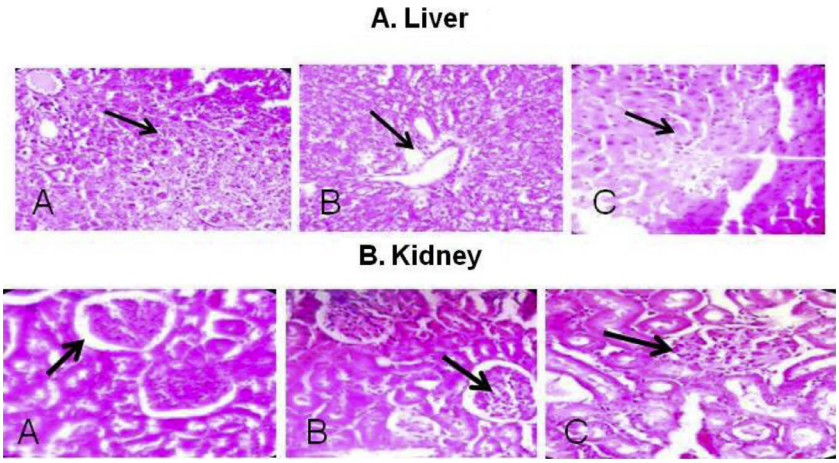

Fig. 2. Representative photomicrographs of tissue sections of liver and kidney of rats treated with *Maerua decumbens* root extract in the oral sub-acute toxicity study (Haematoxylin and eosin stained, X40). Liver; A- normal control, arrow; normal hepatic architecture; B- 400 mg/kg.b.w extract treated, arrow; the portal triad; C- 800 mg/kg.b.w extract treated, arrow; normal hepatocytes. Kidney; A- normal control, arrow; the Bowman's capsule; B- 400 mg/kg.b.w extract treated, arrow; the glomerulus; C- 800 mg/kg.b.w extract treated, arrow; the renal tubules.

Table 3
Effect of root extract of *Maerua decumbens* on body weight of rats in anti-diabetic study.

	Body weight (g)				% change in body weight on 21st day (from day 0)
Normal control	160.3 ± 3.7[a]	173.1 ± 4.6[a]	192.8 ± 5.3[a]	206.3 ± 9.0[a]	28.73
Normal + 400 mg/kg extract	156.9 ± 4.5[a]	174.2 ± 7.0[a]	197.6 ± 10.1[a]	212.2 ± 11.4[a]	35.23
Diabetic control	177.4 ± 1.6[b]	168.5 ± 3.5[b]	157.1 ± 2.7[b]	150.2 ± 5.0[b]	−15.32
Diabetic + 100 mg/kg extract	164.6 ± 6.6[a]	164.7 ± 7.0[b]	169.2 ± 6.7[c]	167.9 ± 6.6[c]	1.97
Diabetic + 400 mg/kg extract	175.6 ± 1.6[b]	181.0 ± 5.3[a]	186.1 ± 6.8[a]	182.1 ± 6.9[d]	3.69
Diabetic + 100 mg/kg metformin	162.1 ± 5.1[a]	163.0 ± 7.3[b]	163.4 ± 11.6[c]	165.5 ± 14.0[b]	2.12

Values are expressed as mean ± SEM; $n = 5$. Means followed by different superscript letters within a column are significantly different at $p < 0.05$ compared to normal and diabetic control.

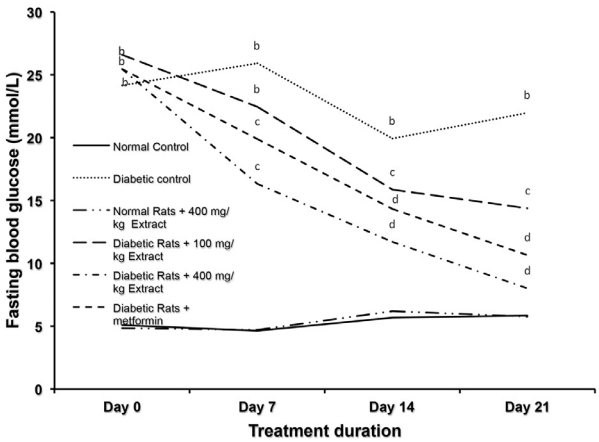

Fig. 3. Effect of *Maerua decumbens* root extract on fasting blood glucose of diabetic and normal rats. Values are expressed as mean ± SEM ($n = 5$). Values with superscript letters b, c and d are significantly different from normal control while values with superscript letters c and d are significantly different from each other and from both normal control and untreated diabetic control *($p < 0.05$).*

Effect of Maerua decumbens root extract on fasting blood glucose of rats in anti-diabetic study

At day 0, that is after five days of administration of streptozotocin (50 mg/kg.b.w) for diabetic induction, diabetic induced rats displayed significant hyperglycaemia with mean values of fasting blood sugar of between 24.2 ± 2.5 and 26.6 ± 2.6 mmol/L (**Fig. 3**) compared to normal rats that had mean normal values ranging between 4.9 ± 0.3 and 5.1 ± 0.4 mmol/L. Oral administration of *Maerua decumbens* root extract for 21 days resulted to a significant dose depen-

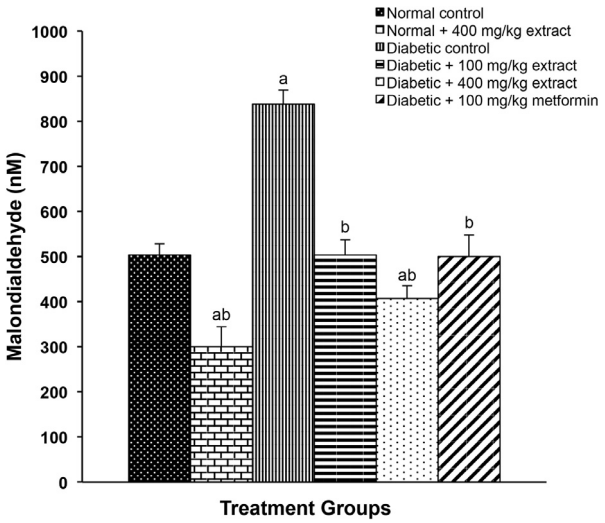

Fig. 4. Effect of *Maerua decumbens* root extract on liver malondialdehyde levels in diabetic and normal rats. Values are expressed as mean ± SEM ($n = 5$). [a] $p < 0.05$ values are significantly different compared with normal control. [b] $p < 0.05$ values are significantly different compared with diabetic control.

dant reduction in fasting blood glucose (Fig. 3) compared to diabetic untreated control. The root extract at high dosage of 400 mg/kg.b.w produced the maximum fall of 62.12% from day 0 in the blood glucose levels in diabetic rats after 21 days of treatment as compared to diabetic control, while low dose of extract (100 mg/kg.b.w) had 45.94% reduction of fasting blood sugar levels. The standard drug, metformin at 100 mg/kg.b.w resulted in a (58.16%) reduction in fasting blood glucose from day 0 (Fig. 3).

Effect of Maerua decumbens root extract on liver malondialdehyde levels in anti-diabetic study

There was significant increase in the malondialdehyde (MDA) levels in liver tissues of diabetic control untreated rats (839 ± 45 nM) compared to normal control rats (504 ± 25 nM) as shown in Fig. 4. Treatment of diabetic rats with metformin (100 mg/kg.b.w), *M. decumbens* methanolic root extract at 100 and 400 mg/kg.b.w caused a significant reduction in the levels of liver MDA (500 ± 48, 504 ± 34 and 408 ± 28 nM) respectively compared to the diabetic untreated rats (839 ± 45). There was also significant reduction in liver MDA observed in the normal rats treated with extract at 400 mg/kg.b.w (300 ± 31) compared to normal untreated rats (504 ± 25).

Discussion

Due to the central role played by medicinal herbs in the management and treatment of diseases in developing countries especially in Sub-Saharan Africa, there is need to carry out toxicological evaluations in order to determine the safety of their continued use. Additionally, there is need to conduct experiments that provide scientific data on therapeutical efficacy of such plants. Despite the wide health-related use of *Maerua decumbens* by various communities in Kenya, to the best of our knowledge, there are no studies available in the literature that has investigated its *in vivo* toxicity and antihyperglycemic efficacy. In the present study, we thus performed the qualitative phytochemical screening and HPLC fingerprinting of the root extract of *Maerua decumbens* in order to ascertain its rich phytochemical content and therefore its medicinal potential. We then evaluated the acute and sub-acute oral toxicity of *Maerua decumbens* root extract in a Wistar rat model in order to provide a scientific basis of its safety to humans. Furthermore, this study also sought to evaluate the antihyperglycaemic effect of *Maerua decumbens* methanolic root extract in streptozotocin (STZ) induced diabetic Wistar male albino rats so as to validate the antidiabetic medicinal claims.

The qualitative phytochemicals screening of the *Maerua decumbens* root extract showed the presence of various phytochemicals such as alkaloids, glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids. Indeed, HPLC fingerprinting of the root extract further confirmed the presence of various phytochemicals with unknown identities. These results have opened an area of interest for further quantitative separation and structural identification of phytochemicals present in the roots of *Maerua decumbens*.

During the 14 days of acute toxicity assessment period, all rats orally administrated with the root extract at a single dose of 2000 mg/kg showed no obvious signs of distress, and there were no noticeable symptoms of either toxicity or mortality indicating that the *Maerua decumbens* extract has no adverse effect on the animals. Based on OECD test guidelines 423 (Acute Oral toxicity–Acute Toxic Class Method), the results of this test allow the substance to be ranked and classified according to the Globally Harmonized System of Classification and Labelling of Chemicals as category 5 with low acute

toxicity hazard, which was the lowest toxicity class [17]. Therefore, it can be concluded that *Maerua decumbens* root extract is tolerated up to 2000 mg/kg.b.w when administered at a single dose. In like manner, a study performed by Al-Afifi et al. [25] using *Dracaena cinnabari* revealed that *Dracaena cinnabari* resin methanol extract can be classified under category 5 when administered at single dose 2000 mg/kg.b.w in accordance with Globally Harmonised System of Classification and Labelling of Chemicals, and this provides a direct relevance for protecting human and animal health.

Noteworthy is that acute toxicity information is of limited clinical application because cumulative toxic effects do occur even at very low doses. Consequently, multiple dose studies are typically useful in evaluating the safety profile of herbal medicines. Therefore, sub-acute oral toxicity test of *Maerua decumbens* root extract was also performed. In toxicity studies, changes in body weight have been used as an indicator of adverse effects of drugs and chemicals. It goes without saying that a decrease in body weight may be an indicator of adverse effects [26], and loss of more than 20% of the animal body weight is regarded as critical and has been defined as one of the humane endpoints in several international guidelines [27]. However, in this study there was stable increase in body weights of all male and female extract treated rats at all doses. The increase in body weight observed during treatment at the high oral dose indicates the nontoxicity of the extract. Furthermore, findings of this sub-acute study showed that the relative organ weights of vital organs (liver, kidneys, heart and spleen) were not adversely affected throughout the treatment in both male and female treatment groups except some incidental significant increases and/or decreases in the kidney, heart and spleen which were non-dose related and could be due to handling inconsistencies of the organs. Therefore, this implies that the presence of the extract in the animal and the various oral concentrations used during the test had little or no impact on the particular organs and provides support on the safety of *Maerua decumbens* root extract. On the other hand, haematological parameters analysis is appropriate in risk evaluation as the haematological system has a higher prognostic value for toxicity [28]. In the present study, most of the haematological parameters were not significantly affected by *Maerua decumbens* extract. However, RBC and its indices, MCV, MCHC, and MCH showed significant increases in the extract treated groups compared to the respective control. There was notably no change in WBC differential count (neutrophils, lymphocytes, basophils and monocytes) which are known to rise during body defence in response to toxic environment [29]. However, the change in haematological parameters was within the normal range as showed elsewhere [30] and did not exceed the toxic levels.

AST and ALT are aminotransaminases which are good indicators of liver function and they are used as biomarkers to study probable toxicity of drugs. Normally, destruction to the liver parenchymal cells will result in an increase of both these aminotransaminases in the blood [31]. On the other hand, elevated levels of serum ALP are reported in liver diseases or hepatotoxicity [32]. The insignificant changes in serum ALP, AST and ALT in male and female extract treated rats in this study at all dosages suggest that sub-acute administration of *Maerua decumbens* extract did not affect the hepatic function in rats. Additionally, the levels of total protein and albumin in serum partly indicate the status of hepatocellular and secretory functions of the liver. Reduction in serum albumin level may suggest infection or continuous loss of albumin [33] while significant low total serum protein may represent a compromised liver's synthetic ability of total protein which may suggest a liver dysfunction [34]. Thus, the insignificant change in serum concentration of albumin in control and extract-treated groups at all dosages used in this study suggest that *Maerua decumbens* extract does not damage the hepatocellular or secretory functions of the liver which in turn indicate non-adverse effects of the extract. There was significant reduction of total proteins in male and female rats treated with the highest-dose (800 mg/kg.b.w) of the extract but there were no significant changes at low dose (400 mg/kg.b.w) when compared to respective normal controls. Indeed, the histopathological evaluation of the liver of normal and all *Maerua decumbens* root extract administered groups in this study during sub-acute toxicity testing showed no signs of toxicity.

Renal dysfunction can be measured by simultaneous measurements of urea and creatinine in serum, and their normal or reduced levels are observed when there is normal functioning of the kidney [35]. Higher than normal levels of serum creatinine and urea are good indicators of renal dysfunction [36]. Thus, the normal or significant decrease in serum creatinine concentrations with concomitant decrease in the serum urea concentration in all the extract treated rats in this study indicates the normal functioning of the kidneys. The liver and kidneys are usually the first casualties of toxic substances and in this study, normal kidney histoarchitecture was observed after the 28 days administration of the extract. These results are in agreement to a previous study regarding non-toxic effects of plant-based crude extracts [37]. Furthermore, the non-toxic effects from our acute and sub-acute toxicity studies of *M. decumbens* root extract in rats validates safety of the roots for the various uses by some local communities in Kenya. These uses include the roots being used for purification of water [13] and various medicinal/food purposes [14]; roots and bark are soaked in warm water and the liquid is drunk for medicinal purposes, roots are used as purgative, roots are boiled and mixed with broth for health and vitality, and roots being chewed to quench thirst. Noteworthy also is that the fruits of *M. decumbens* are edible.

Streptozotocin (STZ) induced diabetes in animal models has been described as a useful experimental tool in the evaluation of novel hypoglycaemic agents [38]. As expected therefore, the STZ-induced diabetic rats in this study had hyperglycaemia after diabetes induction attributable to the effects of injected STZ on their pancreas. Streptozotocin acts by selectively destroying the insulin secreting pancreatic cells of the islets of Langerhans resulting to decrease in the endogenous insulin production and thus affecting glucose utilization by the various tissues and increases gluconeogenesis [7].

The untreated diabetic rats in this study showed drastic reduction in body weight while the normal rats had a steady increase in their body weights. This observation is consistent with clinical presentation amongst type 1 DM patients. Interestingly, the diabetic rats treated with *Maerua decumbens* root extract did not experience similar drastic weight changes compared to untreated diabetic rats. A similar trend was also observed with the metformin treated group which could be sug-

gestive of similar effects between our root extracts and the standard drug, metformin. At a higher dosage (400 mg/kg.b.w), *Maerua decumbens* extract was more effective than the lower dose (100 mg/kg.b.w) of root extract and metformin at 100 mg/kg.b.w that can be attributable to its higher concentration of phytochemicals. Induction of diabetes with STZ is associated with a characteristic loss of body weight due to insufficient insulin and thus the body is prevented from getting glucose from the blood into the body's cells to use as energy [7]. When this occurs, the body starts burning fat and muscle proteins for energy, leading to increased muscle wasting and loss of tissue proteins [38]. The ameliorative effects on body weight reduction observed in the diabetic rats treated with extract and metformin could be therefore protective effects on the islets resulting in better release of insulin and associated better utilization of glucose and reduced utilization of both muscle protein and body fat as compared to the untreated diabetic control.

Blood glucose measurement is one of the most relevant markers used in detecting DM both clinically and experimentally. In this study, the methanolic root extract of *Maerua decumbens* in STZ induced diabetic rats showed blood glucose lowering effect when administered orally for 21 days. The extract at 400 mg/kg.b.w showed a maximum reduction of blood glucose level (62.12%) while extract at 100 mg/kg.b.w and metformin at 100 mg/kg.b.w had 45.94% and 58.16% reduction in fasting blood glucose respectively when compared to the diabetic control. It is interesting to note that despite being crude, our plant extract had similar effects as metformin (100 mg/kg.b.w) at all the measured time points at 400 mg/kg.b.w. The blood glucose lowering effect of *Maerua decumbens* root extract may be attributed to the presence of various phytochemicals that have been associated with hypoglycaemic activity [12]. Several mechanisms such as inhibition of carbohydrate metabolizing enzymes [39], enhancement of glycogen regulatory enzymes expression in the liver and glucose uptake by tissues and adipocytes [40], as well as stimulation of pancreatic insulin release or regeneration process of remnant β-cells [7] have been associated with the antihyperglycaemic effect of antidiabetic medicinal plants**.** On the other hand, metformin, a standard antidiabetic drug, is a biguanide which provides an effective treatment for patients with diabetes by primarily reducing glucose production in liver [41].

In the present study, the effect of *Maerua decumbens* root extract on oxidative stress in STZ induced diabetic rats was also examined. The highest level of liver MDA, an index of lipid peroxidation was recorded in the diabetic untreated group, indicating that these rats were more susceptible to lipid peroxidation compared to normal rats. On the other hand, administration of *Maerua decumbens* root extract as well as metformin in the diabetic rats reduced the diabetes-induced increased liver's lipid peroxide levels and thus reduced the susceptibility of the liver to lipid peroxidation when compared to the untreated diabetic group. Diabetics and experimental animal models of STZ diabetes induction exhibit high oxidative stress due to STZ and persistent chronic hyperglycaemic state which depletes the activity of the antioxidative defence system and thus promotes *de novo* generation of free radicals [42]. STZ and hyperglycaemia increases the production of markers of cell damage related to free radicals, such as MDA and conjugated dienes which may bring about protein damage and inactivation of membrane-bound enzymes [43]. Our results are consistent with numerous reports of increased oxidative stress in the tissues of diabetic rats [7,44]. The decrease in MDA levels in *Maerua decumbens* root extract-treated rats can be attributable to the presence of several phytochemical constituents that we found present in the root extract including flavonoids, alkaloids, glycosides, saponins and tannins which are known natural antioxidants that possess free radical scavenging effects, increases the activities of antioxidant enzymes and has cellular rejuvenating potential [45].

Conclusion

Overall, this study has shown that the orally administered methanolic extract of roots of *Maerua decumbens* did not cause any apparent *in vivo* acute and sub-acute toxicity in Wistar rats as based on the clinical and histological observations as well as on the haematological and serum biochemical indices. Furthermore, we have shown that the phytochemicals-rich *Maerua decumbens* methanolic root extract as also exhibited by the HPLC fingerprints of the phytochemicals has antihyperglycaemic effect in STZ induced diabetic rats since it reduced hyperglycaemia and hepatic lipid peroxidation. However, there is need to elucidate further the mechanisms of action of these antihyperglycaemic effects of the extract and also the chemical structures of the bioactive constituents. This study has provided useful insight on the great potential of *Maerua decumbens* root extract for use as a safe alternative medicine for the management of DM and its complications.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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Declaration of Competing Interest

The authors report no conflicts of interest.

CRediT authorship contribution statement

Richard T. Kiptisia: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Resources, Software, Writing - original draft, Writing - review & editing. **Geoffrey K. Maiyoh:** Conceptualization, Supervision, Formal analysis, Resources, Writing - review & editing. **Benson N. Macharia:** Visualization, Formal analysis, Resources, Writing - review & editing. **Vivian C. Tuei:** Conceptualization, Supervision, Formal analysis, Resources, Writing - review & editing.

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