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Research Article

Evaluation of the Acute and Sub-Acute Toxic Effects of 80% Methanol Rhizome Extracts of *Rumex abyssinicus jacq. (Plygonaceae)* on Histopathology of Liver, Kidney and Some Blood Parameters in Swiss Albino Mice

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Abstract

Background: *Rumex abyssinicus jacq (polygonaceae)* is common medicinal plants used in Ethiopia for various complaints such as wound healing, anti-inflammatory activities, hypertension, liver disease, amoeba and intestinal parasites. So, several studies support the use of this plant for different disease, the toxicity profile was not evaluated yet. Therefore, the aim of the present study was carried out to assess the acute and sub-acute toxicity *Rumex abyssinicus of* rhizome extract on some blood parameters and histopathology of liver and kidney in Swiss albino mice.

Method: Rhizomes of *Rumex abyssinicus* were collected from Gondar area, Northwest Ethiopia. The dried roots extracted with 80% methanol. 25 - 40g weight and 8 - 12 weeks age Swiss albino female mice were randomly divided into one control and three experimental groups. The control group was orally given 0.5 ml of distilled water and treatment groups were given extract by intragastric tube at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight once a day for 4 weeks and then sacrificed. Blood sample was collected from each mouse and examined for hematological and biochemical parameters. Liver and kidney were removed, stained and examined for histopathological effects.

Result: The methanol rhizome extract of *Rumex abyssinicus* was tested for toxicity study. Female Swiss albino mice were randomly divided into control and treatment groups. The doses for acute toxicity study were single doses of 2, 000 mg/kg body weight and sub-acute toxicity study daily administration doses of 100, 200 and 400 mg/kg of extract were used for 28 consecutive days. Hematological parameters and microscopic examination of liver and kidney tissue and body weight of mice were evaluated the test period as well recording the signs of toxicity.

Acute toxicity study did not tell any signs of toxicity; that's why the LD50 was higher than 2, 000 mg/kg. There was no significant change ($p > 0.05$) in general body weight and most of evaluated hematological and biochemical parameters after 28 days of sub-acute treatment. The kidneys and liver of treatment group appear normal in their texture, shape, size or color compared to the control in gross and histopathological examination.

The findings revealed that the 80% methanol rhizome extract of *Rumex abyssinicus* relatively safe in mice.

Keywords: *Rumex abyssinicus; Sub-Acute Toxicity; Histopathological Studies*

Introduction

Herbal medicine frills today as the primary form of medicine for conceivably as much as 80% of the world's population [1]. Usually, a specific part of the plant is formulated into an appropriate preparation and many medicines commonly used today are of herbal source. Indeed, about 25% of prescription drugs contain at least one active ingredient derived from plant material [2].

In the past herbs often represented the unique sources of most drugs and herbal-derived substances remain the basis for majority of the commercial medications used today for the treatment of different illness's [3,4].

Africa is rich in biodiversity which embraces about 6,377 plant species of these more than 4, 000 plant species are used as medicinal plants [5]. Ethiopia is a prime example for a developing country rich in biodiversity, with a millennia old tradition of curers using the rich flora which accounting to about 95% of traditional medicinal preparation in Ethiopia [6] and is probably due to cultural acceptability, accessibility and affordability [7].

Regardless of lack of proper scientific validation and toxicity evaluation, medicinal plants are still mainstay for primary health care in about 75 - 80% of the world population, especially in the developing countries. Hence, in order to improve the quality of primary health care, safety and efficacy of traditional medicines should be scientifically supported. Once the natural plant products pass through toxicity test using scientific methods the safety of the medicine can be guaranteed [8].

Rumex species are medicinal plants commonly used in Ethiopia. The genius *Rumex* includes more than 200 species worldwide and is distributed widely in temperate zones. It is wide spread in tropical Africa, most commonly in the highlands, particularly in central and eastern Africa, including Ethiopia and is 3 to 4 meter tall [9-11].

In photochemical studies on the following constituents have been found in *Rumex* abyssinicus: oxalic acid, chrysophanic acid, chrysophanol, emodine and physcion [12-14].

Many biological activities of this *Rumex* abyssinicus were determined experimentally as anti-direutics and analgesic [15,16]; anti-microbial and anti-inflammatory activities [17]; anti-hehilmentics [18]; antineoplastic activity [19].

Traditionally, *Rumex Abyssinicus* the most common medicinal plants have been used in Ethiopia to treat several human diseases. Regardless of its toxicity, several studies recommend the use of *Rumex abyssinicus* to cure different disease.

Aim of the Study

The aim of this study is therefore, to evaluate the acute and sub-acute toxicity of rhizome extract of *Rumex abyssinicus* on some blood parameters and histopathology of liver and kidney in Swiss albino mice.

Materials and Methods

Plant material collection

The Rhizomes of *Rumex abyssinicus* collected in October 2016 from Gondar area, northwest Ethiopia, located about 740 km from the capital city, Addis Ababa. The plant was then identified and authenticated by Mr. Abiyu Enyew, botanist, Department of Biology, College of Natural Sciences, the University of Gondar, where a voucher specimen (collection number EF001) was deposited for further reference. The rhizomes were cleaned from any irrelevant materials, sliced to smaller pieces and dried at room temperature and then the pieces changed into powdered and formed extracted with a mass 1650g of powdered rhizome was divided in to three batches in a 5Liter conical flask.

Preparation of plant Extraction

1650 gram of powdered rhizome was divided in to three batches in a 5 Liter conical flask then 80% methanol was added to each flask up to a volume sufficient to completely cover the powder inside and left to macerate for 72 hour with occasional shaking. Remacerated two times for the same solvent to exhaustively extract metabolites. The methanols in the extract were removed by Rota vapor and the remaining solution was further dried in an oven with a maximum temperature of 40°c to remove the water. Finally, the dried extract was packed in a plastic bag and stored in desiccators until used. The percentage yield of dried extract was found to be 17.4%. 80% methanol solvent is better solvent to isolate large number of bioactive chemicals than other solvent [20,21].

Experimental animals and administrations of plant extract

A total of 50 female mice weighing 25 - 40g and 8 - 12 weeks of age were used for the studies. For oral median lethal dose (LD_{50}) determination 10 nulliparous and non pregnant female mice were used based on recommendation of OECD 425 [22]. The mice were housed in a group of ten per cage in a standard cage. They were kept under ambient temperature and humidity. Day and night cycle was maintained at 12 h each. Food and drinking water were provided *ad libitum*.

For the sub-acute toxicity study, 40 mice were randomly grouped in to four (group I, II, III and IV), each group containing 10 female mice and received 80% methanol rhizome extract of *Rumex abyssinicus* at a dose of 100, 200 and 400 mg/kg/day, respectively. Group IV animals served as control received distilled water.

Each group of animal was given different doses of methanol rhizome extract of *Rumex abyssinicus* orally using stomach tube. The method of administration for acute toxicity study and LD50 determination of the crude methanol rhizome extract of *Rumex abyssinicus was* following OECD guideline 425 [22]. The extracts were given once after the animals were fasted overnight with a free access for water. Measured the weight of mice and calculated the dose of the test substances based on their body weight, then the test substance was administered accordingly. For the sub-acute toxicity study, the mice in the experimental groups received the crude methanol rhizome extract of *Rumex abyssinicus* and control group were given distill water every day for consecutive 4 weeks. Route of administration was intra-gastric using a ball-tipped stainless steel feeding needle fitted to a 5 ml syringe [23].

Acute toxicity study

The acute toxicity of crude methanol rhizome extract of *Rumex abyssinicus* was determined following OECD guideline 425 [22]. The crude methanol rhizome extract of *Rumex abyssinicus was* administered orally at single doses of 2,000 mg/kg body weight. Once-daily, cage side observations for behavioral changes were made. This included salivation, erection of the hair and diarrhea was observed over a period of two weeks.

Sub-acute toxicity study

The mice in Group I, II and III were treated with the extract at doses of 100, 200 and 400 mg/kg Bwt/day respectively; while mice in Group IV (control) were treated with distill water every day for consecutive 4 weeks. Preparations of the doses and duration of administration for sub-acute toxicity study were based on the guideline document OECD 407 [23].

Body and organ weight measurement

Body weight was recorded prior to the treatment and once a week. Then, the weight recorded before administration of the test substance was considered to be initial body weight. Final body weight was recorded on the last day after 12 hours fasting following administration of test substance OECD 407 [23]. Absolute organ weight of liver and kidney was measured using electronic balance immediately after mice were sacrificed.

Blood collection for hematological and biochemical analyses

Blood sample was collected before mice were sacrificed. The mice were anaesthetized using ketamine. Blood samples were then obtained through cardiac puncture using sterile needle fitted to 5ml syringe and directly introduced in to two groups of test tubes [24]. The test tubes with anticoagulant, ethylene-diaminetetraacetic acid (EDTA) were used to collect blood samples for analysis of hematological parameters while test tubes without anticoagulant were used to collect blood for biochemical estimation. Hematological parameter such as hematocrit (HCT), hemoglobin (Hgb), total counts of RBC and WBC, mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (PLT) were measured. On the other hand, the blood serum was taken to analyze the biochemical parameters that are commonly used for liver and kidney function tests including glucose, total protein, urea, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using Automated Clinical Chemistry Analyzer.

Animal dissection, tissue collection and histological processing

The abdominal cavity of the animals was opened by a vertical midline incision with scissors cut from the neck to pubis. The liver and kidney were gently isolated and immediately weighed [25]. Then, strips of tissue samples were randomly taken [26] from the liver and kidney. Immersed in 10% neutral buffered formalin for the purpose of fixation [26]. After overnight fixation, the tissues were washed with running tap water and dehydrated with graded series of alcohol (70%, 80%, 95%, 100% and 100%: 1/2hr, 1hr, 1hr, $1^{1/2}$ hrs and $2^{1/2}$ hr respectively) The tissues were cleared with two changes of xylene for one and half an hour each, then infiltrated into two changes of melted paraffin wax for one and half an hour that has a melting point of 56°C [24]. The paraffin infiltrated tissues were then placed carefully into squared metallic plate block moulds into which liquid paraffin wax was poured with the help of Electro-thermal Wax Dispenser to form tissue blocks, allowed to harden and label it. The resulting solid paraffin blocks containing the tissue were then removed from the mold and sectioned in ribbons at a thickness of 5µm containing the tissues were allowed to float onto the surface of a warm water bath at 40°c to spread and remove folds in the sections using a rotary microtome. The slides were arranged in slide racks and were placed in an oven with a temperature of 60°C for 10 - 15 minutes. The tissue sections were allowed to cool and dry at room temperature and stained with routine Hematoxylin and Eosin staining method (H and E). The slides to which the tissue sections attached were placed in xylene I for 5 minutes and xylene II for 2 minutes so as to dissolve the paraffin, then immersed in a series of descending alcohol concentrations to remove xylene after which distilled water was used to hydrate the tissue [24]. The slides were mounted by adding a drop of canada balsam mounting medium on the section to cover the microscopic glass with cover slip. This was done with care to prevent bubble formation between the tissue and the glass cover [24]. Finally, the slides were labeled with pertinent identification information and placed in a slide box [27].

For histopathological investigations, stained tissue slides of liver and kidney were examined at different magnifications using light microscope. After examination of histological slides of all groups, photomicrographs of selected samples of liver and kidney from both extract treated and vehicle treated mice were taken using digital camera installed microscope with a magnification of x40 were used for photomicrography for tissue sections [24,27].

Statistical analysis

Data were analyzed using the statistical software package SPSS version 20 for windows program. All the values in the test are presented as mean and standard error of the mean (mean ± SEM). Statistical differences between the means of different groups were evaluated by one-way analysis of variance (ANOVA). P-values < 0.05 were considered significant.

Result

Acute toxicity study and LD_{50} determination

After treatment with the plant extract 2000 mg/kg/body weight of single dose, cage side observations were made for any behavioral changes such as salivation, erection of the hair and diarrhea. Mice did not show any of these behavioral changes except for erection of hair. However, these changes were not apparent after the first 24 hours of the follow-up and no death in female mice during the 14 days period of experiment for acute toxicity. This result indicated that the oral median lethal dose (LD_{50}) is higher than 2,000 mg/kg.

General observations during sub-acute treatment of 80% methanolic rhizome extracts of *Rumex abyssinicus*

Among the mice administered with the repeated doses of the 80% methanolic rhizome extracts of *Rumex abyssinicus* at 100 mg/kg, 200 mg/kg and 400 mg/kg body weight for 28 days, no death was observed throughout the experimental period. However, gentle signs of toxicity such as depression, erection of the hair, loss of appetite and fast breathing were observed, among those mice treated with the crude extract at both doses as compared to the control group.

Effects of the 80% methanolic rhizome extracts of *Rumex abyssinicus* on body weight

No statistically significant ($p > 0.05$) body weight change was observed in the female mice treated with the repeated dose of 100, 200 and 400 mg/kg body weight/day of the rhizome extracts of the *Rumex abyssinicus* as compared to the controls. As indicated in table 1, the mean body weight change in mice treated with the crude methanolic rhizome extract of *Rumex abyssinicus* was lower (0.12, 0.04 and 0.08 gm) as compared to the controls (2.54 gm). However, the differences were not statistically significant ($P < 0.05$) when treatment groups were compared with the control.

Group	Initial weight IWT (in gram)	Final weight FWT (in gram)	Weight difference (FWT-IWT) in gram	% of body weight change
Control	29.06 ± 1.07	31.61 ± 0.61	2.54 ± 0.43	8.74
100 mg/kg/day	34.20 ± 1.36	34.32 ± 0.57	0.12 ± 0.79	3.51
200 mg/kg/day	34.53 ± 0.98	34.57 ± 0.37	0.04 ± 0.21	0.12
400 mg/kg/day	33.36 ± 1.47	33.44 ± 0.61	0.08 ± 0.86	0.24

Table 1: *Comparison of mean body weight change among methanolic rhizome extract of Rumex abyssinicus treated groups and the controls.*

Effects of the 80% methanol rhizome extract of *Rumex abyssinicus* on gross pathology and organ weight

Gross pathology of the liver and kidneys in female mice treated at 100, 200 and 400 mg/kg body weight did not show abnormalities such as in spot, size, texture, color, necrosis and lesion as compared to the controls. Effect of methanol rhizome extract of *R. abyssinicus* did not produce any significant effect on weights of liver and kidneys of mice after the 28 days compared with the group treated with distilled water (Table 2).

Group	Organ weight in grams	
	Liver	Kidney
Control	1.56 ± 0.06	0.19 ± 0 .01
100 mg/kg	1.9 ± 0.10	0.2 ± 0.15
200 mg/kg	1.8 ± 0 .06	0.22 ± 0.01
400 mg/kg	1.6 ± 0 .07	0.18 ± 0.01

Table 2: *The sub-acute effect of rhizome extract of R. abyssinicus on the weight of liver and kidneys of female mice. Values are expressed as Mean ± SEM; *p < 0.05.*

Effects of the 80% methanolic rhizome extract of *Rumex abyssinicus* on hematological parameters of blood

The effect of sub-acute treatment of methanolic rhizome extract of *Rumex abyssinicus on* haematological parameters of blood is shown in table 3. There were a difference resulted between treatment and control groups, but such differences were statistically not significant ($p > 0.05$) of hematological parameters. even if statistically not significant, insignificant reductions in RBC, MCH and PLT at doses (100 mg/kg body weight/day and 200 mg/kg body weight/day in RBC and both 100, 200 and 400 mg/kg body weight/day in MCH and PLT) as well as, WBC, HCT and HGB at 400 mg/kg body weight/day in WBC and 200 mg/kg body weight/day in HCT and HGB were observed in extract treated groups as compared to controls. Though statistically not significant ($p > 0.05$) the values of MCV and MCHC recorded at both doses (100, 200 and 400 mg/kg body weight/day) and HCT and HGB at the dose of 100 mg /kg body weight/day and RBC at the dose of 400 mg/kg body weight/day were found to be increase as compared to the controls.

Hematological parameters	Control	100 mg/kg	% of mean difference	200 mg/kg	% of mean difference	400 mg/kg	% of mean difference
WBC ($x10^3$/**µL**)	4.64 ± 0.11	4.99 ± 0.65	7.54	4.80 ± 0.38	4.3	3.87 ± 0.28	16.59
RBC ($x10^6$/**µL**)	6.17 ± 0.33	5.94 ± 0.38	-3.72	5.93 ±0.36	-3.89	6.21 ± 0.37	6.48
HGB (g/dL)	10.03 ± 0.15	10.91 ± 0.86	8.77	9.71 ± 0.26	-3.19	10.93 ± 0.45	8.97
HCT (%)	30.61 ± 1.12	31.87 ± 1.44	4.12	30.26 ± 1.19	-1.14	33.66 ± 1.38	9.96
MCV (fL)	48.67 ± 0.66	49.64 ± 0.99	1.99	48.96 ± 0.63	0.60	49.27 ± 0.29	1.23
MCH (pg)	17.22 ± 0.27	16.13 ± 0.07	-6.33	16.54 ± 0.16	-3.95	16.59 ± 0.19	-3.66
MCHC (g/dL)	30.48 ± 0.96	32.77 ± 1.22	7.51	31.91 ± 1.30	4.69	31.40 ± 0.89	3.02
PLT ($x10^3$/**µL**)	956.5 ± 12.89	477.6 ± 49.89	-50.07	626.4 ± 37.85	-34.51	687.2 ± 85.67	-28.15

Table 3: *Comparison of hematological parameters in female mice treated with 100 mg/kg, 200 mg/kg and 400 mg/kg doses of the extract with the controls.*
*Values are expressed as mean ± SEM. *P < 0.05, N = 10/group.*

Effects of the 80% methanolic rhizome extract of *Rumex abyssinicus on* biochemical parameters of the blood.

The result in table 4 showed that biochemical parameters in the blood serum of mice treated with methanolic rhizome extract of *Rumex abyssinicus,* total protein, AST, ALT and creatinine were increased in the mice treated with the plant extract at both doses as compared to those of the control mice. However, the increments were not statistically significant at both doses. There were decreases in total bilirubin and glucose levels in mice treated at both doses and 100 and 200 mg/kg bwt/day for urea level, but increases at 400 mg/kg bwt/day as compared to controls.

Biochemical Parameters	Control	100 mg/kg	% of mean difference	200 mg/kg	% of mean difference	400 mg/kg	% of mean difference
Glucose	121 ± 9.12	101.9 ± 11.82	-15.79	107.7 ± 12.48*	-10.99	119.0 ± 10.22	-1.65
Total protein	52.40 ± 1.81	52.5 ± 2.75	0.19	53.70 ± 2.73	2.48	52.60 ± 3.19	0.38
AST (IU/L)	101.40 ± 5.80	108.20 ± 5.46	6.71	104.30 ± 6.06	2.86	109.20 ± 5.10	7.69
ALT (IU/L)	115.30 ± 8.82	131.50 ± 8.91	14.05	132.00 ± 8.95	14.48	122.50 ± 9.52	6.24
Total Bilirubin	0.26 ± 0.11	0.25 ± 0.11	-3.85	0.13 ± 0.07	-50	0.19 ± 0.10	-26.92
Urea (mg/dL)	34.40 ± 1.57	30.80 ± 2.69	-10.47	30.90 ± 2.86	-10.17	34.60 ± 2.86	0.58
Creatinine (mg/dL)	0.21 ± 0.02	0.26 ± 0.02	23.81	0.25 ± 0.02	19.05	0.23 ± 0.02	9.52

Table 4: *Comparison of biochemical parameters between female mice treated with 100, 200 and 400 mg/kg body weight doses of the extract with the control group.*
*Values are expressed as mean ± SEM. *P < 0.05, N = 10/group.*

Effects of the 80% methanolic rhizome extracts of *Rumex abyssinicus* on histology of the liver

Microscopic examination of liver sections from control mice showed the normal architecture of structural units of the hepatic lobules, formed by cords of hepatocytes separated by hepatic sinusoids. The central vein and portal area containing branches of hepatic artery, bile duct and portal veins were maintained with their normal appearance. In comparison to the control, the general microscopic architecture of sections of liver tissue from the mice treated with the extracts at 100, 200 and 400 mg/kg dose body weight/day appeared to be not significantly affected after the 28 days administration.

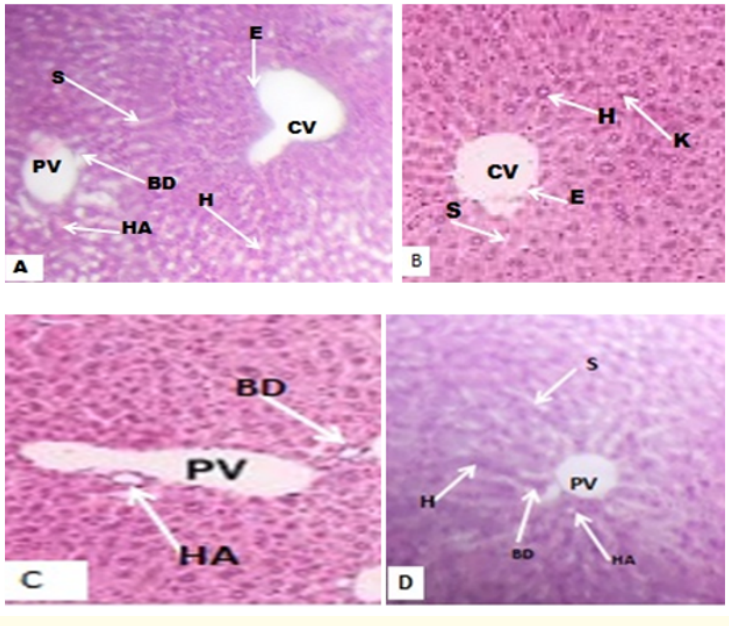

Figure 1: *Photomicrographs of liver of control mouse liver (A) showing no histopathological changes (H & E, x40). (B) Mice treated with 100 mg/kg body weight of R. abyssinicus rhizome extracts showing normal liver cells as compared to the control group (H & E, x40). (C) Mice treated with 200 mg/kg body weight of R. abyssinicus rhizome extract showing normal liver cell (H & E, x40). (D) Mice treated with 400 mg/kg body weight of R. abyssinicus rhizome normal liver cells (H & E, x40). CV: Central vein, H: Hepatocytes, E: Endothelial cells, S: Sinusoids, K: Kupffer cells.*

Effects of the 80% methanolic rhizome extracts of *Rumex abyssinicus* on histology of the kidneys

Histopathological examination of kidney sections of mice treated with the 80% methanolic rhizome extracts of *R. abyssinicus* at both 100, 200 and 400 mg/kg doses indicated no structural disturbance as compared to the control mice. The microscopic architecture of the kidneys in treated mice had similar appearance to that of the controls in which renal corpuscles maintaining their normal size of urinary space and normal tubular structures were observed with no sign of congestion.

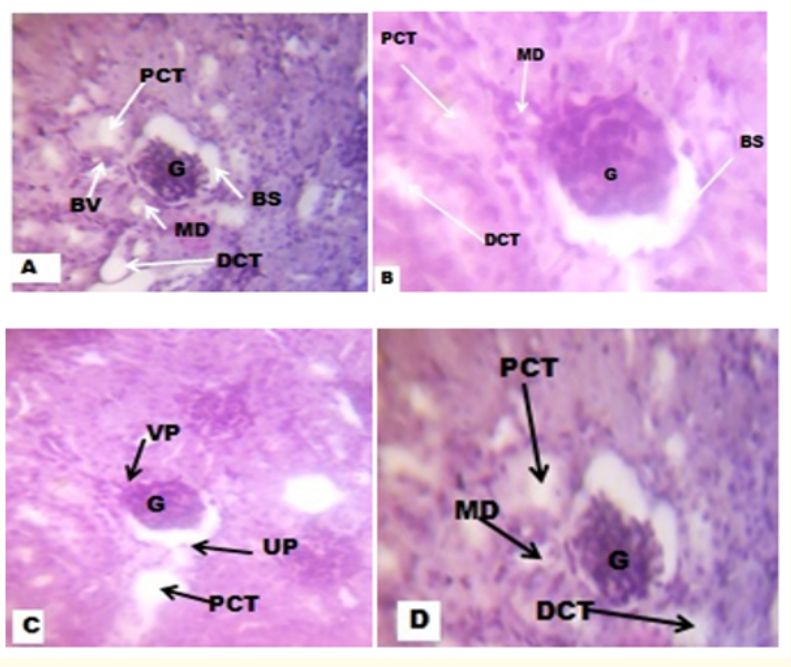

Figure 2: *Photomicrographs of kidney of control mouse kidney (A) showing no histopathological changes (H & E, x40). (B) Mice treated with 100 mg/kg body weight of R. abyssinicus rhizome extract showing no histopathological changes as compared to the control group (H & E, x40). (C) Mice treated with 200 mg/kg body weight of R. abyssinicus rhizome extract showing no histopathological changes as compared to the control group (H & E, x40). (D) Mice treated with 400 mg/kg body weight of R. abyssinicus rhizome extract showing no histopathological changes as compared to the control group (H & E, x40). G: Glomerulus, UP: Urinary Space, PCT: Proximal Convoluted Tubule; DCT: Distal Convoluted Tubule; MD: Macula densa; VP: Vascular Pole.*

Discussion

Herbal medicine has recently attracted attention as health beneficial foods and as source materials for drug preparation. They suggest a potential natural health care approach that spotlight on protecting and repair health [28]. Toxicity profile provide important preliminary data to help select natural preparation with potential health beneficial properties for future work [29]. As part of a permanent screening program searching for natural products with beneficial biological activity properties, the current investigation reports the toxicity of *Rumex abyssinicus* methanol extract.

Accordingly, the methanolic rhizome extracts from the *Rumex abyssinicus* did not induce lethality in mice when administered orally up to doses of 2,000 mg/kg [29]. There were no observed acute signs or delayed toxicity, which is similar with the previous study [30-32].

Daily treatment with both doses of the methanolic rhizome extracts of *Rumex abyssinicus* to female albino mice for a period of 4 weeks did not show any toxicity related morbidities and mortalities. Hematological parameters were also evaluated to obtain further toxicity related information which is not only detected by direct examination of organs and body weight analysis. Studies on hematological parameters can easily reveal abnormalities in body metabolic processes, and the blood profile usually provides important information on the response of the body to injury or lesion, deprivation and stress [33]. Red blood indices such as the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are the most useful indicators in the diagnosis of anemia in most animals [33]. The effect of the methanolic rhizome extract of *Rumex* abyssinicus on MCV, MCH, and MCHC were insig-

nificant in treated group compared to the control which means, the extracts did not cause significant toxicity on the levels of calculated red blood cell (RBC) indices at both doses. Hence, sub-acute treatment with the methanolic rhizome extract of *Rumex* abyssinicus has shown no effect on the size of RBCs and in hemoglobin weight per RBC in mice. This finding is in line with other findings [34,35]. The changes in RBC count, average hemoglobin (Hgb) and hematocrit (HCT) levels of treated group animals were also insignificant ($p > 0.05$) compared with that of the control. In the hematological analysis the white blood cell (WBC) count also was performed. In herbal toxicity studies, increase in WBC may indicate the impact of plant extracts in inducing the immune response of treated animals [35]. On the other hand, significant decrease in the WBC of the blood indicates a decline in the production of leukocytes called leukopenia, means that the body is less able to fight off infections. However, the hematological analysis in this study demonstrated that the estimated total WBC count after sub-acute administration of the formulation was not significantly changed in response to the administered methanolic rhizome extract of *Rumex abyssinicus* at doses of 200 mg/kg compared to the control. This result may indicate that the methanol rhizome extract in this study does not possess chemicals capable of inducing leukocytosis, which is an abnormally high number of WBC in the blood circulation or in suppression of normal production of WBC [36]. Also, similar results were obtained by previous study that found no changes in the WBC count with different groups compared with control groups [35].

In platelet count, thrombocytopenia is a condition of abnormally low number of platelets in the circulation, may result from decreased production or increased destruction of platelets [35]. However, in this study the change in the platelets count as compared to the control group is insignificant in both treated group of mice. This result suggests that rhizome extract of *Rumex* abyssinicus at both doses used in this study has no effect in inducing both thrombocytopenia or thrombocytosis.

Liver is a primary target organ for any toxic substance that entered to the body, especially through gastrointestinal route and suffers first. Because of its wide range of functions, any abnormal change in liver will definitely affect complete metabolism of an animal [37]. The most commonly used serum liver chemistry tests include serum transaminases (alanine aminotransferase (ALT), aspartate aminotransferase (AST)), total bilirubin and glucose. Injuries of liver cells allowing for escape of these enzymes into the bloodstream raises their levels in the blood [38]. In the present study liver function test, there were no significant changes in the serum level of ALT and AST treated with both doses of the methanolic rhizome extracts of *Rumex* abyssinicus in comparison to the controls which, indicates that the methanolic rhizome extract does not cause significant toxic effect or hepatic damage on the liver.

The kidneys regulated different chemicals in the blood and Kidney function test is a collective term for a variety of individual tests and procedures that can be done to evaluate how well the kidneys are functional or not. Therefore, renal function can be assessed by measuring the levels of plasma creatinine, urea and uric acid concentrations [39]. In the present study no significant alteration in the plasma urea and creatinine levels due to *Rumex abyssinicus* treatment was observed. So, plant extracts do not affect the kidneys.

Histopathological examinations provide information to strengthen the findings on biochemical and haematological parameters [35]. The present histological examination indicated that liver sections of mice treated with the methanolic rhizome extract of *R. abyssinicus* at both 100 mg/kg, 200 mg/kg and 400 mg/kg doses did not show focal necrosis, leukocytic cell infiltration and pyknosis. The general histological architecture and its functions were not affected in any of the treated mice as compared to the controls. Therefore, *R. abyssinicus* does not induce any damage to the liver or kidneys at both doses of the present study mice. No statistically significant difference was observed in the weight and structure of the liver and kidney between the control and the treated groups. In agreement with these results, the findings of another study has demonstrated no change in liver and kidney weight, as well as histopathological changes and morphological alterations in liver and kidney of mice at the studied doses [21,35,40].

Conclusion

The acute toxicity test suggests that oral single dose (2000 mg/kg) administration of *Rumex abyssinicus* is virtually non-toxic to albino mice. The sub-acute toxicity study suggests that *Rumex abyssinicus* are do not caused a significant effect in liver, kidney and blood pa-

rameters when administered orally at doses of 100, 200 and 400 mg/kg in albino mice. Therefore, with respect to results from liver and kidney, the 80% methanol rhizome extract of *Rumex abyssinicus* can be considered as relatively safe in Swiss albino mice.

Authors' Contribution

E.F. contributed to conception and design, acquisition of plant material collection, plant extraction, administration of extract and follow up, tissue processing, data entry, data analysis, interpretation of data and preparation of manuscript.

A.M. contributed to plant extraction and revising the manuscript critically for important intellectual concepts and had given final approval of the version to be published.

A.E. was involved in tissue processing, revising the manuscript critically for important intellectual concepts and had given final approval of the version to be published.

B.T. contributed to administration of extract and follows up, tissue processing, data analysis and review manuscript. All co-authors approved their accountability all aspects of the work. All authors read and approved the final manuscript.

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Bibliography

1. Organization WH. "WHO traditional medicine strategy 2002-2005" (2002): 1-61.
2. Saad B and Said O. "Greco-Arab and Islamic herbal medicine: traditional system, ethics, safety, efficacy, and regulatory issues". John Wiley and Sons (2011).
3. Cooper EL. "Drug discovery, CAM and natural products". *Evidence-Based Complementary and Alternative Medicine* 1.3 (2004): 215-217.
4. Saad B., *et al.* "Tradition and perspectives of Arab herbal medicine: a review". *Evidence-Based Complementary and Alternative Medicine* 2.4 (2005): 475-479.
5. WHO. "An Overview of the Traditional Medicine Situation in the African Region" (2003): 1-40.
6. Abebe D. "The role of medicinal plants in health care coverage of Ethiopia: the possible benefits of integration" (2001).
7. Demisse A. "Biodiversity conservation of medicinal plants: problems and prospects. Conservation and Sustainable use of Medicinal Plants in Ethiopia". Proceedings of the National Workshop on Biodiversity Conservation and Sustainable Use of Medicinal Plants in Ethiopia Addis Ababa: IBCR (2001).
8. Godkar PB and Godkar DP. "Textbook of medical laboratory technology". 2nd edition. Mumbai: Bhalani publishing house (2006).
9. Getie M., *et al.* "Evaluation of the anti-microbial and anti-inflammatory activities of the medicinal plants *Dodonaea viscosa, Rumex nervosus* and *Rumex abyssinicus*". *Fitoterapia* 74.1-2 (2003): 139-143.

10. Mulisa E., *et al.* "Evaluation of wound healing and anti-inflammatory activity of the rhizomes of *Rumex abyssinicus* J. (Polygonaceae) in mice". *BMC Complementary and Alternative Medicine* 15 (2015): 341.

11. Vasas A., *et al.* "The Genus *Rumex*: Review of traditional uses, phytochemistry and pharmacology". *Journal of Ethnopharmacology* 175 (2015): 198-228.

12. Fufa F M., *et al.* "Phytochemical Investigation and *In Vitro* Antibacterial Evaluation on Root Extracts of *Rumex abyssinicus*". *Natural Products Chemistry and Research* 04.06 (2016).

13. Fassil Y., *et al.* "Anthracene derivatives from *Rumex abyssinicus*". *Journal of Natural Products* (1985): 48.

14. Zinaye B and Fiseha A. "Phytochemical investigation on the root of *Rumex abyssinicus* (MAKMAKO)". *AAU Electronic Library* (2008).

15. Mekonnen Teshale., *et al.* "Evaluation of the diuretic and analgesic activities of the rhizomes of *Rumex abyssinicus* Jacq in mice". *Journal of Ethnopharmacology* 127.2 (2010): 433-439.

16. Mulisa E., *et al.* "Evaluation of wound healing and anti-inflammatory activity of the rhizomes of *Rumex abyssinicus* J. (Polygonaceae) in mice". *BMC Complementary and Alternative Medicine* 15.1 (2015): 341.

17. Getie M., *et al.* "Evaluation of the anti-microbial and anti-inflammatory activities of the medicinal plants *Dodonaea viscosa, Rumex nervosus* and *Rumex abyssinicus*". *Fitoterapia* 74.1 (2003): 139-143.

18. Raju N and Yesuf E. "Evaluation of anthelmentic activities of *Rumex abyssinicus* JACQ and *Rumex nervosus* VAHL (Polygonacea)". *International Journal of Pharmaceutical Sciences Review and Research* 5.2 (2010): 55-57.

19. Girma B., *et al.* "Effect of *Rumex Abyssinicus* on preneoplastic lesions in dimethylhydrazine induced colon carcinogenesis in rats". *BMC Complementary and Alternative Medicine* 15.1 (2015): 365.

20. Mishra AP., et al. "Bioactive compounds and health benefits of edible Rumex species-A review". *Cellular and Molecular Biology* 64.8 (2018): 27-34.

21. Alebachew M., et al. "Toxicological evaluation of methanol leaves extract of *Vernonia bipontini* Vatke in blood, liver and kidney tissues of mice". *African Health Sciences* 14.4 (2014): 1012-1024.

22. Co-operation OfE. Development. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure: OECD Publishing (2008).

23. No OT. 407: repeated Dose 28-day oral toxicity study in rodents. OECD guidelines for the testing of chemicals Section (2008): 4.

24. Raafat H and Abdelmonem H Hegazy. "Simplified Method of Tissue Processing (Consuming Time and Chemicals)". *Annals of International Medical and Dental Research* 1.2 (2015): 57-61.

25. Sellers RS., *et al.* "Society of Toxicologic Pathology Position Paper: Organ Weight Recommendations for Toxicology Studies". *Toxicologic Pathology* 35.5 (2007): 751-755.

26. Kittel B., *et al.* "Revised guides for organ sampling and trimming in rats and mice--Part 2. A joint publication of the RITA and NACAD groups". *Experimental and Toxicologic Pathology : Official Journal of the Gesellschaft Fur Toxikologische Pathologie* 55.6 (2004): 413-431.

27. Spencer LT and Bancroft JD. "Tissue processing in: Theory and Practice of Histological Techniques". 6th edition: Churchill Livingstone Elsevier limited (2008).

28. Saad B., *et al.* "Safety of traditional Arab herbal medicine". *Evidence-Based Complementary and Alternative Medicine* 3.4 (2006): 433-439.

29. Pour BM., *et al.* "Cytotoxicity and oral acute toxicity studies of *Lantana camara* leaf extract". *Molecules* 16.5 (2011): 3663-3674.

30. Barbosa HM., *et al.* "Acute Toxicity and Cytotoxicity Effect of Ethanolic Extract of *Spondias tuberosa* Arruda Bark: Hematological, Biochemical and Histopathological Evaluation". *Anais da Academia Brasileira de Ciências* (2016).

31. Jothy SL., *et al.* "Acute oral toxicity of methanolic seed extract of Cassia fistula in mice". *Molecules* 16.6 (2011): 5268-5282.

32. Sim KS., *et al.* "Acute oral toxicity of *Pereskia bleo* and *Pereskia grandifolia* in mice". *Pharmacognosy Magazine* 6.21 (2010): 67-70.

33. Raza M., *et al.* "Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice". *Scientia Pharmaceutica* 70.2 (2002): 135-145.

34. Gautam M and Goel R. "Toxicological study of *Ocimum sanctum* Linn leaves: hematological, biochemical, and histopathological studies". *Journal of Toxicology* (2014): 1-9.

35. Hussain T., *et al.* "Acute and subacute oral toxicity evaluation of *Tephrosia purpurea* extract in rodents". *Asian Pacific Journal of Tropical Disease* 2.2 (2012): 129-132.

36. Weingand K., *et al.* "Harmonization of animal clinical pathology testing in toxicity and safety studies". *Toxicological Sciences* 29.2 (1996): 198-201.

37. Paliwal A., *et al.* "Analysis of liver enzymes in albino rat under stress of λ-cyhalothrin and nuvan toxicity". *Biology and Medicine* 1.2 (2009): 70-73.

38. Thapa B and Walia A. "Liver function tests and their interpretation". *Indian Journal of Pediatrics* 74.7 (2007): 663-671.

39. Stark JL. "BUN/creatinine: your keys to kidney function". *Nursing* 10.5 (1980): 33-38.

40. Patel R., *et al.* "Acute and subacute oral toxicity evaluation of *Benincasa hispida* extract in rodents". *Journal of Applied Pharmaceutical Science* 2.8 (2012): 250-253.

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