ORIGINAL ARTICLE

AN EVALUATION OF ACUTE AND SUB-ACUTE TOXICITY OF METHANOLIC ROOT EXTRACT OF *MYRICASALICIFOLIA* A. RICH (MYRICACEAE) ON THE HISTOPATHOLOGY OF THE LIVER, THE KIDNEY AND SOME BLOOD PARAMETERS IN SWISS ALBINO MICE

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ABSTRACT

Background: *Myrica salicifolia is a medicinal plant used for the treatment of various human diseases, like malaria, inflammations, infections, diabetes mellitus and gastrointestinal spasm. The aim of this study was to evaluate the acute and sub-acute toxicity effects of methanolic roots extract of the M. salicifolia on the histopathology of the liver, the kidney and some blood and biochemical parameters in mice model.*

Methods: *Roots of M. salicifolia were collected* ***in October 2016*** *from Gondar area, northwest Ethiopia. The roots were dried and extracted with 80% methanol. Swiss albino female mice weighing 25-40 g and 8-12 weeks old, were randomly divided into four groups (one control and three experimental). The control group received 0.5 ml of distilled water orally, and the treatment groups were given the methanolic root extract of M. salicifolia using oral gavage at the doses of 100, 200 and 400 mg/kg body weight per day for four weeks. The hematological and biochemical analyses were examined after collecting blood samples from the mice. Liver and kidney were removed, stained and examined for histopathological effects. Following the application of standard procedures, the hematological, biochemical and histopathological features of the experimental groups were compared with the corresponding control group.*

Results*: No signs of toxicity and mortality were detected during the acute test evaluation at a dose of 2000 mg/kg, signifying that the oral LD_{50} of the extract was greater than 2000 mg/kg. The measured values of hematological parameters (red blood cells and mean corpuscular hemoglobin concentration) and biochemical parameters (except glucose) of all treated groups increased significantly ($p< 0.05$) compared to the control. Moreover, our histopathological finding hardly indicated any toxicity effect of the root extract in liver and kidney.*

Conclusion: *At the acute toxicity level of administration, the toxicity effect of the plant extract was not detected. Similarly, following the sub-acute test, no significant organ weight and histological changes were identified. Overall, it can be concluded that oral administration of roots of M. salicifolia extract is relatively safe to mice.*

Keywords*: Myrica salicifolia, acute toxicity, sub-acute toxicity, histopathology.*

INTRODUCTION

The trend of using herbal medicine in the treatment of diseases is increasing globally at an alarming rate. Especially in developing countries, the majority of people (80%) rely on traditional medicine as the main resource. Typically, a detailed part of the plant (roots, leaves, fruits, flowers, and seeds) is formulated into a suitable preparation, for example, compressed as tablets or made into pills, used to make infusions, extracts, tinctures, ointments, or creams (1, 2).

M. salicifolia (Myricaceae), known as *Shinet* in Amharic, is a shrub of one meter in height and can grow into a tree of up to 20 meters,. It is widely distributed in the flora of north, east and south Ethiopia. This plant fits in dry and moist agro climatic zones at an altitude ranging from 1,600–3,300 meters above sea level (3, 4).

In Ethiopian folk medicine, *M. salicifolia*is widely used for the treatment of various diseases. The roots

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and bark extracts are used to cure diseases, such as chest congestion, pneumonia, diarrhea, diabetes, hypertension, respiratory diseases, malaria, nervous disorder, respiratory disease, headaches, pain and inflammations(5, 6-9). The barks are chewed for toothache problems, whereas powdered young leaves are used to treat skin infections. On biological models, it was found to exhibit antidepressant, analgesic, antipyretic, antibacterial, antifungal, and anti-inflammatory activities (10-14).

In spite of its extensive traditional uses, to our best knowledge of the literature, only few toxicological investigations have been conducted to date. Hence, the aim of the present study was to identify the toxicological profile of the hydro methanolic root extracts of *M. salisifolia* after a single oral dose (acute toxicity) and 28 consecutive days administration (sub -acute toxicity evaluation).

MATERIALS AND METHODS

Plant material collection and extraction: The roots of *M. salicifolia* were 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 BT001) was deposited for further reference.

The roots were cleaned from any irrelevant materials, dried at room temperature under shadow and crushed to coarse powder. The powdered plant material (672 grams) was macerated in 80% methanol for 72 hours with occasional stirring. The filtrate was separated from the mark by filtration (Whatman No.1, England), and the mark was re-macerated three times. The filtrates were combined and concentrated in a rotary evaporator (Buchi Rota- vapor type R.205, Switzerland). The concentrated extract was further kept in an oven at a temperature not exceeding 40 ^{o}C. Removal of the solvent yielded brown powder (145 grams).

Experimental animals: Adult female mice aged 8-12 weeks and weighing 25-40 grams were obtained from the animal breeding house of the Ethiopian Public Health Research Institution (EPHI), Addis Ababa, Ethiopia. Prior to the commencement of the study, animals were allowed to acclimatize for one week. All experiments were conducted in accordance with the internationally accepted laboratory animal use, care and guideline. During the course of the study, the mice were allowed free access to standard pellets and water *ad libitum* (15, 16).

Acute toxicity test: Ten nulliparous and non-pregnant female mice were equally divided into treatment and control groups with five mice in each group as per the Organization for Economic Commission Development (OECD-425) guideline (16). The extract was administered orally at a dose of 2000 mg/kg and the control group was given 0.5 ml of distilled water. Following the administration procedure, the mice were observed continuously for one h, intermittently for 4 h, over a period of 24 h and for 14 days. Any gross behavioral changes such as loss of appetite, hair erection, laceration, salivation tremor, convulsion, diarrhea, mortality and other signs of toxicity manifestations were recorded and compared with the corresponding control groups.

Sub-acute toxicity study: Forty mice were randomly split into four groups (Group I, Group II, Group III and Group IV) with 10 mice each. Group I, II and III received 80% methanolic root extract of *M. salicifolia* at a dose of 100, 200 and 400 mg/kg, respectively while Group IV (control) received 0.5 ml of distilled water. The extract was administered orally using a standard gavage per day for 28 days (17).

Body and organ weight measurement: Body weight was recorded at the beginning of treatment and once a week thereafter. The weights recorded before the commencement of any experiment and after 12 hours of the last dose administration were considered as initial and final body weights, respectively. Since the liver is the primary organ for metabolism and the detoxification of toxic substances, and the kidney is the blood filtering and excretory organ where the end products of metabolism are removed out of the body, the weights of the liver and the kidney might indicate sub-acute toxicity in the body (16, 17).

Blood collection for hematological and biochemical analyses: Blood samples were collected by cardiac puncture just after the mice were deeply anesthetized. After collection, cervical dislocation was applied to reduce stress and suffering. About 1.5-2 ml of blood was then obtained by using a sterile needle fitted to a 3 ml syringe and directly poured into two test tubes (100 µl each). A test tube containing anti-coagulant ethylene diaminotetra acetic acid (EDTA) was used for the determination of the hematological parameters (WBC, RBC, HGB, HCT, MCH, MCHC and Platelets) using an Automated Hematology Analyzer (Symex- RX, 21, Japan). The other test tube without anti-coagulant was allowed to clot and sera was obtained by centrifuging the blood using an electrical centrifuge (HUMAX-K, HUMAN -Germany). Subsequently, blood chemistry (glucose, urea, creatinine, total protein, ALT, and AST) was conducted to test renal and hepatic functions. Values in the sera were analyzed using an Automated Clinical Chemistry Analyzer (AUTO LAB 18, clinical chemistry analyzer Italy) (18).

Animal dissection, tissue collection and histological processing: The abdominal cavities of the mice were opened by the vertical midline incision from the xiphoid process of sternum to the pubis area. The liver and the kidney were gently isolated; all extraneous tissues like fat were removed and immediately weighted by electronic balance; then, a 5 mm thickness of strips of tissues were randomly taken from the liver and coronary section of the kidney via renal pelvis. The tissue samples were transferred to the labeled test tubes containing 10% formalin in 0.1M of phosphate buffer, pH 7.4. After fixation, tissues were dehydrated in increasing concentrations of alcohol (absolute ethanol 99.7 % El Nasr Pharmaceutical Chemicals, Egypt), cleared with xylene (BDH Laboratory supplies Poole BH15 1TD, England), impregnated and embedded in paraffin wax (Paraffin wax m.pt. 58-60^{0}C, Dongnam petrochemical MFG. Co. Ltd, Korea). Each tissue block was sectioned on Zeiss Microtome (Carl Zeiss Zunch AG, West Germany) at 6- micrometer thickness and collected on to egg albumin coated microscopic slide. Subsequently, sections were deparraffinized, cleared and hydrated and stained with hematoxylin-eosin for 20 minutes. Stained tissues were then dehydrated and cleared in a reverse direction and mounted in pertex (medite GmbH, Wollenweberstrasse12, D-31303 Burgdorf, Germany) and cover slipped. Hematoxylin-eosin stained tissue slides of the liver and the kidney were examined for histopathologic effects of extract by using a light microscope (19, 20).

Statistical analysis: Data were analyzed using the statistical software package SPSS version 20. All values in the test were presented as means ± SEM. The one-way analysis of variance (ANOVA) followed by Tukey's HSD *post-hoc* test were used to compare results among and within groups. Paired *t-test* was also used to compare body weight changes between initial and final results. P-value less than 0.05 was considered as statistically significant.

Ethical consideration: The study was carried out after getting permission from the Ethical Review Board of the University of Gondar (O/V/P/ RCS/05/653/2015). Moreover, all experiments were conducted in accordance with the internationally

accepted laboratory animal use, care and guideline (15).

RESULTS

Acute toxicity and LD_{50} determination: In acute toxicity study, no sign of toxicity (no gross behavioral change) was seen in mice treated with the methanolic root extract of *M. salisifolia* at a dose of 2000 mg/kg. Besides, mortality was not observed at 2000 mg/kg, signifying that the oral LD_{50} was greater than 2000 mg/kg.

Effect of the *M. salicifolia* extract on body weight: As shown in Figure 1, during the four week sub-acute administration, weight change among the experimental groups showed fluctuations. In the first two weeks, the weight of the experimental groups dropped, and then a slight rise was recorded, though not statistically significant (P>0.05).

The sub-acute administrations of the methanolic root extract of *M. salicifolia* caused a slight weight reduction in the mice. The mean weight of each group except the vehicle treated group decreased on the 28th day. However, the reduction was not statistically significant (P>0.05) except in the mice treated with the highest dose (Table 1).

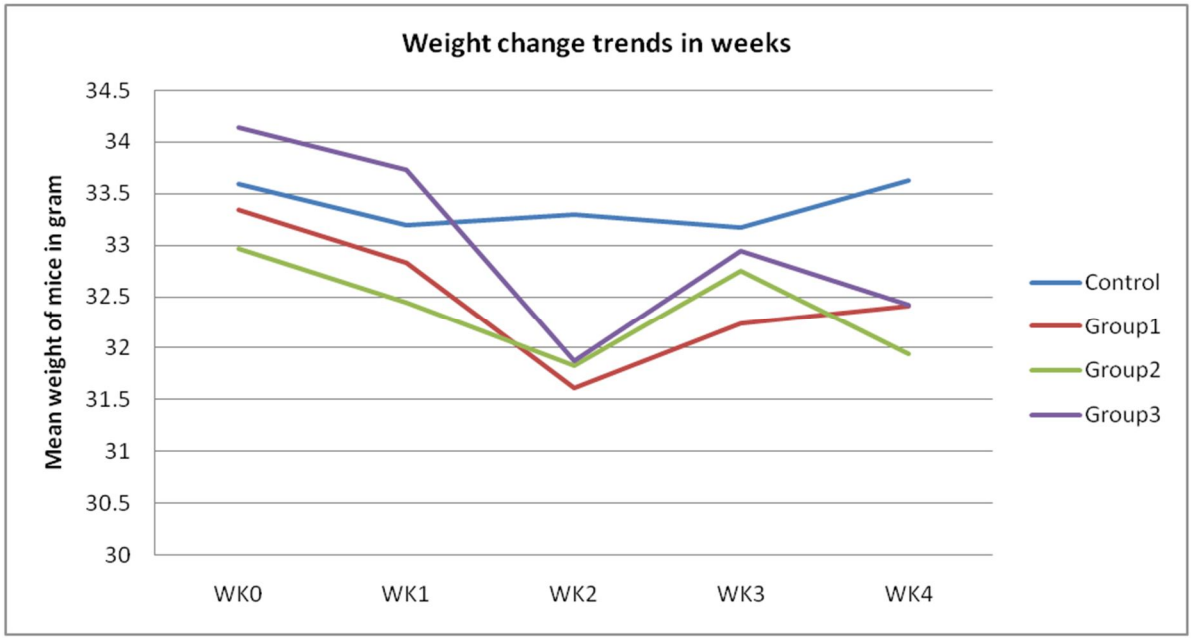

Figure 1: *Body weight of mice after sub-acute administration of the hydroalcoholic root extract of M. salicifolia. Wk0: week zero ; wk1: week one ; wk2: week two ; wk3 : week three ; wk4 : week four*

Table 1: Body weight of mice after sub-acute administration of the hydroalcoholic root extract of *M. salicifolia*

Drug/extract	Dose mg/kg/day	Weight D0 ±SEM	Weight D27 ±SEM	% change
Vehicle	0.5 ml	33.6±.54	33.6±.41	0
M. salisifolia	100	33.34±1.17	32.45±1.16	-2.6
M. salisifolia	200	32.45 ±1.17	31.94 ± 1.28	-1.8
M. salisifolia	400	34.14 ±.64	32.42 ± .81*	-5.0

**The mean weight pre-treatment is statistically significant (P<0.05) compared with post-treatment; data are expressed as means ± SEM for ten mice per group; Weight D0: Weight pre-treatment on day zero; Weight D27: weight post-treatment on 28th day*

The effect of *M. salicifolia* extract on liver and kidney weight: As shown in Table 2, there was hardly any statistically significant weight change in the liver and the kidney of the experimental mice compared to the corresponding control groups.

Effect of the *M. salicifolia* extract on hematological parameters: As shown in Table 3, the effects of sub-acute administration of 80% of methanolic root extract of *M. salicifolia* affected the hematological parameters of treated mice. The White Blood Cell (WBC) counts of the mice treated with 100 mg/kg of *M. salicifolia* extract decreased significantly. The Red Blood Cell (RBC) counts of the extract treated mice, on the other hand, significantly increased at the dose of 100 and 200 mg/kg. Moreover, the Mean Corpuscular Hemoglobin Concentration (MCHC) level declined significantly at all the tested doses when compared to their corresponding controls. Meanwhile, significant increases and decreases were noted in MCH and platelets (PLT) at high (400 mg/kg) doses, respectively.

Effect of the *M. salicifolia* extract on biochemical parameters: The effect of the extract on biochemical parameters was analyzed after sub-acute administration and compared to the control mice (Table 4). The result showed that Alanine aminotransferse (ALT) and protein levels significantly increased ($P<0.005$) and the level of urea significantly decreased ($P<0.005$) in the experimental groups compared to the control group. On the other hand, following the administration of 400 mg/kg of *M. salicifolia* extract, the level of creatinine significantly increased, while glucose level decreased.

Table 2: Mean weight of liver and kidney of mice after sub-acute administration of the hydroalcoholic root extract of *M. salicifolia.*

Dosage group	Mean weight organs in gram			
	Liver	p value	Kidney (single)	p value
Control	1.56± .06		0.19 ± .07	
100 mg/kg of *M. salicifolia*	1.81 ± .11	.165	0.21 ± .01	.444
200 mg/g of *M. salicifolia*	1.62 ± .06	.93	0.20 ± .03	.939
400 mg/kg of *M. salicifolia*	1.63 ± .09	.918	0.21 ± .08	.618

Data are expressed as means ± SEM for ten mice per group

Table 3: Hematological parameter change of mice after sub-acute administration of the hydroalcoholic root extract of *M. salicifolia.*

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg
WBC (K/µl)	4.42 ± 0.8	2.18 ± 0.18*	4.32± 0.6	4.41 ± 0.13
RBC(M/ µl)	6.05 ± 0.22	7.45 ± 0.23*	6.97±0.26*	5.76 ± .14
HGB(g/dl)	10.05 ± 0.23	10.65± 0.55	9.66± 0.52	9.13 ± .69
MCV(fl)	47.21 ± 0.53	49.22 ± 0.62	46.7± 0.61	45.22 ± .1.12
MCH (pg)	17.66 ± 0.31	16.56 ± 0.33	16.87± 0.43	31.86± 0.51*
MCHC (g/dl)	37.3 ± 0.38	32.96± 0.6*	33.2± 0.5*	31.86 ± 0.51*
PLT (K/µl)	732.96 ± 0.64	737.30± 0.38	731.86± 0.51	133.5 ± 0. 46*

**The mean value is significant (P<0.005) compared with the control; data are expressed as means ± SEM for ten mice per a group. WBC: White blood cell; RBC: Red blood cell; HGB: Hemoglobin; MCV: Mean cell volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PLT: platelets; µl: microlitre; K:10^3;M: 10^6; fl: femtolitre; pg: pictogram*

Table 4: Comparison of biochemical parameter results among 80% methanolic root extract of *M. salicifolia* treated groups and control

Blood parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg
AST(IU/L)	118..6 ± 3.7	172± 5.2*	233.7± 5.67*	324 ± 10.25*
ALT(IU/L)	111.1 ± 2.03	217.3± 15.4	264.3± 1.59*	302.6±14.47*
Urea (mg/dl)	40.3 ± 0.92	58.1 ± .52*	47.4± 0.89*	58.1± 2.71*
Bilirubin(mg/dl)	0	0	0.04 ± 0.01	0.09 ± 0.04*
Creatinine (mg/dl)	0.20 ± 0.22	0.23±0.01	0.26 ± 0.01*	0.28±0.01*
Protein (mg/dl)	5.63 ± 0.91	6.12±0.94*	6.79± 0.52*	6.86±0.61*
Glucose (mg/dl)	123.6 ± 3.55	115.3±1.37	101.2 ± 0.77	91.5 ± 2.18*

**The mean value is significant (P<0.005) compared with the control; data are expressed as means ± SEM for ten mice per a group. AST: Aspartate aminotransferase; ALT: Alanine aminotransferse; IU: international unit; mg: milligram; dl: deciliter; L: Liter*

Effect of the *M. salicifolia* extract on the histology of the liver: As shown in Fig 2, the histopathological effect of *M. salicifolia* extract on the liver tissue stained with hematoxylin-eosin was thoroughly investigated. Light microscopic examinations of the liver tissue of the control mice showed a characteristic feature of normal portal triad, radiating hepatic cells and hepatic sinusoids lined by endothelial cells. Mice treated with 80% methanolic root extract of *M. salicifolia* at all tested doses of the extract showed no histopathological changes compared to the control group (Figure: 2A-D)

Effect of the *M. salicifolia* extract on the histology of the kidney: The histopathological effect of hydroalcoholic root extract of *M. salicifolia* was investigated on the histological sections of the kidney stained with hematoxylin and eosin. Light microscopic examinations of the kidney on the control

mice showed a tissue with characteristic features showing normal Bowman's capsule lined with outer parietal layers of squamous capsular cells and inner visceral layer of podocyte cells, urinary space, proximal convoluted tubules, distal convoluted tubules, macula densa and the vascular pole. All treated mice showed no histopathological changes compared to the control group (Figure: 3A - D).

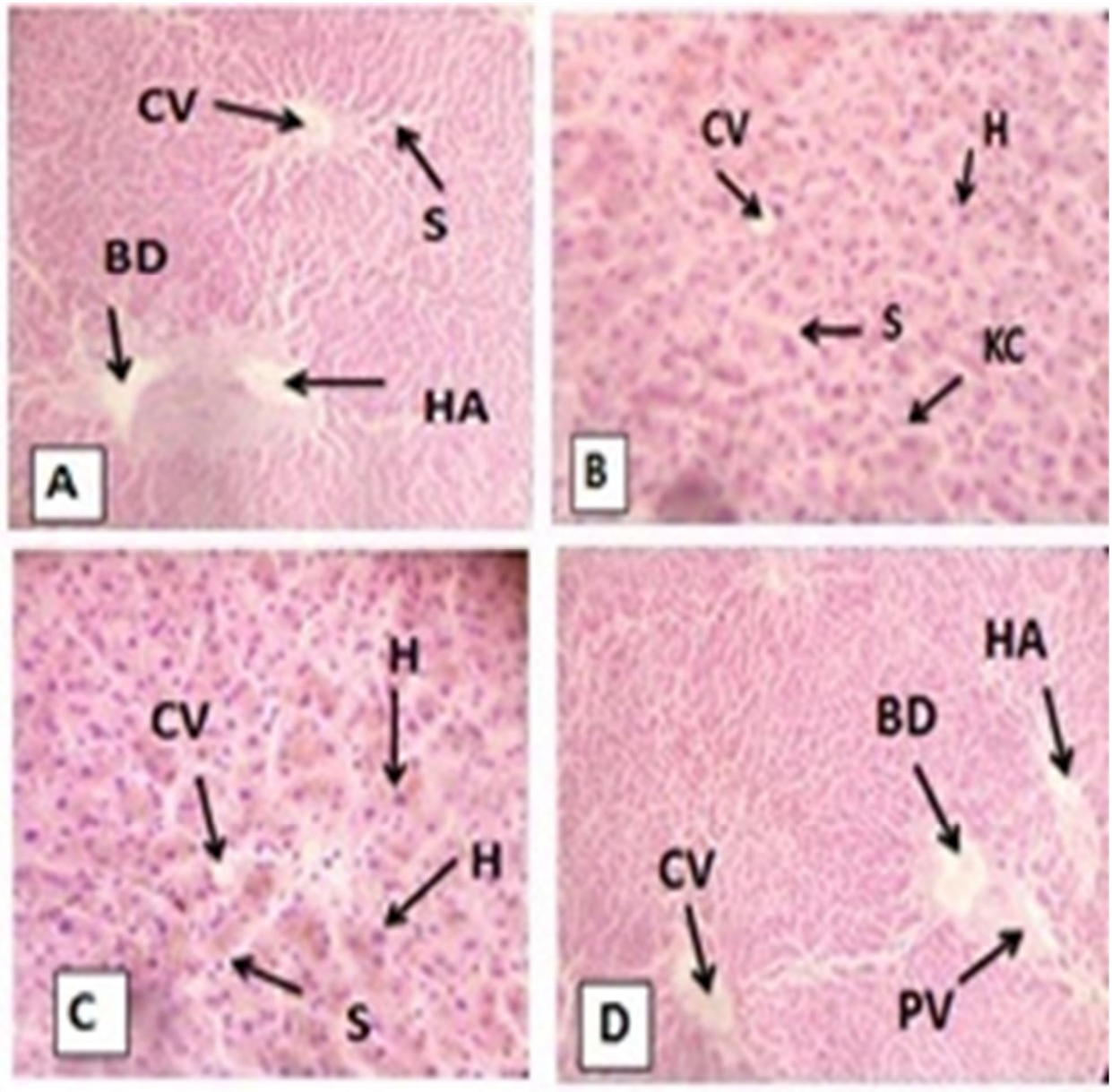

Figure 2: *Photomicrographs of the liver of control mice liver (A) (H and E, x10). (B) Mice treated with 100 mg/kg of M. salicifoliaroot extract. (H and E, x40). (C) Mice treated with 200 mg/kg of M. salicifoliaroot extract. (H and E, x40). (D) Mice treated with 400 mg/kg of M. salicifoliaroot extract (H and E, x10) CV= Central vein, H = Hepatocytes, E= Endothelial cells, S= Sinusoids, KC= Kupffer cells, BD = Bile Duct, PV = portal Vein, HA = Hepatic Artery*

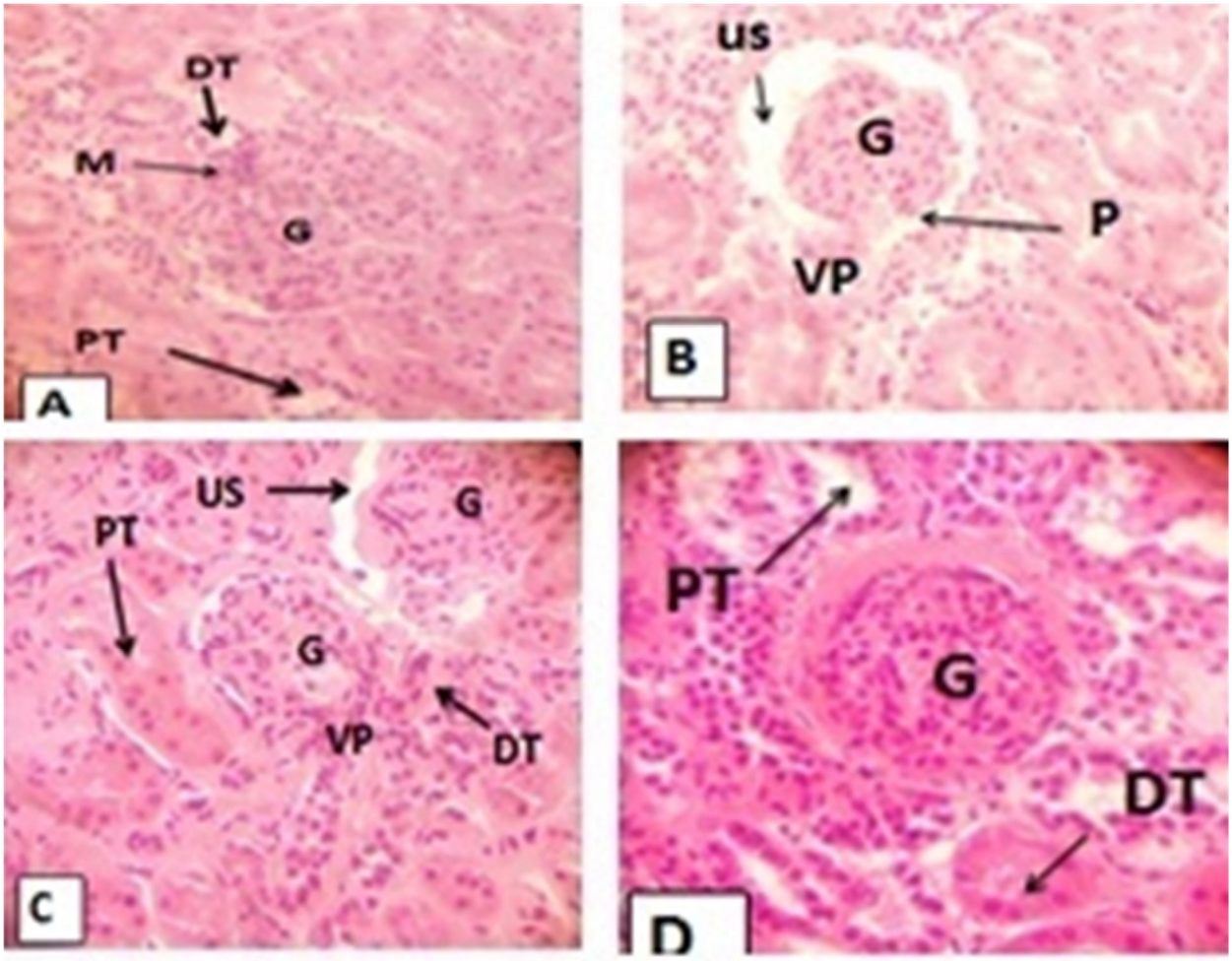

Figure 3: *Photomicrographs of the kidney of control mice (A) (H and E, x40). (B) Mice treated with 100 g/kg of M. salicifoliaroot extract (H and E, x40). (C) Mice treated with 200 mg/kg of M. salicifolia root extract (H and E, x40). (D) Mice treated with 400 mg/kg of M. Salicifolia root extract (H and E, x40). G = Glomerulus, p = Podocytes, US= Urinary space, PT= Proximal convoluted tubule, DT= Distal convoluted tubule, M= Maculadensa, VP= Vascular pole*

DISCUSSION

Globally, many plants, including *M. salicifolia*are commonly engaged by local traditional healers as single or combinations of medicinal plants to have synergic end products for a number of alignments. Toxicity studies are conducted to differentiate toxicity of substances, thereby ensuring their safety. In all conditions, toxic effects are usually manifested either in an acute/sub-acute or a chronic manner that occurs mostly as a result of the time of exposure to a toxic compound by oral ingestion, inhalation or absorption following skin contact (21, 22).

In the present study, a single 80%dose administration of methanolic root crude extract of *M. salicifolia* at 2000 mg/kg to female mice did not show any behavioral changes in all the 14 days of follow up. Therefore, it can be concluded that according to the OECD guidance 425, the extract may be assigned to be the lowest toxicity class 5 (LD_{50}> 2000 mg/kg)(16).

In the first two weeks, the body weights of extract treated groups highly declined, showing slight rises later on. Increases in the body weights of the mice may be allied with luggage compartments of fat more willingly than the toxicological effect of the plant extract (23). On the contrary, reduction in body

weight may be correlated with normal well-designed responses of animals to extracts that result in loss of appetite and low nutrient intake by experimental animals (24). Similarly, no significant weight change was observed in the liver and kidney of the treated mice compared to the control once. This finding is in line with that of a study done on the sub-chronic toxicity of butanolic leaf extract of *Moringa stenopetala* in rats (25). This shows that the plant extract might have caused a non-significant change in their food intake and the utilization of food. Thus, the absence of significant differences in the weight of major detoxifying organs (liver and kidney) provides support to the safety of the plant extract under investigation.

In this study, hematological parameters such as RBC, WBC, MCH, MCHC and platelets were investigated. RBC, MCH and MCHC were significantly different from the control group suggesting that the long-term use of the crude methanolic root extract of *M. salicifolia*has an effect on the rate of production of RBC, implying that there might be a change in the oxygen carrying capacity of the blood and the amount of oxygen delivered to the tissue following the administration (26). The total count of WBC decreased significantly at the lowest dose (100 mg/kg) of the treated group. The notion of our finding was supported by that of a study conducted on the effect of hydromethanolic leaf extract of *Grewiacrenata*(27). The reduction of WBC count may be due to a decline in the immune systems of the animals. Besides, the total count of platelets significantly decreased at the upper dose (400 mg/kg) compared with the vehicle administered group. This finding was similar with that of a research done on the sub-chronic evaluation of hydro -methanolic seed extract of *Coriandrumsativum* in mice that showed a decrease in the platelet counts of treated groups (28). The decrease in platelet count may be due to the ability of the plant extract to interrupt platelet cells production processes.

Meanwhile, the level of AST significantly increased in mice treated with crude methanolic extracts of *M. salicifolia* compared to the corresponding control group. However, a significant elevation of the AST enzyme in serum may not imply liver tissue damage because it is not a specific marker of liver function, rather it may also be found in other vital organs such as the heart, the brain and muscle tissues. However, the level of ALT is a specific marker of hepatocytes action (29). The elevation of the enzymatic values of AST and ALT in the liver of mice might be because of the stabilizing effect of the plant extract on the cell membranes that makes it more conductive to facilitate the action of enzymes and also may be due to the response to the cellular system to offset the stress initiated bythe administration of *M. salicifolia* extract (30). The serum concentration of creatinine and urea in mice treated with methanolic extract of *M. salicifolia* also increased significantly. This may be due to renal function impairment which might result from decreased blood perfusion to the kidney and deranged metabolic processes caused by the extract (31, 32). The concentration of protein in all experimental groups significantly increased. This rise might indicate that the extract has nutritional values for mice. Our finding was supported by the study done on *Moringaoliefera* Lam (Moringaceae) as a food plant with medicinal uses (33). On the other hand, at a higher concentration (400 mg/kg), the serum concentration of glucose in mice treated with methanolic root extract of *M. salicifolia* significantly decreased. The decrease in glucose concentration may suggest ingredients that are found in *M. salicifolia* presumably have hypoglycemic effects. Similarly, a study done on hypoglycemic and hypotensive effect of *Psidiumguajava* Linn (Myrtaceae) leaf aqueous extract on rats reported a significant decrease of glucose concentration, following a high dose administration (34).

A histological examination of liver and kidney tissues of mice treated with 80% of methanolic root extract of *M. salicifolia* did not show any morphological changes in the liver tissue in treated mice compared to their corresponding controls. In contrast, a study conducted on the sub-acute toxicity of the leaves of methanol extracts of *Rhaphidophora decursiva* at a dose of 210 mg/kg showed the presence of an inflammation around the portal area of the liver section in the treated rats (35). This difference might be due to differences in the chemical constituents of plant species and animal types used.

The histological examination of the kidney sections of the treated mice at all tested doses revealed no histological changes compare with the corresponding control group. In contrast, a study done on the acute toxicity of the ethanolic extract of *Enantiachlorantha* stem bark in albino rats showed cortical congestion at a dose of 1000 mg/kg (36). Iilmie and collaborators reported that oral administration of *Mitragnaspeciosa* methanolic leaf extracts at a dose of 200 mg/kg and 500 mg/kg insulated renal tubular degeneration (37). These differences might be due to the duration of administration, dose variation, differences in ingredients of the plant species and animal strains and types. Therefore, histological changes, like tissue necrosis and inflammatory cells infiltration were not observed in the current study.

CONCLUSION

In this study, the acute and sub-acute toxicity of hydroalcoholic root extract of *M. salicifolia* was examined. No sign of toxicity was observed during the acute toxicity study follow-up. Moreover, during the sub-acute oral administration, no significant organ weight changes as well as histological changes were observed at all tested doses. However, a significant change was observed in most of the hematological and biochemical parameters. In conclusion, our study has shown that the plant extract is relatively safe for mice. A further toxicological study such as chronic and sub-chronic evaluation is suggested.

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

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REFERENCES

1. Saad B, Azaizeh H, Said O. Tradition and perspectives of Arab herbal medicine: a review. Evid.Based Complement Alternat Med. 2005;2 (4):475-9.
2. Azaizeh H, Saad B, Cooper E, Said O. Traditional Arabic and Islamic medicine, a re-emerging health aid. Evid.Based Complement Alternat Med.2010;7(4):419-24.
3. Tesema AB, Ann B, Bo T. Useful trees and

shrubs for Ethiopia: identification, propagation and management for Agricultural and Pastoral Communities. 1993. Available from: www.agris .fao.org. Accessed on December, 2017.

4. Negash L. Indigenous trees of Ethiopia: biology, uses and propagation techniques. Addis Ababa: Department of Biology, Addis Ababa University.1995; 285. Available from: www.aau.edu.et. Accessed on July, 2017.
5. Muthaura CN, Rukunga GM, Chhabra SC, Mungai GM, Njagi ENM. Traditional phytotherapy of some remedies used in treatment of malaria in Meru district of Kenya. S. Afr J.Bot. 2007;73(3):402-11.
6. Kirira PG, GMR, Wanyonyi AW, Muregi FM, Gathirwab JW, Muthaurab CN, Tolob F, G.M. Mungaid GM, Ndiegea IO. Anti-plasmodial activity and toxicity of extracts of plants used in traditional malaria therapy in Meru and Kilifi Districts of Kenya. J Ethnopharmacol 2006; 106 (3): 403-7.
7. Lulekal E, Kelbessa E, Bekele T, Yineger H. An ethnobotanical study of medicinal plants in Mana Angetu district, southeastern Ethiopia. J Ethnobiol Ethnomed. 2008 Apr 28;4:10. PubMed PMID: 18442379. Pubmed Central PMCID: 2391147.
8. Ermias Lulekal ZA, Ensermu Kelbessa and Patrick Van Damme. Ethnoveterinary plants of Ankober District, North Shewa Zone, Amhara Region, Ethiopia. J Ethnobiol Ethnomed. 2014, 10:21. 2014;00(21):1-19.
9. Giday M, Asfaw Z, Woldu Z. Medicinal plants of the Meinit ethnic group of Ethiopia: an ethnobotanical study. J Ethnopharmacol. 2009;124 (3):513-21.
10. Kariuki DK, JOM, J. Mugweru, L. O. Kerubo. Antibacterial activity of five medicinal plant extracts used by the Maasai people of Kenya. IJHAMS. 2014;2(7):1-6.
11. N. MT, Macharia D, O AJ. Antimicrobial activities of selected medicinal plants of the Tugens of Koibatek, district, Kenya. Int J. Bioassays. 2016;5(07):4720.
12. Kilonzo M, MC, Patrick A. Ndakidemi. A review on antimicrobial efficacy, toxicity and phytochemicals of selected medicinal plants used in Africa against pathogenic microorganisms. IJB. 2017;10(3):232-50.
13. Njung'e KM, G.; Mwangi, J.W.; Kuria, K.A.M. Analgesic and antipyretic effects of *Myrica salicifolia*(Myricaceae). Phytother Res. 2002;16 (1):73-4.
14. Aberra Geyid et al. Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profile. J Ethnopharmacol 2005; 97(2005):421-7.
15. C Clark JD, Gebhart GF, Gonder JC, Keeling ME, Kohn DF. The 1996 Guide for the Care and Use of Laboratory Animals. ILARJ.1997; 38 (1):41-8.
16. OECD. Guidelines for the testing of chemicals. Test number 425: Acute toxicty test up and down procedure. OECD. 2008. 1-27. Available from: www.oecd.org. Accessed on November 2016.
17. OECD. Guidelines for the testing of chemicals. Test number 407: repeated dosees for 28 days oral toxicity study in rodents .OECD. 2008: 1-8. Available from: www.oecd.org. Accessed on November 2016.
18. Wirth DE, Karaszewska TS, Pyśniak MG. Selected blood serum biochemical indicators in twelve inbred strains of laboratory mice. ANIM SCI PAP REP. 2009; 27(2):159-67.
19. Wai Chin Chong RW, Yongwei TU. A study on tissue processing. IJIIR. 2012; (3):32-40.
20. Abdelmonem Hegazy, Hegazy R. hegazy's Simplified Method of Tissue Processing. AIMDR.

2015;1(2):57-61.

21. Lulekal E, Kelbessa E, Bekele T, Yineger H. An ethnobotanical study of medicinal plants in Mana Angetu District, southeastern Ethiopia. J Ethnobiol Ethnomed. 2008 ;4.
22. Tmbrell JA, Delaney J, Waterfield CJ. Correlation between i*n vivo* and *in vitro* toxic effects of foreign compounds. Comp. Haematol. Int. 1996;6(4):232-6.
23. Harizal SN, Mansor SM, Hasnan J, Tharakan JKJ, Abdullah J. Acute toxicity study of the standardized methanolic extract of Mitragyna speciosa Korth in rodent. J Ethnopharmacol. 2010;131(2):404-9.
24. Rouani H, El-Hilaly J, Israili ZH, Lyoussi B. Acute and sub-chronic toxicity of an aqueous extract of the leaves of *Herniaria glabra* in rodents. J Ethnopharmacol. 2008;118(3):378-86.
25. Musa AH, Gebru G, Mekonnen Y, Debella A, Mekonnen E. Subchronic toxicity studies of butanol fraction of leaves of *Moringa stenopetala* in experimental rats. IJCMPR. 2015;1(10):170-5.
26. Adedapo AA, Abatan MO, Olorunsogo OO. Toxic effects of some plants in the genus *Euphorbia* on haematological and biochemical parameters of rats. Vet. arhiv. 2004;74(1):53-62.
27. Ukwuani AN, Abubakar MG, Hassan SW, Agaie BM. Toxicological studies of hydromethanolic leaves extract of *Grewia crenata.* IJPSDR. 2012;4(4):245-9.
28. Patel D, Devkar R. Acute and sub-chronic toxicological evaluation of hydro-methanolic extract of *Coriandrum sativum* L. seeds. EXCLl Journal. 2012;11:566-75.
29. Gboney PT. Mildly elevated liver transaminase Levels in the Asymptomatic Patient. Am Fam Physician. 2005;71(6):1105-10.
30. Oyewole OI, Malomo SO. Toxicological assessment of oral administration of some antisickling agents in rats. AIBR. 2009;3(2):024-8.
31. Greger R. Loop diuretics. In: Greger R, Knauf H, Mutschler E, eds. Handbook of Experimental Pharmacology; Diuretics. New York: Springer. 1995:PP 221-74.
32. Cameron JS, Greger R. Renal function and testing of function. Oxford textbook of clinical nephrology. 1998; 1(3): 39-69.
33. Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: a food plant with multiple medicinal uses. Phytother res. 2007; 21(1): 17-25.
34. Ojewole JA. Hypoglycemic and hypotensive effects of *Psidium guajava* Linn.(Myrtaceae) leaf aqueous extract. Methods Find Exp Clin pharmacol. 2005;27(10):689-96.
35. Arsad SS, Esa NM, Hamzah H. Histopathologic changes in liver and kidney tissues from male Sprague Dawley rats treated with *Rhaphidophora decursiva* (Roxb.) schott extract. J Cytol Histol. 2014; (S4): http// dx.doi.org/10.4172/2157-7099.S4-001.
36. Adebiyi OE, Abatan MO. Phytochemical and acute toxicity of ethanolic extract of Enantia chlorantha (oliv) stem bark in albino rats. Interdiscip Toxicol. 2013 Sep;6(3):145-51. PubMed PMID: 24678252. Pubmed Central PMCID: 3967441.
37. Iilmie MU, Jaafar H, Mansor SM, Abdullah JM. Subchronic toxicity study of standardized methanolic extract of *Mitragyna speciosa* Korth in Sprague-dawley rats. Front. Neurosci. 2015; 9: 189.