

Methanolic effect of *Clerodendrum myricoides* root extract on blood, liver and kidney tissues of mice

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Abstract

The present study deals with the toxicological investigations of chronic treatment with methanol root extract of *Clerodendrum myricoides* on body weight, hematological and biochemical parameters, and liver and kidney tissue sections. Mice treated with 100mg/kg bw/day of methanol extract showed no behavioral changes. However, there was a general reduction of activity in mice treated with 400mg/kg bw/day methanol extract and LD₅₀ treated mice showed hypoactivity, grooming, prostration, piloerection and irritation during administration towards the last days of the treatment period. The body weight gain difference in the 100mg/kg bw/day methanol extract treated group was not significant, while those of the others were significant as compared with the controls. Hematological results for the RBC count, HCT, MCV, MCH and MCHC in methanol extract treated mice showed no significant changes at both doses of treatments as compared with the controls. However, the value of lymphocytes was found significantly increased at 100 and 400mg/kg bw/day methanol extract. Similarly, HGB was significantly increased at 100 and 400mg/kg bw/day of methanol extract treated groups. On the other hand, WBC and platelets count were significantly decreased after treatment with 400mg/kg bw/day methanol extract. ALT, ALP, AST and urea values were significantly increased respectively at 100mg/kg bw/day and 400mg/kg bw/day methanol extract. Several histopathological changes of liver and kidney were observed in the extract treated mice as compared to the controls. Such histopathological changes observed in both liver and kidneys were inflammations and hydropic degenerations of hepatocytes at both doses of methanol. In addition, in the LD₅₀ treated mice of the extracts there were also hemorrhages and signs in congestion of glomeruli of the kidney.

Conclusion: chronic treatment with *Clerodendrum myricoides* extracts in mice causes reduction in body weight gain, damage to liver & kidney and changes in some hematological & biochemical parameters. It is therefore, suggested that further studies are needed for minimization of the observed side effects, while maintaining the claimed medicinal values of the extract.

Key words: *Clerodendrum myricoides*, root extract, toxicity, mice.

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Introduction

The majority of traditional medicines used in the developing countries have not been evaluated for quality, safety, and efficacy although there are some remarkable claims made for their effectiveness. Some medicinal herbs contain toxic compounds which may cause liver toxicity and cancer; others may cause adverse reactions including allergic, cardiac and irritant effects. Herbal medicines should therefore, need to be assessed for their efficacy and safety. More

than two thirds of the world's plant species, at least 35 000 of which have medicinal value, originate in the developing countries¹. In most developing countries traditional healers, either males or females, are particularly knowledgeable about the recognition and treatment of common diseases. Traditional healers are also skilled traditional botanists and have a great talent for locating the requisite plant from the green vastness that makes up their natural pharmacy. In Latin America and Africa including Ethiopia, the knowledge of traditional medicine has largely remained undocumented and is handed down orally from father to son or mother to daughter². One of the traditional herbs used in different parts of Ethiopia is *Clerodendrum myricoides*. Roots and leaves of *Clerodendrum myricoides* are also used to treat gonorrhoea, rabies, measles, glandular TB, colic, eye disease, malaria, swellings, in the body, wound

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dressings, asthma and as aphrodisiac^{3,4}. This plant is also used for the treatment of pneumonia, dry cough, mental disorder, general malaise (mich), toothache, headache, Qilensa and diuretic^{5,6}.

Methods

Preparation of methanol extract of *Clerodendrum myricoides*

After the roots of the plant were collected from Oromia region Bale, which is 550km away from Addis Ababa Ethiopia, it was rinsed with water to remove dust particles, dried at room temperature, crushed to powder at the Drug Research department of Ethiopian Health and Nutrition Research Institute (EHNRI) and 100g of dry powder was obtained. The dried powder was soaked in 80% methanol and filtered through Whatman filter paper (0.7µm size). It was concentrated in vacuum rotary evaporator at 40°C to remove the methanol and then placed in a water bath to remove water. At the end of these procedures, 4.7g of yield was obtained.

Experimental Animals

The experimental animals used in this study were 40 Swiss albino mice of both sexes, each weighing 25-30g and aged 8-10 weeks. The mice were randomly distributed into four groups (group I, II, III & IV) each with 10 mice (five male and five female) per cage. All mice were maintained on a 12h light/dark cycle, at constant temperature (21°C) and humidity with free access to water and food. They were all acclimatized prior to drug administration.

Group I and II were given 0.5ml/mouse of the Methanolic extract at a dose of 100mg/kg body wt /day and 400mg/kg body wt /day, respectively for 44 consecutive days. The time table for the chronic treatment was according to⁷. Group III was given 0.5ml/mouse of the Methanolic root extract at the dose of 1630 mg/kg body wt /day which was its LD₅₀ as determined by Belay⁸. Group IV were administered 0.5ml/mouse of distilled water and considered as control. In all cases, administrations of the extracts or distilled water were carried out using intragastric catheter. All the doses used for the experimental groups were those found to be effective against plasmodium berghie⁸. Body weight of each animal was recorded just before the first day and after the last day of administration of the extract⁹. Body weight taken on the first day of oral administration was considered initial weight and the weight taken on the last day of administration was considered final weight.

After 24 hours of the last day of extract administration the animals in Groups I, II and IV were sacrificed using diethyl ether inhalation in desiccators Jar. Blood for each animal was immediately withdrawn by cardiac puncture into test tubes with and without EDTA. Blood samples from EDTA containing test tubes were immediately analysed for some hematological parameters. Blood samples from test tubes with no EDTA were allowed to clot and sera were obtained by centrifuging at 5000 revolution per minute for five minutes¹⁰. The sera were used to analyse liver and kidney function tests. Following blood samples collections the right part of the liver and the right kidney were dissected out from mice in Groups I, II and IV. The selection to use the right part of the liver and the right kidney was random. As the mice in Group III died within 24 hours, only the right part of the liver and the right kidney were collected.

Tissue processing

Pieces of tissue samples from the right lobe of liver and the right kidney were immersed in 10% buffered neutral formalin (pH=7.0) overnight at room temperature. After overnight fixation, the tissue samples were washed for 6-8 hours in tap water then after, the tissue samples were dehydrated with graded series of alcohol: one hour each in 70% alcohol, 80% alcohol, 95% alcohol and absolute alcohol-I, and two hours in absolute alcohol-II. The tissues were cleared with two changes of xylene, one hour each. The tissues were then infiltrated with two changes of paraffin wax, for one and half hour each. Upon completion of infiltration, the tissues were embedded in the paraffin wax. All tissue blocks were labeled and placed in refrigerator until sectioned.

Tissue blocks were sectioned with a thickness of 5µm using Leica rotary microtome. After the sections were appropriately spread on a water bath, they were mounted on slides coated with egg albumin to maximize surface adhesion. 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 then cooled dried and stained with routine Hematoxylin and Eosin staining method.

Results

Effects of Methanol root extract of *Clerodendrum myricoides* on:

The behavioral changes of the mice

In the mice that received 100mg/kg body weight/day methanol root extract no noticeable sign of

behavioral change was observed, while in those that were treated with 400mg/kg body weight/day methanol extract there were general reduction in locomotion towards the last week of administration of the drug. On the other hand, mice that received a single dose administration of 1630mg/kg body weight/day methanol extract showed some adverse effects such as reduction of motor activity, sedation, difficulty in breathing and piloerection which were followed by death of the animals after some hours.

The body weight of mice

As shown in table 2, the relative weight gain in mice treated with the methanol extract was lower as compared with the controls. However, such lower body weight gain in mice treated at a dose of 100mg/kg body weight/day (with a gain of 4.08gm) was not significantly different from those of the control group (with a gain of 6.82gm), while this was significant ($p < 0.05$) in the mice treated with a dose of 400mg/kg body weight/day methanol extract (with a gain of 1.18gm).

Table 1: Comparison of body weight gain among methanol root extract of *Clerodendrum myricoides* treated groups, at doses of 100mg/kg body weight/day, 400mg/kg body weight/day, and control mice

Group	Initial weight (in g)	Final weight (in g)	Weight difference (final weight-initial weight) (in g)	% of body weight gain
Control(DW)	26.43±0.86	33.25± 3.26	6.82 ± 3.60	25.8
100mg/kg/day (Methanol)	27.92± 1.53	32.00 ± 3.25	4.08 ± 4.54	14.6
400mg/kg/day (Methanol)	28.53± 1.19	29.74 ± 1.61	1.18 ± 1.35*	4.13

Values are mean ± SEM. $P < 0.05$, N= 10/group

Hematological parameters

As shown in table 3, different values were obtained for the various hematological parameters analyzed in the methanol root extract treated mice as compared with the control mice. Such differences were, however, found to be not statistically significant for RBC count, HCT, MCV, MCH and MCHC.

On the other hand, values for each of HGB and lymphocytes significantly increased at both doses of the methanol extract treated groups. The increments, as compared to the controls, in mice treated with 100 and 400mg/kg body weight/day were 25.63 and 20.63% respectively for the HGB, and 40.55 and 40.19% for the lymphocytes.

WBC count and platelets counts were not statistically different in 100mg/kg body weight/day methanol extract treated mice as compared to the controls. On the other hand, these were significantly decreased at the 400mg/kg body weight/day treated by 48.41 and 28.66% respectively for WBC and platelets. Most of the haematological parameters were decreased at the dose of 400mg/kg body weight/day methanol root extract more than those treated at 100mg/kg body weight/day.

Biochemical parameters

As can be observed from table 4, all the four biochemical parameters studied (AST, ALT, ALP and urea) were found increased in the mice treated with methanol root extract at both doses as compared to those of the control mice. Although the increase of AST at 100mg/kg body weight/day was not statistically significant, the increases in all the others were statistically significant ($p < 0.05$). Moreover, the increases in all the biochemical parameters in the mice treated at the dose of 400mg/kg body weight/day methanol root extract were more than those treated at 100mg/kg body weight/day.

The significant increment ($p < 0.05$) of ALT, ALP and urea in the mice treated with the 100mg/kg body weight/day of the methanol extract were, respectively by 282.97%, 59.25% and 331.16% as compared to the controls. Similarly, the significant increment ($p < 0.05$) of AST, ALT, ALP and urea in the mice treated with the methanol extract at a dose of 400mg/kg body weight/day were, respectively 141.96%, 444.68%, 171.96% and 474.03%.

Table 2: Comparison of hematological results among methanol root extract of *Clerodendrum myricoides* treated groups, at doses of 100mg/kg body weight/day, 400mg/kg body weight/day, and control mice.

Haematological parameters	Control (DW)	100 mg/kg bwt/day (Methanol)	% of mean diff.	400 mg/kg bwt/day (Methanol)	% of mean diff.
RBC (M/UL)	5.89 ± 0.49	5.77 ± 0.91	-2.04	5.51 ± 0.64	-6.45
WBC (K/UL)	6.92 ± 1.44	6.02 ± 2.07	-13.00	3.57 ± 0.95*	-48.41
Platelets (K/UL)	343.30±2.06	348.50±115.48	1.51	244.90±101.75*	-28.66
HGB (g/dl)	11.00 ± 0.71	13.82 ± 2.41*	25.63	13.27 ± 2.42*	20.63
HCT (%)	42.05 ± 5.40	41.07 ± 3.68	-2.33	39.83 ± 8.56	-5.28
MCV (fl)	85.32 ± 3.00	86.88 ± 4.68	1.82	82.06 ± 14.74	-3.82
MCH (pg)	28.78 ± 1.25	30.31 ± 4.19	5.31	26.86 ± 6.09	-6.67
MCHC (g/dL)	34.60 ± 2.12	34.32 ± 1.74	-0.81	34.79 ± 1.81	0.55
Lymphocyte (%)	47.24 ± 5.99	66.40± 17.77*	40.55	66.23 ± 10.92*	40.19

Values are in mean ± SEM. p* < 0.05, n = 10/group.

Table 3: Comparison of biochemical results among methanol root extract of *Clerodendrum myricoides* treated groups, at doses of 100mg/kg body weight/day, 400mg/kg body weight/day, and control group

Biochemical parameters	Control (DW)	100mg/kg body weight/day (Methanol)	% of mean difference	400mg/kg body weight/day (Methanol)	% of mean difference
AST (IU/L)	25.40± 5.21	41.90± 33.158	64.96	61.46± 46.14*	141.96
ALT (IU/L)	9.40± 3.60	36.00± 24.17*	282.97	51.20± 28.38*	444.68
ALP (IU/L)	107.00± 32.34	170.40± 55.39*	59.25	291.00± 86.08*	171.96
Urea (mg/dL)	15.40± 4.81	66.40± 42.53*	331.16	88.40± 39.93*	474.03

Values are in mean ± SEM. p* < 0.05, n = 10/group.

Histopathology of Liver

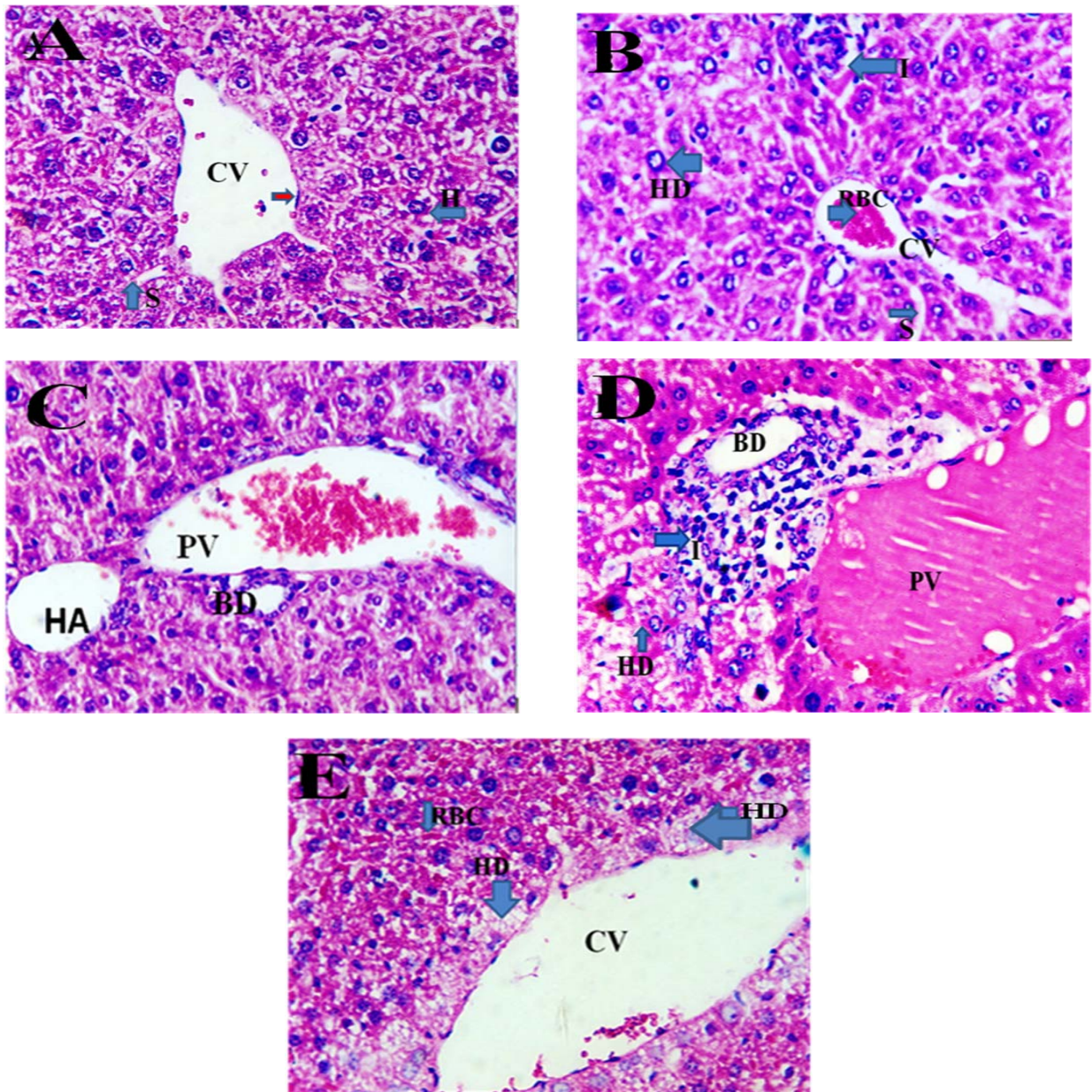
Histopathological examinations of the liver sections under the light microscope revealed that there were several changes in the mice treated with all doses of the methanol root extract as compared with the control group. There were focal inflammations and cytoplasmic vacuolations (hydropic degenerations) of the hepatocytes located in the periphery of liver lobules in the mice treated with 100mg/kg body weight/day methanol root extract (figure 3 B).

In the mice treated with 400mg/kg body weight/day methanol root extract there were remarkable cellular infiltrations in the hepatocytes found around portal triads and hydropic degenerations of the hepatocytes located in the periphery of liver lobules (figure 3 D).

In the liver sections from the mice that received 1630mg/kg body weight/day of the methanol root extract hepatocytes found around the central veins showed severe hydropic degenerations

in which their nuclei were stained pale and pushed to the periphery (figure 3 E).

On the other hand, the normal lobular architecture of the liver were not affected. In the liver sections of the mice from the control group no inflammations and cytoplasmic vacuolations was observed. The lobular architecture and the portal triads were also normal (figure 3 A and C).



Magnifications, all 40×

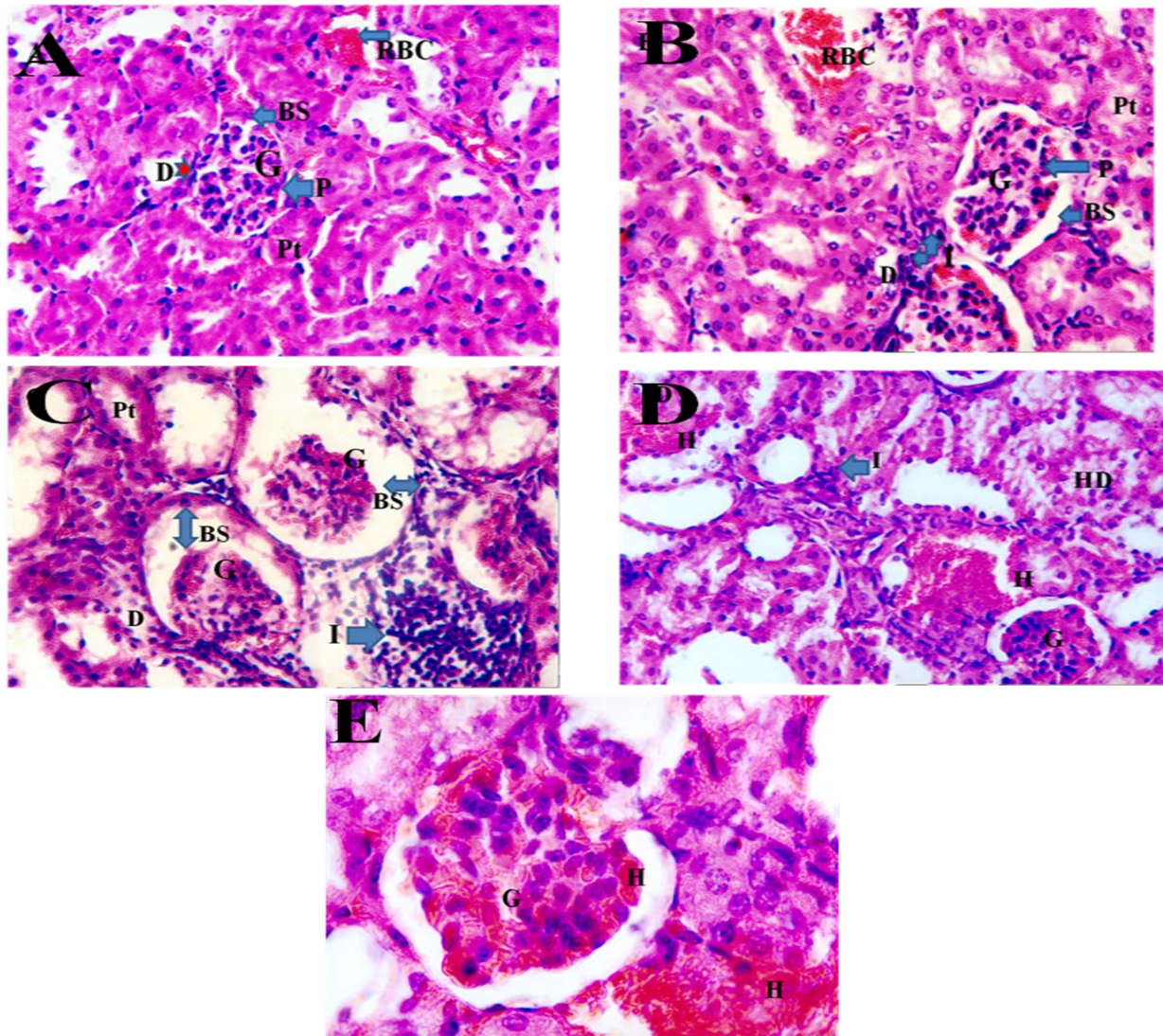
Figure1: Photomicrographs of H and E stained liver sections from control mice, in the regions the central vein (A) & the portal triads (C) as compared with those from mice treated with the methanol root extracts of *Clerodendrum myricoides* at doses of 100mg/kg body weight/day (B), 400mg/kg body weight/day (D) and 1630mg/kg body weight/day (E). While there was no histopathological changes visible in the sections of the control mice (A and C), note the following changes in the sections from the treated mice: inflammations (I) and vacuolar degenerations of hepatocytes (HD) in mice treated at 100mg/kg body weight/day (B); inflammations around bile duct and portal vein (I) and vacuolar degenerations (HD) in mice treated at 400mg/kg body weight/day (D); and severe hydropic degenerations of hepatocytes around central vein (HD) in the mice treated at 1630mg/kg body weight/day methanol root extract E. Red arrow in A indicates endothelial cells. BD in C & D= bile duct, CV in A, B & E= central vein, H in A=hepatocytes, HA in C= hepatic artery, HD in B, D & E= hydropic degenerations, I in B & D= inflammatory cells, PV in C & D= portal vein, RBC in B= red blood cells, S in A & B= hepatic sinusoids.

Histopathology of kidney

Histopathological examinations of the kidney sections using light microscope showed various alterations in the methanol extract treated groups as compared with the control mice. Kidney sections from the mice treated with 100mg/kg body weight/day methanol extract showed cellular infiltrations around distal convoluted tubules and endothelial cells of glomeruli of the kidney (figure 4 B).

Similarly, in the mice treated with the methanol root extract at a dose of 400mg/kg body weight/day

there were cellular infiltrations in the interstitium and in the epithelial cells of Bowman's capsule (figure 4C). In addition, the glomeruli were atrophied and congested. The kidney sections from the mice that received 1630mg/kg body weight/day of the methanol root extract showed haemorrhages, hydropic degenerations of tubules, focal inflammations and signs in congestion of the glomeruli (figure 4 D & E). None of the histopathological changes were described above observed in the kidney sections from the control mice (figure 4 A).



Magnifications, A — D = 40×, E = ×1000

Figure 2: Photomicrographs of H and E stained kidney sections from the control mice (A) as compared with those from mice treated with the methanol root extract of *Clerodendrum myricoides* at doses of 100mg/ kg body weight (B), 400mg/kg body weight/day (C) and 1630mg/kg body weight/day (D and E). While there was no histopathological changes visible in the sections of the control mice (A), observe the following changes in the sections from the treated mice: focal inflammations (I) in mice treated at 100mg/kg body weight/day (B); inflammations around Bowman's capsule (I) and slight shrinkage of glomeruli in mice treated at 400mg/kg body weight/day (C); haemorrhage (H) and signs in congestion of the glomeruli of the kidney in mice treated with 1630mg/kg body weight/day (D and E). Red arrow in A indicates macula densa. BS in B & C= Bowman's space, D in A, B & C= Distal convoluted tubules, G in all micrographs= Glomeruli, H in D & E= Haemorrhage, HD in D= hydropic degenerations, I in B, C & D= Inflammations, P in A= Podocytes, Pt in A, B & C= Proximal convoluted tubules, RBC in A & B= Red blood cell.

Discussion

At the present study it was found that mice treated with 100mg/kg body weight/day of methanol root extracts showed no distinct behavioral changes. However, the 400mg/kg body weight/day methanol root extract treated group showed a general reduction of activity during the last week of the experimental period. Similarly, mice treated with a single dose administration of 1630mg/kg body weight/day (LD_{50}) methanol showed similar multiple behavioral changes like reduction of motor activity, sedation, horripilation, asthenia, grooming, piloerection and difficult to breathe after two hours of the drug administration. In addition, signs of respiratory distress, muscle paralysis and piloerection¹¹ after the treatment of *Securidaca lonapedunculata* extract in rats⁹ also reported hypoactivity after acute and subchronic treatment of *Anacardium occidentale* leaves hexane extract in mice. The observed reduction in the activity and behavioral changes of the mice in higher doses of the extract may be due to the reduction of food intake.

The body weight gain by the 100mg/kg body weight/day methanol extract treated group was statistically insignificant while 400mg/kg body weight/day were significant ($p < 0.05$) as compared with controls. Such changes in the body weight gain as compared to the controls are in line with related studies by other workers which include: toxicity studies following treatments of aqueous extract of *Vernonia amygdalina*¹², hydroalcoholic extract with *Wedelia paludosa* in mice¹³, and methanol extracts of *Casalpinia bonducella* and *Bauhinia racemosa* in mice¹⁴.

Among the mice that gained body weight at the end of the experiment, the body weight gain for the controls was more than those mice treated with the methanol extract. These showed that the extracts may contain compounds with appetite suppressing effect as compared with the controls⁸. Hematological results of the RBC count, HCT, MCV, MCH and MCHC in methanol extracts, treated mice showed no significant changes at both doses of the treatments, while all others were found significantly changed as compared to the controls. The value of lymphocytes was significantly increased at 400mg/kg body weight/day methanol extract. The increased amount of lymphocytes in the extract treated groups may show response to the different tissues facing injurious impacts or sign of underlying problems as the result of the extract treatment. As lymphocytes are responsible for the storage of immunologic memory, a second contact with the drug may even

elicit a more accelerated and increased response and could be a matter of future investigations.

On the other hand, WBC and platelets were significantly decreased after treatment with 400mg/kg body weight/day methanol extract. Decrements in most of the different hematological parameters following extract administration are also found by many other workers, which include. administration of methanol extracts of *Caesalpinia bonducella* and *Bauhinia racemosa* plants in mice¹⁴, *Tithonia diversifolia* leaves administration in rats¹⁵ and feeding diets which contains *Milletia thonningii* in West African dwarf bucks (WAD)¹⁶.

The general tendency of decrement in blood cells count in higher doses of the extract treatments may be due to direct destructive effect of the extracts on these formed elements or impaired production in the hematopoietic tissue (bone marrow) and hence blood cells were reduced in the circulating blood. Similarly HGB was significantly increased in both doses of the methanol extract. This could be due to hemolysis of RBC due to the instability of the compound in RBC, so that the contained hemoglobin is freed into the surrounding medium¹⁷.

In this study, the biochemical parameters investigated showed different values in mice treated with both methanol extracts from those of the control mice. ALT, ALP, AST and urea values were significantly increased in both doses of methanol tract treatments. Similar trends of increments in these biochemical parameters were also observed by other researchers who investigated the effect of several traditional herbs, which include: *Catharanthus roseus* aqueous leaf extract administration in mice¹⁸, *Teucrium polium* (Calpoureh) extract treatment of diabetic male rats¹⁹, *Anacardium occidentale* Linn (Anacardiaceae) leaves hexane extract administration in mice⁹, *Teucrium polium* extract administration²⁰ and *Asparagus pubescens* methanol root extract treatment in rats²¹. The tremendously increased biochemical parameters observed in this study in both methanol extract treated groups may, therefore, reflect liver and kidney damage caused by the extract.

The present study has also investigated of histopathological changes in the sections of liver and kidney. These histopathological changes may be due to the effect of the presence of secondary metabolites in the extracts. These include: alkaloids, flavonoides and phenols⁸ which contain free radical scavenging molecules that can cause substantial biological damage of the mice tissues²². These changes were again similar to the body weight gain

and both haematological and biochemical parameters in methanol extract, and at higher dose than lower dose treatments. There were cytoplasmic vacuolations (hydropic degenerations) in the hepatocytes located towards the periphery of the hepatic lobules in both doses of methanol extracts, and LD₅₀ dose of the extract treated groups. Such cytoplasmic vacuolations are said to occur when the cytoplasm becomes pale and swollen due to accumulation of fluid or lipids as the result of disturbance in lipid inclusions and fat metabolism²³. It could also occur when there is disturbance to the functions of ribosomes, uncoupling of lipid from protein metabolism or cytoplasmic alterations produced to collect the injuries substances in the cell²³. It is interesting that cytoplasmic vacuolations occurred preferentially in hepatocytes located in the periphery of the hepatic lobules and around central veins. Hepatocytes located in the periphery of hepatic lobules are found close to the distributing veins and are believed to be affected more than hepatocytes located away from the incoming blood born drugs, such as the extracts of the present study²⁴. In addition, it is known that hepatocytes lying closest to the central veins (pericentral) are bathed in oxygen-poor and nutrient depleted blood than periportal and midzonal hepatocytes. The other histopathological changes observed were focal inflammations in the liver sections located towards the periphery of hepatic lobules in 100mg/kg body weight/day methanol, around the portal triads in 400mg/kg body weight/day methanol. In addition, such inflammations were observed near the nephrons of the kidney sections of the mice treated with both doses of methanol extracts. Inflammation is a complex reaction to injurious agents involving vascular responses, migration and activation of leukocytes and damaged cells. The unique feature of the inflammatory process is the reaction of blood vessels, leading to the accumulation of fluid and leukocytes in extravascular tissues. The increased inflammatory reaction observed in the present study may, therefore, be associated with the cellular and tissue damage caused by the extract in both liver and kidney, suggesting that these two organs are prone to be damaged by the extracts. Histopathological changes in these two organs were also reported by others following various herbal extract administrations in experimental animals^{9,25-27}.

In the kidney hydropic changes may appear whenever cells are incapable of maintaining ionic and fluid homeostasis as the result of loss of the

function of plasma membrane energy-dependent ion pumps¹⁷. It also occurs in hypoxic injury and various forms of toxic or metabolic injuries¹⁷. It is, therefore, possible that the observed cytoplasmic vacuolations in the hepatocytes and interstitial cells of the kidney may have been caused by one or combinations of the above disturbance as the result of the extract treatments. There was glomeruli atrophy in mice treated with higher dose than lower dose of both methanol root extracts. There were also signs of congestion and hemorrhage in the kidney of the LD₅₀ dose of extracts treated mice. The atrophy of the glomeruli may be due to sluggish circulation in the glomeruli or tissue hypoxia. Similarly, the signs of congestion and hemorrhage of the glomeruli may suggest impaired outflow of venous blood from the tissue and severe vascular injury or depletion of coagulation factors as the dose of the drugs increase.

Conclusion

Chronic treatments with *Clerodendrum myricoides* methanol extracts in mice cause reduction in body weight gain, damage to liver & kidney and changes in some hematological & biochemical parameters in mice irrespective of their sex. Further studies are required to isolate and identify the active constituents of the roots of *Clerodendrum myricoides* and to elucidate the mechanism(s) of their toxic effect.

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