Effect of sub-acute administration of hydromethanolic leaf extract of Ocimum Lamiifolium on the histological framework of the liver and kidney of Swiss albino mice

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Abstract

Background: *Ocimum lamiifolium* Hochst. ex Benth. (Lamiaceae) has been used as traditional remedy for many years. However, its toxicity has not been well studied in order to determine the adverse effect of its application.

Objective: The study was aimed to assess the histopathological effect of oral administration of an extract of *Ocimum lamiifolium* on the Liver and Kidney of mice.

Methods: Twenty-four adult albino mice were allocated into four groups (I, II, III and IV) six mice for each group. Group I was assigned as control, and group II, III and IV were the experimental groups. Each experimental group received 200, 400 and 600 mg/kg respectively of an extract of Ocimum lamiifolium orally for 28 days. At the end of the administration, the mice were sacrificed by cervical dislocation. The liver and the kidney were collected, fixed in 10% formalin and processed for light microscopy.

Results: Gross observations of liver and kidney of both treated and control groups showed no significant difference in shape, texture, size, and colour. While the organ weight measurement showed a dose-dependent reduction in kidney organ weight, but no change was observed in liver organ weight. The histological examination of the liver sections of the mice in treatment group has shown a dose-related mild to severe diffuse swelling of hepatocytes with marked vacuolization, focal microvascular steatosis of hepatocytes and periportal inflammation with mononuclear cell infiltrates compared with the control group. Likewise, the kidney sections of the mice treated at the higher dose (600 mg/kg) showed tubular necrosis, renal tubule vacuolation, and haemorrhage.

Conclusion: This study has shown that oral administration of Ocimum lamiifolium at lower doses of the extract may not have an adverse effect at the cellular level but could be hepatotoxic and nephrotoxic in a pattern when higher doses are consumed and this may have a similar effect in the human. Therefore, appropriate care should be given in the therapeutic use of the plant.

Keywords: *Ocimum lamiifolium, Hydromethanolic, Leaf extract, Sub-acute, toxicity, liver, kidney.*

Introduction

Since ancient time in human history, plants have been played an essential role in all traditional medicines especially in developing countries [1, 2]. In Ethiopia herbal medicine has been used as an integral part of its primary health care [3, 4]. Among the medicinal plants of Ethiopia, *Ocimum lamiifolium* is one of the well regarded and most widely used home medications. This plant is commonly called Hochst. ex Benth (whose vernacular name is 'dama-Kassie (Amharic)) which belongs to the family Lamiaceae. It is woody perennial plant usually having an average height of 0.7-3m high [3, 4]. It is widely used to treat pain, wound, fever, malaria, oropharyngitis and other inflammatory disorders in the Ethiopian population [5, 6, 7]. A comprehensive description and distribution of the plant have been established elsewhere [3, 5, 8].

In view of the fact that traditional healers prescribe and administer decoctions of the leaf without regard to its adverse effects, it is vital to seriously study the key visceral organs (liver and kidney). The liver is an essential organ that involves detoxifying various metabolites, synthesizes proteins, and produces biochemical substances necessary for digestion. Kidneys are also the organs that involve in blood filtration and removal of waste products from the blood [9]. A study on the adverse effect of the herbal medicine is very important, especially for the vital organs like liver and kidney which are often exposed to the toxic component of the decoction of a herbal plant. Some serious adverse reactions, such as nephrotoxicity and hepatotoxicity caused by some plant species have not been completely investigated [10]. Therefore, an experimental study of the liver and kidney is necessary in order to avoid unexpected damage to these vital organs at the cellular level. Ocimum species (family Lamiaceae) have been used extensively in the traditional system of medicine in many countries without the parallel development of national or international standards and methods for evaluating them [11, 12, 13].

The leaf of *Ocimum lamiifolium* has been shown to possess a significant anti-inflammatory activity, antimalarial activity analgesic activities and anti-pyretic activities [5-8, 14]. However, the toxicity of *Ocimum lamiifolium* has not been well studied in order to determine the adverse effect of its application. The aim of this study was to investigate the sub-acute toxicity effects of the extract on histomorphology of the kidney and liver of adult albino mice.

Methods and Materials

Plant collection and authentication of plant materials

The fresh leaves of the shrub were collected from Mekelle city, Ethiopia. The plant was identified and authenticated by Dr. Tadesse Beyene, a plant taxonomist in the Department of Biology, Faculty of natural and Computational Science, Mekelle University and the National Herbarium, Department of Biology, Addis Ababa University, where a voucher specimen of Ocimum lamiifolium (wm001) was deposited [7].

Ethnobotanical information about the plant was obtained from publications of Amabye et al [6] as well as from oral interviews with traditional healers of the local people.

Plant preparation and Extraction

Method of preparation of crude plant extracts was done according to the method described by Kefe et al. [7], a study conducted on antimalarial properties of crude extracts of leaf of *Ocimum lamiifolium*. Accordingly, the fresh leaf of *Ocimum lamiifolium* were washed gently with tap water to clean off extraneous materials and air-dried at room temperature under shade. The dried leaf were cut into pieces and ground to a fine powder. 800 gm of the powdered leaf of *Ocimum lamiifolium* were cold macerated with 80% methanol for 72 hours with intermittent shaking at room temperature. The mixtures were first filtered using muslin gauze and then passed through Whatman grade № 1 filter paper. The filtrate was concentrated in a rotary evaporator at a temperature of 45°C. The water extracts were frozen in a refrigerator overnight and dried in a lyophilizer to get a freeze-dried product. The resulting crude extract was weighed and the percentage yield was expressed as the total mass of dry powder. The crude extract was then kept in sealed plastic vials and stored in a desiccator at room temperature until used.

Quantization of phenols using HPLC

Phytochemical analysis was done by Amabye TG et al. [6], the extracts of the Ocimum lamiifolium species have led to the identification of 6 phenolic compounds (rosmarinic acid, lithospermic acid, vanillic acid, p-coumaric acid, hydroxybenzoic acid, and sinapic acid). Hence, hydroxybenzoic acid was found the most abundant component identified in the extract in comparison with other phenolic compounds and other compounds which are in agreement with previously published qualitative-quantitative analyses of Lamiaceae species [11].

Preparation of experimental animals

Eight to twelve-week-old adult male and female Swiss albino mice, each weighing 20-35gm were used for this study. Twenty-four mice were generously supplied by the animal house of the college of veterinary medicine, Mekelle University, Tigrai-Ethiopia. Throughout the study period, the male and female mice were kept in separate cages and maintained under constant laboratory conditions of temperature (22 ± 2^{o}c) with 12 hours light/dark cycle. All mice were allowed to feed with standard pellet diet and clean tap water. Feeding was maintained by constantly changing the food and water daily and proper sanitation was held by regular cleaning of the cages and the husk was changed every three days. All mice were apparently healthy. In order to minimize any non-specific stress, an acclimatization period of one week was allowed prior to experimentation

Administration of the extract

Administration of the extract was done according to Kefe et al. [7], who reported mice treated at doses of 200, 400, 600 mg/kg leaf extract of *Ocimum lamiifolium* showed antiplasmodial activity. All mice were randomly distributed into four groups (group I, II, III, IV) each containing six mice (three male and three female) per

cage. Group I was used as the control, Groups II, III and IV received the hydromethanolic leaf extract of *Ocimum lamiifolium* at dose levels of 200 mg/kg, 400 mg/kg and 600 mg/kg respectively. The actual dose of the plant extract corresponding to each group was calculated on the basis of body weight. The extract was freshly dissolved in 5 mL distilled water immediately before administration. Animals received their doses daily for 28 days. All the groups of mice were observed twice daily for clinical signs of toxicity until the completion of the experiment. Both the extract and the vehicle were administered orally using gavage feeding, similar to the way used by the traditional healers. According to the information obtained from oral interviews with traditional healers of the local people, the leaf of *Ocimum lamiifolium* is taken for the treatment of malaria, intestinal disorders, eye disease, and cough. The interviewee explained that this plant is traditionally prepared by pounding its leaf and homogenize with pure water and then orally drink a cup (Chilfa in local language) of the decoction which is estimated to be about 6 ml of the solution.

Body weight measurement

The body weight of all animals was taken by using digital electronic balance before commencing the first oral administration (day 1) as initial weight, then weekly (once a week) and at the end of the oral administration of the extract (on day 28) as final body weight. The mean body weights were calculated and used for analysis of body weight progress.

Specimen collection

All mice were sacrificed at the end of the administration using cervical dislocation. Immediately after death, each animal was quickly placed in a supine position on an operating board. The limbs were stretched and fixed. The abdominal cavity was opened by mid-sagittal skin incision and the anterolateral abdominal walls were reflected laterally and the flaps held with pins. At autopsy, liver and kidneys were visually examined for any signs of gross lesions. After the liver and kidney were gently isolated, all extraneous tissues were removed, and immediately weighed on an electronic balance. The data were normalized and expressed per 100 mg body weight to obtain the relative organ weight of each animal. After rinsing with normal saline, sections were taken from each of the harvested organs. The two coronal halves of the right kidney, as well as tissue samples dissected out in a block from the right lobe of the liver were rapidly placed in pre-labeled sample bottles containing fixative and used for histopathological studies. Selection to use the right lobe of the liver and the right kidney were random.

Histological processing

The collected tissue samples from the liver and kidney were soaked up in 10% buffered neutral formalin for 48 hours. After fixation, the tissue samples were washed for 24 hours in running tap water. Then, the tissues were dehydrated with a graded series of ethyl alcohol– in 70% and 90% for 2 hours each and in absolute alcohol I-IV for 1½ hours each. The tissues were cleared with two changes of xylene

for 1½ an hour each. The cleared tissues were transferred to three changes of liquid paraffin wax in an incubator at 60 ^{0}C: wax–I for 1½ hours, wax-II for 2½ hours and wax-III for 24 hours. The wax-impregnated tissues were then embedded in paraffin blocks by placing the tissues in squares of metal plates and carefully pouring molten paraffin over them. After proper orientation of the specimen was confirmed, all tissue blocks were labeled and allowed to harden at room temperature. About 5µm thicknesses of paraffin blocks were trimmed and sectioned with a Leica Rotary Microtome.

The staining solutions were prepared according to the formula given by [15]. After rinsing the slides in distilled water for 5 minutes, sections were stained regressively with Harris hematoxylin for10 minutes. The sections were washed in tap water and dipped into 1% acid alcohol for differentiation and to remove excess stain. The sections were rinsed briefly in running tap water to remove excess acid and halt destain. The slides were then placed in a saturated sodium bicarbonate solution for 3 minutes until the specimen becomes bright blue. The Hematoxylin and Eosin stained sections were dehydrated by dipping into jars containing a series of alcohols: 50% for 5 min, 70%, and 95% for 3 minutes each; and absolute alcohol -I and absolute -II for 3 minutes each. The sections were then cleared in xylene. Finally, the sections were mounted using DPX mountant and glass coverslips.

Microscopy and Photomicrography

Microscopic slides of the liver and kidney tissues were examined carefully under a light microscope at the histology laboratory at Mekelle University. Tissue sections from the treated groups were evaluated for any evidence of histopathological changes with respect to those of the controls. Histological changes in the liver and kidney tissues were observed under X10 and X40 objective lenses. The presence or absence of histopathological changes in the tissue sections was confirmed by, a pathologist at the international clinical laboratory, Addis Ababa. Photomicrographs of selected slides of each of the organs under study were taken under magnification of X100 and X400 using digital camera installed microscope.

Data processing and analysis

All data represented by numbers were analyzed by SPSS 20 statistical software. Data for body weight and organ weights were presented as a mean ± standard error of the mean (S.E.M.). Differences between the treated and control groups were compared by using one-way analysis of variance (ANOVA) followed by Dunnett post-hoc test to determine their level of significance. Differences at $P<0.05$ were considered statistically significant.

Ethical consideration

All mice used for this study were generously supplied by the animal house of the college of veterinary medicine, Mekelle University. Appropriate ethical approval was granted from Mekelle University College of Health Sciences; Health Research Ethics Review Committee (registration number: ERC 1072/2017). All procedures were carried out by well-trained personnel. The animals were

protected from pathogens and placed in an appropriate environment.

Results

Effects of hydromethanolic leaf extract of *Ocimum lamiifolium* on general health and body weight of mice case-side observation of the mice in treatment and control groups was carried out daily for the general assessment of any clinical manifestations all over the study period. Except, in the fourth week, only one mouse treated with 400 mg/kg/day died, the rest of experimental and control mice showed no observed clinical manifestation of extract related toxicity for the consecutive 28 days of administration. Meanwhile, experiential measurement has shown a dose-dependent body weight gain in both the treated and control groups, respectively (Table 1).

Table 1: The pattern of mean body weight change in the different group of the albino mice during the treatment.

Group	Dose (mg/kg) body weight	Initial Body weight	Week-1	Week-2	Week-3	Week-4
I	Control	25.3±1.9	28.7±2.3	30.0±2.4	31.0±2.3	31.8±2.0
II	200	26.4±1.8	28.8±2.5	29.9±2.6	31.1±2.6	32.6±2.8
III	400	24.5±1.5	26.8±1.7	28.0±1.6	28.8±1.8	29.4±2.3
IV	600	24.9±1.4	27.5±1.7	28.4±1.6	28.9±1.8	29.4±1.9

Values are means ±SEM; n=6/group.

However, the mean body weight gain was different between treated and control groups, and the treated group tended to have low mean body weight gain compared to controls ($p<0.05$) (table 2).

Table 2: Comparison of the initial weight and final body weight change before the treatment and at end of the treatment as compared to controls.

Group	**Dose in (mg/kg) body weight**	**Initial weight (in gram)**	**Final weight (in gram)**	**Mean body weight difference (in gram)**	**P-value**
I	Control (DW)	25.3± 1.9	31.8± 2.2	6.6±0.3	R
II	200	26.4+ 1.8	32.6+ 2.8	6.1+1.0	0.4
III	400	24.5±1.5	29.4±2.3	4.9±0.8	0.001*
IV	600	24.9±1.4	29.4±1.9	4.6±0.6	0.0001*

* P value < 0.05 was considered significant; values are expressed as mean ±SEM., n=6/group.

Effects of hydromethanolic leaf extract of *Ocimum lamiifolium* on the weights of liver and kidney of mice

Gross observations of the livers and kidneys did not show any pathological changes in positions, shape, texture, size, and colors. Meanwhile, there was no significant difference in both absolute and relative weights of the liver of the mice in both the treatment and control groups. While, the mean absolute and relative weight of the kidney showed a significant reduction in mice treated at a higher dose (600 mg/kg) ($P< 0.05$) compared to mice treated with a dose of level 200 mg/kg, 400 mg/kg, and the control groups (table 3).

Table 3: Effects of a hydromethanolic leaf extract of *Ocimum lamiifolium on* the absolute and relative weights of liver and kidney of albino mice.

Group	Dose in (mg/kg) body weight	Liver		Kidney	
		Absolute weight (in g)	Relative weight (in g/100g bw)	Absolute weight (in gram)	Relative weight (in g/100g)
I	Control	2.2±0.2	6.8±0.1	0.7±0.5	2.1±0.1
II	200	2.4±0.2	7.4±0.3	0.7±0.1	2.1±0.1
III	400	2.1±0.2	6.8±0.4	0.4±0.1	1.2±0.3
IV	600	1.9±0.2	5.3±1	0.3± 0.1*	0.9±0.2*

*: Significant ($p<0.05$); values are given as Mean ± S.E.M; N= 6/group.

The effects of *Ocimum lamiifolium* hydromethanolic leaf extract on the histopathology of liver

This study has shown dose-dependent toxicity on the histology of the liver. The microscopic observation of liver sections of the control mice shows normal architecture of liver with intact hepatic lobules and portal tract structures (figure 1, A and B). Nonetheless, the liver histology of mice treated with the plant extract at 200 mg/kg shows subcapsular diffused of swollen hepatocytes and binucleated hepatocytes, while the hepatic lobules and portal tract structures were normal (figure 2, A and B). Similarly, diffuse swelling of hepatocytes with marked vacuolization and minimal periportal inflammation with mononuclear cells infiltrates was observed in the liver sections of the mice treated with the plant extract at 400 mg/kg (figure 3, A and B). Microscopic observation of the liver section of the mice treated with the plant extract at 600 mg/kg also showed an extensive diffuse swollen hepatocyte, focal microvesicular steatosis of hepatocytes (fatty changes), and severe periportal inflammation with mononuclear cell infiltrates (figure 4, A and B).

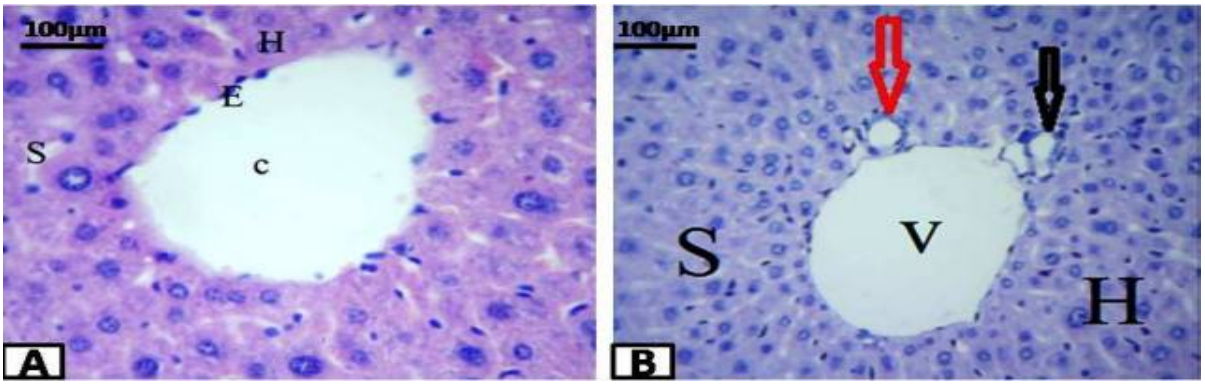

Figure 1: Photomicrographs of H and E stained liver section of mice in the control group (A and B) showing normal histological features around the pericentral and periportal region respectively. Central vein (C) and portal vein (V), hepatic artery (black arrow), bile duct (red arrow), hepatocytes (H), endothelial cell (E), sinusoids (S) –H&E stain ×100 and ×400 respectively.

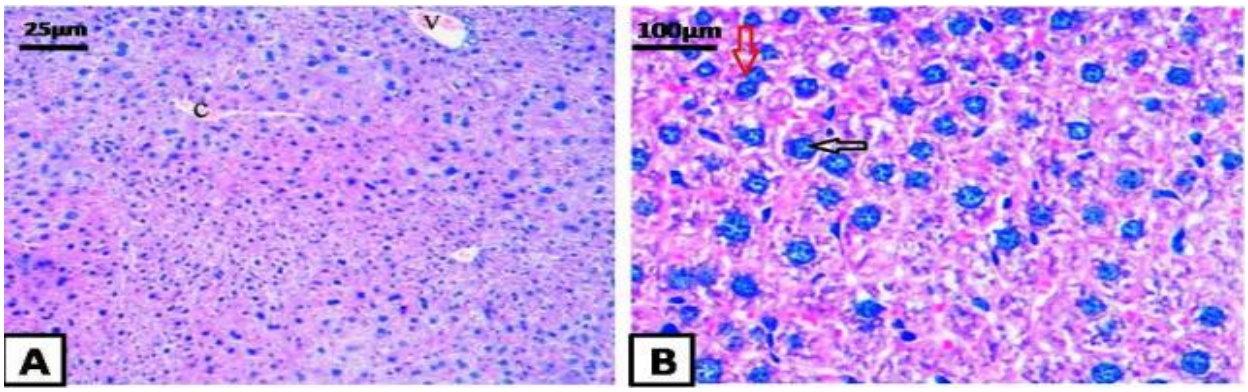

Figure 2: Photomicrographs of H and E stained liver section of mice treated with an extract of Ocimum lamiifolium at 200 mg/kg (A and B). **A**: Normal histological architecture of liver with intact hepatic lobules and portal tract structures (V-portal vein, C-central vein) – H&E stain ×100. **B**: subcapsular diffuse swollen hepatocytes (black arrow) and binucleated hepatocytes (red arrow) were evidenced as compared to the control (H&E stain ×400).

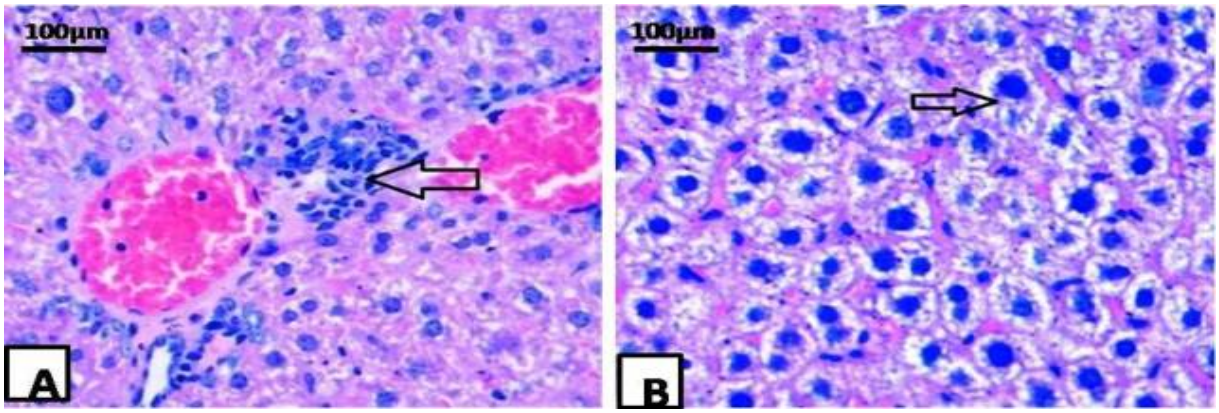

Figure 3: Photomicrographs of H and E stained liver section of mice treated with an extract of Ocimum lamiifolium at 400 mg/kg (A and B). **A**: normal architecture of the liver with intact hepatic lobules, portal tract structures and there is mild periportal inflammation with mononuclear cell infiltrates (arrow) –H&E stain ×100. **B**: Moderate diffuse swollen hepatocytes with marked vacuolation (arrow) were observed in this group –H&E stain ×400.

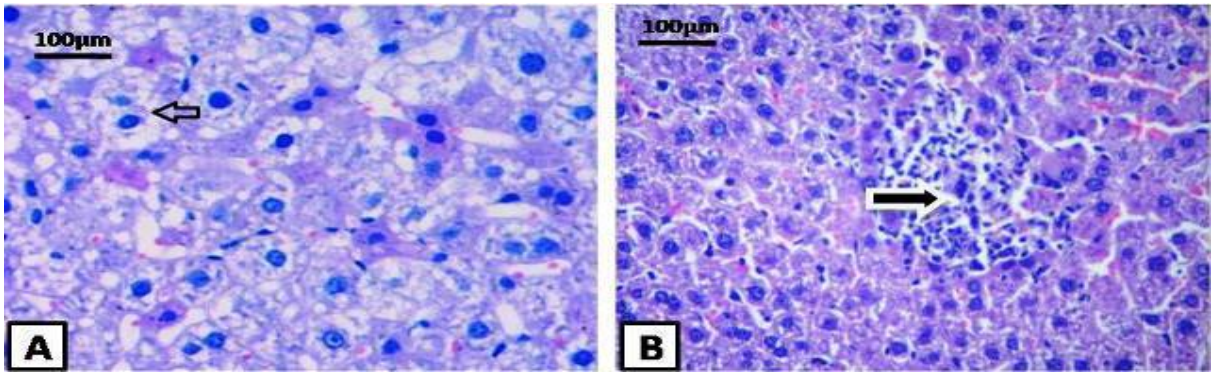

Figure 4: Photomicrographs of H and E stained liver section of mice treated with an extract of Ocimum lamiifolium at 600 mg/kg (A and B). **A**: extensive diffuse swollen hepatocyte, focal microvesicular steatosis of hepatocytes (fatty changes) (arrow) (H&E stain ×100). **B**: severe infiltration of inflammatory cells (arrow) was revealed in this group as compared to the control (H&E stain ×400).

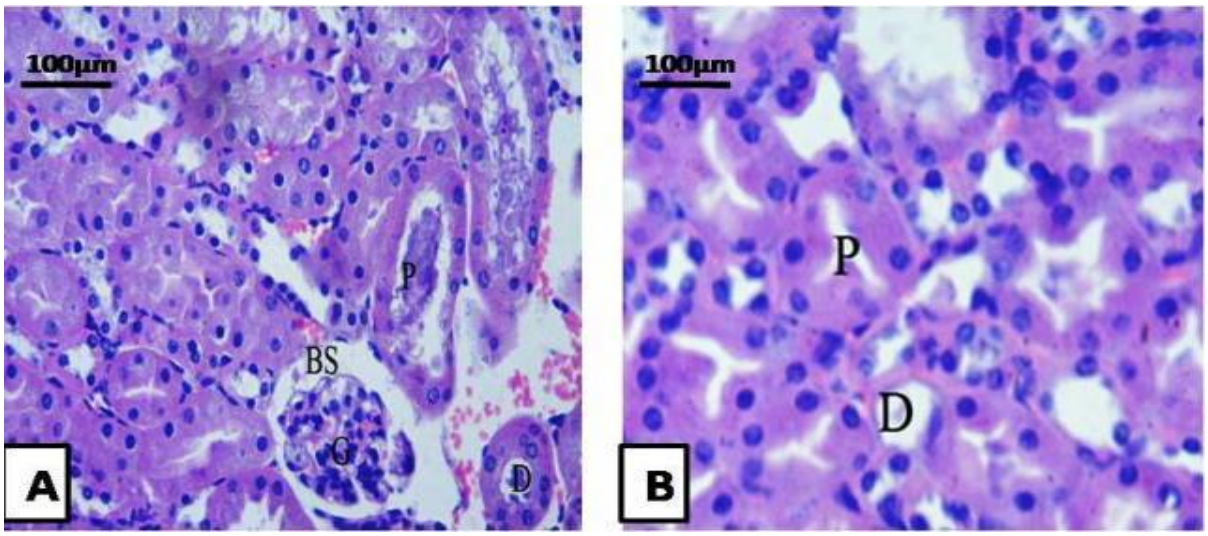

Figure 5: Photomicrographs of H and E stained kidney sections of control mice (A and B), showing normal Glomeruli (G), Bowman's space (BS), Distal convoluted tubule (D), proximal convoluted tubule (P)–H&E stain ×100 and ×400 respectively).

The effects of *Ocimum lamiifolium* hydromethanolic leaf extract on histopathology of kidney

Microscopic observation of kidney section of mice treated with the plant extract at 200mg/kg and 400mg/kg (figures 6 and 7) was comparable with the liver section of mice in control group (figure 5), showing the normal architecture of kidney with intact cortical and medullary structures. The glomeruli are intact with normal looking capillary tufts. There is no tubulointerstitial pathology. However, the kidney sections of the mice treated with the extract at higher dose levels (600 mg/kg) show tubular necrosis, renal tubule vacuolation and haemorrhage (figure 8, A and B).

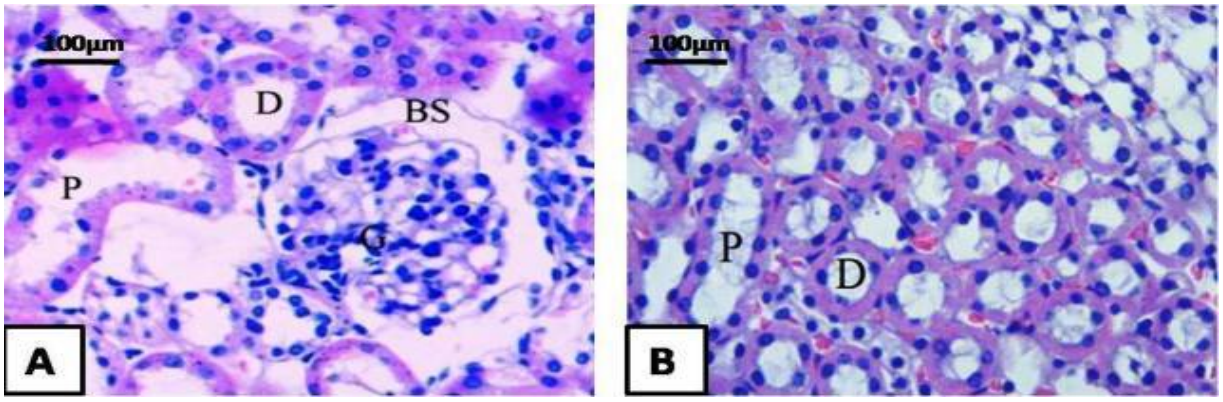

Figure 6: Photomicrographs of H and E stained kidney section of mice treated with an extract of Ocimum lamiifolium at 200 mg/kg (A and B), showing normal Glomeruli (G), Bowman's space (BS), Distal convoluted tubule (D), proximal convoluted tubule (P)–H&E stain ×100 and ×400 respectively.

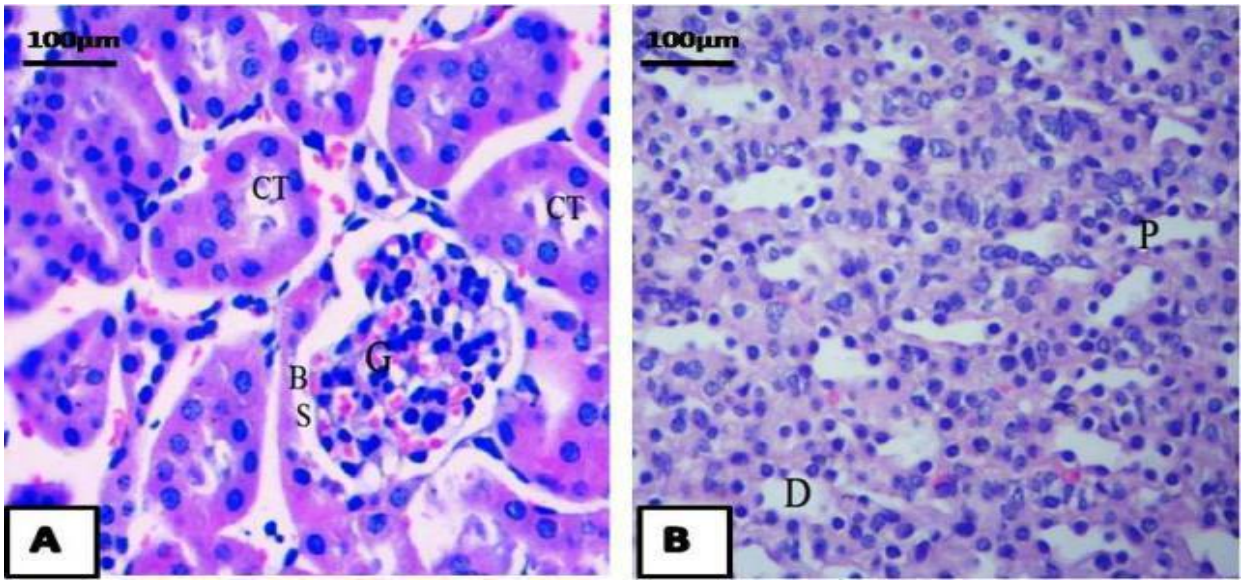

Figure 7: Photomicrographs of H and E stained kidney section of mice treated with an extract of Ocimum lamiifolium at 400 mg/kg (A and B), showing normal Glomeruli (G), Bowman's space (BS), Distal convoluted tubule (D), Proximal convoluted tubule (P), Convoluted tubule (CT)– H&E stain ×100 and ×400 respectively.

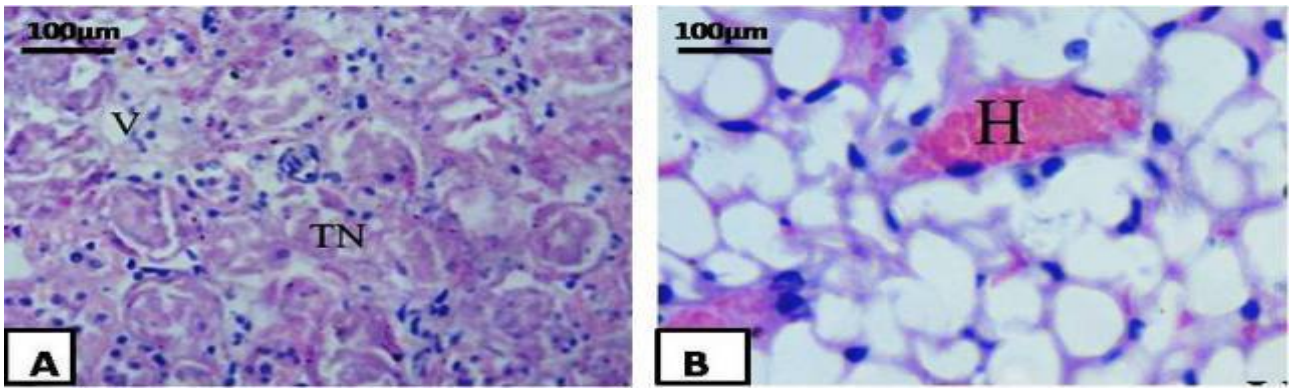

Figure 8: Photomicrographs of H and E stained kidney section of mice treated with an extract of Ocimum lamiifolium at 600 mg/kg (A and B), showing renal tubule vacuolation (V), Tubular necrosis (TN), and hemorrhage (H)–H&E stain ×100 and ×400 respectively.

Discussion

The present study revealed that oral administration of the plant extract has shown a dose-dependent toxicity in the tissue sections of the liver and kidney in line with increased dose regimen. Hence, there was relatively dose-related mild to severe diffuse swelling of hepatocytes with marked vacuolization, focal microvascular steatosis of hepatocytes and periportal inflammation with mononuclear cell infiltrates in the liver sections from the treated group II, III, and IV compared with the control group. Comparable findings were reported elsewhere [16] where oral administration of aqueous extract of *Ocimum gratissimum* to albino rats showed varying degrees of

dilatations of the central vein of the liver which contained cell infiltrates in the treated group compared with the control. Similarly, dose-dependent periportal inflammatory cell infiltrates were reported in liver sections of mice treated with the aqueous extract of *Ocimum gratissimum* [17]. Empirical measurement in this study has shown progressive body weight gain over 28 days of experimental period in both treated and control groups. Notwithstanding, the final mean body weight gain has shown a significant difference between treated and control groups. The treated group tended to have low mean body weight gain compared to the controls group. The observed weight gain difference may indicate that the extract has appetite suppressing chemicals which in turn affects the growth of the animals. In contrast, studies reported that mice treated with the extract of the *Ocimum gratissimum* showed a dose-related decrease in body weight [12, 13, 16]. This contradicting result could be due to study methodology differences such as dose, plant species and chemical composition it contains, type of experimental animal used.

Likewise, our finding showed that the mean absolute and relative weight of the liver in mice treated with plant extract at 200 mg/kg, 400 mg/kg and 600 mg/kg showed no significant variation compared with mice in control group. However, the mean absolute and relative weight of the kidney in mice treated at a higher dose level of 600 mg/kg showed a significant reduction (P= 0.005 and 0.002, respectively) compared with mice treated with plant extract at 200 mg/kg, 400 mg/kg and the control groups, (table 3). This reduction in organ weight may be an indicator for sensitivity of nephrotoxicity at higher dose exposure of the extract.

Moreover, the tubular necrosis, vacuolation, and hemorrhage observed in kidney section of mice treated with 600 mg/kg body weight of the plant extract in this study showed that extract at such higher dose could induce nephrotoxicity at the cellular level. This finding is in line with the study of Adejoke et al. [18] who reported that oral administration of *Ocimum gratissimum* induces vascular congestion and varying degrees of interstitial infiltration by inflammatory cells. Moreover, the finding further supports the results obtained by Efosa, O.B. et.al and Olufemi OJ et.al [12, 19], who reported that the mice treated at a higher dose of the extract of *Ocimum gratissimum* induce nephrotoxicity, thus indicating a deleterious effect at the cellular level.

Comparable findings were reported following administration of higher doses of *Ocimum gratissimum* in a kidney section of albino rats [12, 13, 17]. Based on the aforementioned studies and the present the study, it can be concluded that therapeutic use of *Ocimum lamiifolium* and related species of Ocimum plant decoction at lower doses have no deleterious effect at the cellular level but could be hepatotoxic and nephrotoxic at higher concentrations. Therefore, appropriate care should be given in the medicinal use of the plant.

Limitation of this study

The limitation of this study includes failure to assess the biochemical and hematological

effects of an extract of the *Ocimum lamiifolium*. Thus, this could lead to overestimating or underestimating the effect of *Ocimum lamiifolium* extract administration on liver and kidney tissues.

Conclusions

This study has shown that oral administration of Ocimum lamiifolium at lower doses of the extract may not have an adverse effect at the cellular level but could be hepatotoxic and nephrotoxic in a pattern when higher doses are consumed and this may have a similar effect in the human. Therefore, appropriate care should be given in the therapeutic use of the plant in respect of high dose and long duration of use. Feature studies including sub-chronic and chronic histological and biochemical investigations regarding the current topic is needed to provide adequate evidence of the plant toxicity or safety.

Acknowledgment

Authors are thankful for the School of Pharmacy for facilitating a good working environment during the laboratory work and allowing us to use their laboratory facilities. We extend our deepest gratitude to Dr. Selam G, Department of Pathology, international clinical laboratory, Addis Ababa for her microscopic examinations of the tissue slides and providing insightful comments on the histopathological changes of the tissue sections.

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