Original Article

Evaluation of *Rumex nepalensis* Spreng. Root Extract on Biochemical and Histopathologic Parameters of Mice Liver

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Abstract

Introduction: The *Rumex nepalensis* Spreng. (RN) root has various medicinal uses such as the treatment of abdominal colic, tonsillitis, arthritis, diarrhea, and infertility. The present study evaluated the subacute toxic effects of RN root extract on the histology and biochemical parameters of the liver in mice. **Material and Methods:** A total of 24 male and female mice were used and randomly assigned into four equal groups. Group I (control) received distilled water. Groups II, III, and IV received root extract of RN at 250, 500, and 1000 mg/kg/day for 28 consecutive days, respectively. Data obtained were processed using SPSS statistical software version 20 and presented using tables and graphs, while liver sections were processed and their histopathology microscopically observed. **Results:** Mice treated with 250 and 500 mg/kg/day of the root extract showed no significant changes in body weight in both sexes. Males treated with 1000 mg/kg/day of the extract had significant weight reduction, while the females did not show weight change. Alanine aminotransferase serum levels were significantly increased in all mice treated with 1000 mg/kg/day of the root extract. Histopathological changes such as congestion of portal and central veins, sinusoid dilatation, and Kupffer cell proliferation were observed in the 500 and 1000 mg/kg of the root extract treated groups. Mortality was also noticed in these two groups, though not confirmed as being toxicity related. **Discussion and Conclusion:** Treatment with hydroalcoholic root extract of RN revealed hepatotoxic effects at 500 and 1000 mg/kg of the root extract. This shows that the consumption of high doses of RN may be hazardous.

Keywords: *Biochemical parameters, histological alterations, liver, Rumex nepalensis Spreng., subacute toxicity*

Introduction

The World Health Organization defines traditional medicine (TM) as health practices, approaches, knowledge and skills, based on the theories and beliefs, applied singularly or in combination to diagnose, treat, and prevent illnesses as well as maintain well-being.[1] TM has maintained its popularity in all regions of the developing world and its use is also extended to industrialized countries. In China, for example, traditional herbal therapy accounts for 30%–50% of the total medicinal consumption. In Africa; Ghana, Mali, Nigeria, and Zambia have reported the use of 60% herbal medicine at home for treating children with high fever resulting from malaria.[2] Many other countries, including Ethiopia, use TM/complementary and alternative medicine for health care.[2]

About 80% of Ethiopians use TM due to the cultural acceptability of traditional healers. The popular use of this line of treatment may also be due to the cheaper cost of TM compared to orthodox medicine. Majority of Ethiopians live in rural areas where the health-care coverage is low and existing public-sector resources are limited or unavailable. This difficulty in accessing modern health facilities may propagate TM use.[3,4]

Rumex nepalensis Spreng. (RN), called "Tult" in Amharic and "Shembobaeta" in Tigrigna, is of the genus Rumex. It consists of more than 250 species widely distributed in the world, including Europe, Asia, Africa, and the Americas. It is also widely distributed in different parts of Ethiopia such as Tigray, Amhara, Oromia, and Southern parts of Ethiopia.[5] The roots of this plant, in combination with other medicinal plants, are traditionally used in the treatment of infertility, abdominal

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colic, diarrhea, tonsillitis, and arthritis among a variety of other ailments. They are also used to induce labor and abortions.[6]

Phytochemical screening on RN extracts reveals the presence of anthraquinones, steroids, saponins, and tannins as major classes of constituent compounds. Although published toxicity studies on RN root extract are limited, some of these components of the root extract such as anthraquinones and tannins have been shown to have toxicity on mice organs and liver functions.[7,8]

The safety of several commercially available traditional herbs has recently come into question due to reports of adverse effects and potential hazardous interactions with prescribed orthodox drugs.[9] Renal failure and hepatic dysfunction have been identified, as the most commonly reported adverse effects of herbal medicines misused or abused.[10] Assessments of the toxicities of herbal substances are often conducted experimentally using rodents' livers, kidneys, hematologic parameters, and biochemical enzymes. The current study is aimed at evaluating the toxic effects of crude RN methanolic root extract on mice liver histopathology and its effect on some biochemical parameters.

Material and Methods

Plant collection and extraction

The fresh roots of RN were collected from Mekelle area (Enda Cherkos) about 760 km away from Addis Ababa and authenticated by a botanist in the School of Biological Sciences, Mekelle University, Ethiopia**.** The roots were cleaned, sliced into smaller pieces, dried under a shade at room temperature, and crushed manually to obtain fine powder particles. The powder was extracted with 80% methanol and filtered with Whatman filter paper (no. 1). The marc was remacerated twice using the same solvent. The filtrate was dried under a vacuum oven drier at approximately 40°C for 14 days. This extract was kept in a desiccator at room temperature until use and reconstituted in distilled water to obtain the desired concentration for all pharmacological tests.

Experimental animals

A total of 24 adult Swiss albino mice (12 females and 12 males) between 8 and 12 weeks old, weighing 25 to 30 g each were used. The mice were obtained from the animal house of the Department of Veterinary Medicine, Mekelle University, and acclimatized to a laboratory environment for 5 days. The mice were fed with standard commercial diet and water *ad libitum* at room temperature with 12 h light/dark cycles till the end of the experiment. They were randomly divided into four groups with each group consisting of six mice (3:3, males:females) and separate cages were used for individual sexes.[11] Group I was used as control and received distilled water for 28 days. Groups II, III, and IV were given 250 mg/kg/day, 500 mg/kg/day, and 1000 mg/kg/day of the root extract, each for 28 days, respectively. The test doses were selected according to similar studies conducted on related species.[12] Administration of the extract was done orally with an intragastric tube.

Data collection techniques

The body weight of each mouse was taken using a sensitive digital balance (Mettler Toledo, type PG1003-S, Switzerland). At the end of the experiment, all animals were fasted overnight, anesthetized using diethyl ether, and blood samples obtained by cardiac puncture. The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and Alkaline phosphatase (ALP) levels were analyzed immediately using an automatic biochemical analyzer (Pentra C400, France).

Organ weight measurements and tissue sample

After the experimental mice were sacrificed, their livers were carefully removed and weighed. Random samples of transverse liver sections were preserved in 10% neutral-buffered formalin. For histopathological studies under light microscopy, tissue samples taken from the sacrificed mice were processed; tissue slides of the mice livers were stained in hemotoxylin and eosin (H and E). Photomicrographs of randomly selected slides were taken at a magnification of ×40 using a digital photo camera-fitted microscope (B-380, OPTIKA, Italy).

Data processing and analysis

All data were represented in numerical form, entered and analyzed using SPSS version 20, IBM, New York, USA. All parameters were expressed in mean ± standard error of mean. Treatment over time was compared between control and treated groups by using one-way analysis of variance followed by Tukey's multiple comparison tests. $P < 0.05$ was considered as the level of significance.

Ethical consideration

Ethical clearance was obtained from the research and publication committee of Mekelle University, Medical Faculty with a registration number 1068/2017. All mice were handled according to the Organisation for Economic Co-operation and Development (OECD) guideline 407.[11] All invasive treatments were conducted under anesthesia.

Results

General observations

All male and female mice that were treated with the repeated doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg body weight of RN root extract over 28 days showed extract-related noticeable changes in their general behavior such as depression, piloerection, loss of appetite, and fast breathing compared to the control group.

Effects of *Rumex nepalensis* Spreng. root extract on the body weight of mice

As shown in Table 1, no significant changes were observed in the mean values of the body weights of mice treated with 250 and 500 mg/kg/day body weight of the extract when compared with the controls. Significant decrement was, however, observed in the mean body weight of male mice treated with 1000 mg/kg/day of the root extract at the end of 4th week as compared with the control group. No significant weight decrement was found in the 1000 mg/kg/day root extract-treated female group when compared to the control group.

Effects of *Rumex nepalensis* Spreng. root extract on liver weight

The mean liver weight of male mice treated at 250 mg/kg/day and 500 mg/kg/day as well as female mice treated at 250 mg/kg/day, 500 mg/kg/day, and 1000 mg/kg/day of the root extract did not show statistically significant difference as compared to the controls. However, the mean weight of the male mice livers treated at 1000 mg/kg/day of the root extract was significantly increased ($P < 0.05$) as compared to the mean weight of the controls [Table 2].

Effects of *Rumex nepalensis* Spreng. root extract on hepatic biochemical parameters

There was no significant rise in liver enzymes in all treatment groups compared to the controls except in 1000 mg/kg/day RN root extract-treated male and female mice, respectively [Table 3].

Effect of *Rumex nepalensis* Spreng. extract on the histopathology of the liver

Light microscopic examination of routine H and E-stained liver sections of both male and female mice treated with 250 mg/kg/day RN root extract group showed slight portal venous congestion as well as slight hepatic artery and bile duct dilatation compared to the controls [Figures 1 and 2].

Changes in the 500 mg/kg/day root extract male and female treated groups also showed hepatocyte binucleation, mild central venous and sinusoidal congestion as well as mild dilatation of portal and central veins, hepatic arteries, and bile ducts in contrast to the control group [Figures 1 and 3].

At 1000 mg/kg/day root extract treatment [Figure 4], both male and female groups exhibited more marked portal and central venous congestion, Kupffer cell hyperplasia, cellular infiltration in the central veins, as well as portal vein and sinusoidal dilatation in comparison with the controls [Figure 1].

Discussion

In the current study, daily treatment with doses of RN root extract for a period of 28 days was carried out as per the OECD 407 subacute toxicity guidelines.[11] Subacute toxicity study was performed to elucidate the toxic effects of RN based on the preexisting knowledge on the acute toxicities of individual compounds found in this plant. This was aimed at providing information on the effects of repeated oral exposure and as indication of the need for further longer-term studies and concentration selection.[11] The dosages were chosen based on those used in previous studies of plants in the same genus[12] as well as OECD guidelines.[11]

These treatments showed some toxicity signs in general health such as depression, piloerection, loss of appetite, and fast breathing compared with the control group.

Table 1: The mean body weight of mice treated with root extract of *Rumex nepalensis* Spreng.

Period	Sex	Control Group I	Treatment groups (mg/kg body weight/day)		
			Group II (250 mg/kg)	Group III (500 mg/kg)	Group IV (1000 mg/kg)
Initial weight	Female	26.2±0.40	28.3±0.43	26.1±0.64	27.0±0.92
	Male	29.1±0.63	30.1±0.16	29.6±0.20	28.7±1.00
Week 1	Female	27.2±0.67	27.6±0.97 (1.00)	24.0±1.33 (0.22)	23.7±1.40 (0.17)
	Male	30.1±1.30	28.2±0.92 (0.80)	28.3±0.25 (0.84)	27.1±0.50 (0.37)
Week 2	Female	27.9±0.87	27.9±0.87 (1.00)	26.2±0.65 (0.69) day	25.8±0.92 (0.59)
	Male	31.6±1.47	31.8±0.20 (1.00)	30.6±0.31 (0.98)	27.1±0.30 (0.04)*
Week 3	Female	28.1±0.71	27.9±0.87 (1.00)	26.8±0.65 (0.83) day	26.2±0.58 (0.39)
	Male	34.5±0.68	31.8±0.20 (0.27)	31.7±0.68 (0.24)	27.7±0.12 (0.00)*
Week 4	Female	29.8±0.49	30.0±0.84 (1.00)	26.4±2.15 (0.81) day	28.2±0.50 (0.83)
	Male	36.6±0.73	33.9±0.95 (0.50)	32.2±0.92 (0.07)	30.5±1.65 (0.02)*
Wd (FW-IW) (g)	Female	3.6	1.7	0.3	1
	Male	7.5	3.8	2.6	1.8
Percentage of weight change	Female	13.7	6	1.2	3.7
	Male	25.8	12.6	8.8	6.2

*P<0.05 Values are given as mean±SEM, for each male and female subgroup. The figures in brackets indicate the calculated P values of the treatment groups as compared with the controls. The mean difference is considered significant when P<0.05, (n=6 [3 males and 3 females in each group]). There was significant weight decrement of the males treated with 1000 mg/kg/day in weeks 2, 3, and 4 compared with the controls (P<0.05). Wd: Weight difference, FW: Final weight, IW: Initial weight, SEM: Standard error of mean

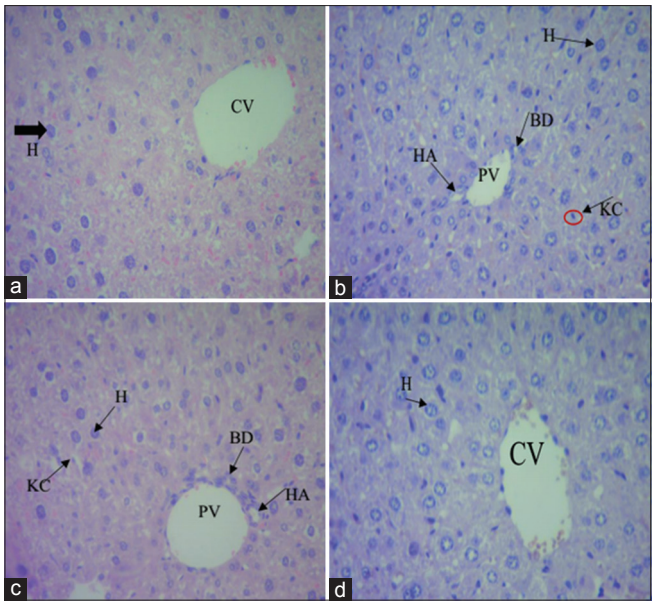

Figure 1: Photomicrographs of liver sections from control mice; both female (a and c) and male (b and d) showed normal hepatic architecture. H: Hepatocytes, BD: Bile duct, HA: Hepatic artery, CV: Central vein, PV: Portal vein, KC: Kupffer cell, (H and E, ×400)

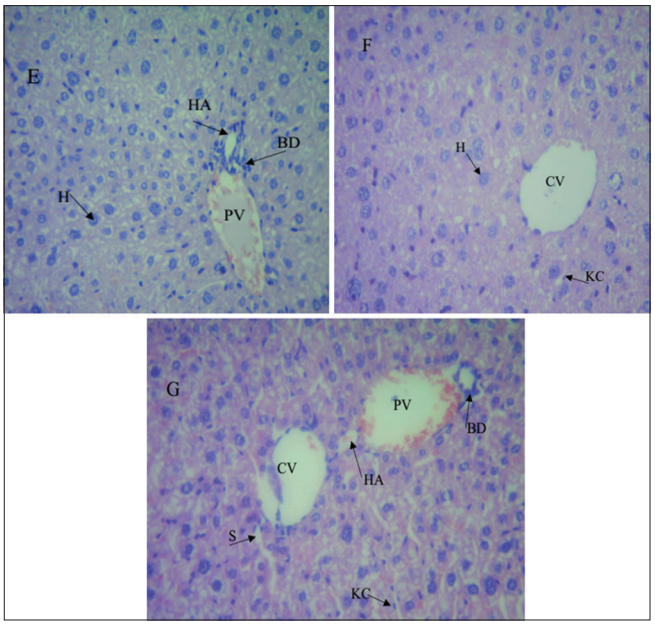

Figure 2: Photomicrographs of liver sections from mice treated with 250mg/kg/day of *Rumex napelensis* Spreng. root extract: male (e and f) and female (g) showing slight dilatation of the hepatic artery and bile duct as well as central vein congestion. Note: H: Hepatocytes, BD: Bile duct, HA: Hepatic artery, CV: Central vein, PV: Portal vein, KC: Kupffer cell, (H and E, ×400)

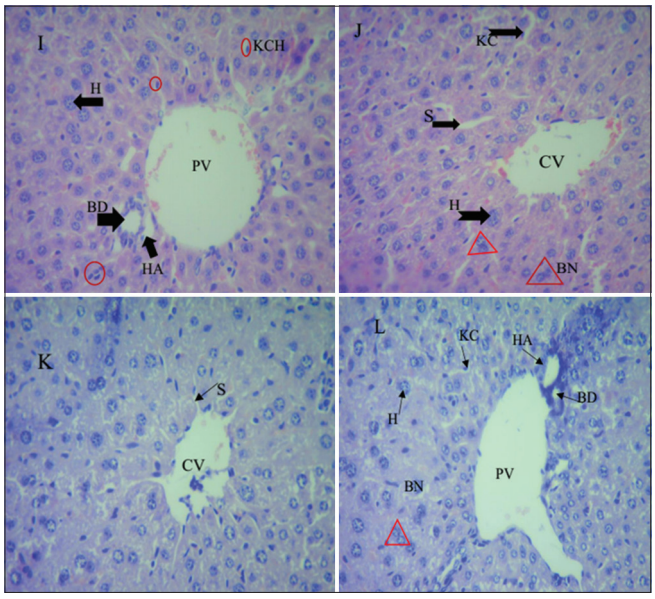

Figure 3: Photomicrographs of liver sections of mice given 500mg/kg/day *Rumex nepalensis* Spreng. root extract, male (i and j) and female (k and l) showing: PV: Portal vein, CV: central vein, HA: mildly dilated and mildly dilated hepatic artery, BD: bile duct. Hepatocytes showed BN: Binucleation, S: Sinusoid, KC: Kupffer cell, KCH: Kupffer cell showed hyperplasia (H and E. ×400)

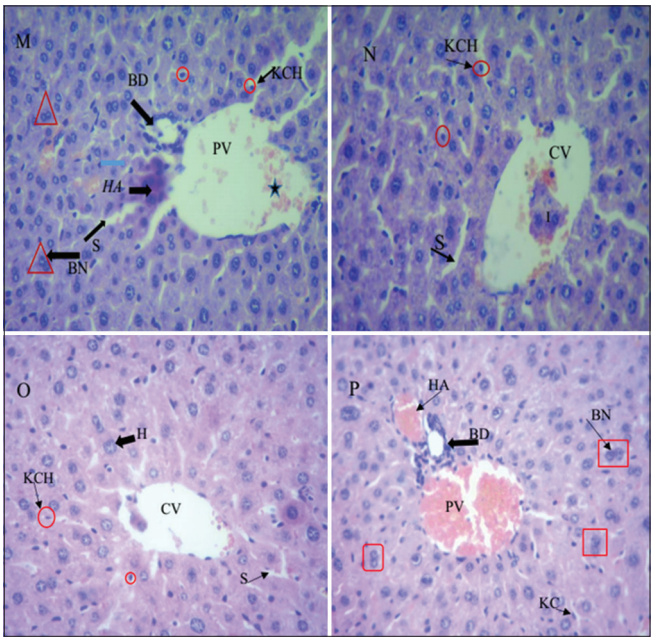

Figure 4: Photomicrographs of liver sections of mice treated with 1000mg/kg/day RN root extract; male (M and N) and female (O and P) showing portal vein (PV) and hepatic artery (HA) congestion and hemorrhage, cellular infiltration in the central vein (CV), sinusoidal dilatation (S), Kupffer cell hyperplasia (KCH) and binucleated hepatocytes (BN), (H and E ×400)

A similar research conducted earlier by Ghosh *et al*.[13] on the psychopharmacological activities of RN root extract in mice and rats revealed that the methanol extract of RN roots altered the behavioral profiles of these animals including reduced exploratory behavioral pattern in the Y-maze and head-dip tests as well as a decline in muscle relaxant activity in rotarod and 30° inclined screen and traction tests. In another work reported by Surjeet *et al*.,[14] methanolic extract of RN leaves administered to albino rats significantly reduced motor coordination in the skeletal muscles. They concluded that RN acts as a skeletal muscle relaxant. We, therefore, speculate that the behavioral changes observed in treated mice in this study may have been due to the toxic component of RN root extract administered to these mice.

The body weights of the extract-treated mice did not show significant changes in both sexes, except in male mice treated with 1000 mg/kg/day of the root extract in weeks 2, 3, and 4 which showed a significant decrease in weight [Table 1]. Changes in body weight are used as an indicator of the adverse effects of drugs and chemicals.[15] In addition, phytochemicals such as tannin and mimosine have been identified as two main antinutrients present in RN. Tannins are bitter-tasting substances, which reduce the digestibility of protein and carbohydrates, including starch and fibers, which in many cases reduce palatability and depress growth in experimental animals.[9,16] It has been considered that foods rich in tannins are of low nutritional value. Chung *et al*.[8] showed in recent findings that the major effect of tannins is not due to their inhibition on food consumption or digestion but due to the diminished efficiency in converting the absorbed nutrients to new body substances. Since tannin has been identified as one of the active substances in RN, the reduction in body weight in mice treated with this extract could be linked to its effects at higher doses.

Organ weight change is an important index for the assessment of toxicity.[17] Liver weights were significantly increased in mice treated with 1000 mg/kg/day of the root extract compared with the controls [Table 2]. This may be due to compensatory cell hyperplasia as a defense mechanism to RN extract exposure. Histopathological sections of mice treated with 1000 mg/kg/day of the extract indicated Kupffer cell hyperplasia and binucleated cells which have negative effects on cell viability and subsequent mitosis.

Table 2: Mean organ weights in *Rumex nepalensis* Spreng. extract-treated mice compared to the controls at 28 days

Group	Treatment (mg/kg/day)	Liver weight (g)	
		Female	Male
I	Control	1.58±0.04	1.79±0.12
II	250	1.56±0.21 (1.00)	1.85±0.08 (1.00)
III	500	1.73±0.09 (0.97)	2.02±0.04 (1.00)
IV	1000	2.04±0.04 (0.14)	2.35±0.14 (0.04)*

*P<0.05 Values are expressed as mean±SEM, n=6. The figures in brackets indicate the calculated P values of the treatment groups as compared with the control. In the males treated with 1000 mg/kg, there was a significant increase in the weight of the liver compared with the controls (P<0.05). SEM: Standard error of mean

Serum ALT, AST, and ALP are well-known enzymes used as biomarkers for predicting possible hepatotoxicity which may be due to bioactive compounds in the body.[18] ALT has been commonly used as a marker to quantify suspected liver cell damage or inflammation.[19] In the present study, the AST and ALP of mice treated at a dose of 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the root extract showed no significant increment, whereas ALT was significantly increased at a dose of 1000 mg/kg/day of the extract in both male and female mice as compared with the controls [Table 3]. The significant increment in the serum levels of ALT, in the present study, was in line with Ferreira *et al*.[20] who reported that serum levels of AST, ALT, and ALP were significantly elevated following treatments of ethanolic leaf extracts of *Rumex abyssinica*. Moreover, it was also consistent with the findings of Tédong *et al*.,[21] in which evaluation of toxicity studies of methanolic extract of *Rumex vesicarius* Linn. showed significant increase in these serum enzymes. In this study, the significant increase of serum levels of ALT in mice treated with the highest dose of extract may be due to a direct destructive effect of the extract on the liver cells.

The liver has fundamental roles in the metabolism of drugs and plant products thus is at a high risk of damage.[22] In the present study, the most common findings in the liver histopathology of male and female mice treated at 500 mg/kg/day of the extract were mild congestion of the central vein and mild dilation of hepatic artery and bile duct [Figure 3]. In addition, in mice administered with 1000 mg/kg/day of the extract, the portal and central veins were more congested, sinusoids were more dilated and Kupffer cells increased in number compared with the control group [Figure 4]. A study conducted on the same species (*R. abyssinica*) plant extract[23] showed gross histopathological changes in the liver in which focal cellular necrosis, congestion, and hemorrhage were observed. Such

Table 3: Serum biochemical parameters of female and male mice treated with *Rumex nepalensis* Spreng. root extract

BP	Sex	Control	Treatment group		
			250 mg/kg (Group II)	500 mg/kg (Group III)	1000 mg/kg (Group IV)
AST (U/L)	Female	105.31±60.80	171.00±33.50 (1.00)	216.50±15.50 (0.98) day	285.00±34.00 (0.59)
	Male	164.00±65.57	172.00±31.53 (1.00)	196.66±52.84 (0.99)	300±30.77 (0.57)
ALT (U/L)	Female	80.66±22.69	100.66±12.99 (0.99)	189.00±19.00 (0.07) day	251.50±32.50 (0.001)*
	Male	39.33±9.76	111.00±25.2390 (0.28)	178.33±14.24 (0.05)	209.5±49.00 (0.002)*
ALP (U/L)	Female	52.25±34.53	188.00±44.73 (0.43)	257.50±12.50 (0.14) day	266.00±34.00 (0.12)
	Male	96.66±31.79	210.66±48.25 (0.62)	223.33±75.10 (0.51)	316±4.00 (0.08)

Values are expressed as mean±SEM. n=6. The figures in brackets indicate the calculated P values of the treatment groups as compared to the controls. *Significant (P<0.05). The mean difference is considered statistically significant at P<0.05. There were significant increases in male and female ALT in Group IV. (P<0.05). BP: Biochemical parameter, SEM: Standard error of mean, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alanine phosphatase

histopathological changes were also reported by other authors following various herbal extract administrations in experimental animals.[24-26]

In the present study, the pathologic changes observed in the histology of the liver could be due to the secondary metabolites that are found in the root extract of RN and may have also induced severe damage to the liver structures.[27]

Conclusion

This subacute toxicity study indicated that the root extract of RN had hepatotoxic effects at 500 mg/kg/day and 1000 mg/kg/day of RN root extract.

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Conflicts of interest

There are no conflicts of interest.

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