

# In vivo Hepatoprotective and in vitro Radical Scavenging Activities of Extracts of *Rumex abyssinicus* Jacq. Rhizome

This article was published in the following Dove Press journal:  
*Journal of Experimental Pharmacology*

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**Background:** Liver diseases contribute a prominent global burden of mortality and morbidity. The current therapies of liver diseases have numerous limitations including severe adverse effects. This denotes that new more effective, safer, and cheaper drugs are required and medicinal plants used in traditional medicines often offer ideal opportunities. Accordingly, the present study aimed to evaluate the in vivo hepatoprotective and in vitro radical scavenging activities of dried rhizome extracts of *Rumex abyssinicus* (*R. abyssinicus*), which is traditionally claimed to provide hepatoprotection.

**Materials and Methods:** Hepatoprotective activity of extracts was evaluated using carbon tetrachloride (CCl<sub>4</sub>)-induced liver injury in mice. Pre- and post-treatment models were employed to test the effect of the extracts and silymarin (standard drug). Serum biochemical markers and liver histopathology were used as parameters to evaluate hepatoprotective activities whereas in vitro radical scavenging activity was tested by 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) assay.

**Results and Conclusion:** Oral administration of CCl<sub>4</sub> (1 ml/kg) significantly ( $P < 0.001$ ) raised the serum levels of liver enzyme markers compared to the normal control group. Pre-treatment with 125, 250, and 500 mg/kg of *R. abyssinicus* extract reduced the serum level of CCl<sub>4</sub>-induced rise in liver enzyme markers with the highest reduction observed at a dose of 500 mg/kg. Likewise, in the post-treatment model, the crude extract and butanol fraction at dose 500 mg/kg reduced levels of liver enzymes. Histopathological examinations revealed lesser liver damage of extract-treated mice compared to the toxic (CCl<sub>4</sub>-treated) controls. The in vitro radical scavenging activity of the different extracts showed concentration-dependent radical scavenging activity. Thus, the results of this study may justify the traditional use of the plant as a hepatoprotective agent.

**Conclusion:** Results of serum biochemical markers and histopathological examinations of CCl<sub>4</sub>-induced mice models, in the present study, show the hepatoprotective potential of extracts from the rhizome of *R. abyssinicus*.

**Keywords:** carbon tetrachloride, hepatoprotective, liver disease, *Rumex abyssinicus*

## Introduction

Liver disease is a cluster term for an array of problems that influences the tissues, structures, and cells of the human liver.<sup>1,2</sup> Generally, liver diseases contribute a prominent global burden of mortality and morbidity.<sup>3</sup> For instance, nearly 2000 cases of acute liver failure occur yearly in the United States.<sup>4</sup> Hospital-based analyses denoted that acute viral hepatitis, chronic hepatitis, hepatocellular carcinoma, and cirrhosis were amenable for at least 12% of medical admissions and over 20% of

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hospital mortality in numerous parts of Africa.<sup>5</sup> Likewise, a clinical study in Ethiopia shows that liver disease accounted for 12% of hospital admissions and 31% of hospital mortality.<sup>6</sup>

One important factor responsible for the development of liver disorder is oxidative stress, often resulting from exposure of the liver to free radicals derived from some xenobiotics and drugs. The most common oxidative liver damage occurs via lipid peroxidation.<sup>7,8</sup> Similarly, CCl<sub>4</sub> causes liver toxicity via free radical-associated damage to liver cells and simulates most human hepatopathologies which makes it a suitable model to assess the efficacy of liver-protective agents.<sup>9</sup> CCl<sub>4</sub> is metabolized in the liver resulting in the formation of free radicals like trichloromethyl and trichloromethyl peroxy radicals, which react with macromolecules such as proteins, lipids, and DNA.<sup>10</sup> Although hepatocytes are protected from free radical-mediated attack by an intrinsic enzymatic and non-enzymatic antioxidant defense system, this antioxidant defense is compromised in certain settings, resulting in oxidative stress.<sup>11–13</sup> In this regard, understanding the impact of oxidative stress and capacity of antioxidants to minimize liver damage may serve an important role in developing more effective hepatoprotective natural products, especially considering that nearly 50% of drugs used in liver diseases are either natural products or their derivatives.<sup>14</sup>

In the present situation, there is an enhanced tendency to discover antioxidants from natural sources as most of the currently available drugs for the treatment of hepatic illnesses are inadequate and have been associated with severe adverse reactions.<sup>14–19</sup> Despite that there have been lots of plant-based medicines used to treat liver ailments,<sup>19</sup> such medicines were not scientifically characterized even in pre-clinical studies using animal models. *Rumex abyssinicus* Jacq. (Polygonaceae), commonly known as “Spinach Rhubarb”, is a medicinal and food additive plant that is widely distributed throughout North Africa, Ethiopia, and in the highlands of tropical Africa.<sup>20,21</sup> The tender shoots and leaves are edible and rhizomes are used to refine butter that give it a rich yellow color, while its roots are utilized for therapy.<sup>20</sup> As a traditional medicinal plant, *R. abyssinicus* roots are claimed to treat various diseases including hypertension, itching skin, vitiligo,<sup>22</sup> toothaches, cancer,<sup>23</sup> malaria, constipation, neuralgia, rheumatism, migraine, ear problems, rabies, scabies, wound, stomachache, typhus, diabetes, and hepatitis.<sup>24,25</sup> Besides, previous studies show that this plant demonstrated different pharmacological activities including anthelmintic,<sup>26</sup> anticancer,<sup>27</sup> wound healing,<sup>28</sup> antibacterial,<sup>29</sup> diuretic, and analgesia.<sup>20</sup> Taking the

above viewpoints into account, the current study was intended to investigate antioxidant and hepatoprotective potential of *R. abyssinicus* root extracts.

## Materials and Methods

### Chemicals

2, 2-Diphenyl-2-picrylhydrazyl hydrate (DPPH), formalin, xylene, Carbon tetrachloride (CCl<sub>4</sub>) (Sigma-Aldrich, Germany), ascorbic acid (S.D. Fine Chemical Limited, India), silymarin (Zhejiang Chemicals Hangzhou, China), methanol (Amaira Petro Chem Pvt. Ltd, France), chloroform (ACS, ISO, Merck), ethyl acetate (Loba Chemie Pvt. Ltd., India), *n*-hexane (Qualikems Fine Chem Pvt. Ltd, India), and *n*-butanol (Faiz Chemical Pvt. Ltd, France). All the chemicals and reagents used were of analytical grade.

### Plant Materials

Rhizomes of *Rumex abyssinicus* were collected from a locality near Gondar city (12.6030° N, 37.4521° E), northern Ethiopia. The plant was authenticated by Mr. Abiyu Enyew (a botanist) at the Herbarium, Biology Department, Faculty of Natural and Computational Science, University of Gondar where a voucher specimen (No. BA001) was deposited.

### Experimental Animals

Healthy female Swiss albino mice (weighing 20–30 g and ages 8 to 12 weeks) were obtained from the animal house of Department of Pharmacology, School of Pharmacy, Mekelle University. Animals were housed in polypropylene cage (6–10 animals per cage), sustained under standard condition (12 h light and 12 h dark cycle; 24°C ± 1°C) and permitted to freely access of standard pellet diet and water ad libitum. After randomize grouping and before initiation of the experiment, animals were acclimatized for 5 days to the laboratory conditions. All procedures used during the study complied with the Guide for the Care and Use of Laboratory Animals<sup>30</sup> and ethical approval to conduct the study was obtained from the Health Research Ethics Review Committee of College of Health Sciences, Mekelle University with ERC number 1024/2017.

### Preparation of Crude Extract and Fractions

Dried powder of *R. abyssinicus* rhizome (600 g) was macerated in 80% methanol for 3 days with manual agitation and occasional shaking using a rotary shaker (BIBBY

Stuart Rotary Shaker S01 UK). At the end of 3 days maceration, the filtrate was separated from the marc (residue), and the marc was re-macerated twice with the same volume of 80% methanol, again each time for 3 days. Extract filtration was made using Whatman No.1 filter paper, and the solvent was evaporated in an oven at 40°C. To prepare solvent fractions, 20 g of the dried crude extract was dissolved in 200 ml distilled water and partitioned into different solvent of increasing polarity as per the procedure described by Otsuka (2006):<sup>31</sup> n-hexane (200 ml x 3), chloroform (200 ml x 3), ethyl acetate (200 ml x 3), n-butanol (200 ml x 3) fractions were prepared by successive solvent-solvent extraction in separating funnel. The filtrates obtained were distilled and concentrated under reduced pressure at a temperature between 40°C and 45°C in a rotary evaporator and dried in an oven (Genlab). All the crude extract and fractions were stored in a refrigerator until used in further studies.

### Acute Oral Toxicity Study

Acute oral toxicity was determined according to the Organization of Economic Co-operation Development (OECD)-425 guidelines.<sup>32</sup> Adult nulliparous and non-pregnant female albino mice were starved for 4 h but with free access to water ad libitum and were acclimatized for 5 days before the study. A total of five animals were employed for this test and received 2000 mg/kg dose of crude extract dissolved in distilled water orally using oral gavages. One animal was dosed in the first day and observed continuously for determination of any sign of toxicity during the first 24 h. Then, the other four animals were dosed similarly since no sign of toxicity was manifested by the first mice. The mice were observed individually for gross behavioral changes at least once during the first 30 min after dosing, periodically with special attention given during the first 4 h of the 24 h, and daily thereafter, for a total of 14 days.

### DPPH Radial Scavenging Activity Assay

The free radical scavenging potential of *R. abyssinicus* crude extract, solvent fractions, and ascorbic acid used as a positive standard control were determined using 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) assay following the procedure described by Braca et al (2001):<sup>33</sup> Briefly, 3 ml of 0.004% methanolic solution of DPPH was mixed with 30 µL of various concentrations (1000, 500, 250, 125, and 62.5 µg/ml) of each test sample (methanol solutions) in different test tubes. After 30-min incubation in the dark at

room temperature, absorbance values were measured at 517 nm using a spectrophotometer (JENWEY6405). Each sample was measured in triplicate and the readings averaged.

### Hepatoprotective Activity Models

#### Pre-Treatment Model

This study was done as per the procedure described by Bhat et al (2014)<sup>34</sup> with some modifications in the technique of blood collection and experimental animals used. Female Swiss albino mice were used for this study and randomly allocated into six experimental groups, each group consisting of six mice. Group I served as a normal control group and received distilled water (10ml/kg, p.o.); group II served as toxic control (CCl<sub>4</sub>) and received distilled water (10 ml/kg, p.o.) while group III received silymarin (100 mg/kg/day, p.o.) as a standard control group; group IV–VI served as extract-treated groups and received three different doses of extract of *R. abyssinicus* (125, 250, and 500 mg/kg/day, p.o.). All treatments were given daily for 7 days. On the 7<sup>th</sup> day of treatments, animals in groups II–VI were fasted overnight and administered with a single oral dose of 1 ml/kg of a freshly prepared CCl<sub>4</sub> in olive oil (1:1 v/v). After 24 h of CCl<sub>4</sub> administration, all the mice were anesthetized by halothane and blood was collected from each mouse from the jugular vein. Serum was separated by centrifugation at 3000 rpm for 5 min and aspirated into test tubes for biochemical analysis.<sup>34</sup> Subsequent to blood collection, each mouse was sacrificed by using the inhaled anesthetic halothane and liver slices were taken for histopathological examination.

#### Post-Treatment Model

In this model, the hepatoprotective activity of *R. abyssinicus* crude extract and the butanol fraction was investigated by using the method described by Wills and Asha (2006)<sup>35</sup> with certain modifications in the dose of CCl<sub>4</sub> and silymarin. Animals were grouped randomly into five groups of six mice. The first group (group I) served as a normal control group and received 1 ml/kg of olive oil on day 1, and distilled water on wards in the rest of the treatment days. Mice in group II (CCl<sub>4</sub> or toxic control group) were administered with a single dose of CCl<sub>4</sub> (1 ml/kg of 1:1 mixture in olive oil) on day 1, and distilled water on wards in the rest of the treatment days. Groups III–V received an oral single dose of CCl<sub>4</sub> (1 ml/kg of 1:1 a mixture in olive oil) on the first day and received *R. abyssinicus* crude extract (500 mg/kg), butanol fraction (500 mg/kg), and silymarin (100 mg/kg), respectively, at 2, 24, and 48 h after CCl<sub>4</sub> administration. All the

animals were sacrificed 72 h after CCl<sub>4</sub> administration. Blood was collected from the jugular vein and serum was separated by centrifugation at 3000 rpm for 5 min.<sup>36</sup> After finishing all the experiments, each mouse was sacrificed under halothane anesthetic.

## Serum Biochemical Analysis

Serum was prepared by centrifugation of blood collected from each mouse. The separated serum was used to measure the liver enzyme markers, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) using blood chemistry analyzer (Humalyzer, Germany).

## Histopathological Examination

Liver tissue was carefully dissected out, washed with 0.9% normal saline solution, and preserved in a 10% formalin solution for fixation. Dipper sections of the tissue were cut using microtome and stained with hematoxylin and eosin dye for microscopic examination with low power field (10X or 40X), the stained sections were examined and photographed under a light microscope (Olympus CHS six headed).

## Preliminary Phytochemical Screening

Preliminary phytochemical screening of *R. abyssinicus* rhizome extract was carried using the method described by Trease and Evans, (1989) and Jones and Kinghorn, (2006) for the presence or absence of phytoconstituents such as anthraquinones, alkaloids, saponins, phenols, flavonoids, tannins, and terpenoids.<sup>37,38</sup>

## Statistical Analysis

For the in vivo hepatoprotective models the result was calculated using the Statistical Package for the Social Science (SPSS) program (Version 21.0) and reported as mean ± standard error of the mean (SEM). The data were analyzed by one-way analysis of variance (ANOVA) and post hoc Tukey's test.  $P < 0.05$  was considered statistically significant and the down shown formula was employed to compute the percentage protection of the extracts:

$$(\%) \text{Protection} = \frac{a - b}{a - c} \times 100 \quad (1)$$

where a is the mean value of the marker produced by hepatotoxin; b is the mean value of the marker produced by toxin plus test sample; c is the mean value of the marker produced by the vehicle control.<sup>36</sup> While for the

in vitro DPPH radical scavenging activity assay, the result was expressed as 50% inhibitory concentration (IC<sub>50</sub>) values, representing the concentration of sample required to scavenge 50% DPPH free radicals, which were calculated from a concentration versus % inhibition graph of each sample.<sup>33</sup> The following formula was used to calculate percentage inhibition:

$$(\%) \text{Inhibition} = \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}} \times 100 \quad (2)$$

where A<sub>blank</sub> is the absorbance of the DPPH, and A<sub>sample</sub> is the absorbance of test samples.

## Results

### Extract and Fractions Yield

The 80% methanol extraction method resulted in a yellowish water-soluble crude extract with a percentage yield of 14% (w/w) while the percentage yields (w/w) of the solvent fractions were 0.6% (petroleum ether), 1.8% (chloroform), 13% (ethyl acetate), 10.5% (butanol), and 47.5% (aqueous).

### Acute Oral Toxicity Study

Administration of the 80% methanolic extract of the dried rhizomes of *R. abyssinicus* at a dose of 2000 mg/kg did not produce any gross behavioral changes, such as loss of appetite, hair erection, lacrimation, tremors, convulsions, salivation, diarrhea, and mortality during the 14 days observation period. Hence, the extract was considered to be safe to proceed in further experimentation.

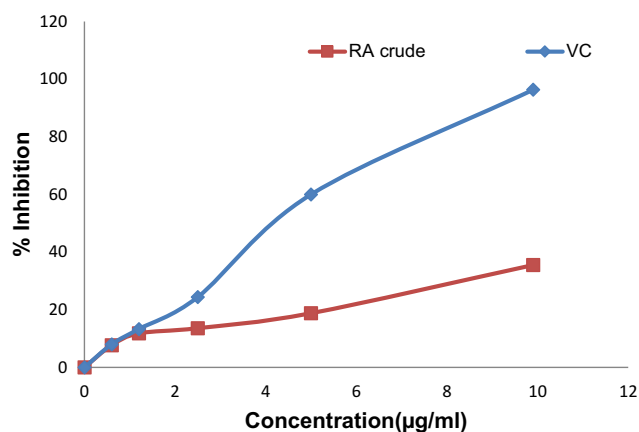
### Radical Scavenging Activity (DPPH Assay)

The crude extract and solvent fractions of rhizomes of *R. abyssinicus* exhibited concentration-dependent radical scavenging activity over the tested concentration ranges (Figures 1 and 2). The IC<sub>50</sub> value of the crude extract was 13.1 µg/ml and that of ascorbic acid was 4.9 µg/ml. Similarly, the different solvent fractions showed different degrees of radical scavenging activities with IC<sub>50</sub> values as shown in Table 1. Of all fractions, the butanol fraction (6.1 µg/ml) was associated with the highest scavenging activity.

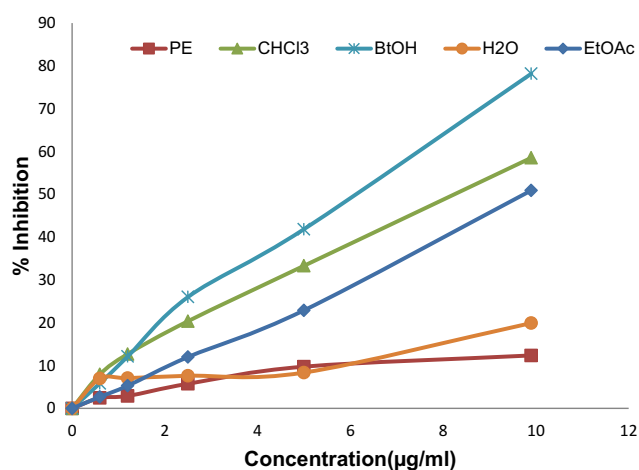
## Hepatoprotective Activity

### Serum Biochemical Analysis in the Pretreatment Model

Hepatoprotective activity of the 80% methanolic extract of *R. abyssinicus* at 125, 250, and 500 mg/kg was determined



**Figure 1** DPPH radical scavenging activity of different concentrations of dried rhizomes of *R. abyssinicus* crude extract (RA-crude) and vitamin C (VC).



**Figure 2** DPPH radical scavenging activity of different concentrations of solvent fractions: petroleum ether (PE), chloroform (CHCl<sub>3</sub>), ethyl acetate (EtOAc), butanol (BtOH), and aqueous (H<sub>2</sub>O) fractions from *R. abyssinicus* extract.

by estimating levels of ALT, AST, and ALP (Table 2) and histopathological examinations (Figure 3). As shown in Table 2, the mean value of ALT of normal animals was  $44.7 \pm 8.9$  U/L, while in the CCl<sub>4</sub> (1 ml/kg)-treated groups it

**Table 1** IC<sub>50</sub> Values of DPPH Radical Scavenging Activity of the Solvent Fractions of *Rumex abyssinicus* in Comparison with Vitamin C

Test Samples	IC <sub>50</sub> (µg/ml)
Vitamin C	4.8
Petroleum ether fraction	34.4
Chloroform fraction	8.0
Ethyl acetate fraction	9.9
Butanol fraction	6.1
Aqueous fraction	24.1

was  $145.3 \pm 11.4$  U/L which was a statistically significant ( $p < 0.001$ ) elevation when compared to the normal control groups. Pretreatment with 80% methanolic extract of *R. abyssinicus* at doses of 125, 250, and 500 mg/kg brought the level of this enzyme to  $121.7 \pm 9.3$  U/L,  $97.6 \pm 7.2$  U/L ( $p < 0.01$ ) and  $93.7 \pm 7.5$  U/L ( $p < 0.01$ ) respectively, while silymarin (100 mg/kg) reduced the level to  $65.5 \pm 7.5$  U/L ( $p < 0.001$ ). The effects of the extract at 250 and 500 mg/kg were statistically significant ( $p < 0.01$ ) and comparable with silymarin. A similar pattern was reflected with AST and ALP (Table 2). Furthermore, in the 500 mg/kg pre-treated mice, the levels of ALT and AST were significantly ( $p < 0.01$ ) reduced but there was no statistically significant difference in the level of ALP. Overall administration of the crude extract of *R. abyssinicus* at a dose of 500 mg/kg reduced the serum ALT (51.3%), ALP (63.8%) as well as the level of AST (73.9%) better than silymarin (69%). On the other hand, pre-treating mice with a dose of 125 mg/kg do not show a statistically significant difference in the reduction of the levels of all enzyme markers. Similarly, in the 250 mg/kg pre-treated mice, no statistically significant difference was seen in the levels of AST and ALP as compared to CCl<sub>4</sub>-treated group.

#### Serum Biochemical Analysis in the Post-Treatment Model

In this study, CCl<sub>4</sub> groups showed significant ( $p < 0.001$ ) increase in serum ALT, AST, and ALP levels compared with the normal control group while treatment of mice with 500 mg/kg of crude extract, butanol fraction, and silymarin significantly lowered ( $p < 0.01$  to  $p < 0.001$ ) these alteration (Table 3). The mean value of ALT of normal control mice was  $67.2 \pm 6.2$  U/L while the level of CCl<sub>4</sub> (1 ml/kg) received group was  $205.6 \pm 28.1$  U/L which is a statistically significant ( $p < 0.001$ ) elevation. Post-treatment with 80% methanolic crude extract of *R. abyssinicus* and its butanol fraction, which has shown a strong radical scavenging activity at the dose of 500 mg/kg, brought the level of this enzyme to  $106.8 \pm 13$  U/L ( $p < 0.01$ ) and  $103 \pm 12.6$  U/L ( $p < 0.01$ ), respectively. These reductions were statistically significant and comparable to silymarin (100 mg/kg), which reduced the level to  $97.1 \pm 9.2$  U/L ( $p < 0.001$ ). For instance, mice treated with butanol fraction of *R. abyssinicus* at a dose of 500 mg/kg had a reduced level of ALT by 74.1% while silymarin reduced ALT levels by 78.4%. Likewise, levels of AST and ALP showed a similar pattern of reduction in the treatment group as compared to CCl<sub>4</sub>-treated group.

**Table 2** Effects of Different Concentrations of *R. abyssinicus* Crude Extract and Silymarin on Serum Biochemical Parameters

Group	Dose	ALT (U/L)	AST (U/L)	ALP (U/L)
DW	10 ml/kg	44.7 ± 8.9	89.4 ± 9.8	92.8 ± 14.1
CCl <sub>4</sub>	1 ml/kg	145.3 ± 11.4 <sup>b**</sup>	226.7 ± 26.2 <sup>b**</sup>	236.3 ± 34.8 <sup>b*</sup>
Silymarin + CCl <sub>4</sub>	100 mg/kg	65.5 ± 7.5 <sup>a**</sup>	131.8 ± 11.5 <sup>a*</sup>	124.3 ± 21.5
RA+ CCl <sub>4</sub>	125 mg/kg	121.7 ± 9.3	226.7 ± 15.1	200.6 ± 27.8
RA + CCl <sub>4</sub>	250 mg/kg	97.6 ± 7.2 <sup>a*</sup>	195.4 ± 18.5	191.2 ± 25.0
RA + CCl <sub>4</sub>	500 mg/kg	93.7 ± 7.5 <sup>a*</sup>	125.2 ± 6.1 <sup>a*</sup>	144.7 ± 31.8

**Notes:** Data presented as mean ± S.E.M. <sup>a</sup>Compared to CCl<sub>4</sub>, <sup>b</sup>Compared with normal control (DW), \**p* < 0.01, \*\**p* < 0.001. One-way ANOVA followed by Tukey test; *n* = 6. **Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; RA, *Rumex abyssinicus*; DW, distilled water; CCl<sub>4</sub>, carbon tetrachloride.

### Histopathological Studies in the Pre-Treatment Model

Histopathological examination of the liver sections under a light microscope revealed that normal control-treated group showed normal liver histology (Figure 3A) whereas the CCl<sub>4</sub>-treated group showed extensive multifocal necrosis, disorganization of the hepatic plate, degenerated nuclei, severe lymphocytic infiltrates, marked cellular swelling, and fatty change (Figure 3B). The liver histopathology of the 125 mg/kg extract pre-treated mice was similar to that of CCl<sub>4</sub>-treated mice (Figure 3C). The liver section from 250 mg/kg extract pre-treated mice showed moderate necrosis and lymphocytic infiltrates (Figure 3D) while the extract at 500 mg/kg resulted in maintained architecture, mild necrosis and mild lymphocytic infiltrates (Figure 3E), which was quite similar to that of silymarin pre-treatments that showed normal hepatic architecture, no necrosis and mild lymphocytic infiltrates in most of the mice (Figure 3F).

### Histopathological Studies in the Post-Treatment Model

Like the pre-treatment model, liver sections from the control group, mice showed normal histology of the liver (Figure 4A) unlike the CCl<sub>4</sub> received group that showed severe periportal necrosis, hepatocyte swelling, degenerated nuclei, marked (severe) lymphocytic infiltration, congested portal vein, and inflammation (Figure 4B). On the other hand, the 500 mg/kg extract treatments showed mild necrosis, mild periportal hepatocytes swelling, mild lymphocyte infiltrate and mild cellular swelling (Figure 4C) while the butanol fraction at a dose of 500 mg/kg prevented necrosis in almost all mice although there were congested blood vessels, mild periportal hepatocyte swelling, and mild lymphocytic infiltrates in the liver sections

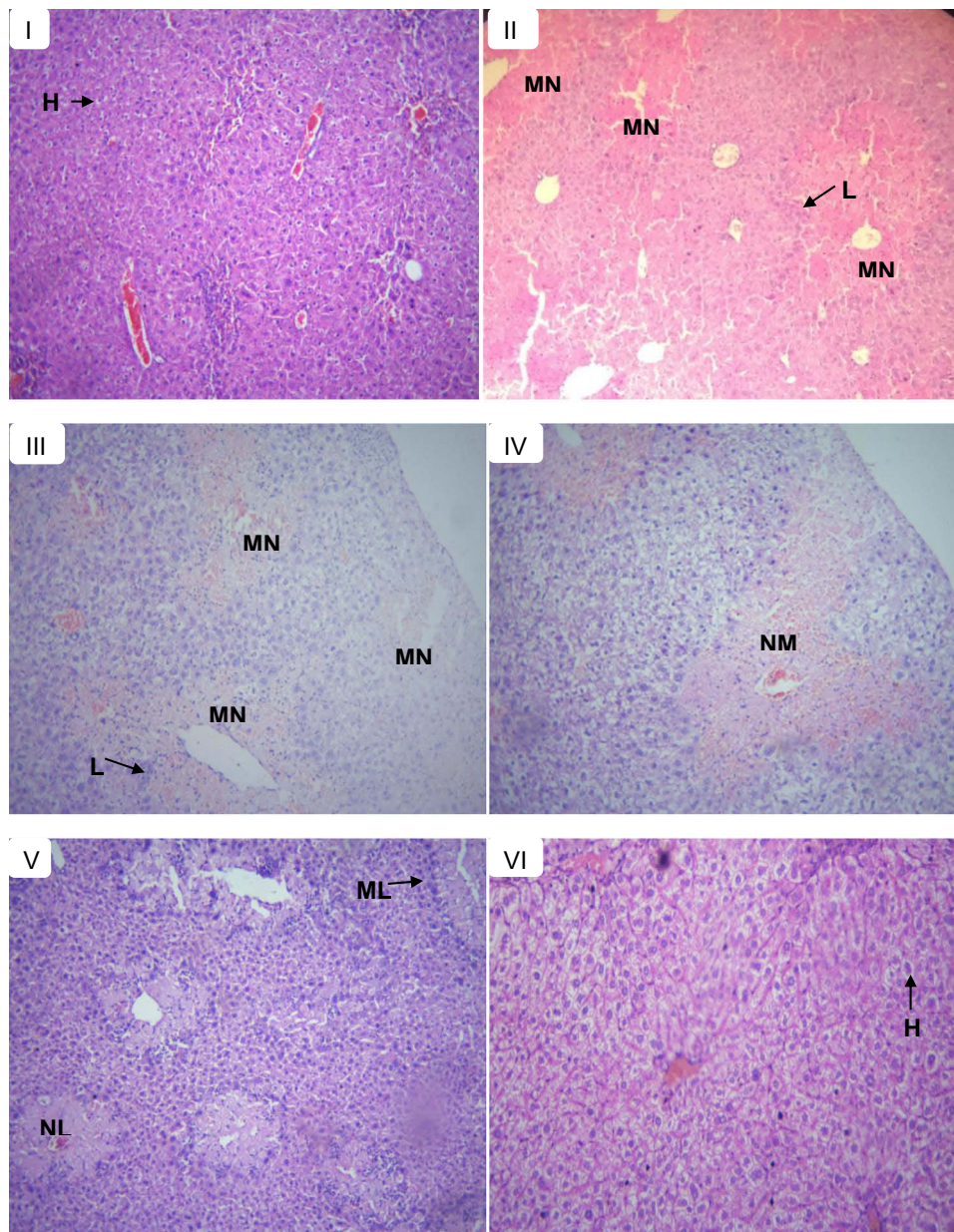
of some mice (Figure 4D). The results obtained from both extract and butanol fraction were similar to silymarin which showed no necrosis, mild lymphocytic infiltrates, dilated portal vein, periportal hepatocytes (Figure 4E).

### Preliminary Phytochemical Screening

The preliminary phytochemical screening of *R. abyssinicus* crude extracts revealed the possible presence of alkaloids, anthraquinones, phenols, flavonoids, saponins, tannins, and terpenoids. Moreover, previous phytochemical studies on *R. abyssinicus* showed the isolation of methyl gallate (a phenolic acid),<sup>39</sup> anthraquinones (chrysofanol, emodin, emodin-8-O-β-D-glucopyranoside, helminthosporin, physcion, and physcion-8-O-β-D-glucopyranoside),<sup>39,40</sup> flavanols (epicatechin, epicatechin-3-O-gallate, and epicatechin-3-O-(4''methyl)gallate),<sup>39</sup> betulone (a triterpenoid), and oleic acid (a fatty acid) from this plant.<sup>29</sup>

### Discussion

In the present study, an attempt has been made to find out the hepatoprotective activity of *R. abyssinicus* extract using pre- and post-treatment mice models of CCl<sub>4</sub> induced hepatotoxicity. Pre-treatment studies were done to investigate the preventive effects while post-treatment studies were done to investigate the efficacy of the test samples in curing liver damage. The dose which produced the greatest effect in the pre-treatment model (500 mg/kg) and the fraction (butanol fraction) which showed the highest DPPH radical scavenging activity was chosen for the post-treatment study. During studies of both pre-treatment and post-treatment models, exposure to CCl<sub>4</sub> (1 ml/kg) resulted in substantial increase in levels of ALT, AST, and ALP within 24 h, and this increment is a hallmark to damage of structural integrity of the liver. As these enzymes occur in the cytoplasm, their release to



**Figure 3** Histopathological section of liver tissues in control and experimental groups of mice in the pre-treatment model; showing normal hepatic cells (H), lymphocytic infiltrates (L), multifocal necrosis (MN), moderate necrosis (NM), mild lymphocytic infiltrates (ML), and mild necrosis (NL). (A) Normal control received with distilled water, (B) toxic control received  $\text{CCl}_4$ , (C) treated with 125 mg/kg extract, (D) treated with 250 mg/kg extract, (E) treated with 500 mg/kg extract, and (F) treated with silymarin 100 mg/kg.

circulation indicates cellular damage or hepatic injury.<sup>41</sup> In both models, the *R. abyssinicus* extracts decreased ALT, AST, and ALP levels. These results are in line with previous reports on different *Rumex* species including *R. vesicularis*,<sup>8</sup> *R. hastatus*,<sup>21</sup> *R. pictus*,<sup>42</sup> and *R. dentatus*.<sup>43</sup> Interestingly, in the pre-treatment model, the dried rhizomes of *R. abyssinicus* at a dose of 500 mg/kg showed comparable hepatoprotective activity

to that produced by 100 mg/kg of silymarin (a well-known plant preparation used as standard positive control and potent hepatoprotective agent in preventing liver pathologies in chemical-induced models). Reversal of serum enzymes by the extracts is an indication for the protection of the liver from  $\text{CCl}_4$ -induced damage. Most of the time, it is regarded as proper when the raised amount of the transaminases restores to normal owing to the healing of

**Table 3** Effect of the 80% Methanolic Extract and Butanol Fraction Obtained from the Dried Rhizomes of *R. abyssinicus* and Silymarin Against CCl<sub>4</sub>-Induced Hepatotoxicity on Serum Biochemical Parameters

Group	Dose	ALT (U/L)	AST (U/L)	ALP (U/L)
DW	10 ml/kg	67.2 ± 6.2	116.6 ± 11.1	128.2 ± 5.8
CCl <sub>4</sub>	1 ml/kg	205.6 ± 28.1 <sup>b**</sup>	347.3 ± 18.8 <sup>b**</sup>	359.8 ± 41.7 <sup>b**</sup>
RA + CCl <sub>4</sub>	500 mg/kg	106.8 ± 13 <sup>a*</sup>	232.6 ± 14.3 <sup>a**</sup>	231.6 ± 10.2 <sup>a*</sup>
BU-RA + CCl <sub>4</sub>	500 mg/kg	103 ± 12.6 <sup>a*</sup>	211.1 ± 6.8 <sup>a**</sup>	224.1 ± 3.3 <sup>a*</sup>
Silymarin + CCl <sub>4</sub>	100 mg/kg	97.1 ± 9.2 <sup>a**</sup>	220.1 ± 11.6 <sup>a**</sup>	180.4 ± 16.6 <sup>a**</sup>

**Notes:** Data presented as mean ± S.E.M. <sup>a</sup>Compared to CCl<sub>4</sub>, <sup>b</sup>Compared with normal control (DW), \*p < 0.01, \*\*p < 0.001. One-way ANOVA followed by Tuckey test; n = 6. **Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; RA, *R. abyssinicus*; BU-RA, butanol fraction of *R. abyssinicus*; DW, distilled water; CCl<sub>4</sub>, carbon tetrachloride.

hepatic parenchyma and the regeneration of hepatocytes.<sup>44</sup> The mechanism by which *R. abyssinicus* extract produced anti-hepatotoxic effects needs further studies. Yet, one possibility could be related to its free radical scavenging activities. Free radicals are associated with various groups of ailments and are responsible for causing heterogeneous pathological events. Antioxidants neutralize the effect of free radicals and thereby protect us from various ailments including liver disease. These agents exert their action either by scavenging reactive free radicals or by enhancing the endogenous antioxidant defense mechanisms.<sup>45</sup> In the DPPH radical scavenging activity assay, the results indicated that *R. abyssinicus* crude extract and its solvent fractions show concentration-dependent radical scavenging activity which was comparable to ascorbic acid. A similar study on extracts of *R. crispus* leaves and seeds reported concentration-dependent antioxidant activity.<sup>46</sup>

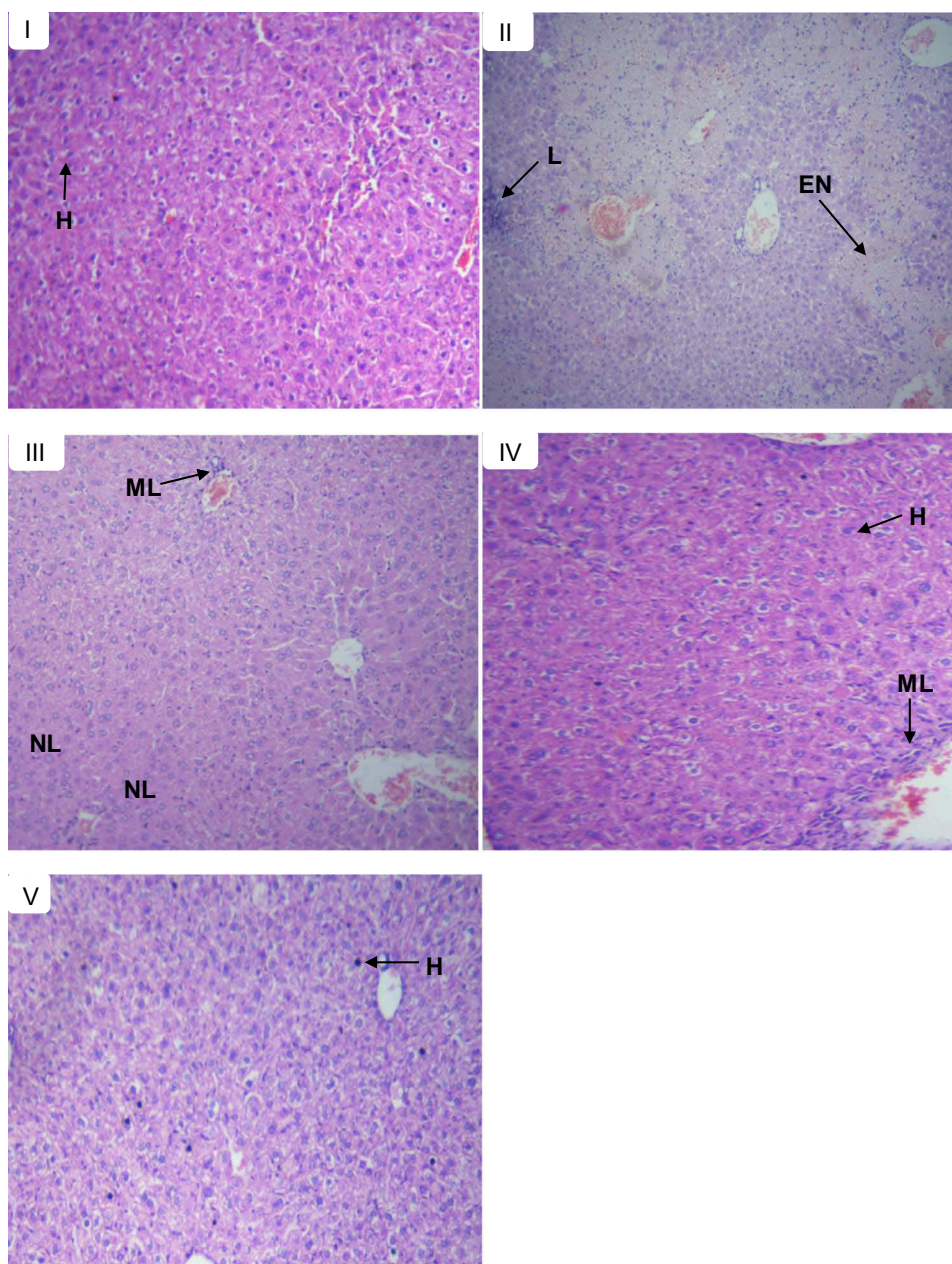
In the current study, the histological studies also showed the hepatoprotective potential of *R. abyssinicus* extracts in addition to the serum enzyme markers. *R. abyssinicus* crude extract at doses of 125 and 250 mg/kg resulted in weak and moderate hepatoprotective activity, respectively, but treatment with the higher dose (500 mg/kg) and silymarin (100 mg/kg) revealed substantial protective effect as compared to the CCl<sub>4</sub>-treated group. Girma et al (2015) showed that *R. abyssinicus* is rich in a number of anthraquinones that showed Cyclooxygenase-2 (COX-2) inhibitory activity and strong anti-inflammatory property which in turn may help the regeneration of hepatocytes.<sup>27</sup> In line with our findings; this report supports the hepatoprotective potential of *R. abyssinicus* extracts as the pharmacological activity produced by plant extracts is usually attributed to the presence of secondary metabolites within them.<sup>47</sup> In the

present study, preliminary phytochemical screening of *R. abyssinicus* extract showed the possible presence of anthraquinones, alkaloids, saponins, phenols, flavonoids, tannins, and terpenoids which were also consistent with previously reported studies.<sup>28,39,48</sup> Especially, flavonoids possess an antioxidant property, which may be useful in the treatment of liver disease.<sup>49</sup> For example, the (-)-epicatechin, a flavanol<sup>50</sup> has been demonstrated to possess hepatoprotective activity. Also, previous studies reported different bioactive anthraquinones in *R. abyssinicus* like chrysophanic acid, chrysophanol, emodin, and physcion,<sup>29,39,51</sup> of which chrysophanol,<sup>52</sup> emodin,<sup>53,54</sup> and physcion<sup>55</sup> did show the ability to scavenge free-radicals implying that may serve as natural antioxidant compounds. In line with this notion, emodin, chrysophanol, and physcion were reported to have a potential liver-protecting activity<sup>55</sup> which could be due to their antioxidant activity.<sup>56</sup> In addition to this, the hepatoprotective activity of emodin has been reported in a number of studies.<sup>56–59</sup> Taken together, these findings indicate that phytoconstituents individually or synergistically may initiate a host of responsible mechanisms for the hepatoprotective activity, which in turn can give a potential clue for the use of this plant in the treatment of liver disease.

## Conclusions

The results of measurements of serum biochemical markers and histopathological examinations from pre-treatment and post-treatment mice models, in the present study, revealed the possible hepatoprotective effects of the extracts of rhizomes of *R. abyssinicus*. These results may have an implicit association with the traditional use of the plant for the treatment of liver disease. The in vitro radical scavenging activity of the extracts may, at least in part, explain the possible hepatoprotective mechanism of the





**Figure 4** Histopathological section of liver tissues in control and experimental groups of mice in the post-treatment model; showing normal hepatic cells (H), lymphocytic infiltrates (L), mild lymphocytic infiltrates (ML), mild necrosis (NL), and extensive necrosis (EN). (A) Normal control received with distilled water, (B) toxic control received  $\text{CCl}_4$ , (C) treated with 500 mg/kg extract, (D) treated with 500 mg/kg butanol fraction, and (E) treated with silymarin 100 mg/kg.

plant. Further work to establish bioactivity guided isolation of lead compound(s) responsible for hepatoprotective activities may be suggested.

## Abbreviations

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase;  $\text{CCl}_4$ , carbon tetrachloride; DPPH, 2, 2-diphenyl-2-picrylhydrazyl hydrate; OECD, Organization of Economic Co-operation Development.

## Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author up one request.

## Ethical Approval

The ethical approval for this study was obtained from the Health Research Ethics Review Committee (HRERC) of College of Health Sciences, Mekelle University with ERC

number 1024/2017. The experimental animals were handled according to the Guide for the Care and Use of Laboratory Animals.

## Acknowledgments

The authors would like to express thanks to the Department of Pharmacognosy and Pharmacology, School of Pharmacy, Mekelle University for providing laboratory facilities for the research work. We also would like to thank Mr. Abiyu Enyew (botanist), for the authentication of the plant material. The principal investigator would like to thank the school of pharmacy, University of Gondar for covering her living expenses throughout the study period.

## Author Contributions

All authors contribute to the conception, data analysis and interpretation, drafting and critical reviewing of the article, gave final approval upon which the final version of the article will be submitted, and agreed to take responsibility and to be accountable for all aspects of the work. BAA conceived the idea, drafted the proposal, and collected the plant materials. BAA and EMA carried out the actual experiments and statistical analysis. YKE and BAA prepared and critically reviewed the final manuscript for publication. MGH and GP were involved in the design of the study, involved at all implementation stage of the work, and revising the manuscript critically for important intellectual content. BS involved in developing and reviewing the proposal. All authors read and approved the final version of the manuscript.

## Disclosure

The authors do not have any conflict of interest to disclose.

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