Hepatoprotective effect of hydromethanol extract of *Otostegia integrifolia* benth leaves in isoniazid and rifampicin induced Swiss albino mice

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ARTICLE INFO

ABSTRACT

Introduction: Drug-induced liver injury is the most common cause of acute liver failure. Off-Target effect "hepatotoxicity "frequently detected during clinical examination of patients on *anti*-Tb medication particularly isoniazid (INH), and rifampin (RMP). However, there is no any treatment option against isoniazid and rifampicin induced hepatotoxicity. It is, therefore, necessary to search for effective affordable and safe drugs from medicinal plants for the prevention of liver toxicity caused by isoniazid and rifampicin. The aim the current study is to evaluate hepatoprotective effect of hydro methanol extract from *Otostegia integrifolia* leaves in isoniazid and rifampicin-induced hepatotoxicity in Swiss albino mice.

Methods: O. integrifolia leaves powder was macerated in hydromethanol and thirty Swiss albino mice 29.0–40.6 g were grouped in to five groups. Group I were given 20 ml/kg distilled water, group II were given 100 mg INH and 150 mg RIF per kg body weight. Group III, group IV, and group V were given 200 mg extract, 400 mg extract, and 100 mg of *N*-acetyl cysteine respectively per kg 1hr before induction with 100 mg INH plus 150 mg RIF per kg. The treatments were followed for 14 days. On the 15th day, all mice were anaesthetized with diethyl ether; blood samples were collected for the assessment liver enzyme and function test.

Results: Group II mice's serum ALT, AST and total bilirubin levels were significantly increased and serum total protein and albumin levels were significantly decreased as compared with group I mice. The groups of mice treated with *O. integrifolia* at a dose of 400 mg/kg and *N*-acetyl cysteine AST, ALT and total bilirubin level were significantly decreased; and total protein and albumin levels were significantly ($P < 0.05$) increased as compared with group II. The liver index of the group IV showed decreased ($P < 0.05$) as compared to the group II.

Conclusion: Evidence from our study revealed that the hydromethanol extract of O. integrifolia has a hepatoprotective effect against isoniazid and rifampicin-induced hepatotoxicity in Swiss Albino mice. This protective effect of O. integrifolia extract may be based on its metal ion reducing power, free radical scavenging activity, and anti-inflammatory activity and could be used as a potential therapeutic option.

1. Introduction

Liver is the most important organ in xenobiotic metabolism/detoxification, in which exogenous lipophilic xenobiotics or drugs are converted to hydrophilic compounds via biochemical processes catalyzed by the p450 enzyme system. Recently, more than 1000 drugs produce direct toxicity or via production of toxic (reactive) metabolites, which, leading to changes in protein conformational structure, DNA mutation, or actuating lipid peroxidation, subsequently results to hypersensitivity reaction or drug induced liver injury (DILI) [1,2].

Tuberculosis (TB) continues to be the leading health problem all over the world. With the increment of human immunodeficiency infection

(HIV) patients, an increment within the frequency of tuberculosis got to be a major issue around the world. In the treatment of TB, the short course combination chemotherapy of isoniazid (INH), rifampin (RIF), pyrazinamide, and ethambutolare extremely effective and chosen as first-line anti-tubercular drugs due to their unique physicochemical properties and efficacy. However, among many more off-target effect, hepatotoxicity is a potential and serious acute or chronic sequale of isoniazid (INH), and rifampin (RIF), drugs in clinical practice of Tb treatment [3,4]. Isoniazid and rifampicin exact hepatotoxicity mechanisms are not well known [5]. However, those drugs can promote formation of reactive oxygen species (ROS) and induce dysregulated exuberrent inflammation, oxidative stress induced liver injury [6]. INH

and RIF actively induce CYP2E1, which plays a great role in INH-mediated hepatotoxicity by stimulating the formation of hydrazine via the amidase pathway [7,8]. Hydrazine can reduce the antioxidant level of the cell via binding with the glutathione (GSH) sulfhydryl group [9,10], and results oxidative stress and cell death. INH increases the peroxidation of membrane lipid by the formation of ROS and its metabolite act as a peroxidation stimulator. Peroxidation of accumulated lipid leads to the formation of toxic reactive aldehyde byproducts and downstream effects, such as impaired membrane integrity and mitochondrial dysfunction [6,11]. In other way co-administration of isoniazid and rifampicin metabolites stimulates Kuffer cells which cause activation of proinflammatory cytokines receptors [12,13]. Collectivelly, oxidative stress and inflammation are interdependent processes, one of which can be easily induced by another and results cell death [14]. Excpet discuntination of the offending drug, by far there is no any treatment option used to tackle antituberculosis drug induced hepatotoxicity as well as liver failure. The search for drugs that can be used to prevent liver toxicity due to anti-tuberculosis drugs remains important. Studying the hepatoprotective effect of medicinal plants has great clinical importance [15].

There are several kinds of medicinal plants with hepatoprotective and antioxidative activities. One of those plants, *Otostegia integrifolia* which has shown its potential to be therapeutic in averting several diseases in Ethiopia. *O. integrifolia* is under *Otost*egia genus and Lamiaceae family. It is endemic to Ethiopia, Eritrea, and Yemen. It is commonly known as Abyssinian rose, by its Amharic name "Tinjut" (ጥንጁት) an erect perennial shrub with long oval grey green leaves. It has green –white colored flowers and fruits that are small nutlets within the calyx [16]. Studies have shown that, it is also used for the treatment of many diseases such as hyperglycemic, ophthalmia and malaria, and used in preventing many kinds of sickness and disorders like stomachache, vomiting, nausea, diarrhea, and dysentery because its antioxidant and antibiotic properties [17,18]. Moreover, its hydro alcoholic leaf extract has been demonstrated that antimalarial [16], antihypertension [19], antidiabetic [20], analgesic, anti-inflammatory [21], *anti*-leishmaniosis [22], antimicrobial and antioxidant [18] activities in the past. The study from the same genus *Otostegia persica* indicated the presence of antioxidants and antiinflammatory compounds like quercetin, morin, isovitexin, and kaempferol [23]. Moreover, effectiveness of *O. persica* in protective of liver against toxicant [24] could indicate that *O. integrifolia* (in the same genus) may also be a potential hepatoprotective agent that has to be investigated. To the best of our knowledge, no scientific study has been done to evaluate the potential of *O. integrifolia* as a hepato-protectant in INH and RIF-induced hepatotoxicity. Therefore, considering the high incidence of tuberculosis as well as hepatitis in developing countries and the insufficiency of any cheap and effective modern drug-protecting liver, the present study aimed to investigate the hepatoprotective activity of hydromethanol extract of *O. integrifolia* leaves in INH and RIF-induced hepatotoxicity in Swiss albino mice by assessing biochemical parameters.

2. Materials and methods

2.1. Equpmentes and reagents

To conduct this research, different types of chemicals and reagents were used. These were, ferric chloride (FeCl₃) (BDH chemical pool), potassium iodide (BDH chemical pool), iodine (BDH chemical pool), sodium nitrite (NaNO₂) (BDH chemical pool), Hydrochloric acid (BDH chemical pool), sulfuric acid (H₂SO₄) (BDH chemical pool), sodium hydroxide (NaOH) (BDH chemical pool), nitric acid (HNO₃) (BDH chemical pool), Isoniazid and Rifampicin combnation drugs, *N*-acetyl cysteine, 99.5 % methanol, ethanol, formalin, xylene, paraffin, diethyl ether (BDH chemical pool), distilled water, and sodium chloride (NaCl) (BDH chemical pool). Automated chemistry analyzer (humastar 200), whatman no-1 filter paper, gavages (oral feeding syringes), oven (made

in France), desiccators (2 L), flasks (2 L,1 L, 0.5 L), funnel, rotary evaporator (yomato, made from America), lyophilizer (FD-12 made from China), centrifuge scientific LTD (made in West Sussex U.K) and electrical weight balance (ADG 3000 L ADAM).

2.2. Collection and extraction of Otostegia integrifolia leaves

Fresh *O. integrifolia* leaves were collected at Maksegnit Woreda, Central Gondar Zone, Amhara Region, Ethiopia (Alt.:2133 m, Lat.: 12° 39′ N and Long.: 37° 55' E) and the plant was Authenticated by the University of Gondar Department of Biology Botanist and a voucher number ባዮ/ጅ/870/11/2014 has been given and deposited in herbarium of the department. The collected leaves of the plant were transported to the University of Gondar Department of Biochemistry laboratory and the fresh plant leaves were washed with distilled water to remove dirt and soil, and dried under shade and optimal ventilation. The dried leaves were powdered by using electrical grinder. The 600 g of powder was macerated for 72 h with an automatic shaker [25] with a 1 g m powder to 7 ml of 80 % methanol ratio for the first extraction. The resulting liquid extract was filtered using a Whatman no. 1 filter paper and the residue was re-extracted for the second and third time by adding another fresh solvent (5 ml of 80 % methanol to 1 g m powder ratio). The fluid extracts were combined and concentrated in a rotary evaporator under reduced pressure at 40 °C and 70 rpm and then its remaining part was placed in an oven at 40 °C. The concentrated result was frozen in a deep refrigerator and dried in a lyophilizer and kept in a refrigerator (4 °C) with browen bottle [21]. The extract product was used for phytochemical test after two days and for hepatoprotective assay after nine days.

2.3. Phytochemical screening

Preliminary qualitative phytochemical screening of the plant extract was carried out by using the standard procedures [26,27] for detection of the present of secondary metabolites like saponins, flavonoids, alkaloids, tannins, phenols, and glycosides were tested by using color forming and precipitate forming substances.

2.4. Experimental animals

Mouse is a commonly used model for the studies on INH [28]. In the current study, healthy male Swiss albino mice weight (initial) between 29.00 and 40.6 g and with no prior drug treatment was used. The Mice used for the study were obtained from University of Gondar College of Medicine and Health Science School of Pharmacy, Pharmacology Department. The animals were housed in polypropylene cages under standard conditions, maintained on a 12:12 h light-dark cycle at ambient room temperature (22 ± 2 °C), and have free access to food (pellet diet) and water being ad libitum up to the date of experimentation. The mice were acclimatized to the laboratory conditions for about seven days before the beginning of the experiments. All animal handling and care was done as per the guidelines set by the National Research Council of the National Academies press, Washington, D.C., USA s [29].

2.5. Grouping of experimental animals and treatment protocol

In this study, 100 mg/kg isoniazid and 150 mg/kg rifampicin combination drug were used for induction of hepatotoxicity by slight modification of the previously described 100 mg/kg INH plus 200 mg/kg RIF dose by Guo YX et al. [30]. The dose (200 mg/kg and 400 mg/kg) of methanol extract of the *O. integrifolia* leaves was selected according to acute toxicity study results conducted by Endale, An et al. [16]. The calculated dose of the plant extract was dissolved in 20 ml/kg of distilled water and administered orally to each animal of treatment groups 1 h before induction of isoniazid plus rifampicin. *N*- Acetyl cysteine enhancing intracellular glutathione via elevated intracellular cysteine,

and this can scavenge peroxynitrite and hydroxyl radicals as well as convert hydrogen peroxide to water. 100 mg *N*- Acetyl cysteine per kg weight was used as reference in this study [31–33].

Thirty mice initially weighted between 29.00 and 40.6 g were selected and then coded one to thirty randomly and grouped into five groups' six mice in each group randomly and then groups were assigned one to five randomly.

A. Mice in Group I (Normal control group) were treated with 20 ml/kg distilled water.
B. Mice in Group II (Negative control group) were treated with 100 mg INH plus 150 mg RIF combination per kg body weight.
C. Mice in Group III (Experimental group) were treated with 200 mg hydromethanol extract of *O. integrifolia* per kg body weight of mice 1 h before induction with 100 mg INH plus 150 mg RIF combination per kg body weight.
D. Mice in Group IV (Experimental group) were treated with 400 mg hydromethanol extract of *O. integrifolia* per kg body weight of mice 1hr before induction with 100 mg INH plus 150 mg RIF combination per kg body weight.
E. Mice in Group V (Positive control group) were treated with 100 mg *N*-acetyl cysteine/kg body weight 1hr before induction with 100 mg INH plus 150 mg RIF combination per kg body weight. The treatments were given orally once per day at 4:00 a.m. (local time) for 14 consecutive days using oral gavage.

All experimental test were conducted two or three or four times from x-y 2022.

2.6. Body weight and liver index

Body weights of mice were measured by using electrical balance before starting the treatment at day zero and at the end of treatment at day 15th. And also liver index were calculated after liver cut out from mice at day 15th based on the following formula.

$$Liver\ index\% = \frac{liver\ weight}{bodyweight\ at\ day\ 15} x\ 100\%\qquad(34)$$

2.7. Blood sample collection

At the end of the treatment, animals were fasting overnight and were anaesthetized by inhaled diethyl ether 1.9 % (0.08 ml/Liter of container volume) soaked in cotton wool in a desiccator [34]. Immediately each animal was placed in a supine position on the operating board. The extremities of the animals were stretched and fixed on a dissecting board. The abdominal cavity was opened, and a 1–1.5 ml blood sample was withdrawn from each mouse by cardiac puncture on the left-hand side of mice using a sterile needle of a 3 ml syringe. The blood sample was transferred to a sterile test tube and allowed to clot. After 30 min the test tube was centrifuged at 3000 rpm for 15 min to separate the serum. Finally, the separated serum sample was transported to University of Gondar Leishmaniosis Research and Treatment Center Laboratory for biochemical analysis.

2.8. Biochemical assay

Biochemical assays were conducted at the University of Gondar Leishmaniosis Research and Treatment Center Laboratory by using commercially available assay kits used as per the protocols provided by the manufacturers (Roche Diagnostics and the Roche/Hitachi Analyzer) to measure the liver damage marker enzymes (ALT and AST) and other proteins, such astotal protein, albumin and bilirubin in the serum samples.

2.9. Statstical analysis

Data were entered with epidata version 4.6 and transferred to the SPSS version 26 program to analyze and one-way ANOVA followed by Tukey post-hoc test for comparisons between groups were used. And also paired *t*-test was used to analyze the mean difference in initial (day 0) and final body weight (day 15) of groups of mice. Data presented in the study were expressed as mean ± standard error. The difference was considered significant with the value of $P < 0.05$.

3. Results

3.1. Phytochemical screening tests

The phytochemical screening tests indicate that the hydromethanol extract of the *O. integrifolia* leaf contains glycosides, flavonoids, saponin, triterpenoids and phenolic acids (Table 1).

3.2. Effect of O. integrifolia extract in body weight of INH and RIF induced mice

The initial body weight of mice ranged from 29 to 40.6 with 35.23 ± 3.53 mean initial body weight. Statistically significant difference has been observed in the mean initial body weight of group I (Normal control) (34.30 ± 1.81) and Group V (*N*-acetyl cysteine) mice (35.67 ± 1.19) when compared with their final mean body weight (Table 2). However, no significant difference was observed between group II (INH and RIF), group III (Extract 200 mg/kg) and group IV (Extract 400 mg/kg) mices' mean initial body weight and their mean final body weight (day 15th).

3.3. Effect of O. integrifolia extract in liver index of INH and RIF induced mice

Group II mice treated only with INH and RIF (7.39 ± 0.19) has been shown a significant ($P < 0.001$) elevated liver index as compared to normal control group I mice (5.93 ± 0.31) which were treated only with distilled water and with group V (*N*-acetyl cysteine control). The liver indexes of group IV mice (6.2 ± 0.29) treated with *O. integrifolia* leaves extract at a dose of 400 mg/kg plus INH and RIF were shown significantly ($P < 0.05$) decreased as compared to group II mice. Even though mice in group III (6.70 ± 0.12) were treated with extract 200 mg/kg plus INH and RIF, they showed a slight decrease liver index as compared to negative control group II mice (Fig. 1).

The results were expressed as mean ± SEM: ANOVA followed by Tukey's post hoc test, (n = 6). "A" statistically significant (p < 0.01) difference as compared with "b", "c" describes statistically significant (p < 0.05) difference as compared with "b". (n = 6).

Table 1
The result of phytochemical screening of hydro-methanol extract of *O. integrifolia* leaves.

Phytochemical constituent	Result
Alkaloid	–
Flavonoids	a
Glycosides	a
Saponins	a
Tannins	–
Triterpenoids	a
Steroids	a
Phenolic acid	a

[a] Stands for the presence of phytochemicals, - stands for the absence of phytochemicals.

Table 2

Effect of hydromethanol extract of O. integrifolia on body weight.

Groups	Initial body weight	Final body weight	Mean body weight difference	p-value
Group I (Normal control)	34.30 ± 1.81	36.05 ± 1.61	−1.75	0.006[a]
Group II (INH and RIF)	34.86 ± 0.97	34.49 ± 1.08	0.37	0.40
Group III (Extract 200 mg/kg)	36.27 ± 1.91	36.58 ± 2.08	−0.32	0.393
Group IV(Extract 400 mg/kg)	35.20 ± 1.39	35.78 ± 1.35	−0.58	0.234
Group V (*N*-acetyl cysteine Control)	35.67 ± 1.19	37.08 ± 1.39	−1.42	0.006[a]

The results were expressed as mean ± SEM, Paired *t*-test was used for comparing the mean of initial and final bodyweight, (n = 6), the value with "a" superscript describes $p < 0.05$.

3.4. Effect of O. integrifolia leaves extract in serum ALT level

The serum ALT level has been increased significantly ($p < 0.001$) in INH and RIF treated group (84.00 ± 12.97) as compared to normal control group-I (35.00 ± 2.92) mice. The serum ALT level in INH and RIF +400 mg/kg leaves extract treated mice (42.67 ± 5.35) has decreased significantly ($p < 0.001$) as compared to INH and RIF only treated mice. Serum ALT level of group IV mice show near to *N*-acetyl cysteine treated mice serum ALT level. Extract 200 mg/kg + INH and RIF treated mice showed significantly increase ($p < 0.05$) serum ALT level (61.83 ± 6.74) as compared with normal control group of mice and significantly decrease ($p < 0.05$) as compared with negative control group mice (Table 3).

3.5. Effect of O. integrifolia extract in serum AST level

Serum AST level in group-II (175.50 ± 21.64) mice has been increased significantly ($p < 0.001$) as compared to group-I mice (50.16 ± 15.11) (Table 4). Serum AST levels of group IV mice (94.83 ± 19.26) and *N*-acetyl control group mice (76.16 ± 14.70) have been significant decreased ($p < 0.01$) as compared with group-II mice. There is no significant serum AST level difference between group I, IV, and V mice. Serum AST level of group III mice (131.17 ± 25.93) have been significantly ($p < 0.01$) increased as compared with group-I mice and decrease as compared with INH and RIF only treated group II mice.

3.6. Effect of O. integrifolia leaves extract in serum TP level

The mice given INH and RIF showed a statistically significant decrease in serum total protein level (4.68 ± 0.29) as compared to normal control group mice (6.44 ± 0.19) and the serum total protein level of group IV mice (6.07 ± 0.40) was showed a significant increment as compared with group II. The serum total protein level of group IV mice was similar to that of *N*-acetyl cysteine drug treated mice (6.21 ± 0.18) [4]. However, the serum total protein level of group III mice (5.53 ± 0.26) treated with 200 mg/kg extract plus INH and RIF showed a statistically significant ($p < 0.05$) elevation as compared with drug only

Table 3

Effect of hydromethanol extract of O. integrifolia leaves on serum AST and ALT levels.

Groups	ALT (IU/L)	AST (IU/L)
Group I (Normal control)	35.00 ± 2.92[a]***	50.16 ± 15.11[a]***
Group II (INH and RIF)	84.00 ± 12.97	175.50 ± 21.64
Group III (Extract 200 mg/kg)	62.83 ± 6.67[ab]*	131.17 ± 25.93[ab]*
Group IV (Extract 400 mg/kg)	42.67 ± 5.35[a]**	94.83 ± 19.26[a]**
Group V (*N*-acetyl cysteine control)	37.01 ± 2.5[a]***	76.16 ± 14.70[a]***

The results were expressed as mean ± SEM: ANOVA followed by Tukey's post hoc test, (n = 6). Values with "a" indicate that statistically significant difference with group II; Values with "ab" superscript describe that statistically significant difference with group I and II. *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$).

Table 4

Effect of hydromethanol extract of *O. integrifolia* leaves on serum total protein, total bilirubin and albumin levels.

Groups	Total Protein (g/dl)	Albumin (g/dl)	Total Bilirubin (mg/dl)
Group I (Normal control)	6.44 ± 0.19[a]**	3.43 ± 0.23[a]***	0.52 ± 0.1[a]**
Group II (INH and RIF)	4.68 ± 0.29	2.46 ± 0.12	0.92 ± 0.09
Group III (Extract 200 mg/kg)	5.53 ± 0.26[ab]*	2.78 ± 0.13	0.71 ± 0.2
Group IV (Extract 400 mg/kg)	6.07 ± 0.40[a]	3.13 ± 0.15[a]*	0.56 ± 0.06[a]*
Group V (*N*-acetyl cysteine control)	6.21 ± 0.18[a]**	3.21 ± 0.19[a]*	0.54 ± 0.03[a]*

The results were expressed as mean ± SEM: ANOVA followed by Tukey's post hoc test, (n = 6). Values with "a" superscript indicate that statistically significant difference with group II, Values with "b" superscript indicate that statistically significant difference with group I × $p < 0.05$, **$p < 0.01$.
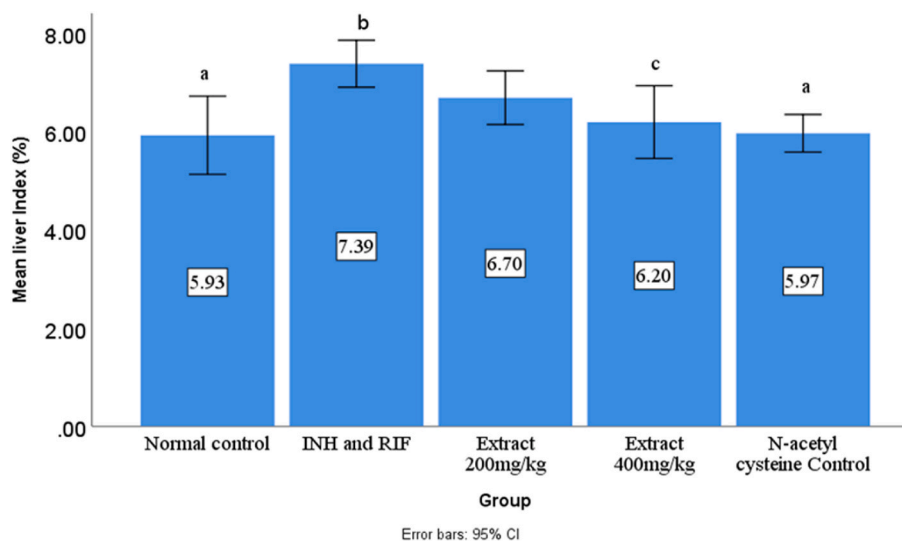

Fig. 1. Effect of hydromethanol extract of Otostegia integrifolia leaves on liver index.

treated mice. A significant decrease (p < 0.05) in serum total protein level has been shown in group III as compared to group I.

3.7. Effect of O. integrifolia leaves extract in serum albumin level

Serum albumin level was found to be decreased (P < 0.01) in group II mice (2.46 ± 0.12) as compared with group I mice (3.43 ± 0.23). An increased (P < 0.05) serum albumin level has been observed in mice treated with O. integrifolia extract at a dose of 400 mg/kg plus INH and RIF (3.13 ± 0.15) as compared to drug only treated mice. O. integrifolia extract-treated mice at a dose of 400 mg/kg have shown a slight similarity to *N*-acetyl cysteine-treated mice (3.21 ± 0.19). Even though O. integrifolia extract at a dose of 200 mg/kg has slightly increased serum albumin level (2.78 ± 0.13) as compared to drug-only treated mice.

3.8. Effect of O. integrifolia leaves extract in serum total bilirubin level

Significant (p < 0.01) increment of serum total bilirubin level (0.92 ± 0.09) was shown in group II mice as compared with normal control group mice (0.52 ± 0.1). However, significant decrement of serum total bilirubin level was shown in *O. integrifolia* leaves extract at a dose of 400 mg/kg plus INH and RIF induced group of mice (0.56 ± 0.06) as compared with group II (INH and RIF) mice (0.92 ± 0.09). There was statistically no significant difference in serum total bilirubin levels between group I, IV and V mice. There was no statistically significant decrement of serum total bilirubin level in group III (200 mg/kg) mice (0.71 ± 0.2) has been shown as compared to INH and RIF only treated group of mice.

4. Discussion

The most frequent off-target effects of *anti*-TB drugs hepatotoxicity or drug induced liver injury (DILI) [35]. DILI is spectrums of three clinicopathologic presentation: hepatocellular injury (injury to hepatocytes), cholestatic injury (toxicity to biliary tree that disrupts bile flow), or a mixed pattern of injury [36].

In the present research study, mice hepatotoxicity was induced by oral administration of isoniazid and rifampicin combination drugs daily for 14 days. After 14-day treatment, significant increment was observed in the levels of ALT (p < 0.001), AST (P < 0.001) and total bilirubin (p < 0.01) in negative control group mice when compared with normal control group mice. This is in accordance with previous reports showing an increase in ALT, AST and total bilirubin content in INH and RIF treated humans and experimental animals [30,37,38]. Thus, the increase in the activity of ALT and AST clearly showed as marked hepatocellular damage.

Furthermore *O. integrifolia* extract treatment at a dose of 200 mg/kg (Group III) showed a significant lowering effect in serum ALT and AST levels as compared with the INH and RIF treated group of mice (group II). It could also be suggested that the administration of *O. integrifolia* extract may preventive role of hepatocyte damage which might be related with to the maintenance of plasma membrane integrity hepatocyte, thereby suppressing the leakage of enzymes through membranes. However, it has been shown a significant increase (P < 0.05) in ALT and AST levels as compared with a normal control group. Whereas, a dose of 400 mg/kg extract showed significant reduction of serum ALT and AST level (p < 0.01) as compared with INH and RIF treated group of mice. At the dose of 400 mg/kg extract, there was no significant increase in serum AST and ALT levels as compared with normal control group and *N*-acetyl cysteine treated group mice. In agreement with this, a study conducted in by Ref. [39] on *Ajuga parviflora* leaves in INH and RIF induced rats [40], on *Ballota glandulosissima* aerial parts and [41] on *O. persica* aerial parts in CCL$_4$ induced liver injury rats. Those plants are in the same Lamiaceae family. Moreover, a significant decrease (p < 0.01) ALT and (P < 0.05) AST level was observed in the *N*-acetyl cysteine treated mice as compared to only the INH and RIF treated mice. This ALT and AST

level of *N*-acetyl cysteine treated group result is similar to those previously done by Ref. [42].

Serum level of AST and ALT decreasing effect of methanol extract of *O. integrifolia* as compared with group II might be due to good metal ion reducing power and high free radical scavenging activity of *O. integrifolia* extract. Those activities of *O. integrifolia* were conducted in vitro studies [18]. Free radicals and metal ions are initiators for membrane lipid peroxidation [43]. Isoniazid can metabolize into reactive intermediates, which can initiate the formation of other free radicals and lipid peroxidation by abstracting a bisallylic hydrogen atom from unsaturated fatty acids [44]. Lipid peroxidation can cause for released of AST and ALT from the cytoplasm and mitochondria of the cells to the blood. Or it may be because of their secondary metabolites which can inhibit the expression of CYP2E1 proteins. Like flavonoids have hepatoprotective activity by inhibiting the expression of CYP2E1, which produces free radicals and ROS to promote OS and lipid peroxidation (LPO), by scavenging free radicals and by inhibiting enzymes which are participating in free radical formation and inflammation [45]. *N*- acetyl cysteine have ability to prevent hepatic damage by membrane stabilization through inhibiting membranes damage [33].

Furthermore, the present study pointed out a notable decrease in serum total protein level and albumin level in INH and RIF only treated group of mice as compared with the normal control group. *O. integrifolia* treated group of mice at a dose of 200 mg/kg showed a significant decrease in serum total protein as compared with normal control group mice and a slight increase in serum total protein and albumin level as compared with negative control group mice. Whereas the *O. integrifolia* at a dose of 400 mg/kg showed near to *N*- acetyl control group serum total protein level and albumin level as compared with only INH and RIF treated group of mice. The result of this finding is in line with the previous study conducted on *O. persica* [41] and *Ajuga parviflora* [39]. This may be due to the suppressing proinflammatory cytokine like IL-6 signaling pathway in the liver by the secondary metabolites of the plant extract. Since, one factor in decreasing albumin synthesis is the response of the liver to proinflammatory cytokine [46]. During an injury, the body focuses on producing proteins involved with the immune system, such as C reactive proteins. Under these types of stress, the liver synthesizes more immune-related proteins and fewer albumins [47]. But this study total protein result have been not in line with the study conducted by Ref. [15] on *Vitex negundo*. This may be because of different in dose, followed time and model.

Bilirubin is transported to the liver hepatocytes by albumin for detoxification. One of the possible causes of serum bilirubin level elevation is liver injury, which decreases the efficiency of the liver to produce albumin and detoxify bilirubin [48]. In this study serum bilirubin levels in drug only induced mice showed a significant (P < 0.01) increase as compared with a normal control group of mice. This study result is in agreement with the data of other authors showing that RIF produced competition for the elimination of bilirubin by the liver [49] and a combination of INH and RIF resulted in a higher rate of inhibition hepatic clearance of bile, an increase in lipid peroxidation in the hepatocyte, and cytochrome P450 involved in the synergistic effect of RIF and INH [30,50,51]. Whereas *O. integrifolia* leaves extract induced mice challenged with INH and RIF (G IV) did not show a significant difference as compared with the normal control group. The plant extract at a dose of 400 mg/kg showed a significantly decreased (p < 0.05) serum bilirubin level as compared with a negative control group mice. This study result is consistence with the study conducted by Refs. [15,39,41]. This might be because of the hepatic preventive effect of the secondary metabolites which leads to decrease lipid peroxidation and inhibit the activity of CYP2E1.

Moreover, our study showed that liver index for the INH and RIF only treated group mice was increased (7.39 %) concerning the normal control group (5.93 %); this increase was significant (p < 0.01). The increased liver index in the isoniazid and rifampicin group may be due to fatty changes in the liver because of altering of metabolic function of

liver cells. This result is in agreement with the data of other authors showing that repeated administration of rifampicin (50 mg/kg i. p. Daily for 6 days) increased liver weight, microsomal cytochrome P-450 and heme contents in mice [52]. However, liver index values were less in the *N*-acetyl cysteine control group (5.97 %) (p < 0.01), as well as the *O. integrifolia* treatment groups 200 mg/kg (6.7 %) (p > 0.05) and 400 mg/kg (6.2 %) (0.05) as compared with a negative control group. This result is in line with previously conducted by Ref. [53] on *Cnidoscolus chayamansa*. *Cnidoscolus chayamansa* have similar phytochemical compositions with *O. integrifolia*. This may be because the mitochondria in liver cells continue to carry out all of their tasks, which includes lipid metabolism.

Isoniazid and rifampicin consumed negative control group mice slightly decreased in body weight at day 15. This may be due to impair body's metabolic system because of liver injury [8]. However, significant (p < 0.01) difference between the initial and final body weight (day 15) of the normal control and *N*-acetyl cysteine groups mice was shown. Slightly increased in body weight of experimental groups of mice have occurred, this may be the protective effect of the extract against INH and RIF induced metabolic alter.

This study phytochemical screening test result showed that *O. integrifolia* leaves hydromethanol extract contains flavonoids, phenols, terpenoids, saponins, steroids and glycosides secondary metabolites. This finding has been also expressed previously [18,22]. Its hepatoprotective activity of the *O. integrifolia* leaves extracts may be due to the presence of those secondary metabolites. Since, flavonoids like quercetin show improve nonalcoholic fatty liver disease by reducing lipid peroxidation, inflammation, and oxidative stress [54]. Quercetin, morin, isovitexin, and kaempferol flavonoids are identified in *O. persica* [23]. Morin acts as an inhibitor of acute liver damage by blocking the expressions of inflammatory cytokines and mediators including TNF-α, IL-1β, IL-6, and iNOS [55]. Quercetin showed hepatoprotective effect in D2M-induced NAFLD by inhibiting expression of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α, by antioxidant and improved lipid metabolism [54]. Also Phenols like curcumin has hepatoprotective effect by antioxidant and antiinflammatory activity [56]. Oleanolic and ursolic acid are terpenoids showed hepatoprotective in antitubercular drug-induced liver damage [57]. The effect of *O. integrifolia* on serum level of lipid profile, and liver tissue antioxidant enzyme activates needs to be analyzed.

5. Conclusion

Novel theraputic drugs are almost always developed from plant origin, and then *Otostegia integrifolia* leaves hydromethanol extract clearly proofs the protective role against INH and PIF induced hepatocellular damage. Especially at a higher dose (400 mg/kg) serum level of liver membrane damage biomarker and liver function biomarker have been relatively near to the *N*-acetyl cysteine treated control group of the study mice. Hence, *Otostegia integrifolia* leaves hydromethanol extract at 400 mg/kg dose have a hepatoprotective effect against isoniazid and rifampicin induced toxicity in Swiss albino mice. This hepatoprotective effect of *O. integrifolia* may be due to its metal ion reducing power, free radical scavenging, lipid peroxidation inhibition and anti-inflammatory activities.

Data availability

Data support these findings are confined within the manuscript and will share up on request to the corresponding author.

Ethical approval and considerations

Ethical clearance was requested before the experiment starts and it was obtained from the University of Gondar, College of Medicine and Health Science, School of Medicine research review and ethics committee with reference number SOM/1737/2022. Then the research was performed as per the agreement. The uses of animals and all activities in this experimental study were carried out according to the regulation of animal care [29].

Author contribution statement

Endalkachew Gugsa: Participated in conception, study design, execution, and acquisition of the study. Tadesse Asmamaw Dejenie participated in the original writing of the manuscript; participated in the Supervision of the study work. The also actively participated in the article's critical review, gave final approval of the version that would be published, selected the journal to which the article would be submitted, and agreed to be held accountable for all aspects of the work. Tewodros Shibabaw Molla participated in the Supervision of the study work. The also actively participated in the article's critical review, gave final approval of the version that would be published, selected the journal to which the article would be submitted, and agreed to be held accountable for all aspects of the work. Tesfahun Bekele participated in the Supervision of the study work. The also actively participated in the article's critical review, gave final approval of the version that would be published, selected the journal to which the article would be submitted, and agreed to be held accountable for all aspects of the work.

Abbreviation

INH	Isoniazid
RIF	Rifampicin
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase

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