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Ameliorative property of the ethanolic extract of *Tragopogon graminifolius* DC. on carbon tetrachloride-induced hepatotoxicity in mice: A pharmacological examination

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ABSTRACT

Hepatotoxicity is the most common disorder with severe effects on quality of life. *Tragopogon graminifolius* DC. has been used in Iran as an astringent and bleeding inhibitor, wound healer and gastro-protector agent. To our knowledge, there are little evidence at hand on hepatoprotective activity of *T. graminifolius*. The present study was carried out to assess hepatoprotective property of *T. graminifolius* ethanolic extract on carbon tetrachloride (CCl_4) induced hepatotoxicity in mice for 45 consecutive days. Fifty male mice were divided into five groups (n=10). Group 1 (control) received 1 ml/kg olive oil intraperitoneally (i.p.) and distilled water orally; Group 2 (untreated) received CCl_4 (50% in olive oil, 1 ml/kg; i.p.); Groups 3, 4 and 5 received CCl_4 and 30, 90 and 270 mg/kg of *T. graminifolius* (T30, T90 and T270), respectively. At 45th day, the mice were killed, dissected, then blood and liver samples were collected for biochemical and stereological parameters analysis. Biochemically, *T. graminifolius* at all doses (especially T270) could significantly ($p \leq 0.05$) reduce the raised levels of cholesterol, low density lipoprotein (LDL), triglyceride, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), total and conjugated bilirubin, and increase the decreased levels of high density lipoprotein (HDL), total protein, albumin, superoxide dismutase (SOD) and catalase (CAT). Stereologically, different doses of *T. graminifolius* (especially T270) decreased the weight and volume of the hepatic structures as compared to the untreated group significantly ($p \leq 0.05$). The obtained results indicate that *T. graminifolius* ethanolic extract, potential applications as a therapeutical supplement or medicine, after confirming clinical trial studies.

Conflicts of Interest: Declared None
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Keywords
Tragopogon graminifolius DC., Ethanolic extract, Hepatoprotective property, Carbon tetrachloride

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INTRODUCTION

Liver dysfunctions or injuries have been recognized as serious health problems [1]. Especially acute and chronic liver injuries resulted from exposure to drugs, toxic chemicals and virus infiltration from infection or ingestion, have

gained more attention in recent years [2]. Carbon tetrachloride (CCl_4) is a toxic substance for most organs of the body such as brain, testes, kidneys, heart and liver [3]. Furthermore, several documented case studies have reported CCl_4 induced hepatic disease by changing antioxidant status in animals and humans [3]. Findings from different ethno medicinal plants screening have revealed their antioxidant and protective activities against CCl_4 by increasing the concentrations of antioxidant enzymes [4].

Medicinal plants are popular remedies used by most people [5-8]. The impression of ethno medicinal plants in prevention, control and treatment of diseases are irrecusably [9-13]. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments [11-13]. *Tragopogon graminifolius* DC. grows widely in western parts of Iran and is important as an ethno medicinal plant [14]. *T. graminifolius* has long been used to treat poisoning and as a gastro-protector, a wound healer, astringent and bleeding inhibitor in Iranian traditional medicine [14]. It is also used for healing digestive bleeding and pulmonary and digestive ulcer [15]. Active constitutes of *Tragopogon* genus are flavonoids including; apigenin, isoorientin, isovitexin, lucenin, luteolin, orientin, quercetin, vitexin, swertisin and vicenin-1 and 2 [16,17].

High prevalence of hepatotoxicity in the whole world has drawn the attention of researchers in finding remedial and preventive methods to control and treat the disease. In this regard, we attempted to study the hepatoprotective property of *T. graminifolius* ethanolic extract leaf on the treatment of hepatotoxicity in mice.

MATERIALS AND METHODS

Plant collection and extraction

T. graminifolius was collected from Kermanshah city (geographical coordinates: 34.3277° N, 47.0778° E), Kermanshah province, Iran. Then, the leaves of the plant were dried in shadow and after grinding, each time 100 gr of the obtained powder was dissolved in 1000 cc ethanol and put in Soxhlet extractor for 8 h (Total 1000 gr). The collected extract was filtered by Whatman filter paper no 1 and steamed into a glass container at the solvent temperature. The remaining dried extract was poured into a glass container and weighed. The powder of the obtained extract was weighed as required depending on the dose [5].

Animals

Male Balb/c mice weighing between 38-40 gr were procured from laboratory animal center of Kermanshah University of Medical Sciences. The animals were housed in an air-conditioned room (22±2 °C) with 12 h light/dark cycle and had free access to the standard pellet diet and water *ad libitum* conditions during the study. Animal studies were approved by the Local Research Ethics Committee of Razi University, Kermanshah, Iran with the ethical code of 397-3-001.

Experimental design

In the present study, a total of 50 mice were used. The mice were divided into five groups each containing ten mice each. Group I served as control, received 1mL/kg olive oil intraperitoneally and 0.5 mL distilled water through gavage. Group II served as untreated group, received 1 ml/kg CCl_4 (CAS Number 56-23-5, Merck company, Germany) mixed with olive oil in the ratio of 1:1, intraperitoneally and 0.5 mL distilled water orally. Group III, IV and V received CCl_4 mixed with olive oil in the ratio of 1:1 intraperitoneally and 30, 90 and 270 mg/kg of *T. graminifolius* (T30, T90 and T270) through gavage, respectively. The animals treated twice a week for 45 consecutive days. At the end of 45th day of the treatment, animals in all groups were euthanized by ketamine HCl (40 mg/kg) [4]. Then blood samples were drawn from mice heart and inserted in serum tubes for determination of cholesterol, low density lipoprotein, high-density lipoprotein (HDL), triglyceride, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), total protein, albumin, and total and conjugated bilirubin. All the above mentioned parameters were measured by available commercial kits (Pars Azmun CO, Iran) according to their procedures. Also, capacity of antioxidant enzymes was assessed by determining the activities of superoxide dismutase (SOD) and catalase (CAT) in the whole liver for each group (n=5) using the procedures reported by Abei (1974) and Martin *et al.* (1987) [18,19].

Stereological study

Liver of each group (n=5) was dissected out and washed with ice cold saline to remove blood. The livers were then weighed and immersed in 10% neutral buffered formaldehyde. The liver volume was obtained from liver dipping method in water. After 72h fixation, the livers were cut using the orientator method. Totally, 7–10 slabs were collected from each liver. The slabs were embedded in paraffin and sections (5 µm thicknesses) were prepared and stained by hematoxyline and eosin stain.

Volume density of the liver structures including hepatocytes, sinusoids, central veins, portal veins, hepatic arteries, and bile ducts were estimated with point counting rule briefly as follow: one section from each liver was used. The images of microscopical fields from each section were projected on point probe (frame 15cm×15cm) by video projector via microscope equipped with a camera (Dinocapture ver.5, dino-lit.com 30.5 mm) attached to the computer.

At the total magnification of 2000×, points that hit desired structures were counted and volume density was estimated using the following formula:

$$Vv = P_{structure} / P_{reference}$$

where $P_{structure}$ and $P_{reference}$ were the numbers of points falling the structure's profile and on the reference space, respectively. 10-14 microscopic fields were examined in each liver. The absolute volume of the structures was esti-

mated by multiplying the fractional volume by the final volume of the liver to prevent the reference trap [20,21].

Statistical analysis

Data expressed as mean ± SD and were analyzed by one way ANOVA and Duncan's test. P≤0.05 was considered significant.

RESULTS

Effect of T. graminifolius ethanolic extract on the levels of liver biochemical parameters

The estimated values of liver biochemical parameters are presented in Figures 1 to 5. CCl_4 - induced toxicity enhanced the levels of cholesterol, LDL, triglyceride (Fig. 1), ALP, AST, ALT, GGT (Fig. 2), total and conjugated bilirubin (Fig. 3) and decreased the levels of HDL, total protein, albumin (Fig. 4), SOD and CAT (Fig. 5) in comparison with the control group, significantly (p≤0.05). Different doses of *T. graminifolius* ethanolic extract considerably (p≤0.05) improved all above mentioned parameters in comparison with the untreated group. No remarkable (p≤0.05) difference was observed among T30, T90, T270 and the control groups in the level of triglyceride. T90 and T270 significantly (p≤0.05) regulated the levels of total and conjugated bilirubin like those observed in the control group.

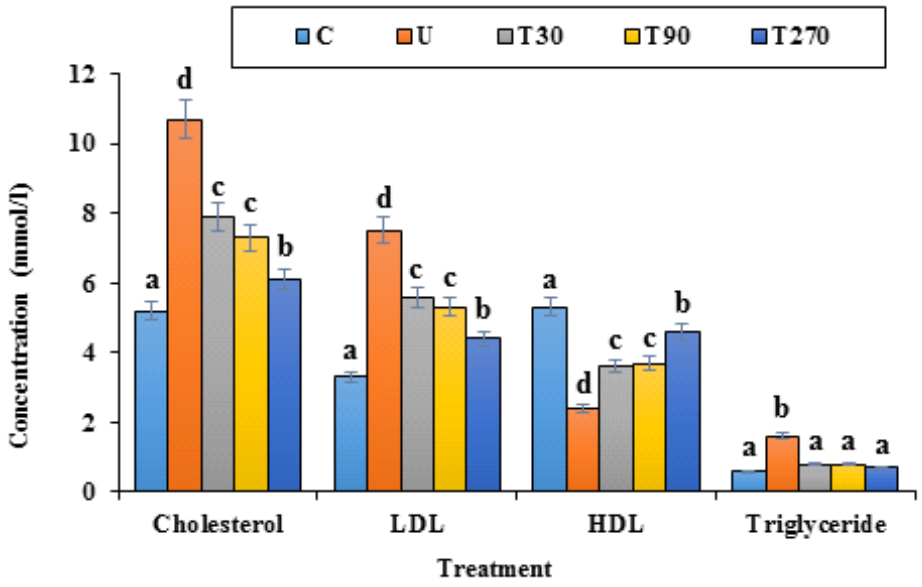

Figure 1. Cholesterol, LDL, HDL and triglyceride levels in several groups. C: Control, U: Untreated, T: *Tragopogon graminifolius*, LDL: Low-density lipoprotein, HDL: High-density lipoprotein. Non-like letters show a remarkable change between the several groups (p≤0.05).

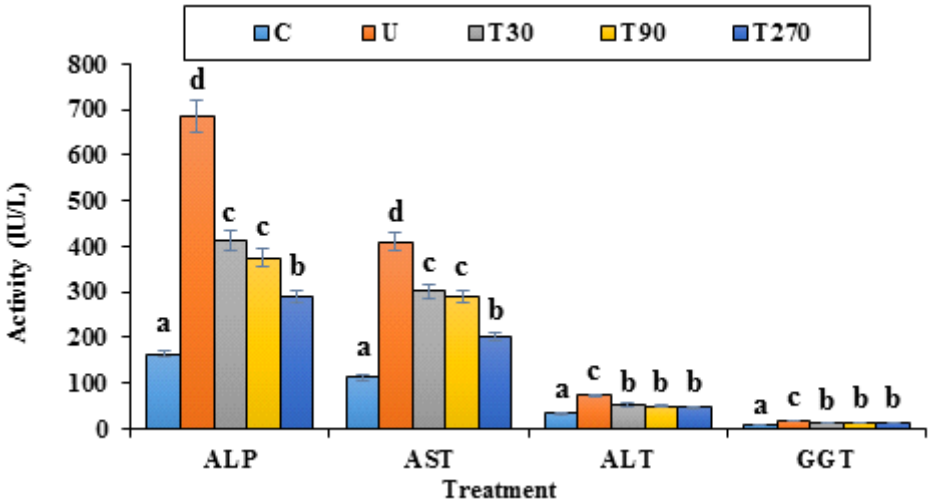

Figure 2. ALP, AST, ALT and GGT levels in several groups. C: Control, U: Untreated, T: *Tragopogon graminifolius*, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transferase. Non-like letters show a remarkable change between the several groups (p≤0.05).

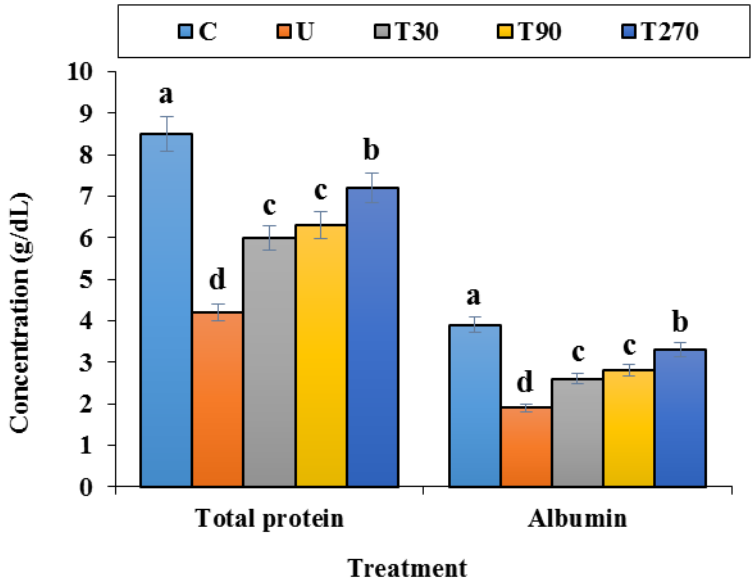

Figure 3. Total protein and albumin levels in several groups. C: Control, U: Untreated, T: *Tragopogon graminifolius*. Non-like letters show a remarkable change between the several groups ($p \leq 0.05$).

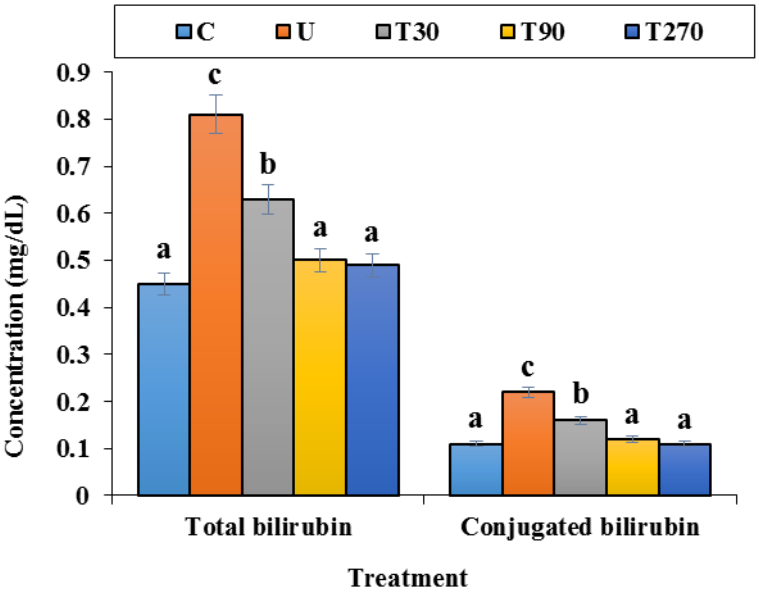

Figure 4. Total and conjugated bilirubin levels in several groups. C: Control, U: Untreated, T: *Tragopogon graminifolius*. Non-like letters show a remarkable change between the several groups ($p \leq 0.05$).

Effect of T. graminifolius ethanolic extract on the levels of stereological parameters

Administration of *T. graminifolius* ethanolic extract at all doses could significantly ($p \leq 0.05$) ameliorate the liver weight and volume compared to the untreated group (Figs. 6 and 7). No significant difference was noticed ($p \leq 0.05$) between T30 and T90 groups in the monitored parameters.

The volumes of hepatocytes, central veins, sinusoids, portal veins, hepatic arteries and bile ducts increased significantly ($p \leq 0.05$) in the untreated mice group in comparison with the control ones (Figs. 8, 9 and 10). Administration of *T. graminifolius* ethanolic extract at all doses could significantly ($p \leq 0.05$) decrease the volumes of the mentioned structures volumes. Also, gavage of T30 could significantly ($p \leq 0.05$) reduce the volume of hepatic arteries similar to that of the control group. There was no significant difference in the volumes of portal veins and hepatic arteries ($p \leq 0.05$) between T90 and the control groups. T270 could significantly ($p \leq 0.05$) decrease the volumes of portal veins, hepatic arteries and bile ducts similar to the control group.

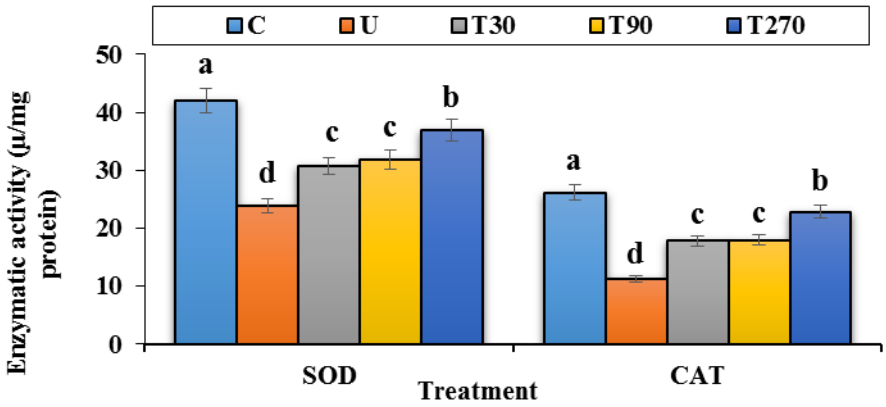

Figure 5. Liver SOD and CAT levels in several groups. C: Control, U: Untreated, T: *Tragopogon graminifolius*, SOD: Superoxide dismutase, CAT: Catalase. Non-like letters show a remarkable change between the several groups (p≤0.05).

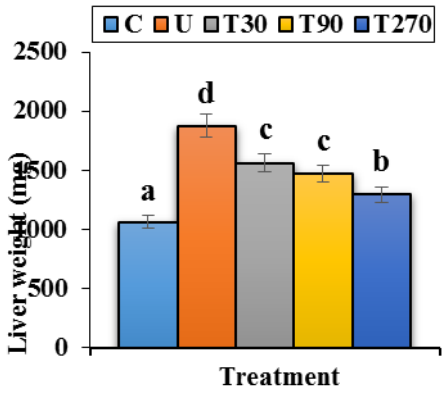

Figure 6. Liver weight in several groups. C: Control, U: Untreated, T: *Tragopogon graminifolius*. Non-like letters show a remarkable change between the several groups (p≤0.05).

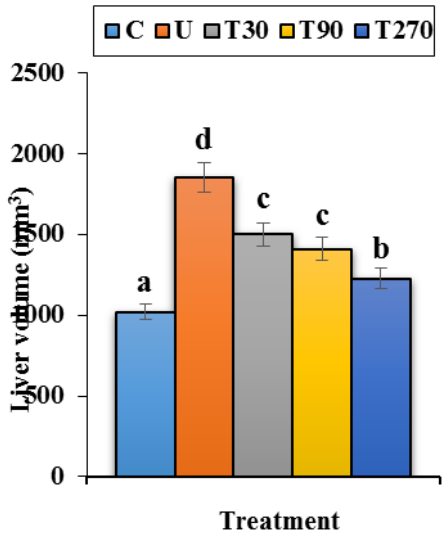

Figure 7. Liver volume in several groups. C: Control, U: Untreated, T: *Tragopogon graminifolius*. Non-like letters show a remarkable change between the several groups (p≤0.05).

DISCUSSION

The remedial benefits of herbal medicine have been rec ognized for centuries by clinical experience and practice [22-26]. They have the immense effect on the management and remedy of every disease such as hepatotoxicity [4,27]. Considerable number of ethno medicinal plants are consumed for their hepatoprotective properties, including; include *Fagonia schweinfurthii*, *Vitex glabrata*, *Astragalus kahiricus*, *Zingiber officinale* Roscoe, *Cissus quadrangularis*, *Feronia limonia*, *Terminalia paniculata*, *Melastoma malabathricum* L, *Ficus religiosa*, *Feijoa sellowiana*, *Garcinia indica*, *Daucus carota*, *Moringa oleifera* Lam, *Abelmoschus manihot* and *Acacia nilotica* Linn [27].

In this experimental study, hepatoprotective potential of *T. graminifolius* ethanolic extract at several doses was determined on CCl_4-induced hepatotoxicity in mice model.

In the recent study, obtained *in vivo* data revealed that animals treated with CCl_4 exhibit a substantial (p≤0.05) re-

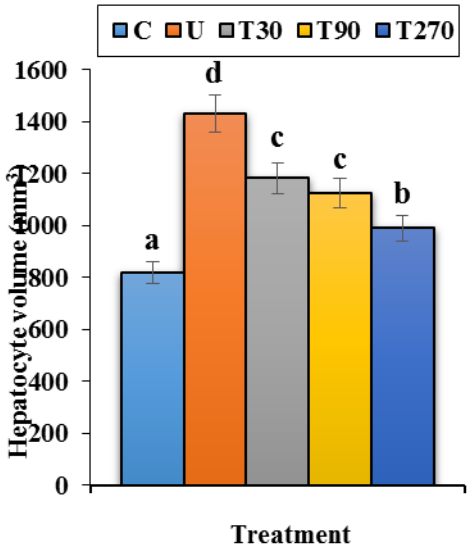

Figure 8. Hepatocyte volume in several groups. C: Control, U: Untreated, T: *Tragopogon graminifolius*. Non-like letters show a remarkable change between the several groups (p≤0.05).

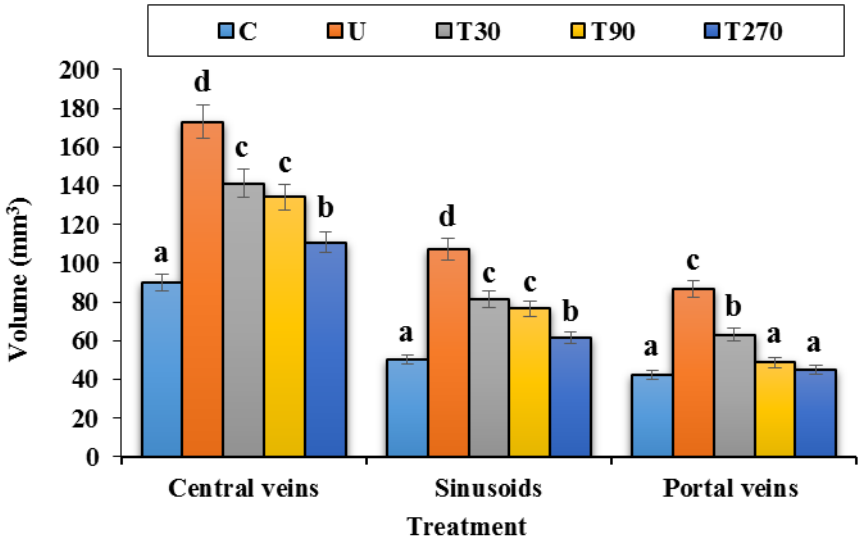

Figure 9. Central veins, sinusoids and portal veins volumes in several groups. C: Control, U: Untreated, T: *Tragopogon graminifolius*. Non-like letters show a remarkable change between the several groups ($p \leq 0.05$).

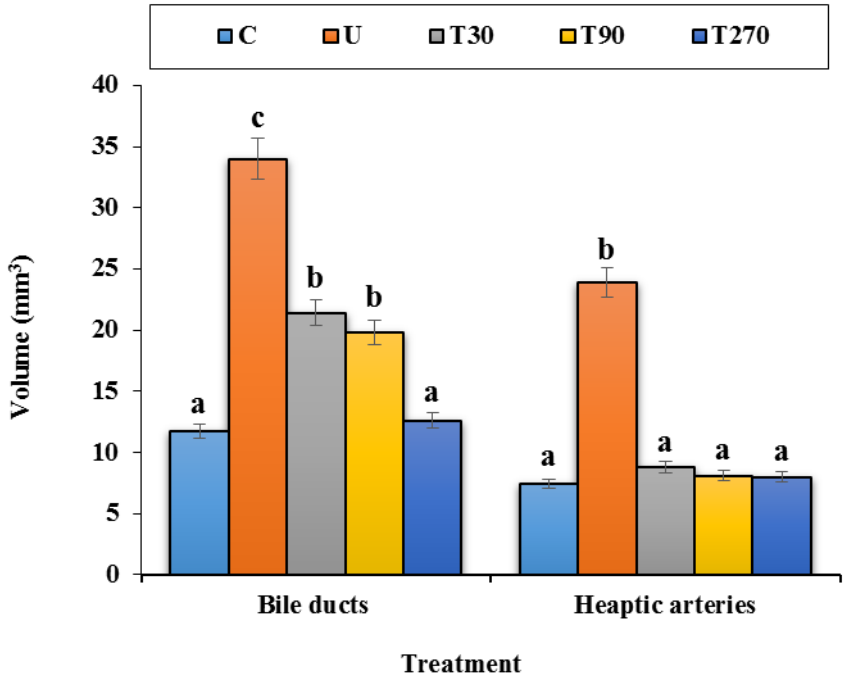

Figure 10. Bile ducts and hepatic arteries volumes in several groups. C: Control, U: Untreated, T: *Tragopogon graminifolius*. Non-like letters show a remarkable change between the several groups ($p \leq 0.05$).

duce in the levels of liver antioxidant enzymes such as SOD and CAT when comparing to the control group animals. *T. graminifolius* ethanolic extract enhanced the levels of SOD and CAT at all doses (especially 270 mg/kg) with ameliorating of liver function. In a study by Jadhav et al, similar hepatoprotection activity was reported against CCl_4 while using silymarin (a reference drug) at a concentration of 200 mg/kg; an effect analogous to 250 mg/kg dose of *Tragopogon* genus [28].

The extension of hepatic damages is assessed by the elevated serum levels of cytoplasmic enzymes as well as histological examination [29,30]. The increased serum parameters levels such as cholesterol, LDL, triglyceride, ALP, AST, ALT, GGT, and total and conjugated bilirubin have been attributed to the damaged structural integrity of the liver [29,30]. Administration of CCl_4 produces liver damage in mice as manifested by the rise in serum parameters levels of ALP, AST and ALT [4,30]. Also in a previous study by Recknagel et al., on the mechanism of CCl_4-induced hepatotoxicity it was shown that endogenous antioxidants play a

crucial role in detoxifying the reactive toxic derivatives of CCl_4 and that liver necrosis begins when antioxidant stores are markedly depleted [29]. In this study, the raised levels of cholesterol, LDL, triglyceride, ALP, AST, ALT, GGT, and total and conjugated bilirubin decreased following administration of different doses of *T. graminifolius* ethanolic extract (especially 270 mg/kg) and the observed changes can be attributed to the antioxidant compounds present in the plant. Antioxidant property of *Tragopogon* genus aerial parts has been already reported *in vitro* [31]. Flavonoids including apigenin, isoorientin, isovitexin, lucenin, luteolin, orientin, quercetin, vitexin, swertisin and vicenin-1and 2 are the antioxidant compounds of *Tragopogon* genus [32,33]. These constituents demonstrated the protective effect against HCl/ethanol-induced gastric ulcer and healing property against acetic acid-induced chronic gastric ulcer with inhibition of gastric tissue lipid peroxidation [34]. Yan et al. revealed Quercetin-3-O-α-D-glucuronopyranoside as a compound present in *Tragopogon* genus extract with a protective effect on gastric mucus against indomethacin-induced ulcer by rising gastric mucus secretion, decreasing of myeloperoxidase (MPO) and free radical production, inhibiting the expression of intercellular adhesion molecule protein and down-regulation of the pro-inflammatory cytokines [35].

Other results of the present study revealed that the liver of CCl_4-treated mice show remarkable hypertrophy which leads to an increase in weight and volume of the hepatic structures. The pathogenesis of liver hypertrophy can be attributed to the overproduction of oxygen-free radicals following administration of toxins such as CCl_4, which is expressed in response to cytokines [36]. These changes were alleviated significantly in all doses of *T. graminifolius* ethanolic extract (especially 270 mg/kg). Thus, these results suggest *T. graminifolius* ethanolic extract use to ameliorate hepatic structural changes due to CCl_4-induced toxicity. Agree with this experiment, in a study indicated that ethno medicinal plant decrease the volumes of hepatocytes, central veins, sinusoids, portal veins, hepatic arteries and bile ducts in CCl_4-induced hepatotoxicity in mice [4]. The hepatoprotection by *T. graminifolius* ethanolic extract may be due to antioxidant property of the phytochemicals which decrease the oxidative stress imposed by CCl_4 and other like anti-inflammatory and analgesic potentials inhibiting the inflammatory hepatic damage [3,37].

CONCLUSION

From the observations and monitored parameters, it can be concluded that all doses of *T. graminifolius* ethanolic extract (especially T270) has a hepatoprotective activity against hepatic structural changes induced by CCl_4 in mice. Additional clinical trials studies would be needed to justify the potential of the plant as a hepatoprotective agent in the human.

CONFLICT OF INTEREST

The authors declare that this research does not have any conflict of interest with anyone or any institute.

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