Comparative Clinical Pathology (2019) 28:1197–1203
https://doi.org/10.1007/s00580-018-2828-9

ORIGINAL ARTICLE

Histopathological and biochemical effects of aqueous extract of *Tragopogon graminifolius* on the liver tissues of Wistar rats fed with high-fat diet

Mohammad Mahdi Zangeneh[1,2] · Saman Salmani[1] · Akram Zangeneh[1,2] · Reza Khedri[1] · Mohammad Saeid Zarei[1]

Received: 4 August 2018 / Accepted: 25 September 2018 / Published online: 22 October 2018

Abstract

The fatty liver disease is one of the most usual metabolic diseases among people around the world. Appropriate strategies for treating this disease are prioritized by each country. The purpose of the new research was to survey the therapeutical property of aqueous extract of *Tragopogon graminifolius* on the high-fat diet-induced fatty liver disease in Wistar male rats. In this study, 60 rats were used. A total of ten rats were chosen as the negative control, and the rest of them were treated with a high-fat diet for 4 months. Then, the animals were randomly divided into six subgroups, including negative healthy control, untreated negative control, and four groups receiving the aqueous extract of *T. graminifolius* at 20, 40, 80, and 160 mg/kg concentrations. After 2 months, the rats were sacrificed, and the blood and liver samples of them were collected for biochemical and histopathological parameter analysis. All doses of *T. graminifolius* could significantly ($p \leq 0.05$) decrease the raised levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin, and glucose and increased HDL, total protein, albumin, SOD, and CAT as compared to the untreated group. Also, aqueous extract of *T. graminifolius* decreased the degree of hepatic steatosis as compared to the untreated group. In conclusion, the obtained results demonstrated the hepatoprotective effect of *T. graminifolius* aqueous extract.

Keywords *Tragopogon graminifolius* · Aqueous extract · Hepatoprotective effect · Fatty liver · High-fat diet

Introduction

Nonalcoholic fatty liver as a metabolic disorder has involved human beings globally and is usually followed by extreme obesity, increased blood lipid, and diabetes type II (Ganz et al. 2014; Jacobs et al. 2002; Day 2011). Triglyceride and cholesterol are important biologic lipids whose overconsumption through food results in hyperglycemia (Zhang et al. 2016; Day 2006) and hypercholesterolemia (Yao et al. 2016). Studies have shown that a high-fat diet leads to hepatic steatosis (Ganz et al. 2014; Xiao et al. 2013). The nonalcoholic fatty liver is characterized by the accumulation of triglycerides in liver cells due to stratification of free fatty acids and glycerol. It is accompanied by a series of histopathologic changes varying from steatosis to cirrhosis (Haga et al. 2015; Flora et al. 1998; Tamayo and Diamond 2007; Shaker et al. 2010). Formerly, it was believed that steatosis is a simple phenomenon free of side effects. In any case, it is evident now that fatty liver is dependent upon factors such as vulnerable oxidative stress and can lead to steatohepatitis, which is characterized by necrosis, inflammation, fibrosis, and cirrhosis (Bosisio et al. 1992). However, although steatosis may lead to complete hepatic failure, no appropriate and ideal treatment has been established for it yet (Tamayo and Diamond 2007), and adopted methods only treat or control the risk factors. The possible pharmacologic treatments include antioxidants, insulin sensitizers, hepatic protectors, or lipid-reducing factors (Bundy et al. 2008). Since there are numerous pharmaceutical plants with antioxidant and anti-inflammatory properties, their application can be effective in the prevention and treatment of steatohepatitis in high-fat diet cases (Yao et al. 2016).

✉ Mohammad Mahdi Zangeneh
m.mehdizangeneh@yahoo.com

[1] Department of Clinical Science, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran

[2] Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran

In Iranian traditional medicine, plants have been the foundation of inhibition and treatment of several diseases (Hagh-Nazari et al. 2017; Ghashghaii et al. 2017; Zhaleh et al. 2018). One of the most important herbal medicines which are widely used is *Tragopogon graminifolius* from *Plantae* kingdom, *Asterales* order, *Asteraceae* family, *Cichorioideae* subfamily, *Cichorieae* tribe, and *Tragopogon* genus. The plant is widely distributed in Iraq, Turkey, and Iran. *T. graminifolius* is a good source of low-cost food and is a perfect part of Iranian diet (Farzaei et al. 2014a; Farzaei et al. 2014b; Farzaei et al. 2015). It applied as a medicinal plant has been used for its antioxidant, anti-inflammatory, antibacterial, wound healer, and bleeding inhibitor activities (Tenkerian et al. 2015; Zeeni et al. 2014). *T. graminifolius* has a long history of use in traditional medicine, but there is a little evidence to reveal; it is useful to prevent and cure fatty liver. We attempted to survey the therapeutical effect of *T. graminifolius* on the high-fat diet-induced fatty liver in rats.

Materials and methods

Plant extract preparation method

T. graminifolius was collected from Kermanshah city. The leaves of the plant were dried in shadow, and after grinding, each time 300 g of the obtained powder was dissolved in 3000 cc of distilled water and put in Soxhlet extractor for 8 h. 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 and dissolved in normal saline.

Experimental design

This experimental study was conducted on 60 Wistar male rats with the weight of 200 ± 5 g that were kept in individual cages for 10 days to adapt to the environment. During the experiments, the temperature of the animal house was adjusted at 22 ± 3 °C under a 12-h dark/light cycle. A total of ten rats were chosen as the negative control, and the rest of them were treated with a high-fat diet for 4 months. The rats with fatty liver were then divided into five groups, ten rats in each group: (1) fatty diet, (2) fatty diet plus 20 mg/kg of *T. graminifolius*, (3) fatty diet plus 40 mg/kg of *T. graminifolius*, (4) fatty diet plus 80 mg/kg of *T. graminifolius*, and (5) fatty diet and 160 mg/kg of *T. graminifolius*. Different concentrations of extract were administered via gavage for 2 months. To consider gavage stress, distilled water was administered to the control group every day. After 2 months of gavage, the rats were sacrificed. Blood samples were taken from the rats' heart to analyze biochemical parameters. The capacity of antioxidant enzymes was evaluated by determining the activity of superoxide dismutase (SOD) and catalase (CAT) in the livers of each group ($n = 5$) using the procedures reported by Abei (1974) and Martin et al. (1987). Also, the rats' liver ($n = 5$) was subjected to microscopic analysis. The histological changes were rated based on fat accumulation in liver: 0 = no steatosis, 1 = steatosis in less than 25% of hepatocytes, 2 = steatosis in 26–50% of hepatocytes, 3 = steatosis in 51–75% of hepatocytes, and 4 = steatosis in more than 75% of hepatocytes (Mohammadifar et al. 2018).

Fatty diet preparation

Rats diet powder (28%), butter, (28%), egg yolk (19%), sucrose (14%), and egg white (11%) were mixed to prepare the fatty diet. The obtained powder was dried in 100 °C oven for 30 min and was given to the rats as a pellet. The fatty diet was prepared weekly and stored in the refrigerator (Mohammadifar et al. 2018).

Statistical analysis

The quantitative data were analyzed by SPSS-21 software using one-way ANOVA followed by Duncan test. To determine the normality of data, Kolmogorov-Smirnov test was applied. To analyze the histological data, the Kruskal-Wallis test was run. $p \leq 0.05$ was considered significant.

Results

Effect of *T. graminifolius* aqueous extract on the degree of hepatic steatosis

As shown in Table 1, the degree of hepatic steatosis enhanced in untreated rats compared to the control ones. Several groups

Table 1 The degrees of hepatic steatosis in several groups

Groups	The degrees of hepatic steatosis					*p*
	0	1	2	3	4	
C	10	0	0	0	0	**
UD	0	4	3	1	2	*
TG20	1	4	4	1	0	*, **
TG40	3	5	2	0	0	*, **
TG80	4	4	2	0	0	*, **
TG160	6	3	1	0	0	**

C control, *UT* untreated, *TG Tragopogon* graminifolius

*Significant difference between the control group and other groups

**Significant difference between the untreated group and other groups

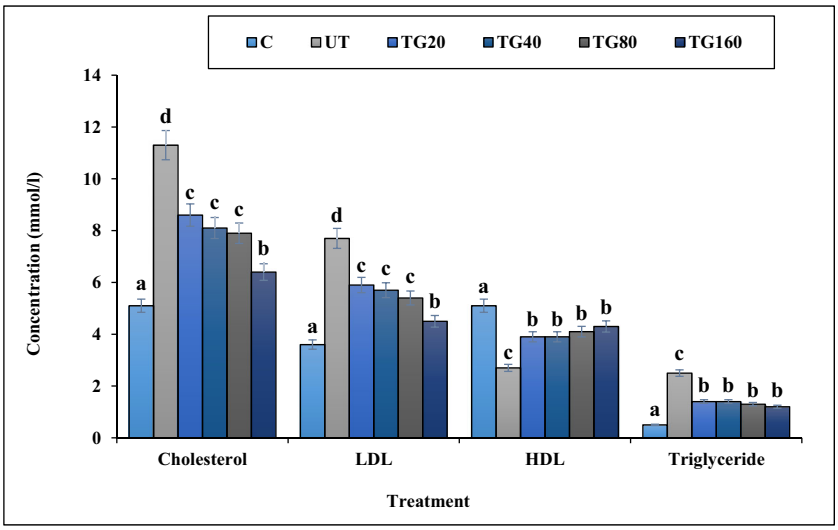

Fig. 1 Cholesterol, LDL, HDL, and triglyceride levels in several groups. C control, UT untreated, TG *Tragopogon* graminifolius, LDL low-density lipoprotein, HDL high-density lipoprotein. Non-like letters show a remarkable change between the several groups ($p \leq 0.05$)

of *T. graminifolius* aqueous extract could decrease it. There were not remarkable changes in the degree of hepatic steatosis between TG160 and control groups.

Effect of *T. graminifolius* aqueous extract on the levels of biochemical parameters

High-fat diet-induced fatty liver, increased significantly ($p \leq 0.05$) the concentrations of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin, and glucose and decreased significantly ($p \leq 0.05$) the concentrations of HDL, total protein, and albumin as compared to the control group. Several doses of *T. graminifolius* aqueous extract could significantly ($p \leq 0.05$) ameliorate the above parameters. There were not remarkable changes ($p \leq 0.05$) between all doses of *T. graminifolius* and control group in the levels of GGT, albumin, and total and conjugated bilirubin. Also, administration of TG80 and TG160 could significantly ($p \leq 0.05$) improve the concentration of ALT similar to the control group. No significant changes ($p \leq 0.05$) were found between TG160 and control groups in the level of total protein (Figs. 1, 2, 3, 4, and 5).

Effect of *T. graminifolius* aqueous extract on the levels of antioxidant enzymes

The levels of SOD and CAT were significantly ($p \leq 0.05$) reduced in the untreated group. The treatment with *T. graminifolius* aqueous extract significantly ($p \leq 0.05$) ameliorated them. There were not remarkable changes ($p \leq 0.05$) in the level of CAT between several doses of *T. graminifolius* and control group. The concentration of SOD was significantly ($p \leq 0.05$) increased in TG160 and were similar to the control group. No remarkable changes ($p \leq 0.05$) were found between TG20, TG40, and TG80 in the levels of antioxidant enzymes (Fig. 6).

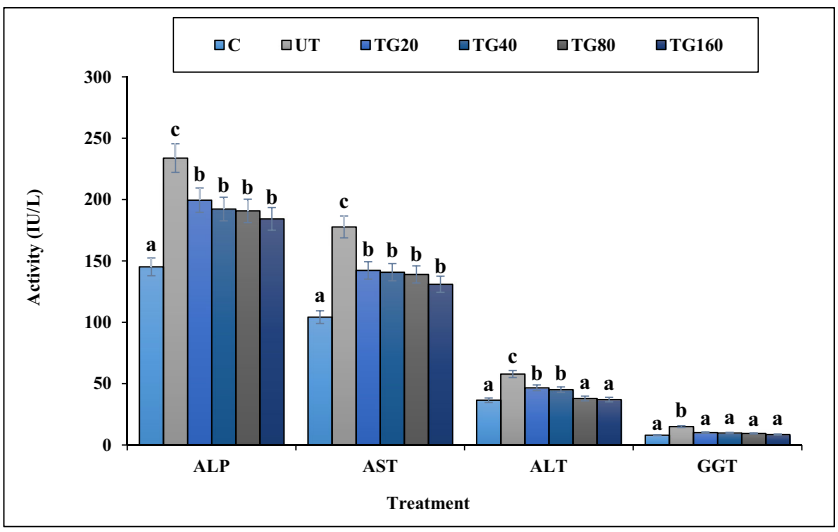

Fig. 2 ALP, AST, ALT, and GGT levels in several groups. C control, UT untreated, TG *Tragopogon* graminifolius, ALP alkaline phosphatase, AST aspartate aminotransferase, ALT alanine aminotransferase, GGT gamma-glutamyl transferase. Non-like letters show a remarkable change between the several groups ($p \leq 0.05$)

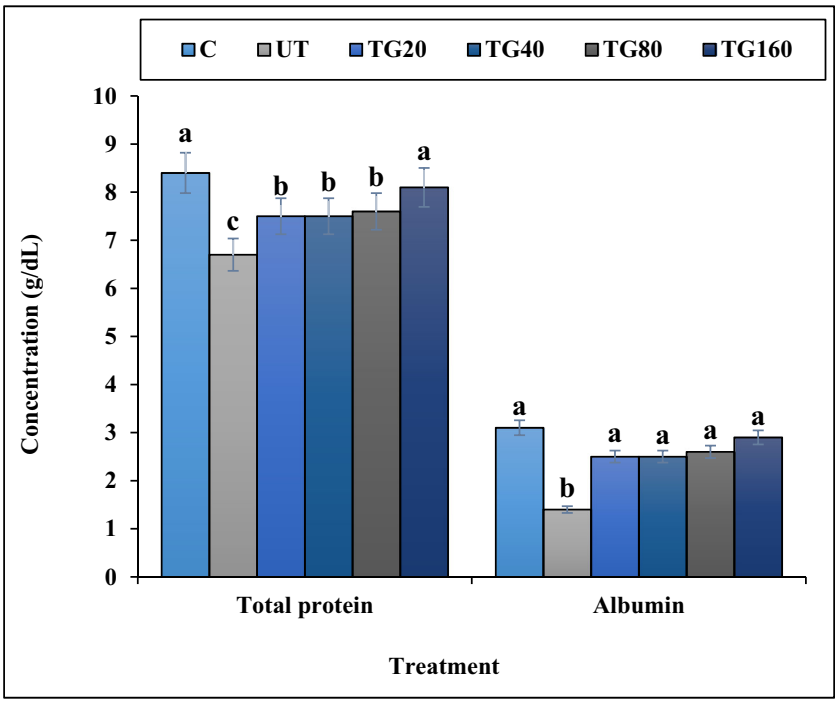

Fig. 3 Total protein and albumin levels in several groups. C control, UT untreated, TG *Tragopogon* graminifolius. Non-like letters show a remarkable change between the several groups ($p \leq 0.05$)

Discussion

The remedial effects of the traditional medicine have been realized for centuries by clinical practice and experience (Hamelian et al. 2018; Farzaei et al. 2018; Sayyedrostami et al. 2018). They have the strong activity on the prevention, control, and treatment of every disease such as fatty liver (Sabzghabaee et al. 2013; Hemmati et al. 2015; Nazni et al. 2006; Lee et al. 2010). A list of medicinal plants used for their remedial properties on fatty liver includes *Alisma orientalis* Juzep, *Alisma plantago-aquatica* Linn, *Amomum tsao-ko* Crevost et Lemaire, *Anemarrhena asphodeloides* Bunge, *Alpinia katsumadai* Hayata, *Artemisia capillaries* Thunb, *Bupleurum scorzonerifolium* Willd, *Carthamus tinctorius* L, *Cinnamomum tamala* Nees, *Citrus reticulata* Blanco, *Coptis chinensis* Franch, *Crataegus pinnatifida* Bunge, *Curcuma rcenyujin* Y, *Fructus aurantii*

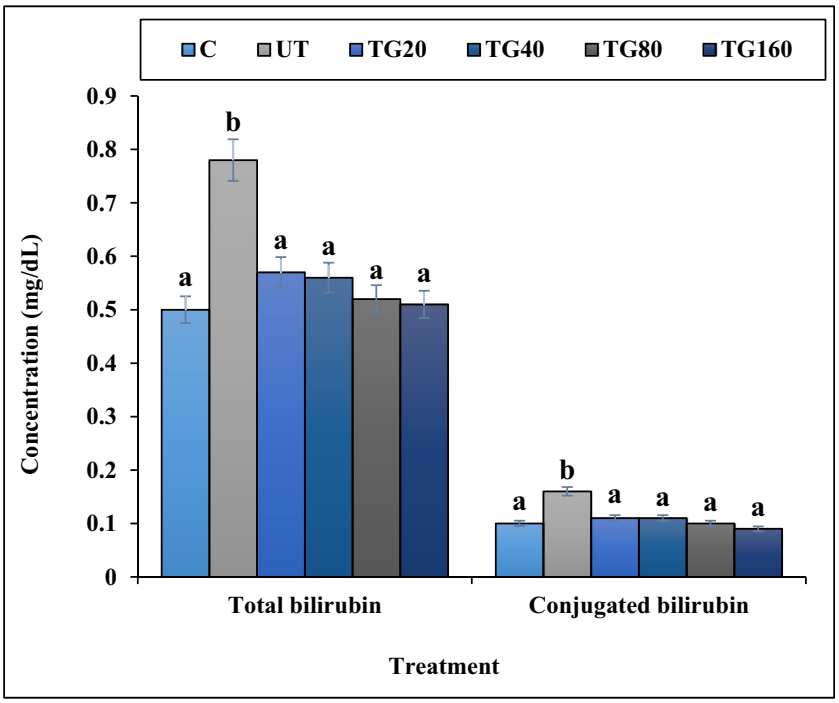

Fig. 4 Total and conjugated bilirubin levels in several groups. C control, UT untreated, TG *Tragopogon* graminifolius. Non-like letters show a remarkable change between the several groups ($p \leq 0.05$)

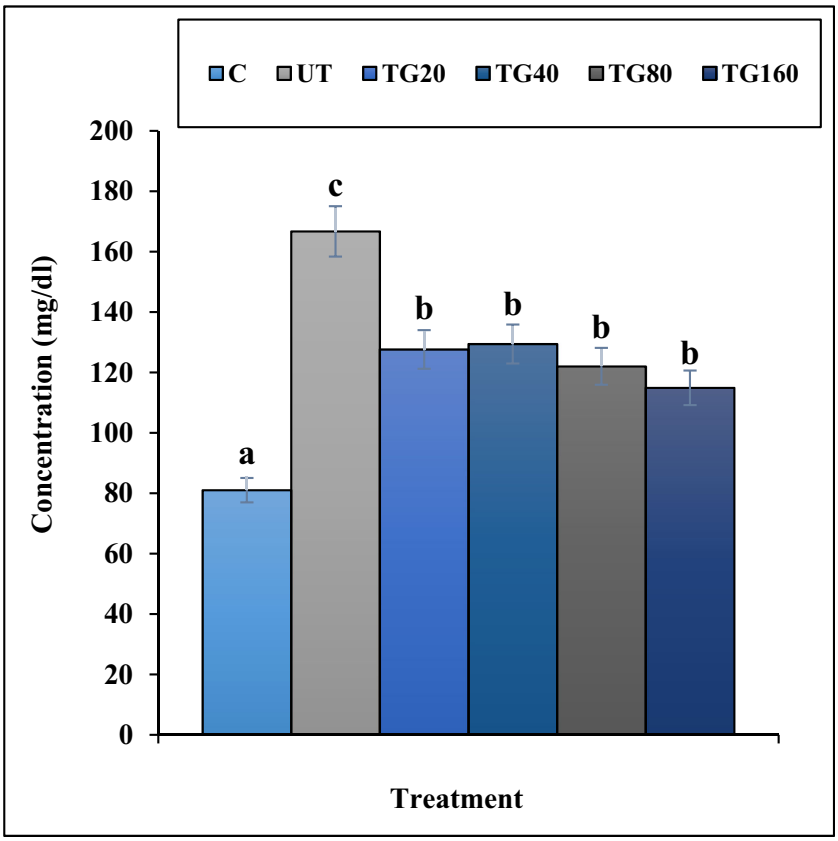

Fig. 5 Glucose levels in several groups. C control, UT untreated, TG *Tragopogon* graminifolius. Non-like letters show a remarkable change between the several groups ($p \leq 0.05$)

Immaturus, *Gardenia jasminoides* Ellis, *Glycyrrhiza uralensis* Fisch, *Grataegus pinnati fida* Bge, *Heteropogon contortus* P, *Hypericum japonicum* Thunb, *Lotus leaf* Tea, and *Panax pseudoginseng* var. notoginseng (Yao et al. 2016). In Iranian traditional medicine, people used *T. graminifolius* to prevent and treat fatty liver disease.

The biochemical results of the recent study demonstrated that high-fat diet enhanced significantly ($p \leq 0.05$) the levels

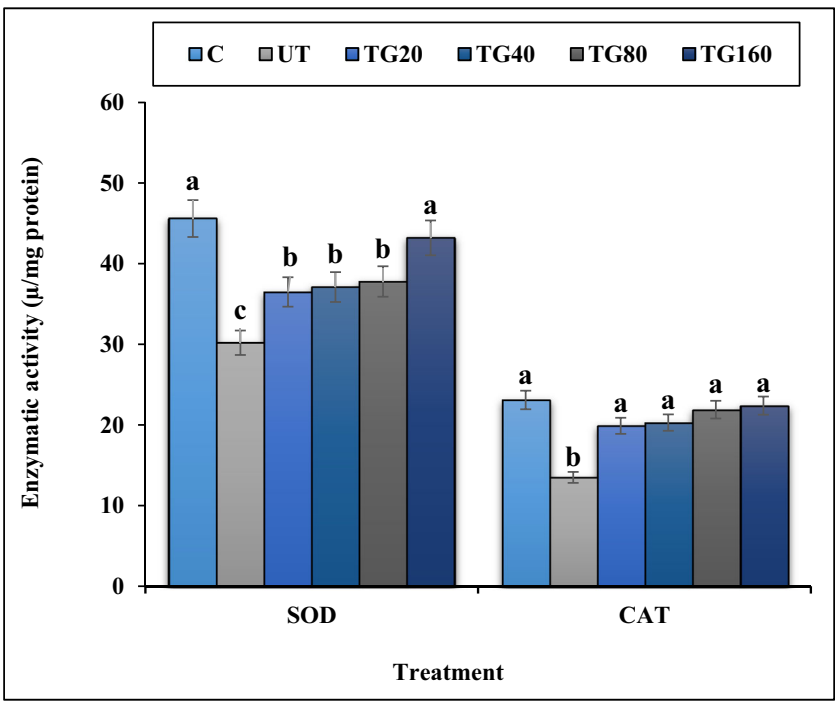

Fig. 6 Liver SOD and CAT levels in several groups. C control, UT untreated, TG *Tragopogon* graminifolius, SOD superoxide dismutase, CAT catalase. Non-like letters show a remarkable change between the several groups ($p \leq 0.05$)

of ALP, AST, ALT, GGT, and total and conjugated bilirubin and reduced significantly ($p \leq 0.05$) the level of total protein and albumin as compared to the control group. Therefore, this diet caused severe hepatic toxicity. In spite of hepatotoxicity property of high-fat diet, the treatment with aqueous extract of *T. graminifolius* could significantly ($p \leq 0.05$) improve the levels of the above parameters. In a study indicated that *T. porrifolius* (Another species of the *Tragopogon* genus) reduced the raised levels of hepatic biochemical parameters includes ALT, AST, and LDH as compared to the CCl_4-treated group (Tenkerian et al. 2015). In the study of Sadeghi et al. (2018) revealed the hepatoprotective property of the 25, 50, and 100 mg/kg dose of *T. graminifolius* hydroalcoholic extract against high-fat diet-induced hepatotoxicity. In the previous study, the levels of AST and ALT reduced as compared to the untreated group.

Antioxidant enzyme concentrations of our study indicated that a high-fat diet decreased significantly ($p \leq 0.05$) the levels of SOD and CAT. But, the treatment with aqueous extract of *T. graminifolius* could significantly ($p \leq 0.05$) improve the levels of SOD and CAT. In the study of the Tenkerian et al. (2015) reported that the *T. porrifolius* with increasing the degradation of free radicals, increased the concentrations of CAT, SOD, and GST. In the other study showed that the hydroalcoholic extract of *T. graminifolius* with increasing of the levels of the MPO, LPO, and total thiol eliminated the complications of a high-fat diet. Also, in the previous study revealed that the *T. graminifolius* were rich of antioxidant compounds includes *p*-coumaric acid, caffeic acid, catechin, ferulic acid, and gallic acid, so it was normal that the plant had antioxidant effects (Sadeghi et al. 2018).

In the study, *T. graminifolius* reduced the concentrations of cholesterol, LDL, triglyceride, and the degree of hepatic steatosis and increased the level of the HDL as compared to the untreated group. In the study of the Zeeni et al. (2014) revealed that the *T. porrifolius* with 50, 100, and 200 mg/kg doses decreased the levels of the cholesterol, LDL, and triglyceride in rats with fatty liver. In the other study indicated the hepatoprotective properties of hydroalcoholic extract of *T. graminifolius* with 25, 50, and 100 mg/kg doses in animals fed with high-fat diet. In the previous study reported that the *T. graminifolius*, with increasing the level of lipid peroxidation, reduced fatty liver complications (Sadeghi et al. 2018).

Conclusion

From the study, it concludes that aqueous extract of *T. graminifolius* at all doses (especially TG160) revealed significant hepatoprotective effects. This extract also demonstrated improvement in histopathological and biochemical parameters and so might be of value in fatty liver treatment. Further research is necessary to determine the exact dose of aqueous extract of *T. graminifolius* for treatment of fatty liver in human.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethic approval All institutional and national guidelines for the care and use of laboratory animals were followed.

References

Abei H (1974) Catalase. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Academic Press, New York, pp 673–684

Bosisio E, Benelli C, Pirola O (1992) Effect of the flavanolignans of Silybum marianum L. on lipid peroxidation in rat liver microsomes and freshly isolated hepatocytes. Pharmacol Res 25(2):147–154

Bundy R, Walker AF, Middleton RW, Wallis C, Simpson HC (2008) Artichoke leaf extract (Cynara scolymus) reduces plasma cholesterol in otherwise healthy hypercholesterolemic adults: a randomized, double blind placebo controlled trial. Phytomedicine 15(9):668–675

Day CP (2006) From fat to inflammation. Gastroenterol 130:207–210

Day CP (2011) Non-alcoholic fatty liver disease: a massive problem. Clin Med 11(2):176–178

Farzaei MH, Khanavi M, Moghaddam G, Dolatshahi F, Rahimi R, Shams-Ardekani MR, Amin G, Hajimahmoodi M (2014a) Standardization of *Tragopogon graminifolius*DC. Extractbasedonphenolic compounds and antioxidant activity. J Chem. https://doi.org/10.1155/2014/425965

Farzaei MH, Rahimi R, Attar F, Siavoshi F, Saniee P, Hajimahmoodi M, Mirnezami T, Khanavi M (2014b) Chemical composition, antioxidant and antimicrobial activity of essential oil and extracts of *Tragopogon graminifolius*, a medicinal herb from Iran. Nat Prod Commun 9(1):121–124

Farzaei MH, Ghasemi-Niri SF, Abdolghafari AH, Baeeri M, Khanavi M, Navaei-Nigjeh M, Abdollahi M, Rahimi R (2015) Biochemical and histopathological evidence on the beneficial effects of *Tragopogon graminifolius* in TNBS-induced colitis. Pharm Biol 53(3):429–436

Farzaei MH, Zangeneh MM, Goodarzi N, Zangeneh A (2018) Stereological assessment of nephroprotective effects of *Trachyspermum ammi* essential oil against carbon tetrachloride-induced nephrotoxicity in mice. Int J Morphol 36(2):750–757

Flora K, Hahn M, Rosen H, Benner K (1998) Milk thistle (Silybum marianum) for the therapy of liver disease. Am J Gastroenterol 93(2):139–143

Ganz M, Csak T, Szabo G (2014) High fat diet feeding results in gender specific steatohepatitis and inflammasome activation. World J Gastroenterol 20:8525–8534

Ghashghaii A, Hashemnia M, Nikousefat Z, Zangeneh MM, Zangeneh A (2017) Wound healing potential of methanolic extract of *Scrophularia striata* in rats. Pharm Sci 23(4):256–263

Haga Y, Kanda T, Sasaki R, Nakamura M, Nakamoto S, Yokosuka O (2015) Nonalcoholic fatty liver disease and hepatic cirrhosis: comparison with viral hepatitis-associated steatosis. World J Gastroenterol 21(46):12989–12995

Hagh-Nazari L, Goodarzi N, Zangeneh MM, Zangeneh A, Tahvilian R, Moradi R (2017) Stereological study of kidney in streptozotocin-induced diabetic mice treated with ethanolic extract of *Stevia rebaudiana* (bitter fraction). Comp Clin Pathol 26(2):455–463

Hamelian M, Zangeneh MM, Amisama A, Varmira K, Veisi H (2018) Green synthesis of silver nanoparticles using *Thymus kotschyanus* extract and evaluation of their antioxidant, antibacterial and cytotoxic effects. Appl Organomet Chem. https://doi.org/10.1002/aoc.4458

Hemmati M, Zohoori E, Mehrpour O, Karamian M, Asghari S, Zarban A, Nasouti R (2015) Anti-atherogenic potential of jujube, saffron and barberry: anti-diabetic and antioxidant actions. EXCLI J 14:908–915

Jacobs BP, Dennehy C, Ramirez G, Sapp J, Lawrence VA (2002) Milk thistle for the treatment of liver disease: a systematic review and meta-analysis. Am J Med 113(6):506–515

Lee TY, Chang HH, Lo WC, Lin HC (2010) Alleviation of hepatic oxidative stress by Chinese herbal medicine Yin-Chen-Hao-Tang in obese mice with steatosis. Int J Mol Med 25(6):837–844

Martin JP, Dailey M, Sugarman E (1987) Negative and positive assays of superoxide dismutase based on hematoxylin autoxidation. Arch Biochem Biophys 255(2):329–336

Mohammadifar M, Behnam M, Talaei SA, Khamechian T, Mehran M, Taghizadeh M (2018) Evaluation effect of *Silybum Marianum, Cynara scolymus L and Ziziphus jujube Mill* combination extract on nonalcoholic fatty liver in rats. Iran J Endocrinol Metab 19(6):410–418

Nazni P, Vijayakumar TP, Alagianambi P, Amirthaveni M (2006) Hypoglycemic and hypolipidemic effect of Cynara scolymus among selected type 2 diabetic individuals. Pak J Nutr 5(1):147–151

Sabzghabaee AM, Khayam I, Kelishadi R, Ghannadi A, Soltani R, Badri S, Shirani S (2013) Effect of Zizyphus jujuba fruits on dyslipidemia in obese adolescents: a triple-masked randomized controlled clinical trial. Med Arch 67(3):156–159

Sadeghi A, Bahramsoltani R, Rahimi R, Farzaei MH, Farzaei F, Siavosh-Haghighi ZM, Abdollahi M (2018) Biochemical and histopathological evidence on beneficial effects of standardized extract from *Tragopogon graminifolius* as a dietary supplement in fatty liver: role of oxidative stress. J Diet Suppl 15(2):197–206

Sayyedrostami T, Pournaghi P, Ebrahimi Vosta-Kalaeea S, Zangeneh MM (2018) Evaluation of the wound healing activity of *Chenopodium botrys* leaves essential oil in rats (a short-term study). J Essent Oil Bear Plants 21(1):164–174

Shaker E, Mahmoud H, Mnaa S (2010) Silymarin, the antioxidant component and Silybum marianum extracts prevent liver damage. Food Chem Toxicol 48(3):803–806

Tamayo C, Diamond S (2007) Review of clinical trials evaluating safety and efficacy of milk thistle (Silybum marianum [L.] Gaertn.). Integr Cancer Ther 6(2):146–157

Tenkerian C, El-Sibai M, Daher CF, Mroueh M (2015) Hepatoprotective, antioxidant, and anticancer effects of the *Tragopogon porrifolius* methanolic extract. Evid Based Complement Alternat Med 2015:1–10. https://doi.org/10.1155/2015/161720

Xiao J, Fai So K, Liong EC, Tipoe GL (2013) Recent advances in the herbal treatment of non-alcoholic fatty liver disease. J Tradit Complement Med 3(2):88–94

Yao H, Qiao YJ, Zhao YL, Tao XF, Xu LN, Yin LH, Qi Y, Peng JY (2016) Herbal medicines and nonalcoholic fatty liver disease. World J Gastroenterol 22(30):6890–6905

Zeeni N, Daher CF, Saab L, Mroueh M (2014) *Tragopogon porrifolius* improves serum lipid profile and increases short-term satiety in rats. Appetite 72:1–7

Zhaleh M, Sohrabi N, Zangeneh MM, Zangeneh A, Moradi R, Zhaleh H (2018) Chemical composition and antibacterial effects of essential oil of *Rhus coriaria* fruits in the west of Iran (Kermanshah). J Essent Oil Bear Plants 21(2):493–501

Zhang R, Yu Y, Hu S, Zhang J, Yang H, Han B, Cheng Y, Luo X (2016) Sesamin ameliorates hepatic steatosis and inflammation in rats on a high-fat diet via LXR5 and PPAR5. Nutr Res 36(9):1022–1030