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Protective Effect of *Trogopogon Graminifolius* Hydroalcoholic Extract against Acute Ethanol Induced Liver Damages in Rat

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Ethanol induces different side effects on tissues and organs and it is associated with biochemical, physiological, and pathological alterations in the liver. *Tragopogon graminifolius (TG)* is traditionally used for the treatment of gastrointestinal disorders. The aim of present study was to investigate *TG* effect against ethanol induced liver enzyme (ALT, AST and ALP) and nitric oxide (NO) secretion in male rat. In this experimental study, 30 male Wistar rats (210 ±10 g) have been randomly divided into the experimental, ethanol and control groups (6/group). The experimental groups were received one of *TG* hydroalcoholic extract (50, 100 and 150 mg/kg) for 15 days orally. One ml of distilled water was given to ethanol and control group daily. At the day of 15th, ethanol (5 g/kg) was given to all groups orally except control group. After one hour, cardiac blood sample was collected and serum levels of ALT, ALP, AST and NO concentration were measured. Data were analyzed by one way-ANOVA test. Ethanol increased ATL, AST, AP and NO in the serum of male rat. Pretreatment with *TG* extract prevent increasing the serum level of ALT ($p=0.001$), ALP ($p=0.004$) and NO ($p=0.000$) significantly during ethanol consumption. Moreover, serum AST decreased in experimental groups ($p>0.05$). *TG* extract has protective effect on ethanol-induced liver toxicity in rats and also verified claims of traditional medicine about hepatoprotective function of *TG*.

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Introduction

Liver is a vital organ with numerous important functions and liver diseases are serious health problem. Proteins and enzymes of liver are highly important biomarkers for the diagnosis and assessment of the normal function of tissues, organs and whole body [1]. Changes in cell membrane integrity lead to significant changes in liver enzyme. For example, alanine amino transferase (ALT) and aspartate amino transferase (AST) are commonly used for the diagnosis of liver diseases, hepatocellular damage and increased permeability of liver cells, whereas alkaline phosphatase (ALP) is involved in extrahepatic or intrahepatic obstruction [2].

Alcohol is one of the most commonly abused drugs worldwide and can cause alcoholic liver, cirrhosis, fibrosis and hepatic steatosis. Alcohol can't store in the body and it must metabolize in liver as soon as possible [3], so there is close relation between alcohol consumption and liver damage. Alcoholic liver is a complex and multifactorial disease with its incomplete understood etiology. Ethanol consumption leads to the generation of reactive oxygen species (ROS) and enhance oxidative stress in many tissues, especially the liver. Ethanol-induced oxidative stress plays a major role in liver injury. One of the most sensitive parts to alcohol is cell membrane. Alcohol caused membrane damages and enzymatic (ALT, AST, ALP) leakage to blood plasma [4].

Nitric oxide (NO) is an important signaling molecule and a pluripotent gaseous free radical that has been identified in many tissues and it regulates the function of many organs in the body. NO has complex behavior in pathophysiological condition and involved in several hepatic diseases. In the liver, like many other organs, NO has many actions and can be derived from multiple cellular sources [5]. So it could be consider as a marker of liver function.

Using herbal remedies for prevention and treatment of many diseases is growing worldwide. Plant extracts have a long history in the treatment of human disease. Also, many studies have been done on medicinal plants to find new remedies for the prevention and treatment of liver disease. Furthermore, it seems that herbal drugs are safe, clinically effective, and relatively cheaper. *Tragopogon graminifolius* (*TG*) known as "sheng" from compositae family is widely consumed as a green vegetable. *TG* was suggested as a beneficial plant for liver dysfunction, and used traditionally for poison elimination, aseptic property, wound healing, liver and stomach protection [6].

Our pervious study was shown that *TG* was non-toxic up to 2000 mg/kg and showed protective effect against ethanol induced gastric ulcer in rat [7]. Flavonoids are the main active constituents of *Trogopogon* genus [8], and its other constitute include gallic acid, ferulic acid, caffeic acid, p-coumaric acid, Catechin, quercetin, swertisin, apigenin, luteolin, and lucenin. Furthermore, teriterpene saponins, and also vitamins such as C, K and E have been detected in this genus [9].

There are many reports on protective and treatment effect of herbal or natural remedy on liver cytotoxicity. Pre-treatment with the flowers extract of *Calotropis gigantea* decreased the levels of serum enzyme markers, thus suggesting that the extract possessed compounds that protected the hepatocytes from alcohol-induced liver injury and subsequent leakage of enzymes into the circulation [10]. Also, Ghanbari et al. showed that a neutral compound, royal jelly improves the serum levels of AST, ALT, and ALP in STZ-induced diabetes [11].

Nedaii *et al.* [12] carried out an experiment on protective effects of *TG* extract on liver of rats treated with CCl4. They found that *TG* is able to improve the adverse effects of tetrachloride carbon, including reduced liver enzymes and total antioxidant capacity. Also a study was reported hepatoprotective effect of Dill extract against CCl4 induced liver toxicity in rat [13]. The aim of the present study was to investigate *TG* protective effect against ethanol induced liver enzyme (ALT, AST and ALP) and Nitric Oxide (NO) changes of male rat.

Materials and Methods

Plant

TG was collect from Kermanshah province in the west of Iran and authenticated by Dr. F. Attar (Department of Biology, Faculty of Sciences, University of Tehran), and a voucher specimen (No.43603) deposited in the central herbarium of Tehran University.

Extract preparation

Arial parts of *TG* were dried in shadow at room temperature and were powdered. 100 g of powder were minced in 600 ml 70% ethanol. After 48 hours, the extract was filtered and ethanol was evaporated. The extract was weighted (6.73 g) and kept in refrigerator [7].

Animals

Male Wistar rats (210±10 g) were used in this study. Animals were kept under standard laboratory conditions (23±2˚C, 12 light and 12 dark cycles and standard humidity) and had free access to water and food ad libitum. The ethic committees for animal study accept the protocol of present study. The ethanol (Merck, Germany) was used for induction liver damage.

Experiment design

Thirty Wistar male rats were divided into 5 groups (n=6) including: Group 1 and Group 2: received 1cc/kg distilled water (DW), Groups 3-5: received *TG* hydroalcoholic extract (50, 100 and 150 mg/kg) [7]. The extracts and water were given orally for 15 consecutive days. At the 15th day, ethanol (5 g/kg) was given to all groups except group 1 (positive control) by gavage [14]. After one hour, animals were anesthesayzed and blood was collected from their heart. Serum was isolated after centrifugation at 2500 rpm and stored at -20^{ºC} for biochemical analysis.

Determination of serum parameters

Serum parameters including ALT, AST, and ALP were evaluated as described previously via diagnostic kite [11]. Serum level of NO was determined using calorimetric Griess reaction as described previously [15]. Also, the AST/ALT ratio was calculated.

Data analysis

The values are presented as the means ±standard deviation (SD) and One-way ANOVA with tukey post hoc test was used for data analysis. P values less than 0.05 were considered significant. Results are expressed as mean ± standard error (SEM).

Results

Ethanol increased serum level of ALT, AST, AP and NO significantly ($p<0.05$). Pre-treatment with TG extract inhibit serum level increasing of ALT ($p= 0.001$), AP ($p= 0.004$), and NO ($p= 0.000$) significantly, but AST decreasing was not significant (table 1, figure 1A-D). *TG* extracts inhibit ALT level increasing dose dependently and 150 mg/kg of *TG* kept serum ALT near to control level. The AST/ALT ratio were increased 3 times in ethanol groups compare to control and this ratio decreased by *TG* extract in dose dependant manner (table 1).

It reaches near to control group (6.51) in 150 mg/ kg of *TG*. Also *TG* extracts, decrease serum level of NO dose dependently and alcohol consumption cannot increase it even to normal (control) level (figure 1B). Pre-treatment with *TG* extract decreased serum level of AP in 50 and 100 mg/kg extract concentrations.

Table 1. The serum values ±SD of ALT, AST, AP and NO in controls and experimental groups.

Groups	ALT (IU/L)	NO (µM)	AP (IU/L)	AST (IU/L)	AST/ ALT ratio
Control (DW)	47.14±2.93	17.58±1.42	332±15.55	201±16.47	4.46
DW+ Ethanol	67.57±3.8	22.32±2.94	387±31.17	857±89.17	12.68
TG 50 mg/Kg + ethanol	59±4.89	10.41±2.34	248±29.7	551±63.2	9.34
TG 100 mg/Kg + ethanol	57.64±5.01	7.02±1.2	236±22.97	584±58.9	10.13
TG 150 mg/Kg + ethanol	42.29±4.15	6.83±1.26	311±21.43	267±43.2	6.31
P=	0.001	0.000	0.004	>0.05	--

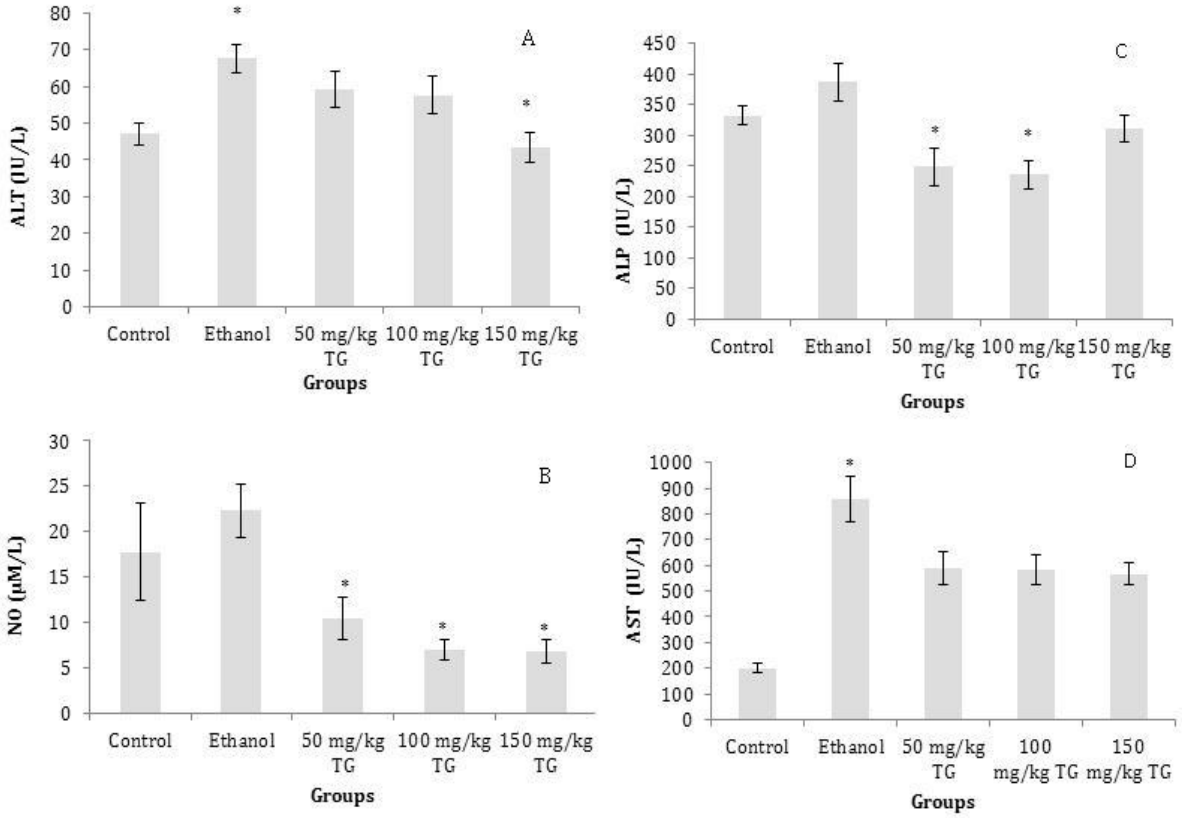

Fig. 1. Serum level of ALT (A), NO (B), AP (C) and AST (D) in control, ethanol and 50, 100, and 150 mg/kg *TG* extracts. Differences between groups were significant for ALT (p= 0.001). AP (p= 0.004), NO (p= 0.000) compare to ethanol groups. AST in ethanol group increased significantly, it decreased in extract groups (p>0.05).

Discussion

Oral administration of ethanol leads to a significant increase in serum levels of liver biomarkers (ATL, AST, and ALP) and NO in male rat. *TG* hydroalcoholic extract was found to protect hepatocytes against ethanol induced toxicity in rat, and pretreatment with *TG* inhibits

serum level elevation of ALT, ALP and NO significantly during ethanol consumption and decreased serum level of AST in pretreated experimental groups. Although important progress has been made in better understanding of liver disorder induced by alcohol, the current therapy still is not effective. Herbal and natural remedies possess a long history in the prevention and treatment of human disease and many plant extracts have been used for hepatic protection against alcohol-induced toxicity.

To our knowledge, this is the first report on the protective effect of *TG* against ethanol induced hepatic damages. Many studies have examined the protective effect of total plant extract, herbal mixture, and active compounds of herbal plants on hepatic disorders [10-13]. *T. graminifolius* has been used for gastrohepatic disorder in Iranian folk medicine [16] and a recent experiment on protective effects of TG extract on liver of rats treated with CCl4 showed that *TG* is able to improve the adverse effects of carbon tetrachloride, including reduced liver enzymes and total antioxidant capacity [12].

ROS, as a common by-product of oxidative biochemical and physiological processes, is involved in numerous physiological and pathophysiological processes. Furthermore, higher concentrations of ROS can result in cell damage through oxidative modification of proteins, lipids and DNA and, therefore, plays a major role in the pathogenesis of a variety of human diseases [17]. Kim et al. [18] have reported that liver injury is associated with the increase of ROS generation in liver.

The current study demonstrated that alcohol leads to a significant increase in NO in treated subjects as compared to the control group, highlighting the role of alcohol in increased production of free radicals.

Many natural and synthetic compounds could use to induce liver injury; selection of ethanol in present study was based on importance of ethanol consummation in human community and strong needs to protect liver damages against ethanol. Although a recent study was reported hepato-protective effects of *TG* extract on acute toxication of CCl4 in rat liver [12], but there isn't common consumption of CCl4 by human. Also, Souli et al. used a simple method of acute ethanol-induced oxidative stress in rat liver [14] and we select their model for our study.

Ethanol-induced oxidative stress plays a crucial role in alcoholic liver disease. Alcohol induced production of reactive oxygen species (ROS) and decrease antioxidant level. One of the most sensitive parts to alcohol is cell membrane. Elevated ROS and free radicals together with the inhibition of the antioxidative system by ethanol can generate a state of oxidative stress and lead to cell damage through various mechanisms. Therefore, alcohol caused membrane damages and enzymatic (ALT, AST, AP) leakage to blood plasma. ALT, AST and AP are key enzymes of liver function. Moreover, AST/ALT ratio is useful in medical diagnosis to differentiate between causes of liver damage, or hepatotoxicity and AST/ALT ratio of 2:1 or greater is suggestive of alcoholic liver disease[19]. In present study, this ratio reached to 3:1 in ethanol-induced liver damage animal and it decease near to normal rat in 150 mg/kg *TG* extract.

NO is a mediator of inflammation process and regulates the function of many organs in the body. NO has complex behavior in pathophysiological condition and involved in several hepatic diseases [5]. Pretreatment with all *TG* doses of our study inhibit serum NO elevation by alcohol. This effect could contribute to antioxidant compound in TG. Previously, we evaluated gastroprotective activity of *TG* hydroalcoholic extract which indicates the strong anti-ulcer activity of *TG* extract and was attributed to phenolic components and antioxidant mechanism of action [7]. Polyphenols exert their antioxidant effect by reducing oxidative stress. Antioxidant and free radical scavenging activity of *TG* are responsible for hepatoprotective function of *TG* which has been proven, previously [20]. Thus, phenolic constituents of TG are the main active components which exert biological functions including antioxidant and hepatoprotective activity.

Conclusion

TG showed hepatoprotective effect against ethanol-induced liver toxicity and traditional consumption of this herb in liver disorder was

confirmed. More studies are recommended to determine TG active components and its complete mechanisms of hepatoprotective function.

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Conflict of Interests

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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