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Antioxidative and hepatoprotective effects of hydroalcoholic extract of *Artemisia absinthium* L. in rat

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ABSTRACT

Introduction: *Artemisia absinthium* L. (AA) is a large, diverse genus of the family Asteraceae. AA has long been used as customary herbal medicine in world for the treatment of gastric pain, cardiac stimulation, improvement of memory and for the restoration of declined mental function. The aim of present study was to evaluate the hepatoprotective effects of AA on some factors reflecting the development of oxidative toxic stress in plasma.

Methods: Twenty male rats were equally divided in to 4 groups (5 rats each). Group I acted as control (received normal salin). Treatment groups were II, III and IV which were given *Artemisia* 10, 50 and 100 mg/kg/day respectively only by gavage for 24 hours. After treatment, blood specimens were collected. Liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) with total antioxidant power (TAP) and total thiol groups (TTG) concentrations were measured.

Results: Levels of ALT, AST and TTG were decreased in the group II compared to the control (group I). ALT and AST in 50 mg/kg group was observed compared with control group. Also, TTG increased in *Artemisia* 50 mg/kg group compared to control group.

Conclusion: Results suggests that alcoholic extract of *Artemisia* can ameliorate liver toxicity in rats through reducing the serum levels of ALT, AST, and oxidative damage.

Implication for health policy/practice/research/medical education:
Artemisia absinthium is able to ameliorate liver toxicity by reducing oxidative damage and might be beneficial in patients using toxic agents.

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Introduction

An imbalance between endogenous antioxidant defense system and reactive oxygen species (ROS) level in the body causes oxidative stress, which is associated with overproduction of free radicals from various liver disorders, such as alcoholic liver damage, non-alcoholic fatty liver disease and drug-induced liver injury (1,2). The liver is an important target of the toxicity of drugs and xenobiotics (3). Recently, natural agents with improved effectiveness and safety profiles as a therapy for liver disease have been extensively sought because the administration of drugs sometimes meets with limited therapeutic success and is usually associated with serious complications, especially the long-term use (4,5). ROS derived from many sources influence macromolecules in the liver result in hepatotoxicity (6). In general, ROS can be scavenged by detoxifying systems within the body, such as glutathione, glutathione peroxidase, and catalase (7). However, quantities of ROS that overwhelm the capacity of the body's defence system lead to a disturbance of homoeostasis in ROS production and antioxidant defence. Which in turn eventually damages biological molecules and key cellular components and processes such as lipid peroxidation, enzyme inactivation, and oxidative DNA damage (8). Dietary antioxidant

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supplementation may help to restore this balance eventually inhibition of hepatotoxicity (9,10). Therefore, plant products or alternative medicines that could limit ROS-mediated injuries are essentially needed to protect aid in the protection the liver from against all types of possible damage (11,12). *Artemisia* is a large, diverse genus of the family Asteraceae. *Artemisia absinthium* (AA), which is also known as 'sweet wormwood' and 'Qinghao' has traditionally been used in traditional Chinese medicine for treatment of fever and chills (13). In particular, *Artemisia* and its derivatives have been used clinically in the treatment of drug-resistant malaria while they were reported to have several bioactive functions including antitumor and anti-inflammatory activities (14-16). In addition, coumarins, flavonoids, and other terpenoids constituents present in AA are also reported to have significant pharmacological activities such as antitumor and antibacterial activities that contribute to the therapeutic effects of the herb (17,18). The aim of the present study was to evaluate the hepatoprotective effects of AA on some factors reflecting associated with the development of oxidative toxic stress in plasma.

Materials and methods

Chemicals and reagents

Dithiobis-(2-nitrobenzoic acid) (DTNB) and Tris base, 2,4,6-tripyridyl-s-triazine (TPTZ) from Merck Chemical Co. (Tehran) and alanine aminotransferase (ALT) & aspartate aminotransferase (AST) kit from Parsazemon Co. (Tehran) were used in this study.

Plant material and preparation of extract

Aerial parts of the plant AA were procured from herbs stores. The taxonomic identity of the plant material was authenticated by Herbarium Unit of the Faculty of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran with a deposition specimen holder (No: 220). The dried aerial parts of the plant were milled to a fine powder using an electric blender. The methanol extract was prepared by extracting 50 g of the powdered plant material using soxhlet apparatus (20 hours). Thereafter, the resulting methanol extract was reduced in vacuo (40°C), freeze dried and stored at 4°C until further used. The yield was 11.69% (w/w).

Animals and treatments

20 Wistar rats (200 ± 20 g) were purchased from the Animal Center of Pastor Institute, Wuhan University, Wuhan, China. They were housed on 12 hours light–dark cycle at 25 ± 2°C in relative humidity of 30%-60%, and maintained on a standard pellet diet and water ad libitum. The study received clearance from the Institutional Animal Ethical Committee of the Hamadan University of Medical Sciences, Hamadan, Iran.

The experimental animals were divided into 4 equal groups: Group I acted as control (received nothing). Treatment groups were II, III and IV which were respectively given *Artemisia* 10, 50 and 100 mg/kg/day by gavage for 24 hours (Group I (control), while group II was given Artemisia (10 mg/kg/day). Animals of groups III received only Artemisia (50 mg/kg/day). Group IV was given Artemisia (100 mg/kg/day) with gavage for 24 hours. After treatment, blood specimens were collected.

Serum biochemical analysis

Blood specimens were collected from the abdominal aorta. After centrifuging at 3000 g for 15 minutes, the serum was separated and stored in -80°C. The serum levels of AST and alanine transaminase ALT were determined by using autoanalyzer.

Determination of antioxidative biomarkers

Assay of total antioxidant power

Total antioxidant power (TAP) was measured by ferric reducing ability of plasma (FRAP) method. This method is based on the ability of plasma in reducing Fe^{3+} to Fe^{2+} in the presence of TPTZ. The reaction of Fe^{2+} and TPTZ gives a complex with a blue color and maximum absorbance in 593 nm (19).

Assay of total thiol groups

To evaluate the plasma total thiol groups (TTG), DTNB was used as a reagent. DTNB reacts with thiol molecules and creates a yellow complex which has good absorbance at 412 nm in spectrophotometer (20).

Statistical analysis

All experiments were done in triplicate and results were reported as mean ± SEM (n = 5). The data were analyzed by one-way analysis of variance (ANOVA). Statistically significant effects were further analyzed. Means were also compared using Tukey's multiple range tests. Statistical significance was determined at $P < 0.05$.

Results

Table 1 shows the mean ± SE of variables related to either oxidative stress or liver function in the animals tests. A significant increase ($P = 0.041$) in TTG was observed in the group II as compared to control group (group I). ALT of animals-treated with *Artemisia* 50 mg/kg were significantly ($P = 0.046$) lower than that of control group. Levels of ALT in the group II 50 mg/kg *Artemisia* were significantly ($P = 0.046$) lower than those of the control group (group I). Also, 50 mg/kg *Artemisia*, reduced AST, with respect to the control group ($P = 0.035$). No significant differences were observed in the TAP between the groups.

Discussion

This study was aimed to evaluate the hepatoprotective effects of AA on some factors reflecting the development of oxidative toxic stress in plasma. Administration of hydroalcoholic extract of AA would improved the liver

Table 1. Liver function and antioxidative parameters in animal blood test

Groups n = 5	TAP (umol/ml) Mean ± SE	TTG (nmol/ml) Mean ± SE	ALT (U/ml) Mean ± SE	AST (U/ml) Mean ± SE
Control	2.1 ± 0.02	0.12 ± 0.02	24.5 ± 3.8	52.6 ± 13
Artemisia 10 mg/kg	2.4 ± 0.3	0.14 ± 0.02	22.4 ± 2.1	37.1 ± 8.8
Artemisia 50 mg/kg	2.8 ± 0.4	0.77 ± 0.07[a]	16.3 ± 1.6[a]	19.2 ± 2.3[a]
Artemisia 100 mg/kg	2.6 ± 0.7	0.28 ± 0.11	17.9 ± 2.3	31.4 ± 7.8

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; TAP, total antioxidant power; TTG, total thiol groups.
[a] Significantly different from control group at $P < 0.05$.

function and the level of oxidative stress parameters such as TTG in blood. Free radicals are the initiators of a redox reaction cascade, which may lead to changes of the chemical structure of biological macromolecules, such as proteins, lipids and DNA, or trouble of human cell metabolism or even tissue injury (21). In vitro and in vivo studies reported the antioxidant capacity of several species of medicinal plants, acting at cellular level, through cell growth stimulation, membrane potential stabilizing or at molecular level, through ROS scavenging, lipid peroxidation, etc. (22). Also, for the evaluation of liver injury serum concentrations of the most commonly used biochemical markers, ALT and AST were determined. Increased levels of these specific hepatic marker enzymes after chemical or immunological intoxication, compared to control rats, indicated considerable hepato-cellular damage and resulting leakage of cytosolic contents into the systemic circulation (23). AA extract seems to be able to preserve the structural integrity of the hepatocellular membrane, as can be seen with the evident reduction of serum ALT and AST activities of pretreated rats in both experimental models. While previous study showed that 80% aqueous-methanolic extract of *A. absinthium* was effective against CCl4-injury at the concentration of 500 mg/kg (24), our results indicated improving liver function in 50 mg/kg of AA. Previous studies showed the reduction of serum ALT and AST levels (25,26). We elucidated the probable mechanisms of hepato-protection investigating further the effects of AA on the liver antioxidant status. The liver is a principal organ involved in generation of ROS induced by drugs and toxic chemicals (27). In this study, our results suggested that AA could exert its antioxidant or radical scavenging activities thus preventing the formation ROS. AA administration was shown to put off decrease TTG concentration in experimental animals. AA by directly scavenging the free radicals and improving liver function in rats may increases in the glutathion (GSH) content in the rat liver (13). The previous studies showed antioxidant properties of AA, too (15). In addition, to more clarify possible mechanisms of hepato-protective activity of AA, future studies are required investigating the molecular and cellular mechanisms in oxidative toxic stress pathways.

Authors' contributions

All authors contributed to the conception of the work, conducting the study and approval of the final version of the manuscript, and agreed for all aspects of the work.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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