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ORIGINAL ARTICLE

Hepatoprotective Effect of Hydro-Alcoholic Extract of *Zataria multiflora* Boiss to Acute Liver Damage in Mice Exposed to Carbon Tetrachloride-Induced Toxicity

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ABSTRACT
Zataria multifloraBoiss (ZM) belongs to Lamiaceae families that can provide an important source of phenolic compounds with antioxidant effects on human diets. The present study was performed with the goal to investigate the hepatoprotective potential of the hydro-alcoholic extract of Zataria multiflora against carbon tetrachloride-induced liver damage in mice.This experimental study was performed on 36 male mice (25±5) gr. The mice were randomly divided into six groups of six animals in each group. The animals were concurrently intraperitoneally administered with proper materials every day during a period of seven days. Twenty-four hours after the last injection, necropsy was done; the blood samples were collected. The next step the serum activity of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) was measured. Lipid peroxidation was evaluated in liver homogenates. A significant increase of GSH, MDA, and Liver enzymes' levels, including ALT, AST, and ALP was observed between the bunches receiving carbon tetrachloride + diverse dosage of ZM hydro-alcoholic extract and group three, which just received carbon tetrachloride ($p<0.05$). The effective dose (400 mg/kg) reduces the enzymes' ALT, AST, and ALP as 82.83±7.985, 86.17±8.909, 121.67±12.485, respectively. The results revealed that hydroalcoholic extract of Zataria multiflora has a protective effect on liver toxicity induced by carbon tetrachloride.
Keyword: *Zataria multiflora, Carbon Tetrachloride, Hepatotoxicity, Antioxidant,MDA, GSH*

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INTRODUCTION

Liver diseases are considered of the global issues accompanying high deaths grade [1].One of the most common locations for toxins Accumulation is liver receiving a high volume of blood circulation based on weight unit and as a result; it is exposed to poisonous substances. Since this organ is the location almost for body biochemical pathways, it plays a vital role in implementing and regulating body hemostasis and has further metabolic and detoxification functions, making it more sensitive to toxic substances [2]. Carbon Tetrachloride as an important hepatotoxic gets metabolized by P450 cytochrome system after entering the body and generates free radicals through metabolizing enzymes. During Carbon Tetrachloride metabolism, two toxic compounds, including trichloromethyl (CCL_3) and proxy tetrachloromethyl ($OOCCL_3$) – radical [3] result in non-saturated acids' peroxidation and calcium hemostasis disorder ultimately, cell death and because of creating acute or chronic toxicity, it causes some damages like necrosis, lipid change, and hepatic cirrhosis. Free radicals result from carbon

tetrachloride with hepatocyte membrane destruction and increased enzyme's activity of the above mentioned. The same factor caused the enzymes naturally located in cellular cytosol getting into a bloodstream, and these enzyme's activity increases can indicate the liver damage type and level [4].

Using medicinal plants for treating diseases has been common for a long time in human communities and for about half a century ago, plants were considered of the most important sources to treat pains. The necessity behind fundamentally and realistically analyzing traditional medicine and medicinal plants has been concentrated for an extended time in our country. It based academic communities and in recent years, the requirement of investigating medicinal plants have attracted excessively attention. During the overdue decade, a large number of plant products and food compounds have been examined as hepatoprotective [5].

This plant with the vernacular name of Avishan-e-Shirazi (Shirazithyme) belonging to Lamiaceae family is vulgar of Iran, Afghanistan, and Pakistan [6]. *Zataria multiflora* is a valuable medicinal plant of this family. Zataria genus is of Lamiaceae family with a shrub species known as *Zataria multiflora* boiss. [7]. Its raw material contains volatile oil, including phenol, Thymol, and Carvacrol as the supplements of Flavonoids activity. Its essential oil or extract contains Terpinene, phenol, and aliphatic compounds with diverse vital activity [8]. Polyphenol, particularly flavonoid compounds with their antioxidant possessions have a protective effect against hepatotoxic damage. Flavonoids oxidation by free radicals resulted in produce some radicals with lower activity and higher steadiness. These increased reactions within the hydroxyl group existing in Flavonoids defuse the radicals [9].

Since broad studies have been conducted on other effects of this plant, the present study has been designed to determine the effects of ZM (considering the type of the compounds available in this plant, especially types of Flavonoids) that has Hepatoprotective and anti-radical effects against carbon tetrachloride-induced Hepatotoxicity(as a model creating Hepatotoxicity).

MATERIALS AND METHODS

Plant material extraction procedure

The aerial parts of the ZM were collected from Mazandaran, north of Iran, systematically identified and approved by Biology Department of Mazandaran University of Medical Science, (deposited in Herbarium of Mazandaran University, director: Dr.EmranHabibi). The ZM was powdered in an electric chopper. The extraction was carried out through the multistage maceration of dry plant powder in ethanol 70% for 48 hours at room temperature. After the extraction, ethanol was evaporated by rotary evaporator at 40ºC and was dried using a freeze-dryer at - 50ºC The efficiency of extraction was 10%. The extract was constructed in distilled water before use. [10]

Laboratory animals

The male mouse with a regular weight of 25±5g was used for this assay. They were procured from the animal breeding laboratories of Faculty of Pharmacy Sari (Mazandaran, Iran) and had a free approach to food and water, and were persevered in a qualify temperature (20 ± 25ºC) and light cycle (12 h light and 12 h dark).

Experimental design

The animals were divided into six groups. Each consisting of six mice as intraperitoneal(IP) injection, Group 1 standard normal saline as a regular mouse, group two received Olive oil, group 3 received 10% carbon tetrachloride (one ml/kg, Ip); groups 4-5 received Zatariamultiflora hydro-alcoholic extract in doses of 200 and 400 (mg/kg body weight (b.w.),Ip) relatively and carbon tetrachloride (one ml/kg, Ip), group 6 legal on which contained vitamin C (one mg/Kg (b.w.)) as a reference drug.

Throughout the experiments, local principled guidelines were considered for taking care of laboratory animals. Twenty-four hours after the last injection, mice were sacrificed by an overdose of diethyl ether, and Surgery was performed in accordance with ethical principles. Blood samples were withdrawn, collected in tubes and After 30 minutes of safekeeping at an ambient temperature for incubation, then were centrifuged at 1500 × g for 15 minimums to obtain plasma. Plasma samples were used to determine the aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) activities. On the other hand, the liver of each mouse was promptly removed and used to determine the tissue levels of malondialdehyde (MDA) and glutathione (GSH) [10].

Biochemical assays

Standard Biochemical kits, ELITEK and RA-1000 autoanalyzer were used to measure the AST, ALT, and ALP activities in plasma. The methodology described by Kurtel *et al* was used to measure malondialdehyde. Malondialdehyde reacts with thiobarbituric acid to a temperature of 100 °C, to produce a pink colour. By butanol and Tris buffer was extracted. Absorption at a wavelength of 490 Nanometer (nm) was read using ELISA [10]. The glutathione-based compounds containing sulfhydryl groups with

DTNB Changes in absorbance at 412 nm was measured on a spectrophotometer and reduced glutathione was used to obtain a standard curve [11].

Statistical analysis

The acquired data were analyzed by one-way ANOVA followed by the Tukey's post-hoc test and $p < 0.05$ were considered statistically significant.

RESULTS

According to the table (1) listed findings, carbon Tetrachloride has decreased the reduction glutathione. It, Furthermore, has increased the enzymes ALT, AST, ALP, & MDA. Using hydro-alcoholic extract of *Zataria multiflora* after carbon tetrachloride toxicity results in a glutathione level rise compared with the toxin receiving group. About the increased enzymes, ALT, AST, ALP also, it decreased them with a meaningful difference and MDA level as the oxidative stress marker has declined compared with a toxin receiving group that as the extract dose increased shown in the table, the decline in 400 mg dose has been more significant.

The results gained by measuring the mouse liver enzymes (ALT, AST, & ALP) levels after ZM extract injection as 200mg/kg revealed that the data have a significant difference in the outcomes based on measuring the mouse liver's glutathione after carbon tetrachloride injection as the positive control group ($P<0.05$). The results of the mouse liver enzymes (ALT, AST, & ALP) levels measurement after injecting ZM extract as 400, g/kg indicated that the data have a significant difference on the results completed through measuring the mouse liver's glutathione of post-carbon tetrachloride injection ($P<0.05$). Thus the extract has led to the liver enzymes' drop in the group receiving toxin and according to the table (1), the liver enzymes' variations among the study groups have been illustrated. The liver glutathione measurement after 400 mg/ kg ZM injection suggested that the data had a significant difference in the liver glutathione measurement after carbon tetrachloride injection ($P<0.05$).

Regarding the results of MDA measurement that considered as an index for oxidative stress assessment, according to the table (1) included data, it has been very high after carbon tetrachloride injection. Comparing the results of MDA measurement between the extract receiving groups as 200 and 400 mg/kg + carbon tetrachloride with the positive control group' results revealed that both groups had a significant statistical difference ($P<0.05$).

DISCUSSION

In the current research, we have surveyed *Zataria multiflora* induced effects on carbon tetrachloride led to hepatotoxicity and as seen compared with the control group, ZM extracts have resulted in a significant glutathione level increase, and consequential liver enzymes level decrease. To exonerate this process, the antioxidant impact of ZM can be stated that via neutralizing body-free radicals. It has reduced glutathione consumption level of the body. Regarding the comparison of the group receiving vitamin C, this hypothesis can be arranged for that the extract plays the role of vitamin C (since similar to this vitamin, it probably increases glutathione and has antioxidant like role) and plays the role in converting the glutathione oxide form (GSSG) to its reduced form (GSH) [12,13 and 14].

Furthermore, it can be said that this plant extracts, compared with the positive control group (carbon tetrachloride) have decreased liver enzymes and glutathione, the result that can be attributed to phenolic compounds available to the plant extracts commonly as Carvacrol.

About enzymes level, the indicator of liver destruction, it can be mentioned that when liver cells are damaged and consequently, its available enzymes are released into serum; these enzymes level increments in blood [14].

The enzymes' ALP, AST & ALT levels in the mice that receiving ZM hydroalcoholic extract as 200 and 400 mg/kg have declined compared with the group receiving carbon tetrachloride and at the same time as the dose increases. These enzymes' levels go up and finally, it is revealed ZM extract plays a protective role against carbon tetrachloride-induced liver destruction while ZM extract concentration rises; this custody gets higher. It considerably prevents the level decrease the reason of which can be antioxidant compounds like phenol and flavonoid in ZM neutralizing toxic compound, and the antioxidant produced by carbon tetrachloride.

In the present research, especially the significant difference of lipid peroxidation in the group receiving the extract compared to the group receiving carbon tetrachloride vividly displays that this plant has antioxidant property and naturally. This property of the reduced produced free radicals and liver damage has decreased, too. As a result, liver enzymes' activity has got lower as the tissue repaired. Instinctive antioxidants are found almost in all plants, microorganisms and even in all animals' tissues. The majority of natural antioxidants belong to phenolic compounds [15].

Although the intracellular possessive mechanisms greatly decrease ROS induced damage [16], due to abundant production of these free radicals, the presence of other protective channels, particularly foodstuff and natural antioxidants is really useful for a human. Antioxidants are able to reduce lipid peroxidation and Hepatocyte necrosis considerably protects the liver against destruction. That is why in the recent decade, a large number of herbal products and food ingredients have been analyzed as hepatoprotective.Today, despite plenty of advances in modern medications, no drug exists to be able to stimulate and reconstruct damaged liver. This issue has motivated some pharmacologists to probe for the potential of hepatoprotective activity in such plants and based on traditional herbal medicine, since many plant compounds, including phenol, possess antioxidant properties [17].

In the research done by Sibghatullah Sangi *et al.* on the hepatoprotective effect of Oleuropein, thymoquinone and date in the carbon tetrachloride induced toxicity in rat showed that these substances significantly overhaul liver tissue and enzyme destruction [18]. In the review paper by Sajed *et al*. on the effects and pharmacological valuable ingredients of ZM suggested that these plant properties were 100% antibacterial, antioxidant, anti-inflammatory and pain killing and its extract contained Carvacrol and Thymol [19].Thus regarding the present study results, ZM hydroalcoholic extract can be benefited as hepatoprotector. On the other hand, more comprehensive assessment of another animal biochemical and pathological circumstances additionally using the extract effective compounds can give more conclusive results. In order that it is potential further generalizing the data to the human community.

Table 1.ZM hydro-alcoholic extract preparation results on the liver enzymes' activity of the animals exposed to CcI4 induced toxicity

Groups	ALT(IU/L)	GSH nmol/g liver	AST(IU/L)	MDA nmol/g liver	ALP(IU/L)
1.normal saline (negative control)	36.5±14.209	0.979±0.112	48.17±10.342	0.194±0.074	52.67±12.176
2.olive oil (marker)	103.33±15.552	0.841±0.149	103.00±6.356	0.268±0.064	107.50±7.259
3.Carbon Tetrachloride	184.67±16.269	1.016±0.398	129.00±13.755	1.170±0.085	20.027±221.67
4.Carbon Tetrachloride + extract 200mg/kg	111.17±14.162	1.112±0.108	9.004±106.67	0.765±0.163	19.146±172.17
5.Carbon Tetrachloride + extract 400 mg/kg	82.83±7.985	0.529±0.062	86.17±8.909	0.259±0.095	12.48±121.67
6.vitamin C	56.5±9.731	1.122±0.169	9.559±88.83	0.160±0.046	77.67±15.642

Results are expressed as mean ± SD.

CONCLUSION

The hydro alcoholic extract of *Zataria multiflora* has the protective effect against the grave liver damage and hepatoprotective mechanisms of this extract on CCL4-induced acute liver damage might be due to decreased lipid peroxidation. More studies are needed to determine supplemental mechanisms involved in the hepatoprotective effects of this plant.

COMPETING INTEREST

The authors have declared that no competing interest exists.

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