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Evaluation of the Beneficial Effects of *Zataria Multiflora* Boiss in Halothane-Induced Hepatotoxicity in Rats*

Ocena korzystnego działania *Zataria multiflora* na hepatotoksyczność wywołaną halotanem u szczurów

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Abstract

Background. Halothane is one of the most important anesthetics. In some studies, hepatic injury was occasionally induced in animal models as a result of the interaction of halothane exposure. *Zataria multiflora* is a valuable medicinal plant in the *Labiatae* family that is distributed only in Iran, Pakistan and Afghanistan.
Objective. In this study, we attempted to evaluate the beneficial effects of *Zataria multiflora* Boiss in halothane-induced hepatotoxicity in rats.
Material and Methods. In the present study, thirty-two Wistar albino rats, weighing 180–200 g (3.5–4 months old), were divided into four different groups for 7 days as follows: control animals (Group C), which did not receive any treatment, the second group (Group Z) which received *Zataria multiflora* extract (800 p.p.m. in drinking water) during the experiment period (7 days), the third group (Group H) which was exposed to an anesthetic gas mixture (100% oxygen and 2% halothane, v/v) in a vaporizer using the semi-closed method for 2 hours on the 6th day, and the fourth group (Group T), *Zataria multiflora*-treated animals which received 5 days of pretreatment with *Zataria multiflora* and were exposed to the anesthetic gas mixture for 2 hours on the 6th day, and then *Zataria multiflora* was continued through the 7th day.
Results. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and total bilirubin (T-BIL) were significantly increased ($p < 0.05$) by halothane anesthesia (Group H) as compared with group C. All of the aforementioned parameters except AST were significantly decreased ($p < 0.05$) in group T in comparison with group H.
Conclusions. Based upon our results, *Zataria multiflora* extract may have a prophylactic effect in the prevention of halothane hepatotoxicity complications (**Adv Clin Exp Med 2011, 20, 1, 23-29**).

Key words: rat, halothane, *Zataria multiflora*, liver.

Streszczenie

Wprowadzenie. Halotan jest jednym z najważniejszych środków znieczulających**.** W niektórych badaniach uszkodzenie wątroby wywołano przypadkowo na modelach zwierzęcych w wyniku oddziaływania halotanu. *Zataria multiflora* jest cenną rośliną leczniczą z rodziny *Labiatae***,** która występuje tylko w Iranie, Pakistanie i Afganistanie.
Cel pracy. Ocena korzystnego działania rośliny *Zataria multiflora* na uszkodzenie wątroby u szczurów wywołane przez halotan.
Materiał i metody. W przeprowadzonym badaniu, trwającym 7 dni, 32 albinotyczne szczury szczepu Wistar o masie 180–200 g (3,5–4-miesięczne) zostały podzielone na 4 różne grupy na 7 dni w następujący sposób: zwierzęta z grupy kontrolnej (grupa C), które nie otrzymywały żadnego leczenia, druga grupa (grupa Z), która otrzymała ekstrakt *Zataria multiflora* (800 ppm w wodzie pitnej) podczas eksperymentu (7 dni), trzecia grupa (grupa H), która została poddana narkozie mieszaniną gazów znieczulających (100% tlenu i 2% halotanu, *v/v*) w parowniku metodą częściowo zamkniętą na 2 godz. przez 6 dni, a w czwartej grupie (grupa T) były zwierzęta, którym podawano *Zataria multiflora*; wstępnie otrzymywały *Zataria multiflora* przez 5 dni i 6. dnia poddano je działaniu mieszaniny gazów znieczulających przez 2 godz. , a 7. dnia kontynuowano podawanie im *Zataria multiflora*.

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Wyniki. Stężenie aminotransferazy asparaginianu (AST), aminotransferazy alaninowej (AlAT), dehydrogenazy mleczanowej (LDH), fosfatazy alkalicznej (ALP) i stężenie bilirubiny całkowitej (T-BIL) były znacznie większe ($p < 0,05$) pod wpływem znieczulenia halotanem (grupa H) w porównaniu z grupą C. Wszystkie wyżej wymienione wskaźniki, z wyjątkiem AST, były istotnie mniejsze ($p < 0,05$) w grupie T w porównaniu z grupą H.

Wnioski. Na podstawie wyników badań autorzy uważają, że ekstrakt *Zataria multiflora* może wywierać korzystne działanie w zapobieganiu powikłaniom uszkodzeń wątroby wywołanych halotanem (**Adv Clin Exp Med 2011, 20, 1, 23-29**).

Słowa kluczowe: szczur, halotan, *Zataria multiflora*, wątroba.

Halothane is one of the most important anesthetics commonly used for anesthetizing humans and animals, and is metabolized up to 20% by the cytochrome P450 microsomal system in the liver [1, 2]. Halothane is metabolized to trichloroacetic acid, which may undergo reductive metabolism to produce hepatotoxins during hypoxia. In some studies, hepatic injury was occasionally induced in animal models as a result of the interaction of halothane exposure, induction of liver enzymes, and hypoxia, especially after multiple halothane exposures over short periods. Accumulation of volatile metabolites and other free radicals in hepatic cellular components may increase cellular degeneration of the liver [2] and increase the plasma activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), and affect some other biochemical and hematological parameters in laboratory animals [3]. Several procedures have been used to protect the liver from damage by administering antioxidants such as β-carotene [4], vitamin C [5, 6], vitamin E and Selenium-vitamin E combination [3].

Many herbal concoctions are said to be effective in chronic inflammatory conditions. *Labiatae* are generally known for their various effects such as analgesic and anti-inflammatory activities [7], antioxidant [8], hepatoprotective [9] and hypoglycemic actions [10]. *Zataria multiflora* is a valuable medicinal plant in the Labiatae family that is distributed only in Iran, Pakistan and Afghanistan [12]. It is extensively used for medicinal and condimental purposes in these countries. This plant, with the vernacular name of "Avishan Shirazi" in Iran, has several traditional uses such as antiseptic, anti-fungal, antispasmodic, analgesic, antitussive, anti-inflammatory, antioxidant, antinociceptive, hypoglycemic and carminative properties [11, 14, 15]. There are also commercial pharmaceuticals with formulae based on *Zataria multiflora* essential oil. The chemical compositions of extracts have been extensively characterized in Iran [11, 16] and Pakistan [17]. The extract contains thymol, carvacrol [13, 17] zatrinal, oleanolic acid, betulic acid, rosmarinic acid [18] and also monoterpenoids, sesquiterpenoids, p-cymene and γ-terpinene [15, 18].

From these reported activities for this plant and regarding the above-mentioned points about halothane, we hypothesized that premedication by *Zataria multiflora* before anesthesia may reduce the hepatotoxicity of halothane. In this study, we attempted to evaluate the beneficial effects of *Zataria multiflora* Boiss in halothane-induced hepatotoxicity in rats.

Material and Methods

Preparation of Total Extract

Zataria multiflora Boiss tops at the full flowering stage (June and July) were collected from plants growing wild in Firoozabad, Fars province, Iran. The taxonomic identification of plant materials was confirmed by the Agricultural Research Centre of Shahid Bahonar University of Kerman and voucher specimens were deposited in the Herbarium of the School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran (KUMS). The tops of the plant were dried in the shade, ground in a grinder with a 2 mm in diameter mesh, and about 200 g of dry powder extracted with 85% methanol using the percolation method for 48 hours. Extracts were concentrated in a vacuum rotary evaporator (Buchi, Switzerland) and were left to dry in a desiccator.

Animals

Thirty-two male Wistar albino rats were purchased from the Animal Laboratory of the Kerman University of Medical Sciences, Kerman, Iran and kept in the Center for Laboratory Animal Care at the Veterinary Medicine Department of Shahid Bahonar University of Kerman for 1 week before treatment. The rats weighed 180–200 g and were the same age (3.5–4 months old). The experimental animals were randomly divided into four groups of eight animals and were housed in standard polypropylene cages with wire mesh top, at 21°C in a 12 h/12 h dark-light cycle. During the study, the animals received water and pellet food (Javaneh Khorasan co, Iran) *ad libitum* plus a vitamin and mineral mix containing vitamins A, D_3, E, K, B_1, B_2, Ca-pantothenate, nicotinic acid, pyridoxine, folic acid, biotin, cholic acid, Mn, Mg, Zn, Fe, Cu, Co, I and Se. All ethical considerations concerning using animals were considered carefully.

Test Method

The study was comprised of four different groups for 7 days as follows: control animals (Group C), which did not receive any treatment, the second group (Group Z) which received *Zataria multiflora* extract (800 p.p.m. in drinking water) during the experimental period (7 days), the third group (Group H) which was exposed to an anesthetic gas mixture (100% oxygen and 2% halothane, v/v) in a vaporizer (Ohmeda, Fluotec 4) using the semi-closed method for 2 hours on the 6th day, and the fourth group (Group T), *Zataria multiflora*-treated animals, which received 5 days pretreatment with *Zataria multiflora* (800 p.p.m. in drinking water) and was exposed to the anesthetic gas mixture (100% oxygen and 2% halothane, v/v) for 2 hours on the 6th day, and then *Zataria multiflora* was continued through the 7th day.

Sampling of Blood and Plasma

The blood of each animal was taken by cardiac puncture on the 8th day at 8–10 o'clock. All blood was collected aseptically using sterile 5 ml syringes and poured into tubes with and without the disodium salt of ethylene diamine tetra acetic acid (EDTA) as an anticoagulant. The blood (1 ml) in the tubes containing the anticoagulant was used for an analysis of hematological parameters and another portion of the blood was centrifuged (Shimifan, Iran) at 3000 g for 10 minutes at room temperature. Sera were then harvested using disposable pipettes and transferred into 1.5 µl sterile micro tubes (Eppendorf).

Biochemical and Hematological Assays

A spectrophotometer (Shimadzu, Japan) was used to measure the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and total bilirubin (T-BIL) (Ziest chem, Iran), in the serum samples. The values of erythrocyte counts (RBC), packed cell volumes (PCV), hemoglobin (Hb) concentration, mean corpuscular volumes (MCV), mean corpuscular hemoglobin (MCH), leukocyte counts (WBC), neutrophil (NEU, percent) and lymphocyte rates (LYM, percent) were determined according to the methods described in Schalm's Veterinary Hematology of Jain (1986).

Statistical Analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA) for multiple comparisons of all groups. Means and their standard errors (SE) were calculated using the $SPSS_{16}$ program. A value of $p < 0.05$ was considered statistically significant.

Results

Biochemical and hematological parameters and their comparisons among all groups are presented in Tables 1 and 2.

As shown in Table 1, serum levels of AST, ALT, ALP, LDH and T-BIL were significantly increased ($p < 0.05$) by halothane anesthesia (Group H) in

Table 1. Comparison of enzyme levels and biochemical parameters in all groups*

Tabela 1. Porównanie stężenia enzymów i wskaźników biochemicznych we wszystkich grupach

Parameters (Wskaźniki)	Group C (Grupa C)	Group Z (Grupa Z)	Group H (Grupa H)	Group T (Grupa T)
AST (IU × L^{-1})	$134 \pm 5.58^{a,b}$	$137.6 \pm 6.01^{c,d}$	$183.2 \pm 3.92^{a,c}$	$165.2 \pm 2.15^{b,d}$
ALT (IU × L^{-1})	$37.2 \pm 2.49^{a,b}$	$47.6 \pm 4.53^{c,d}$	$189.2 \pm 8.72^{a,c,e}$	$153.8 \pm 5.66^{b,d,e}$
ALP (IU × L^{-1})	345.6 ± 10.72^{a}	352.4 ± 14.03^{b}	$445.8 \pm 8.93^{a,b,c}$	379.4 ± 3.48^{c}
LDH (IU × L^{-1})	279.4 ± 53.25^{a}	328.2 ± 50.11^{b}	$589.2 \pm 51.37^{a,b,c}$	312.8 ± 62.88^{c}
T-BIL (mg × dl^{-1})	0.388 ± 0.036^{a}	0.468 ± 0.037^{b}	$0.671 \pm 0.065^{a,b,c}$	0.41 ± 0.034^{c}

* Data are expressed as means ± SE; statistical significance with respect to each group has been shown by a, b, c, d and e.
Group C – control animals.
Group Z – rats which received *Zataria multiflora* extract.
Group H – rats which were exposed to halothane.
Group T – rats which received pretreatment with *Zataria multiflora* and were exposed to halothane.

* Dane są wyrażone jako średnia ± SE; wartość statystyczna względem każdej grupy oznaczona a, b, c, d, e.
Grupa C – grupa kontrolna.
Grupa Z – szczury, którym podawno ekstrakt *Zataria multiflora*.
Grupa H – szczury, które były narażone na działanie halotanu.
Grupa T – szczury, którym najpierw podano ekstrakt *Zataria multiflora*, a potem były narażone na działanie halotanu.

Table 2. Comparison of some hematological parameters in all groups*

Tabela 2. Porównanie niektórych wskaźników hematologicznych we wszystkich grupach

Parameters (Wskaźniki)	Group C (Grupa C)	Group Z (Grupa Z)	Group H (Grupa H)	Group T (Grupa T)
Hb (g × dl^{-1})	2.2 ± 0.34^{a}	1.8 ± 0.37^{b,c}	3.9 ± 0.33^{a,b}	3.04 ± 0.16^{c}
PCV (%)	34 ± 0.7^{a}	34.4 ± 0.51^{b}	39 ± 0.89^{a,b,c}	35.2 ± 0.8^{c}
RBC (× 106 x µl^{-1})	4.9 ± 0.33	4.1 ± 0.64	4.1 ± 0.62	4.2 ± 0.6
MCV (fl)	69.2 ± 3.26	93.2 ± 13.73	99.8 ± 15.95	92 ± 13.4
MCH (pg)	4.74 ± 0.9	4.92 ± 1.38	9.64 ± 1.7	7.69 ± 1.12
WBC (× 103 × µl^{-1})	4 ± 0.94	3.9 ± 0.66	4.6 ± 0.91	3.6 ± 0.69
NEU (%)	18 ± 3.39	23 ± 2.7	29.2 ± 1.31	22.2 ± 5.81
LYM (%)	82 ± 3.39	77 ± 2.7	70.8 ± 1.31	77.8 ± 5.81

* Data are expressed as means ± SE; statistical significance with respect to each group has been shown by a, b and c.
Group C – control animals.
Group Z – rats which received *Zataria multiflora* extract.
Group H – rats which were exposed to halothane.
Group T – rats which received pretreatment with *Zataria multiflora* and were exposed to halothane.

* Dane są wyrażone jako średnia ± SE; wartość statystyczna względem każdej grupy oznaczona a, b, c.
Grupa C – grupa kontrolna.
Grupa Z – szczury, którym podano ekstrakt *Zataria multiflora*.
Grupa H – szczury, które były narażone na działanie halotanu.
Grupa T – szczury, którym najpierw podano ekstrakt *Zataria multiflora*, a potem były narażone na działanie halotanu.

comparison with group C. All of the aforementioned parameters except AST were significantly decreased ($p < 0.05$) in group T in comparison with group H. There was no significant difference in the concentration of all enzymes and T-BIL between groups C and Z ($p > 0.05$).

As shown in Table 2, the values of PCV and Hb were significantly increased ($p < 0.05$) in group H in comparison with group C, but there was no significant difference in other hematological parameters ($p > 0.05$) (Table 2). There were no significant differences in all hematological parameters except PCV between group T and group H and there were also no significant differences between groups C and Z in hematological parameters ($p > 0.05$) (Table 2).

Discussion

Flavonoids, as the main constituent of *Zataria multiflora* extract, are a class of plant phenolics with significant antioxidant and chelating properties. Their positive effects come from their ability to inhibit lipid peroxidation, chelate redox-active metals and attenuating other procedures involving reactive oxygen species [21]. There is evidence that flavonoids have anti-phosphodiesterase activity and thus could elevate intracellular levels of cyclic nucleotides [23]. Recent studies indicate that both cAMP and cGMP can diminish oxidative stress in many biological systems and diseases [24]. Sharififar and colleagues [25] showed that oxidation of linoleic acid was effectively inhibited by *Zataria multiflora* extract. It seems that this activity is mostly related to the presence of the phenolic compounds such as flavonoids and phenolic acids in this extract.

The hepatotoxicity induced by halothane in the present study agrees with a previous study [2]. All potent inhalation anesthetics are capable of causing hepatocellular injury by reducing liver blood flow and oxygen delivery. Localized hypoxia may damage hepatocytes directly and/or perhaps result in the production of reactive intermediary compounds from agent biotransformation. These compounds then act to produce hepatocellular damage via an autoimmune-mediated reaction or some other as-yet-undescribed processes [1]. Halothane is an important human and veterinary anesthetic, which produces free radicals during biotransformation [2]. Occasionally, these free radicals and other reactive species cause the oxidation of biomolecules (e.g. proteins, amino acids, lipids, and DNA) which leads to cell injury [26]. Halothane is metabolized to trichloroacetic acid, which may undergo reductive metabolism to produce hepatotoxins during hypoxia. Recent studies indicate that the trifluoroacetate metabolite of halothane combines with a hepatic protein, resulting in the formation of a hapten. The trifluoroacetate hapten is subsequently attacked by serum antibodies, causing hepatitis [1].

Susceptibility to hepatic damage varies from species to species. Rat liver microsomes, which contain the cytochrome P450, will bind to the reductive me-

tabolites of halothane and if these microsomal enzymes were pre-induced, hepatotoxicity results [27].

The purpose of the present study was to investigate the probable protective effects of *Zataria multiflora* on liver enzymes and to determine some other hematological parameters in the halothane anesthesia of rats. Determination of serum levels of hepatic enzymes is commonly used for detection and evaluation of hepatic diseases. The interpretation of elevated values of enzymes in plasma is dependent not only on the tissue and site of origin but also on the half-time of clearance of the enzymes [30]. The half-life in the circulation is about 47 hours for ALT and about 17 hours for total AST [28]. High serum levels of AST, ALT and LDH are usually indicative of liver damage in animals [28] and humans [29]. An increase in ALT serum levels is, therefore, more specific to liver damage. In the liver, ALT is localized solely in the cellular cytoplasm, whereas AST is both cytosolic (20% of total activity) and mitochondrial (80% of total activity) [28]. ALP levels are used as a valuable test of hepatic excretory function and, among the tests available for testing of biliary obstruction, the serum ALP test is preferred [30]. Cholestasis may also result in a progressive liver disease – biliary cirrhosis [31]. ALP and bilirubin levels are routinely assessed and the level of GGT is often measured as an additional aid toward diagnosis in particular situations because of its high sensitivity but low specificity [28]. Liver ALP is mobilized most rapidly into blood and its levels in plasma may increase at early periods of liver damage [2]. ALP half-life in the circulation is about 1 week [28]. Serum levels of total bilirubin (T-BIL) are increased due to degeneration of hepatic cells because of retention of direct bilirubin [30]. In about 80% of patients with ischemic injury, the serum bilirubin level is low, and lactate dehydrogenase (LDH), a marker of ischemic damage, may reach very high concentrations [28]. The present study showed that serum levels of these enzymes and T-BIL increased in group H, and pretreatment with *Zataria multiflora* decreased serum levels of these parameters significantly ($p < 0.05$) when the rats were exposed to the halothane. Our results are in agreement with the results of other studies performed using chemical antioxidants such as vitamin C, E and Selenium and hepatotoxic substances [2, 32]. Karakilcik et al. (2005) determined that the activities of AST, ALT and ALP enzymes were significantly increased ($p < 0.05$, $p < 0.01$, and $p < 0.05$, respectively) by halothane anesthesia, but decreased ($p < 0.05$ in all parameters) with the administration of vitamin C. Selenium, vitamin E and their combination may prevent the increase of liver enzymes after halothane anesthesia [32]. It seems that *Zataria multiflora* can efficiently scavenge free radicals before they can initiate lipid peroxidation, and contribute to the stability of cellular and basal membranes. Results of the present study showed that there were no significant differences in serum levels of all enzymes and T-BIL between groups C and Z ($p > 0.05$), although the mentioned factors in group Z were higher than group C.

Halothane anesthesia significantly increased the values of packed-cell volume (PCV) and hemoglobin (Hb) concentrations ($p < 0.05$). There is hypoxia during halothane anesthesia and production of free radical species may increase via the reductive metabolism of halothane in hypoxia [33]. The results of the present study show that red blood cell (RBC) counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), white blood cell (WBC) counts, Neutrophil (NEU) and lymphocyte (LYM) rates were not statistically influenced. However, Karakilcik [2] showed that the values of RBC, PCV, Hb, and neutrophil rates were significantly increased by halothane anesthesia. WBC counts and lymphocyte rates were significantly decreased by halothane anesthesia, but the values of RBC, WBC, LYM and NEU rates were significantly decreased with the administration of vitamin C. However, the MCV, MCH, MCHC were not significantly influenced by halothane anesthesia or administration of vitamin C.

Ashtaral Nakhai et al. [34] evaluated the benefits of different concentrations (400, 600 and 900 p.p.m.) of *Zataria multiflora* through drinking water in experimental models of inflammatory bowel disease in mice. They showed that the beneficial effects of *Zataria multiflora* (at a dilution 900 p.p.m.) were reliable. Based on their results, the present study was conducted to evaluate the beneficial effects of *Zataria multiflora* (at a dilution 800 p.p.m.) through drinking water in halothane-induced hepatotoxicity in rats. According to the results, it seems that a lower concentration (at a dilution 1:600 or lesser) of *Zataria multiflora* should be assessed in later studies for evaluation of the beneficial effects in halothane-induced hepatotoxicity in rats.

In conclusion, we have determined that halothane anesthesia affected some liver enzymes and some other biochemical and hematological parameters. *Zataria multiflura* may prevent the increase in some of these biochemical and hematological parameters after halothane anesthesia. Based upon our results, *Zataria multiflora* extract may have a prophylactic effect in the prevention of halothane hepatotoxicity complications. Proper clinical investigations should also be carried out to confirm the same activity in human disease. However, there is a need for more detailed studies in order to assess the possible relationships between antioxidants and halothane hepatotoxicity.

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