Advanced Herbal Medicine, 2017; 3(2): 29-35.

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Heracleum persicum extract improves cyclophosphamide-induced liver toxicity and oxidative stress in male rats

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Received: 19/Jun/2016 Accepted: 26/Jul/2016

ABSTRACT

Background and aims: Cyclophosphamide (CP), is a widely used cytotoxic alkylating agent with antitumor and immunosuppressant properties. In spite of its therapeutic importance, a wide range of adverse effects including reproductive toxicity has been demonstrated following CP treatment in humans and experimental animals. This drug has serious side effects such as inducing genotoxic effects, renal and hepatic damage Therfore The current report was designed to investigate the possible protective effect of *Heracleum persicum* against cyclophosphamide(CP)-induced hepatotoxicity in rats.

Methods: In this experimental research, 30 male albino Wistar rats, with body weights of 180-200 g were obtained. The animals were randomly assigned into five groups of 6 in each.Group 1 (control) group 2 (only receiving cyclophosphamide) and groups 3, 4, 5 (receiving cyclophosphamide with different doses of methanol extract of *H. persicum*). In order to induce liver toxicity in groups 2, 3, 4 and 5, CP was administered as a single dose (0.5 mg/kg), intraperitoneally and methanol extracts (0.5, 1 and 2 mg/Kg) wereadministered by gavage in 24-h cycles over a 21-day period.

Results: The results showed that administration of CP induced hepatic damage associated with significant increase in the serum marker enzymes aspartate and alanine transaminases (AST, ALT) and alkaline phosphatase (ALP) level in the CP treated group in comparison with the control ($P<0.05$). In addition, it was revealed that CP-administration cause a significant decrease ($P<0.05$) in activity of catalase (CAT) and superoxide dismutase (SOD). However, groups which received the extract of *H. persicum* in association with CP represented significantly improved parameters.

Conclusion: The results revealed that the methanol extract of *H. pesicum* has hepatoprotective effect against cyclophosphamide(CP)-induced toxicity in rats.

Keywords: Heracleumpersicum, Antioxidant enzymes, Cyclophosphamid, Liver toxicity.

Original article

INTRODUCTION

The liver as a vital organ in the body is primarily responsible for the metabolism of endogenous and exogenous organic compounds. It plays a crucial role in drug elimination and detoxification. Liver damage may be caused by xenobiotics, alcohol consumption, malnutrition, infection, anemia and medications.[1] Cyclophosphamide (CP), is

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a widely used cytotoxic alkylating agent with antitumor and immunosuppressant properties. It is used for treatment of chronic and acute leukemia, multiple myeloma, lymphomas, rheumatic arthritis and systemic lupus erythematosus as well as in preparation for bone marrow transplantation.[2] CP undergoes bioactivation by hepatic microsomal cytochrome P450 mixed function oxidase system to active metabolites that enter the circulatory system. Phosphoramide mustard and acrolein are two active metabolites of cyclophosphamide.[3] The antineoplastic effects of cyclophosphamide are associated with phosphoramide mustard, whereas acrolein is linked to toxic side effects like cell death, apoptosis, oncosis and necrosis.[4] In spite of its therapeutic importance, a wide range of adverse effects including reproductive toxicity has been demonstrated following cyclophosphamide treatment in humans and experimental animals.[5] However, this drug has serious side effects such as inducing genotoxic effects, renal and hepatic damage. Hereby, limiting its therapeutic use is suggested.[6,7] Its cytotoxic effects result from the reactive metabolites that alkylate DNA and form a variety of DNA adducts that sufficiently alter DNA structure or function, leading to formation of chromosome aberrations and micronuclei.[8,9] This antitumor agent is also able to generate active oxygen species such as superoxide anions and hydroxyl radicals that induce oxidative stress and inhibit the activity of antioxidant enzymes in several tissues. The use of antioxidants mitigates the side effects associated with CP treatment and more efficient and comfortable therapy can be achieved.[10] In recent years, considerable attention has been devoted to medicinal plants particularly rich in polyphenols, mainly flavonoids and phenolic acids which exhibit antioxidant properties due to their hydrogen-donating and metal chelating capacities as potential chemopreventive agents.[11] The phenolic compounds have demonstrated protective effects against deleterious effects of genotoxic carcinogens by scavenging reactive oxygen species (ROS) and enhancing host antioxidant defense systems.[12] It is known that many plant infusions have a large number of these molecules. *Heracleum persicum* is a plant of Umbelliferae family (Apiaceae) and it is scientifically named as *Heracleum persicum*.[13] Persian's *Heracleum persicum* is "Angdan". However, some people call it "Anjudan". *Heracleum persicum* has 2 types. One type is an aromatic one that is named as *Heracleum persicum* and Golapar. The second kind is a funky plant that is called anghoze, angozhd, fat fingers, infected Haltit and black Haltit.[14] *H. persicum* (HP) belonging to the genus Heracleum with more than 120 species in the world is one of the largest genera of the Umbelliferae family. This genus is widely distributed in Asia and is represented by 8 species in the flora of Iran, three of which (H.rechingeri, H.gorganicum (*H. persicum*) and H.anisactis) are endemic.[15,16] The Persian name for the *H. persicum* is Golpar and is used as flavoring agent and spice for food in many parts of Iran. In some areas of the country, Golpar is used as a flavoring agent for making pickles. The fruits and leaves of this genus are also used as antiseptic, carminative, digestive and analgesic in the Iranian folk medicine.[17] Some reports indicate the presence of 6 furanocumarins and flavonoids in the fruts of *H. persicum*.[18] Some isolated furanocoumarin have antioxidant functions. Therefore, *H. persicum* can be used as a source of antioxidant.[19] The current study was designed to evaluate the protective influences of *H. persicum* against CP-induced hepatotoxicity in rats.

METHODS

Drugs and chemicals: Cyclophosphamid, ehylenediaminetetraaceticacid (EDTA), hydrogen peroxide (H_2O_2), thiobarbituric acid

(TBA), solvents and other salts were obtained from Merck (Darmstadt, Germany).

Fruits of H.percicum growing wild in Iran were collected in May 2013 from Babol, Mazandaran province (north of Iran). The aerial parts of the plant were gently washed in tap water and completely dried under room temperature (25±2 °C) for 2 weeks protected from direct heat or sunlight. The powdered plant material (1000 g) was extracted with methanol (MtOH) (80%), at room temperature (RT) overnight. The extraction was repeated three-times and the solvent was evaporated in vacuum, and dried extracts were stored at 4°C until use.[20]

In this research, 30 male albino Wistar rats, with body weights of 180-200 g were obtained from the experimental animal care centre of Faculty of Pharmacy, Tabriz University of Medical Sciences. Animals with 15 weeks old were housed in colony cages (6 rats per cage) at an ambient temperature of 25±2 °C with 12 h-light and 12 h-dark cycle. The rats were fed normal diets purchased commercially from vendors and also had free access to water and libitum. The animals were allowed to acclimatize to the laboratory environment for one week and then randomly allocated into five groups 6 in each: Group 1 (control), group 2 (only receiving cyclophosphamide) groups 3,4,5 (receiving cyclophosphamide with different doses of HPME). In order to induce liver toxicity in groups 2,3,4 and 5, CP was administered as a single dose (0.5 mg/kg), intraperitoneally and methanol extract (0.5, 1 and 2 mg/Kg) was administered by gavage in 24-h cycles over a 21-day period.[21,22]

At the end of the experiment, rats from each group were killed under diethyl ether anesthesia. Blood samples were collected and left to coagulate, then centrifuged and supernatant were quickly removed and kept at -20 °C till use. Liver samples were quickly removed, cleaned and washed in ice-cold saline solution. Frozen liver tissue was ground in liquid nitrogen and suspended in a homogenization buffer consisting of 4 ml of 100 mM potassium phosphate buffer (pH 7.4), containing 150 Mm KCl and 0.1 mM EDTA and centrifuged at 12000 ×g for 15 min at 4 °C. The supernatant was used to assay activity of antioxidant enzymes.

For assaying of antioxidant enzymes, Catalase (CAT) activity was determined according to the Aebi method.[23] The rate of H_2O_2 decomposition was followed by monitoring absorption at 240 nm. One unit of CAT activity is defined as the amount of enzymes required to decompose 1μmol of hydrogen peroxide in 1 min. The enzyme activity was expressed as μmol H_2O_2 consumed/min/mg protein. Superoxide dismutase (SOD) activity was estimated according to the method of Winterbourn.[24] The developed blue color in the reaction was measured at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50% and the activity was expressed as U/mg protein.

To assay of serum aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), the cell membrane is compromised, when the hepatocytes are damaged, allowing release of cytosolic proteins and serum enzymes such as ALT, AST and ALP into the circulation.[25] To evaluate the efficacy of HP against the CP challenge, the activities of serum hepaticmarker enzymes ALT, AST and ALP were assayed in serum using standard kits from Pars Azmun Company of Iran as units/litre (U/L).

RESULTS

According to Table 1, experimental group 2 received only cyclophosphamide-induced activation of cyclophosphamide metabolites

and the effect of this drug on DNA resulted in reduceing meiotic division and damageing cells and lossing of functional integrity of cell membrane in liver. In the experimental groups of 3,4 and 5 serum liver marker enzymes, AST, ALT, and ALP activities, there were significant differences between the studied groups (P<0.05). CP-administered rats showed a significant increase in serum ALT, AST, and ALP activities when compared to control rats. Regarding the experimental groups 3,4 and 5 increase in dose of *H. persicum* led to decrease in the mentioned parameters compared to control group. Therefore, an increase in the dose of Heracleum persicum decreased the number of cells damaged. Treatment with dosages of 1mg and 2 mg of HPHE significantly reduced these parameters. However, it was significant only in the dose of 2 mg/kg group even rather than control group. According to the Table 1, activities of SOD and CAT were decreased significantly in the experimental groups when compared to the control group (P<0.05). Regarding the experimental groups 3,4 and 5 increase in dose of *H. persicum* led to increase in the mentioned parameters compared to the control group.

Table 1: Average of serum ALT, AST and ALP and average of antioxidant enzymes of normal, Cp control and CP treated with H.Percicum

Group	AST(U/L)	ALT (U/L)	ALP(U/L)	CAT(U/L)	SOD(U/L)
1	101.67±12.5	150±19.69	249.33±28.88	117.94±4.35	12.23±1.32
2	197.5±12.02	172.33±17.80	599.33±63.28	63.42±5.17	6.83±0.42
3	158.33±16.07	134±6	547.67±39.8	78.02±8.56	7/91±0.28
4	131.67±23.24	101.25±10.24	377.33±18.33	88.07±5.32	10.85±0.38
5	86.33±8.32	87.33±2.51	250.67±15.82	104.152±8.68	11.75±1.18

Values are Mean ± SE of 6 animals. Means which share different superscript symbol(s) are significantly different (P<0.05); HPME: H. persicum methanolic extract, CP: cyclophosphamid and CP + HP: cyclophpsphamid + H. persicum extract.

DISCUSSION

Cyclophosphamide is an alkylating agent with an active metabolite that leads to DNA cross-linking. It has been widely used for treatment of cancer patients although it has several side effects, mainly bone marrow toxicity and severe infection.[26] In the current study, CP treatment demonstrated significant hepatotoxic effects as confirmed by the increased serum liver markers, ALT, AST, and ALP. The abnormal high levels of these marker enzymes observed in our study are the consequence of CP-induced liver dysfunction and damage of the hepatic cells. The increased levels of these enzymes and metabolites in the serum could be attributed to the activity of acrolein. Acrolein also produces oxidative stress resulting in a decrease in the activities of antioxidant enzymes and in an increase in lipid peroxidation and the production of intracellular ROS such as superoxide anion

radicals, hydroxyl radicals, and singlet oxygen. These reactive oxygen and nitrogen species damage cellular lipid, proteins, and DNA.[27] Kumar et al. reported that the increased levels of serum enzymes are indicative of cellular damages and loss of functional integrity of cell membrane in liver.[28] The reversal of increased serum enzymes in CP-induced hepatotoxicity by HPME supplementation may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing efficacy. The present data also showed that CP-induced liver toxicity disturbs actions of antioxidant enzymes (SOD and CAT) in liver. These enzymes could destroy the peroxides and play a significant role in providing antioxidant defenses to an organism. In the enzymatic antioxidant defense system, SOD and CAT are the two important scavenging enzymes that remove superoxide radicals (O_2^-) and hydrogen peroxide, respectively.[29] Moreover, CP metabolism produces highly reactive electrophiles and the decreased value the antioxidant enzymes activity in CP-treated group most probably due to the electrophilic burden on the cells and also due to the formation of acrolein, which deplete SOD and CAT content.[30] Decrease in SOD and CAT activities after CP administration may be due to inadequacy of antioxidant defenses in combating with ROS production. The current study is in agreement with the reports of Rajasekaran et al. and Tripathi and Jena who reported that CP-induced hepatotoxicity is associated with oxidative stress caused by the reduction in the antioxidant enzymes.[31,32] Antioxidunt activity of some furanocoumarins isolated from *H. persicum* has been reported by souri et al, and they reduce free radicals.[19.33] The presence of high phenolic and flavonoid content has contributed directly to the antioxidant activity by neutralizing the free radicals.[34] The extract of *H. persicum* acts as the receiver of free radicals and thus protects cells from oxidative stress induced by cyclophosphamide use.[35]

CONCLUTION

In conclusion, the current study demonstrated that *H. persicum* protected against CP-induced hepatotoxicity via potentialing the antioxidant defense system. The positive effect of *H. persicum* on antioxidant enzymes activity is most probably due to the high contents of flavonoids and polyphenol components of *H. persicum*, which were probably involved in the healing process of free radical mediated diseases. The increase in anti oxidant enzyme and serum marker enzyms following administration of the methanol extract of *H. persicum* may signify the positive effects on the haemopoietic system of experimental rats and might be capable of improving liver toxicity induceb by drugs. The extract of *Heracleum persicum* acts as the receiver of free radicals and thus protects cells from oxidative stress induced by cyclophosphamide used. The considerable biological activities of *H. persicum* essential oils make them good condidates to develop natural derived therapeutics. This study shows, for the first time, that the theory behind using *H. persicum* as a repressor of the liver toxicity, as mentioned in traditional medicine, might be true. However, concrete evidence for introducing this plant as an effective drug in the field of hepato protectivity would need further investigation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENTS

We would like to thank all individuals who collaborated in this study.

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How to cite the article: Rostampur S, Hosseinpour Feizi MA, Banan Khojasteh SM, Daluchi F. *Heracleum persicum* extract improves cyclophosphamide-induced liver toxicity and oxidative stress in male rats. Adv Herb Med. 2017; 3(2): 29-35.