Journal of Ethnopharmacology 190 (2016) 387–395

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Pharmacological safety evaluation of a traditional herbal medicine "Zereshk-e-Saghir" and assessment of its hepatoprotective effects on carbon tetrachloride induced hepatic damage in rats

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ARTICLE INFO

Article history:
Received 27 December 2015
Received in revised form
7 June 2016
Accepted 14 July 2016
Available online 15 July 2016

Keywords:
Traditional herbal medicine
CCl_4-induced liver damage
Hepatoprotective effect
Oxidative stress

Chemical compounds studied in this article:
Quercetin (PubChem CID: 5280343)
Rutin (PubChem CID: 5280805)
Kaempferol (PubChem CID: 5280863)
Emodin (PubChem CID: 3220)
Gallic acid (PubChem CID: 370)

ABSTRACT

Ethnopharmacological relevance: "Zereshk-e-Saghir" (ZES), one of the traditional herbal medicines in old manuscripts of Persian hakims, has been used for the treatment of liver disorders. This current study is aimed to evaluate ZES effects on animal model to investigate its safety and hepatoprotective activity.
Materials and methods: ZES was prepared according to a traditional method by blending aqueous extracts of *Berberis vulgaris* L., with fine particles of other plants including *Rosa damascene* Mill, *Cichorium intybus* L., *Cucumis sativus* L., *Portulaca oleracea* L., *Rheum palmatum* L., and *Nardostachys jatamansi* DC.. The lethality of ZES was determined in male NMRI mice. Acute organ toxicity of ZES (750 and 1500 mg/kg for 15 days, orally) was evaluated by measuring the cell blood count, liver marker enzymes, creatinine, antioxidant status and histopathological examinations in rats. CCl_4-induced liver toxicity was used to examine the hepatoprotective effects of the preparation. The rats were pretreated with 250, 500, 750 and 1500 mg/kg ZES by gavage for 15 days. At day 16, the rats were intraperitoneally injected 1 ml/kg CCl_4 in olive oil. Forty-eight hours after CCl_4 injection, the animals were sacrificed and their liver samples and blood were collected for determination of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase (ALT, AST, and ALP), histopathological examinations and antioxidant status.
Results: Treatment of the mice with a single dose of ZES up to 2 g/kg did not cause mortality. Treatment of the rats with doses of 750 and 1500 mg/kg for 15 days showed no significant hematotoxicity and hepatotoxicity. Treatment of the rats with ZES reduced the increased serum levels of ALT, AST, and ALP induced by CCl_4 at the doses of 250, 500, and 750 mg/kg. This was almost confirmed by histopathological examinations. Pretreatment with ZES also decreased lipid peroxidation and maintained the levels of glutathione and total antioxidant capacity.
Conclusions: The present in vivo study revealed that the long term usage of ZES was safe for organs in laboratory animals. Meanwhile, prescribing the traditionally-recommended dose of ZES can be probably used against the liver injuries induced by xenobiotics. Further studies in other models of liver injuries are recommended for finding the exact hepatoprotective mechanism of ZES.

1. Introduction

Detoxification is one of the vital operations of the liver in the human body to clear foreign and naturally occurring substances including chemicals and xenobiotics. This process produces reactive intermediates such as reactive oxygen species (ROSs) which induce oxidative stress and liver injuries (Halliwell, 1991; Wiseman and Halliwell, 1996). Oxidative stress is an imbalance

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http://dx.doi.org/10.1016/j.jep.2016.07.043
0378-8741/

between prooxidant and antioxidant in the body (Rahal et al., 2014).

Because of production of ROSs and induction of oxidative stress, carbon tetrachloride (CCl_4) induced hepatotoxicty has been a widely used animal model for investigation of liver injuries induced by xenobiotics. Metabolism of CCl_4 by phase I cytochrome P450 produces reactive free radicals, decreases hepatic glutathione content and initiates a chain lipid peroxidation in the hepatocyte membrane (Jaeschke et al., 2002; Recknagel, 1967).

Although there are endogenous defensive antioxidant mechanisms to limit the adverse effects of ROSs, the excessive production of ROSs overwhelms these mechanisms and leads to oxidative stress. Here, the considerable importance of free radical scavengers called antioxidants to protect body is highlighted (Devasagayam et al., 2004; Ulicna et al., 2003). Synthetic antioxidants are cost-effective and efficient, but there are concerns about the safety of these products in long term usage (Brewer, 2011; Pokorny, 2007). So, there have been interests in finding natural antioxidants in recent years. Plants are rich sources of antioxidants whose antioxidant activities could be related to the presence of bioactive constituents such as total polyphenols, total flavonoids, and compounds with Fe^{2+}-chelating activity (Barreira et al., 2008).

Herbal preparations have attracted the attention of clinicians, and more than 700 mono- and poly-herbal preparations have been derived from more than 100 plants in different forms such as decoction, tincture, tablets and capsules for clinical use (Ram, 2001). Although botanical drugs have been iteratively recommended in complementary and alternative medicine for treating the liver disorders, there is not enough evidence for their efficacy and safety recommended by World Health Organization (WHO) for traditional herbal medicines (Stickel and Schuppan, 2007; World Health Organization, 2002).

Traditional Iranian Medicine (TIM) or Persian Medicine dates back to over 8000 years BC, and it is a branch of Arabic Unani medicine with a rich and long history of practicing and studying medicine for diagnosis, prevention and treatment of diseases from ancient times to the present (Jorjani, 2011; Rezaeizadeh et al., 2009). Pharmacotherapy has been playing a key role in this therapeutic system. Several available manuscripts from glorious Persian hakims recommended herbal preparations such as hepatotheraputic agents (Aghili Khorasani Alavi Shirazi, 2011; Jorjani, 2011). One of these herbal medicines is called “Zereshk-e-Saghir” (ZES). This preparation is a combination of micro fine particles of seven herbal plants as follows: *Berberis vulgaris* L. (Berberidaceae), *Rosa damascena* Mill (Rosaceae), *Cichorium intybus* L. (Compositae), *Cucumis sativus* L. (Cucurbitaceae), *Portulaca oleracea* L. (Portulacaceae), *Rheum palmatum* L. (Polygonaceae) and *Nardostachys jatamansi* DC. (valerianacea).

Although the herbal preparation ZES has been used to treat the liver disorders in folk remedy, still there has been no experimental study on its hepatoprotective effects and safety. Therefore, the antioxidant and hepatoprotective effects of ZES on CCl_4-induced acute liver injury model in rats. Meanwhile, acute organ toxicity of the preparation was studied to determine its safety.

2. Methods and materials

2.1. Chemicals

All the chemicals and reagents used in this study were of analytical grade purity. Carbon tetrachloride, ethylenedinitrilotetraacetic acid, ketamin, xylazine, formalin solution (neutral buffered, 10%), trichloloroacetic acid, *n*-butanol, acetic acid glacial, tris-buffered saline, sodium acetate trihydrate, hydrogen chloride, iron (III) chloride hexahydrate, and iron (II) Sulfate heptahydrate were purchased from Merck Company. Malondialdehide, 5, 5′-dithio bis-2-nitrobenzoic acid and 2, 4, 6- tripyridyl-s-triazine were obtained from Sigma-Aldrich. Gallic acid and emodin were obtained from Carl Roth Company.

Table 1
Characteristics of ZES preparation.

Plant	Family	Used part	Amount (% w/w)
Berberis vulgaris L.	Berberidaceae	Fruit	39.2
Rosa damascena Mill	Rosaceae	Petal	19.7
Cichorium intybus L.	Compositae	Seed	11.7
Cucumis sativus L.	Cucurbitaceae	Seed	11.7
Portulaca oleracea L.	Portulacaceae	Seed	11.7
Rheum palmatum L.	Polygonaceae	Rhizome	4
Nardostachys jatamansi DC.	Valerianaceae	Rhizome	2

2.2. Herbal preparation

2.2.1. Preparation of “Zereshk-e-Saghir” (ZES)

The characteristics of the herbal preparation, ZES, are shown in Table 1.

Local plants were collected from farms in Kerman province (30.3°N, 57.0°E) from April to August 2013. All the used species were authenticated by an herbalist. Voucher specimens for *Berberis vulgaris* L. (KF1629), *Rheum palmatum* L. (KF2003), *Nardostachys jatamansi* DC. (KF2001), *Rosa damascena* Mill (KF1362), *Cichorium intybus* L. (KF1157), *Cucumis sativus* L. (KF1391) and *Portulaca oleracea* L. (KF1512) were deposited in the Herbarium of Pharmacognosy Department, Kerman University of Medical Sciences, Kerman, Iran. Quality control tests were carried out on the plant samples in accordance to British Pharmacopoeia (2013). Phytochemical analysis by using thin-layer chromatography (TLC) indicated presence of flavonoids including quercetin, kaempferol, and rutin, and anthraquinones (emodin) in the plants which are in consistent with previous studies (Bhandari et al., 2007; Bladt and Zgainski, 1984; Street et al., 2013; Zhou et al., 2015).

For preparation of ZES, all the used parts of plants were powdered by ball-mill® and sieved to obtain homogenous particles (mesh no. 30). The obtained powder was mixed with aqueous extract of *B.vulgaris* which was prepared by maceration method and dried with freeze dryer. The proportion of each plant is shown in Table 1. This preparation was made in the Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran. For pharmacologic studies, ZES was given to animals by mixing with oxymel syrup as vehicle according to the traditional recipe. OS was prepared by dissolving 50 g sugar in 100 ml distilled water which was added to 50 ml vinegar. The mixture was boiled to the final volume of 100 ml.

Presence of emodin in methanolic extract of *R. palmatum* L. and ZES was confirmed by TLC (Bladt and Zgainski, 1984). ZES was standardized on the basis of emodin by UV-spectrophotometer at 435 nm (Sucheta et al., 2011) which was equivalent to 432.0 ± 1.1 mg emodin/100 g ZES. Also total phenolic content (Altunkaya and Gokmen, 2008) of ZES was determined by Folin-Ciocalteau reagent and spectrophotometric determination (1232.1 ± 1.6 mg eq. G. A/100 g ZES).

2.2.2. Aqueous herbal extract

For measuring the antioxidant effect of the herbal plants by ferric reducing ability of plasma (FRAP) assay, aqueous extracts were prepared using the following method. The powdered part of the plants were macerated in distilled water (1/5, %w/v) for 6 h (h), and this process was repeated 4 times at room temperature for 24 h. The collected extracts were freeze dried, and finally the