Sage leaves (*Salvia miltiorrhiza*) extract attenuates hepatic injury in Isoniazid induced hepatotoxicity in rats

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Abstract: Oxidative stress is suggested as a mechanism underlying diabetes mellitus complications. The main objective of this study was to investigate the effect of sage leaves extract on oxidative stress of hepatic tissue in isoniazid-induced diabetic rats. The lipid peroxidation product, malondialdehyde (MDA) and reduced glutathione (GSH) content was measured to assess free radical activity in the liver tissues. The enzymatic activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) were measured as indicators of antioxidation in liver tissue. Wistar rats were made diabetic with a single injection of STZ (75 mg/kg i.p.). Rats were randomly separated into four groups, of 10 animals each: Group 1, healthy control rats; Group 2 non-diabetic rats treated with 50 mg/kg b.w./day intraperitoneal injection of sage extract; Group 3, diabetic rats; Group 4, diabetic rats treated with sage extract (50 mg/kg b.w./day, i.p.) for 8 weeks. At the end of experiment, MDA contents of the liver tissue in Groups 3 was found to be significantly increased as compared with Group 1 ($P<0.05$) and liver MDA level in Group 4 were significantly decreased as compared with Group 3 ($P<0.05$). The GSH, SOD, CAT and GSH-Px contents of the liver in Group 3 were significantly decreased as compared to Groups 1 ($P<0.05$) and were increased in Group 4 as compared to Group 3 ($P<0.05$). The results obtained, demonstrated that sage extract alleviate oxidative stress of hepatic tissue in streptozotocin-induced diabetic rats.
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1. Introduction

Diabetes mellitus is a serious metabolic disorder which is a major source of ill health all over the world and its incidence is expected to increase by 5.4% in 2025 (Kim et al., 2006). Diabetes mellitus is characterized by hyperglycemia and is associated with disturbances in carbohydrate, protein and fat metabolism which occurs secondary to an absolute (type I) or relative (type II) lack of insulin (Alberti and Zimmet, 1998). Oxidative stress occurs when the balance between oxidant and antioxidant systems shifts in favour of the former leading to the generation of free oxygen radicals. Reactive oxygen species are involved in the pathogenesis of many diseases including hypoxia, hypercholesterolemia, atherosclerosis, hypertension, ischemia reperfusion injury and heart failure (Taniyama and Griendling, 2003; Wilcox and Gutterman, 2005). It has been shown that patients with diabetes mellitus have increased oxidative stress and impaired antioxidant defense systems, which appear to contribute to the initiation and progression of diabetes-associated complications (Maritim et al., 2003). There is convincing experimental and clinical evidence that the generation of reactive oxygen species is increased in both types of diabetes. Normally, the level of oxidative stress is modulated by antioxidant defense systems (Saxena et al., 1993). Diabetes-linked alterations in antioxidant defense system enzymes such as catalase, glutathione peroxidase, superoxide dismutase have been demonstrated (Maritim et al., 2003). The negative impact of diabetes on the retinal, renal, nervous and cardiovascular systems is well recognized, yet little is known about its effect on the liver (Lipscombe and Hux, 2007; Orasanu and Plutzky, 2009). However, Lipid peroxidation and antioxidant status of hepatic tissue were studied by Feillet-Coudray and associates in experimental diabetes (Feillet-Coudray et al., 1999). Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease. More attention has been paid to the protective effects of natural antioxidants against chemically induced toxicities (Frei and Higdon, 2003). Herbal formulations with a simultaneous antioxidant effect would thus be more useful in the management of diabetes mellitus. Their use alone or in combination with oral hypoglycemic agents or insulin may help in the better control of blood glucose level in diabetic subjects. Natural antioxidants strengthen the endogenous antioxidant defenses from reactive oxygen species (ROS) and

restore and optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. Salvia miltiorrhiza, also known as red sage, Chinese sage, tan shen, or danshen, is a perennial plant in the genus Salvia, highly valued for its roots in traditional Chinese medicine (Clebsch and Carol, 2003). Native to China and Japan, it grows at 90 to 1,200 m (300 to 3,900 ft) elevation, preferring grassy places in forests, hillsides, and along stream banks. The specific epithet miltiorrhiza means "red juice extracted from a root" (Sutton, 2004). Salvia miltiorrhiza has been widely used in China and, to a lesser extent, in Japan, the United States, and other European countries for the treatment of cardiovascular and cerebrovascular diseases. In China, the specific clinical use is angina pectoris, hyperlipidemia, and acute ischemic stroke (Zhou et al., 2005; Wu et al., 2007; Cheng, 2007). A patented Chinese herbal medicine has successfully completed Phase II clinical trials in the United States and will soon begin Phase III investigations, raising the possibility that it could become the first Traditional Chinese Medicine (TCM) product to obtain drug approval from the US Food and Drug Administration (FDA). The product, Compound Danshen Dripping Pill (also referred to as Cardiotonic Pill), is produced by Tianjin Tasly Pharmaceutical Co. Ltd. in Tianjin, China. It contains the extract of the root of danshen as well as extract of the root of notoginseng (Panax notoginseng; known as sanchi or tien-chi ginseng), and synthetic borneol, an active ingredient that replaces the more expensive natural borneol found in cardamom, ginger, and other spices (Lindsay, 2010). Results from animal and human studies support the use of Danshen for circulatory disorders to some extent because it is known to decrease the blood's ability to clot in at least two ways. First, it limits the stickiness of blood platelets. It also decreases the production of fibrin, the threads of protein that trap blood cells to form clots. Both these effects help to improve blood circulation. In addition, chemicals in danshen may relax and widen blood vessels, especially those around the heart. In animal studies, chemicals in danshen may also have protected the inner linings of arteries from damage. Some other research suggests it may increase the force of heartbeats and slow the heart rate slightly. The aim of present study was to evaluate hepatoprotective effects of sage leaves (Salvia miltiorrhiza) extract against isoniazid induced hepatotoxicity in rats.

2. Materials and Methods

2.1. Experimental plan

This experimental study was carried out in Islamic Azad University Research Center and all procedures and works on animals was conducted under Animal Rights Monitoring Committee of Islamic Azad University Research Center.

80 Wistar rats, 4-6 weeks old, weighing 180-200g were purchased from Pastor Institute, Karaj, Iran. Management and husbandry conditions were identical in all groups with 12/12 h light/dark cycle at 21±2°C. Food and water were provided ad libitum. Animals were divided into the 4 identical groups with 20 rats in each.

2.2. Induction of diabetes Mellitus

Diabetes was induced by intravenous injection of isoniazid (Sigma, St. Louis, Mo, USA) into the tail vein at a dose of 200 mg/kg body weight. Isoniazid was extemporaneously dissolved in 0.1 M cold sodium citrate buffer, pH 4.5. After 18 h, animals with fasting blood glucose levels greater than 120-250 mg/dl were considered diabetic and then included in this study (Gupta et al, 2005). Fasting blood glucose was estimated by using one touch glucometer (Accu-chek sensor) of Roche Diagnostics, Germany.

2.3. Animals

The duration of experiment was 8 weeks. The rats were randomly divided into 4 groups (20 rats each) as the following: Group 1, healthy control rats received isotonic saline solution (ISS, 10 ml/kg) intraperitoneally; Group 2 non-diabetic rats were treated with 50 mg/kg b.w. /day intraperitoneal (i.p.) injection of sage extract; in Group 3, diabetic rats administered by ISS (10 ml/kg) was given through Intraperitoneal (i.p.) route; Group 4, diabetic rats were treated with sage leaves extract (50 mg/kg b.w. /day, i.p.) for a period of 8 weeks. The animals of different groups were sacrificed under light anesthesia (diethyl ether) 1 day after the end of the treatment.

2.4. Measurement of antioxidant activity:

The rat's liver were removed immediately and washed in normal saline and homogenate 10% prepared in 1.15% w/v of potassium chloride. The homogenate was centrifuged in 7000 ×g for 10 minutes at 4˚C and supernatant were used for measurement of oxidative stress by estimation of reduced glutathione (GSH) and determination of malondialdehyde (MDA) as well as antioxidant enzymes (AOE) such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). GSH, MDA, SOD, CAT and GSH-Px were measured by using commercially available kits according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Reduced glutathione (GSH) content was determined according to Sedlak (Sedlak and Lindsay, 1968). GSH reacts with 5,5′-dithiobis-2-nitrobenzoic acid, and the absorbance spectra of the product have a maximum absorbance at 410 nm. The results were expressed as µmol/gwt.Liver homogenate MDA levels were expressed as nmol MDA per mg protein and antioxidant activity was expressed as arbitrary units per mg protein. Degree of lipid peroxidation in liver tissue homogenates was determined in terms of thiobarbituric acid reactive substances (TBARSs) formation by following the protocol of Esterbauer and Cheesman (Esterbauer and Cheesman, 1990). SOD activity was measured by Nishikimi method (Nishikimi et al., 1972) and was modified by Kakkar method (Kakkar et al., 1984). Each unit of SOD activity was determined as required enzyme concentration for prohibition of creation color at 1 minute, under study conditions. CAT activity was measured by Claiborne method and was based on hydrogen peroxide breakdown. GSH-Px activity was measured by Rotruck method (Rotruck et al., 1973) and was expressed as micromole of GSSG /minute/milligram of protein, based on blew reaction:

$$2H2O+GSSG \rightarrow H2O2+2GSH$$

2.5. Statistical analysis

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean ± SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. $P<0.05$ was considered statistically significant.

3. Results

The sage leaves extract produced significant hypoglycemic effect in normal ($P<0.05$) and diabetic ($P<0.01$) rats after 8 weeks of administration.

Figures 1-5 show the effects of sage leaves extract on antioxidative activity of liver tissue in diabetic rats. MDA contents of the liver tissue in Groups 3 was found to be significantly increased as compared to Group 1 ($P<0.05$) and liver MDA level in Group 4 were significantly decreased as compared to Group 3 ($P<0.05$). The GSH, SOD, CAT and GSH-Px contents of the liver in Group 3 were significantly decreased as compared to Groups 1 ($P<0.05$) and GSH, SOD, CAT and GSH-Px activity were increased in Group 4 as compared to Group 3 ($P<0.05$).

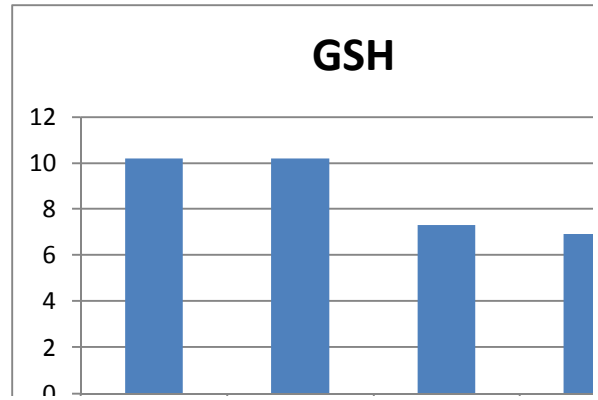

Fig 1: Comparison of the effect of sage leaves extract on liver GSH content among the experimental groups (mean±SEM).

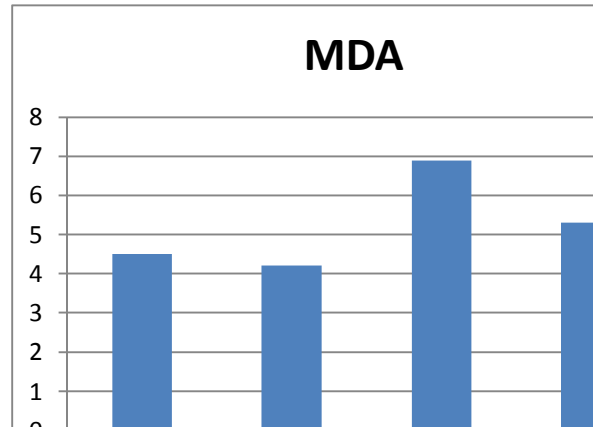

Fig 2: Comparison of the effect of sage leaves extract on liver MDA content among the experimental groups (mean±SEM).

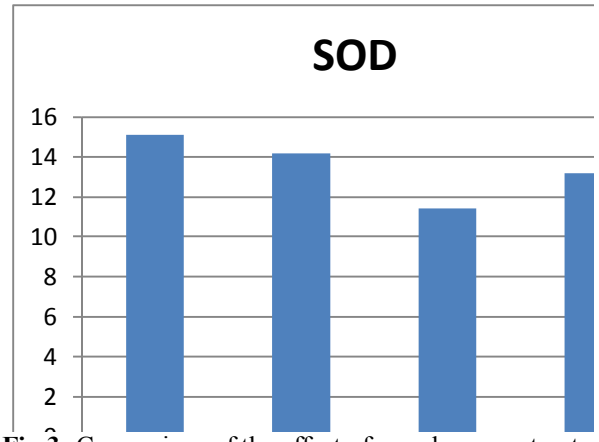

Fig 3: Comparison of the effect of sage leaves extract on liver SOD activity among the experimental groups (mean±SEM).

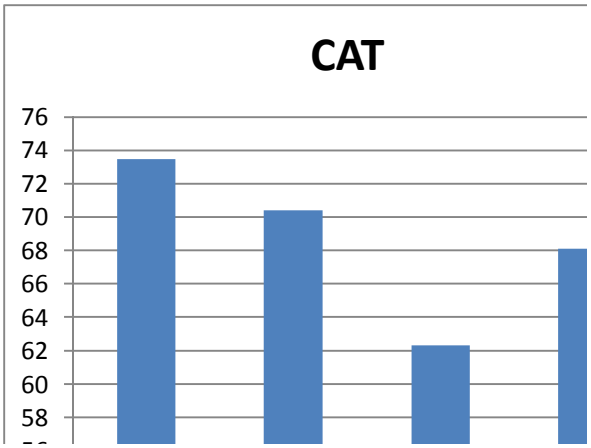

Fig 4: Comparison of the effect of sage leaves extract on liver CAT activity among the experimental groups (mean±SEM).

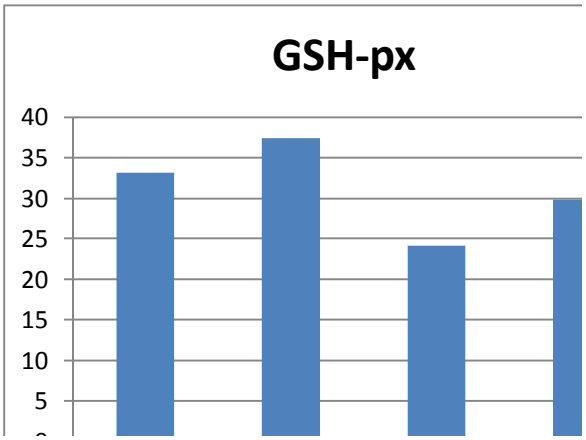

Fig 5: Comparison of the effect of sage leaves extract on liver GSH-Px activity among the experimental groups (mean±SEM).

4. Discussion and Conclusion

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling.

During metabolism of INH, hydrazine is produced directly (from INH) or indirectly (from acetylhydrazine). It is evident that hydrazine plays a role in INH-induced liver damage. It can cause moderate abnormalities in serum transaminases leading to hepatotoxicity; hence the measurement of serum transaminases is often advocated during INH administration, to assess the extent of INH-induced hepatotoxicity (Mitchell et al., 1976). Administration of INH significantly elevated levels of AST, ALT, ALP, LDH and bilirubin, due to damaged structural integrity of the liver because these are cytoplasm in location and are released into circulation after cellular damage. Riomonabant treatment with INH prevented the INH-induced perturbations in the activities of AST, ALT, ALP, LDH, TB, DB, Sodium and potassium ions in both the serum and liver tissue. It is likely that the INH-derived hepatotoxic metabolites, namely acetyl.isoniazid and acetyl hydrazine would have been trapped by rimonabant (Vaghasiya et al., 2009).

Ou et al., (2012) showed the pathological changes in multiple organs in the two treated groups were relieved to different degrees ($P<0.05$ and $P<0.01$, respectively), they showed the expression levels of Bax and NF-κB proteins, and apoptotic indexes of multiple organs were reduced ($P<0.05$ and $P<0.01$, respectively). Also, they declared that contents of amylase, GPT, GOT, BUN, and CREA in the two treated groups were significantly lower than those in model control groups ($P<0.05$ and $P<0.01$, respectively). The expression level of ICAM-1 protein in the lungs (at 3 and 12 h) in the dexamethasone treated group was significantly lower than that in the Salvia miltiorrhiza treated group ($P<0.05$). The serum contents of CREA (at 12 h) and BUN (at 6 h) of the Salvia miltiorrhiza treated group were significantly lower than those in the dexamethasone treated group ($P<0.05$). They concluded that both dexamethasone and Salvia miltiorrhiza can reduce the inflammatory reaction, regulate apoptosis, and thus protect multiple organs of rats with SAP.

Sferra et al., (2012) demonstrated that combined oral administration of Boswellia and Salvia extracts improved the course and macroscopic findings of DMN-induced chronic hepatitis-associated fibrosis. The histological severity of the hepatic fibrosis showed a marked improvement following treatment and was associated with a reduction in the hepatic expression of alpha-SMA, collagen I-III, CTGF, TGF-beta1, Smad3, and Smad7. Their data demonstrated that co-treatment of Boswellia plus Salvia extracts is effective in preventing hepatic fibrosis in DMN-induced chronic hepatitis. The anti-fibrotic properties are mainly related to Salvia extracts and appear to be mediated by the inhibition of the TGF-beta1/Smad3 pathway.

Li et al., (2013) during their study found that treatment with danshen aqueous extract reduced body weight gain, improved serum lipid profiles, and prevented formation of fatty liver induced by HFD

and OVX. In addition, danshen could increase endothelial-dependent vasorelaxation and displayed vasoprotection in OVX rats fed with HFD, primarily by stimulating nitric oxide (NO) production, up-regulating the mRNA expression of endothelial NO synthase, and down-regulating the mRNA expression of tumor necrosis factor α, intercellular cell adhesion molecule-1, and vascular cell adhesion molecule-1 in the isolated aortas. They concluded that for the first time that danshen aqueous extract could protect OVX rats fed with HFD from endothelial dysfunction. Its effect may be related to its abilities to normalize serum lipid profiles and enhance NO availability in the vascular system. Our findings indicate that danshen aqueous extract could be a promising natural supplement for postmenopausal women for preventing CVD.

Zhao et al., (2012) showed that after suffering from ischemia/reperfusion, the W/D of every specimen increased in different degree ($P<0.05$, $P<0.01$). In plasma, the values of SOD decreased but MDA increased obviously ($P < 0.05$, $P < 0.01$). The level of IL-1, IL-6 and TNF-alpha-a in plasma were increased ($P<0.05$, $P<0.01$). After LI/R, infiltration of inflammatory cells, broaden interstitial around muscle fiber and disordered arrangement of muscle fibers could be seen under microscope. However, Compared with LI/R group, W/D and levels of serum inflammatory factors in SM group were all lower, the values of SOD in plasma increased but MDA in plasma failed down. Pathological changes in skeletal muscle were improved. They concluded that limb ischemia/reperfusion can lead to multiple organ edemas; Salvia miltiorrhiza can prevent the edema in some degree by anti-oxidation and anti-inflammation. In conclusion, can state that sage has hepatoprotective activity against hepatotoxicant like isoniazid and it is due to its active component which called Salvinorin and it is a flavonoid in nature.

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