

Full Length Research Paper

***Salvia sahendica* prevents tissue damages induced by alcohol in oxidative stress conditions: Effect on liver and kidney oxidative parameters**

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Oxidative stress plays an essential role in the development of alcohol liver disease. The present study represents the protective effect of a polyphenolic rich extract of *Salvia sahendica* against liver and kidney damages in alcohol-induced oxidative stress. Measurement antioxidant activity by scavenging of 1,1-diphenyl-2-picrylhydrazyl radical showed that methanolic extract of *S. sahendica* possesses the highest antioxidant activity. Treatment of experimental rats with ethanol for one month showed an increase in activities of liver marker enzymes, lipid peroxidation marker (TBARS) with significantly lower activity of enzymatic and non-enzymatic antioxidants in rat liver and kidney compared to those in normal rats. Treatment alcohol rats with *S. sahendica* at a dose of (0.1 g/kg) significantly reduced the level of lipid peroxidation and liver marker enzymes and restored the enzymic and non-enzymatic antioxidants levels in liver and kidney of treated rats. The results of this study strongly indicate that *S. sahendica* has potent hepato-protective effect against alcohol induced tissues damage in experimental animals. This study also suggests that possible mechanism of this activity may be due to the presence of flavonoids and phenolics compound(s) in the methanolic extract of *S. sahendica* which may be responsible to hepato-protective activity.

Key words: *Salvia sahendica*, alcohol, antioxidant, lipid peroxidation, oxidative stress.

INTRODUCTION

Reactive oxygen species (ROS) including hydrogen peroxide (H₂O₂), singlet oxygen (O₂[•]), superoxide radicals (O₂^{•-}), and hydroxyl radicals (OH[•]) derived from the metabolism of oxygen in aerobic systems are produced in an imbalance conditions between generation and elimination of ROS (Gutteridge, 1994). ROS provide a wide potential condition for causing damages of cellular com-

ponents such as DNA, protein and lipid.

Accumulation of oxidative damages in intracellular macromolecules is an essential factor in pathogenesis of many diseases such as respiratory distress syndrome, ischemia/reperfusion injury, renal failure, rheumatoid arthritis, local or systemic inflammatory disorders, diabetes, atherosclerosis, cancer and neurodegenerative diseases (Aruoma et al., 1991; Emerit et al., 2004). A highly complex antioxidant protection system including antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)), and diet-derived antioxidants like ascorbic acid, vitamin E, carotenoids, polyphenols and other low weight molecular compounds such as -lipoic acid are served as primary lines of defense in destroying free radicals (Block, 1991; Wang and Luo, 2007). Recently, several studies have showed the role of oxidative stress on developmental alcohol-mediated cytotoxicity, possibly via the formation of

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Abbreviations: ALT, Alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BHT, butylated hydroxytoluene; CAT, catalase; DPPH, 1,1-diphenyl-2-picrylhydrazyl; GPx, glutathione peroxidase; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase.

free radicals (Chen and Sulik, 1996; Heaton et al., 2000; Collins et al., 1998). Alcohol-induced oxidative stress is linked to the metabolism of ethanol involving both microsomal and mitochondrial systems. Ethanol metabolism is directly involved in the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These form an environment favorable to oxidative stress. Ethanol treatment results in the depletion of GSH levels and decreases antioxidant activity. It elevates malondialdehyde (MDA), hydroxyethyl radical (HER), and hydroxynonenal (HNE) protein adducts. These cause the modification of all biological structures and consequently result in serious malfunction of cells and tissues (Kumar and Vasudevan, 2007). Alcoholics often have lower levels of key antioxidant molecules such as vitamin C and vitamin E and a decreased antioxidant status (Lieber, 2000). Because pro-oxidant production is also increased during alcohol consumption, there is an imbalance between pro-oxidants and antioxidants in alcoholics leading to oxidative stress. Oxidative stress could be involved in triggering a vicious cycle of pathology in alcoholic livers by increasing the transcription of pro-inflammatory cytokines such as NF- κ B (Arteel, 2000). Indeed, recent work showed that mice deficient in the TNF receptor, TNFR1, did not develop alcohol-induced liver injury when exposed to enteral ethanol for 4 weeks (Yin et al., 1999). Thus, therapies against oxidants (compounds with antioxidant activities) could be useful for treatments of human alcoholic hepatitis.

These days, scientific interests in the functional components of food, with health protecting potencies, are increasing mainly due to their various biological activities and low cytotoxic effects (Silva et al., 2005). Thus, the daily consumption of functional dietary components (mainly from plant sources), which have an antioxidant effect, may provide health benefits associated with preventing damages due to biological degeneration.

Salvia is one of the most widespread members of the *Lamiaceae* (Labiatae) family. It features prominently in the pharmacopoeias of many countries throughout the world from the Far East, through Europe and very different places and several of the almost thousand *Salvia* species have been used in many ways, e.g., essential oils used in perfumery. *Salvia* species especially *Salvia officinalis* are an important source of antioxidants used in food industry and have wider implications for the dietary intake of natural antioxidants (Kintzios, 2000). From 900 *Salvia* species which are distributed in the world, about 17 species are endemic of Iran (Mozafarian, 1996). *Salvia sahendica* extract (SSE) (endemic of Iran) is traditionally used for antibacterial, anti-fungi purposes and treatment of Dyspepsia in many part of Iran (Lotfipour et al., 2007). In addition, the essential oils and various extract of *S. sahendica* were found to have antioxidant potential (Salehi et al., 2007). Regarding the ability of antioxidant compounds to reduce Diseases induced by oxidative stress, we proposed that the polyphenolic-rich extract (methanolic) of *S. sahendica* might possess

significant capacities to reduce tissue injuries induced by alcohol in an *in vivo* conditions as well. For that case, we planned to evaluate the effect of methanolic extract of *S. sahendica* on alcohol induced oxidative stress in rat.

MATERIAL AND METHODS

Chemical

Ascorbic acid, Butylated hydroxytoluene (BHT), ethanol, gallic acid, and potassium persulfate were purchased from E. Merck, Darmstadt, Germany. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent was purchased from Sigma chemical Co. (St. Louis, MO, USA). Catechin was obtained from Fluka. All other reagents were of analytical reagent grade.

Plant material

Aerial parts of *Salvia sahendica* (Boiss.) Buhse, were collected from Tabriz province, Iran during spring. A voucher herbarium specimen (MPH-848) was deposited in the herbarium of Medicinal Plants and Drug Research Institute, Shahid Beheshti University (G.G), Tehran, Iran. The plant aerial parts were air-dried, protected from direct sunlight, and then powdered. The powder was kept in a closed container in cold room.

Preparation of plant extract

The powdered plant material (100 g) was extracted three times with various organic solvent including methanol, acetone, chloroform, and ethanol at room temperature overnight. All the extracts were combined and concentrated under reduced pressure on a rotary evaporator and the volume was adjusted to 100 ml. Each extraction was evaporated to dryness under reduced pressure. All the extracts obtained were used in antioxidant measurements and the methanolic extract as polyphenolic-rich extract with powerful antioxidant activity was used in further studies.

Determination of total phenols and flavonoids levels

The total phenolics content of the plant extract was determined according to the Folin and Ciocalteu procedure method (Slinkard and Singleton, 1977). The FC reagent oxidizes phenols in plant extract and changes in the dark blue color ($\lambda = 765$ nm) are monitored by UV visible. Each sample (0.5 ml) was mixed with 2.5 ml FCR (diluted 1:10, v/v) followed by 2 ml of Na_2CO_3 (7.5%, v/v) solution. The absorbance was then measured at 765 nm after incubation at 30°C for 90 min. A calibration curve, that is, absorbance versus gallic acid concentration, was taken. Results were expressed as gallic acid equivalents (mg gallic acid/g dried extract).

Determination of the flavonoid content was achieved using the method described by Zhishen et al. (1999), by addition of aluminum chloride reagent to the solution containing the extract. Each sample (0.5 ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a NaNO_2 solution (15%). After 6 min, 0.15 ml of aluminum chloride (AlCl_3) solution (10%) was added and allowed to stand for 6 min, then 2 ml of NaOH solution (4%) was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then determined at 510 nm versus prepared water blank and concentrations of flavonoids were deduced from a standard curve and calculated in mg catechin equivalent.

DPPH free radicals scavenging activity assay

Radical scavenging capacity (RSC) was determined according to the technique reported by Blois (1958). An aliquot of 1.5 ml of 0.25 mM DPPH solution in ethanol and 1.5 ml of extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm with a Varian spectrophotometer. The DPPH radicals scavenging activity was calculated according to the following equation:

$$\text{RSC} = [(A_0 - A_1/A_0) \times 100]$$

Where A_0 is the absorbance of the control (blank, without extract) and A_1 is the absorbance in the presence of the extract or standard sample.

Animals

Adult male albino Wistar rats (150–200 g), 5-7 months old were obtained from Pasteur institute, Tehran, Iran. The rats were housed in plastic cages under conventional condition and were allowed free access to food and water *ad libitum*. The study was conducted in accordance with the "Guide for the Care and Use of Laboratory Animals".

Oral administration of alcohol and plant extract

The extract and alcohol solutions were administered by gavages (i.g.) to various rat groups for one month as followed:

- i) Normal rats orally received 30 % glucose (isocaloric to ethanol) as group 1.
- ii) Normal rats orally received plant extract (0.1 g/kg body weight) as group 2.
- iii) Normal rats orally received 20 % ethanol (4.0 g/kg body weight) as group 3.
- iv) Normal rats orally received 20 % ethanol with plant extract (0.1 g/kg body weight) as group 4.

Preparation of serum and tissue for biochemical assay

Blood was collected from rat heart with anticoagulant and separate plasma by centrifuge at 2000 g for 15 min. Each liver and kidney was quickly removed from the sacrificed, washed with ice cold saline solution, minced and homogenized in 50 mM phosphate buffer, pH 7.4 and centrifuged at 3000g for 15 min at 4°C. The supernatant was used for all the assays.

Measurement of serum marker enzymes activity

The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assayed spectrophotometrically according to the standard procedures using commercially available diagnostic kits (Pars Azmoon, Tehran, Iran) and an auto-analyzer (Vita Lab Selectra 2, Helsinki, Finland). Gamma glutamyl transferase (GGT) activity was determined according to Rosalki and Rau method (Rosalki and Rau, 1972).

Measurement antioxidant defense system factors

Catalase (CAT) activity was measured according to Aebi's method (Aebi, 1984). The enzyme activity was expressed as k (s mg pro-

tein)⁻¹, where k is the rate constant of the first order reaction of CAT. Superoxide dismutase (SOD) activity was measured according to method of Kakkar et al. (1984). A one unit of enzyme activity was expressed as 50% inhibition of NBT reduction/ (Unit/mg protein). Reduced glutathione (GSH) was determined by the method of Ellman (1959) briefly, to 0.5 ml supernatant, 0.5 ml Ellman's reagent (19.8 mg of 5, 5' dithiobisnitro benzoic acid, DTNB, in 100 ml of 0.1% sodium nitrate) was added and the absorbance read at 412 nm. GSH was expressed as mg/100 g tissue.

Glutathione peroxidase (GPx) activity was measured by Ellman's method (1959). Briefly, reaction mixture contained 0.2 ml of 0.4 M phosphate buffer pH 7.0, 0.1 ml of 10 mM sodium azide, 0.2 ml of tissue homogenate (homogenate on 0.4 M phosphate buffer, pH 7.0), and 0.2 ml glutathione, 0.1 of 0.2 mM H₂O₂. The content was incubated at 37°C for 10 min. The reaction was arrested by 0.4 ml of 10% TCA and centrifuged. Supernatant was assayed for glutathione content by using Ellmans reagent. Ascorbic acid (vitamin C) concentration was measured by Omaye et al., (1979) method. To 0.5 ml of sample, 1.5 ml of 6% TCA was added and to 0.5 ml of DNPH reagent (2% DNPH and 4% thiourea in sulfuric acid (9 N)) mixed with and incubated for 3 h at room temperature. After incubation, 2.5 ml of 85% sulfuric acid and color developed was read at 530 nm after 30 min. Vitamin E level was determined based on Derai's methods (1984) after preparation of lipid extract (Folch et al., 1957). To 0.5 ml of lipid extract, 1.5 ml ethanol, 2.0 ml of petroleum ether were added and centrifuged. The supernatant was evaporated to dryness at 80°C, to that added 0.2 ml of 2,2 - dipyrindyl (2%) and, 0.2 ml of ferric chloride (0.5%), kept in dark for 5 min and then 4 ml butanol was added. The color developed was read at 520 nm.

Non-enzymatic lipid peroxidation

The extent of lipid peroxidation was estimated by measuring the product of thiobarbituric acid reactive substances (TBARS) in the rat tissue homogenate using Mahakunakorn et al. (2007) method. 0.1 ml tissue homogenate (phosphate buffer, pH 7.4) was treated with 0.2 ml TBA-TCA-HCl reagent and mixed thoroughly. The mixture was incubated at 37°C for 15 min and centrifuged for 10 min at 1000 g. After centrifugation, the absorbance of supernatant was measured at 535 nm. For measurement hydroperoxides level, 0.1 ml of tissue homogenate was treated with 0.9 ml of Fox reagent (88 mg butylated hydroxytoluene (BHT), 7.6 mg xylenol orange and 9.8 ml of methanol and 10 ml 250 mM sulfuric acid) and after incubated at 37°C for 30 min the absorbance was measured at 560 nm.

Statistics

The significance of differences between the control and the test groups was established by the paired Student's t-test with the significance level set at $P < 0.05$.

RESULTS

The antioxidant activity of various extracts is probably due to its phenolic contents. The phenolic compounds exhibit considerable free radical scavenging activities, through their reactivity as hydrogen- or electron-donating agents and metal ion chelating properties (Rice-Evans et al., 1996). The total phenolic and flavonoid contents of different extracts of *S. sahendica* were determined by using Folin-Ciocalteu colorimetric and AlCl₃ methods, separately. The total phenolic content of each *S. sahendica* extract was reported in Table 1. The results showed

Table 1. Total phenolic^a, flavonoid^b and antioxidant activity determined by scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH)^c of different extracts of *S.sahendica*.

Extracts	Total phenolic	Total flavonoid	DPPH (IC ₅₀)
Methanol- extract	64.5 ±3.4	35.21 ± 2.1	17.14 ± 1.04
Acetone- extract	60.2 ±1.8	31.16 ± 1.4	18.50 ± 2.11
Chloroform- extract	48.13±2.1	25.46 ± 1.80	22.30 ± 1.37
Ethanol- extract	31.13±1.8	19.27±0.54	25.12±1.23
BHT	-	-	21.30±1.43
Ascorbic acid	-	-	3.81±0.14

Each value represents the mean ± SD (n=3).

a, Total phenolic content was expressed as mg gallic acid equivalents/g dried extracts.

b, Total flavonoid content was expressed as mg catechin equivalents/g dried extracts.

c, IC₅₀ values were expressed as µg/ml.

Table 2. Effect of methanolic extract of *S. sahendica* on hepatic enzymes in the serum of normal and experimental animals.

Groups	Control	Control+MESS	Ethanol	Ethanol + MESS
AST (IU/L)	70.25±3.67	72.43±3.25	157.24±8.63 ^a	85.26±6.03 ^b
ALT (IU/L)	24.83±2.87	22.44±5.48	59.21±1.06 ^a	28.47±5.65 ^b
ALP (IU/L)	78.47±5.13	76.32±2.14	134.87±9.54 ^a	89.21±7.25 ^b
CGT(IU/L)	2.14±0.15	1.99±0.11	5.45±0.54	2.54±0.24 ^b

Each measurement has been done at least in triplicate and values are the means ± SD for eight rats in each group. MESS means "methanolic extract of *S. sahendica*"

a, Significantly different from normal (P < 0.05).

b, Significantly different from ethanol-treated group (P < 0.05).

that various *S. sahendica* extracts contained phenolic compounds in the following order: Methanol extract > Acetone extract > Chloroform extract > Ethanol extracts. In addition, the total flavonoid contents of different *S. sahendica* extracts were reported as mg catechin equivalent per g dried extract. The results, as presented in Table 1, showed that the flavonoid contents of various extract of *S. sahendica* were followed in the above order. Based on these data, it can be concluded that the methanolic extract of *S. sahendica* possesses high content of phenolics and flavonoids.

The antioxidative capacities of different extracts of *S. sahendica* were also determined by comparing with the activities of known antioxidants, such as BHT by the *in vitro* assays; DPPH, The DPPH radical is a stable organic free radical with an absorption maximum band around 517 nm and thus, it is a useful reagent for evaluation of antioxidant activity of compounds (Maksimovic et al., 2005). Using this method, the result indicated that the methanolic extract of *S. sahendica* had the highest antioxidant capacity with a value of 17.14 µg/ml. The chloroform and ethanolic extracts showed lower activity with values of 22.30 µg/ml and 25.12 µg/ml, respectively (Table 1). Based on this data, the methanolic extract of *S. sahendica* as polyphenolic-rich extract with powerful antioxidant potential was used in *in vivo* examinations.

In order to explore the effect of methanolic extract of *S. sahendica* on serum biochemical factors of normal and experimental rats, the activity of three major enzymes including (AST, ALT, ALP and GGT) was evaluated. The results reported in Table 2 show that ethanol administration significantly (P < 0.05) increases the activities of ALT, ALP, AST and GGT, while the activities of these factors decreases in treated rats with methanolic extract of *S. sahendica* along with ethanol when compared with corresponding control group.

Table 3, shows that the level of peroxidation products such as TBARS and lipids hydroperoxide in tissues of control and experimental rats. A significant elevation in the levels of lipid peroxidation markers were observed in ethanol fed group when compared to control rats. Administration of methanolic extract of *S. sahendica* along with ethanol caused a significant decreased in the level of TBARS and lipid hydroperoxide in liver and kidney when compared with alcohol administrated rats.

Table 4, represents the level of antioxidant defense system factors in liver and kidney of different rat groups. The levels of vitamin E, vitamin C and GSH were significantly reduced in ethanol treated rats compared to control rats. Orally administration of plant extract significantly (P < 0.05) restored the levels of non-enzymatic antioxidants in both rat tissues; kidney and liver. The activities

Table 3. Effect of *S. sahendica* methanolic extract on the levels of lipid peroxidation in tissues of normal and experimental animals.

Groups	Control	Control+MESS	Ethanol	Ethanol + MESS
TBARS Liver	0.76±0.05	0.70±0.05	1.90±0.14 ^a	0.87±0.03 ^b
(mM/100 g tissue) Kidney	1.28±0.14	1.67±0.81	2.32±0.18 ^a	1.45±0.07 ^b
Hydroperoxides Liver	89.96±3.24	87.54±2.87	156.32±9.18 ^a	100.26±8.07 ^b
(mM/100 g tissue) Kidney	76.21±4.87	73.24±4.48	140.25±11.06 ^a	85.98±5.75 ^b

Each measurement has been done at least in triplicate and values are the means ± SD for eight rats in each group. MESS means "methanolic extract of *S. sahendica*"

a, Significantly different from normal (P < 0.05).

b, Significantly different from ethanol-treated group (P < 0.05).

Table 4. Changes in the concentration of reduced glutathione (GSH), vitamin C, vitamin E and the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-S- transferase (GST) in liver and kidney of control and experimental animals.

Groups	Control	Control+MESS	Ethanol	Ethanol + MESS
GSH (mg/100 g tissue)				
Liver	43.35±2.67	46.43±2.12	23.54±2.13 ^a	37.26±2.43 ^b
kidney	37.23±1.87	39.34±2.34	21.24±1.76 ^a	32.30±2.65 ^b
Vitamin C (µmol/mg tissue)				
Liver	1.54±0.13	1.43±0.14	0.84±0.13 ^a	1.22±0.03 ^b
Kidney	1.10±1.07	1.15±0.06	0.19±0.04 ^a	0.89±0.05 ^b
Vitamin E (µmol/mg tissue)				
Liver	1.64±0.11	1.53±0.13	0.89±0.03 ^a	1.32±0.09 ^b
Kidney	0.78±0.07	0.74±0.06	0.39±0.07 ^a	0.69±0.08 ^b
CAT (U/mg protein)				
Liver	73.45±3.67	78.56±4.54	56.34±4.13 ^a	69.27±3.93 ^b
Kidney	34.63±3.02	39.64±4.34	25.87±1.26 ^a	30.34±4.25 ^b
SOD (U/mg protein)				
Liver	10.65±1.34	11.43±2.12	4.34±0.13 ^a	8.16±0.78 ^b
Kidney	11.56±0.47	12.55±1.34	8.13±1.55 ^a	10.32±2.15 ^b
GPx (U/mg protein)				
Liver	9.35±0.67	10.12±0.12	5.43±0.34 ^a	8.64±0.76 ^b
Kidney	7.47±0.69	7.65±0.47	4.32±0.68 ^a	6.35±0.62 ^b
GST (U/mg protein)				
Liver	5.88±0.44	6.03±0.12	3.54±0.26 ^a	4.76±0.43 ^b
Kidney	4.68±0.87	5.34±0.39	3.03±0.76 ^a	4.12±0.43 ^b

Each measurement has been done at least in triplicate and values are the means ± SD for eight rats in each group. MESS means "methanolic extract of *S. sahendica*"

a, Significantly different from normal (P < 0.05).

b, Significantly different from ethanol-treated group (P < 0.05).

of antioxidant enzymes (SOD, CAT, GPx and GST) in liver and kidney were also given in Table 4. A significant decrease in the activities of these antioxidant enzymes were observed in alcohol-treated rats compared with normal rats. But, methanolic extract of *S. sahendica*-treated alcoholic rats exhibited significant increase in the activities of enzymatic antioxidants compared to untreated alcoholic rats (P < 0.05).

DISCUSSION

Oxidative stress is increasingly suspected to contribute to the initiation and progression of many diseases, including those caused by alcohol exposure. Cellular systems are protected from ROS-induced cell injuries by an array of defenses composed of various anti-oxidants with different functions. When the ROS present in the cellular system

overpower the defense systems, they will cause oxidative stress or cell injury, leading to the development of diseases. It has been revealed that during ethanol metabolism free radicals are generated (Lieber, 1997; Nordman, 1994). It plays a major role in ethanol-induced oxidative stress, which may be additionally enhanced by depletion in antioxidant defense system and in consequence by an imbalance between oxidants and antioxidants. Regarding the presence of free radicals and oxidative steps in alcoholic disease process, compounds with antioxidant activities have been examined in order to reduce or stop injuries induced by alcohol. In recent years dietary plants with antioxidative property have been the center of focus. It is believed that these plants can prevent or protect tissues against damaging effect of free radicals (Osawa and Kato, 2005). In addition, it has been shown that dietary supplementation with natural antioxidants such as, vitamins E and flavonoids attenuated the oxidative stress induced by ethanol (Pari and Suresh, 2008; Sailaja and Setty, 2005; Marino et al., 2004; Freitas et al., 2004).

Polyphenolic compounds are widely distributed in plants and known to be excellent antioxidants *in vitro*. They have the capacity to reduce free-radical formation by scavenging free radicals and protecting antioxidant defenses. In our study, the phenolic level estimation of various *S. sahendica* extracts reveals that methanolic extract of plant contains considerable amount of polyphenolic compounds. For evaluation of antioxidant capacity of various extracts of plant, DPPH assay was performed. The DPPH radical is a stable organic free radical with an absorption maximum band around 515–528 nm and it is a useful reagent for evaluation of antioxidant activity of compounds (Maksimovic et al., 2005). The highest scavenging activity was found for the methanolic extract of *S. sahendica* with an IC₅₀ value of 17.14 g/ml. It can be concluded that the content of polyphenolic compounds of methanolic extract of *S. sahendica* could be responsible for the radical scavenging activity in alcohol toxicity.

Liver damages were assayed by biochemical studies. Four separate liver enzymes including aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT), which are known together as transaminases and alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT), which are known together as cholestatic liver enzymes are the reliable indices of liver function. The increased levels of serum enzyme such as AST and ALT indicate the increased permeability and damage and/or necrosis of hepatocytes (Goldberg and Watts, 1965). The membrane bound enzymes like ALP and GGT are released unequally in to bloodstream depending on the pathological phenomenon (Sillanauke, 1996). Based on our results, the chronic ethanol consumption caused a significant increase in the activities of AST, ALT, ALP and GGT, which could be due to severe damage to tissue membrane. The decreased activities of these enzymes after treated rats by plant ex-

tract indicate the hepato-protective effect of methanolic *S. sahendica* extract.

Free radicals or reactive oxygen species such as hydroxy ethyl radical, superoxide and hydroxy radicals which formed in ethanol mediated process are responsible for alcohol induced oxidative stress (Nordmann et al., 1992; Hoak and Pastorino, 2002). They have a great potential to react rapidly with lipids, which in turn leads to lipid peroxidation. Changes in NAD⁺/NADH ratio, induction of CYP2E1 formation of 1-hydroxy ethyl free radicals, acetaldehyde protein adducts formation, ethanol mediated mitochondrial damage, endotoxin derived activation of kupffer cells and decrease in the cellular antioxidant defense are the main pathways implicated in ethanol-induced oxidative stress (Sergent et al., 2001). Treatment with plant extract strongly decrease the level of lipid peroxidation compared to alcoholic rats, which may be due to its free radical scavenging potential induced by ethanol. It is known that various species of *Salvia* has inhibitory effect in lipid peroxidation induced by Fe⁺² and Cu⁺² by its free radical scavenging potential (Zupkó et al., 2001). Because of central role of transition metals in lipid peroxidation process, our observations confirm the ability of *S. sahendica* extract to scavenging free radicals and inhibition of lipid peroxidation damage in alcohol toxicity.

The glutathione peroxidase system consists of several components, including the glutathione peroxidase and glutathione reductase and the glutathione (GSH) and reduced nicotinamide adenosine dinucleotide phosphate (NADPH) effectively remove hydrogen peroxide (Wu and Cederbaum, 2003). GSH, an essential component of this system, serves as a cofactor for glutathione transferase, which helps remove certain drugs and chemicals as well as other reactive molecules from the cells. Moreover, GSH can interact directly with certain ROS (hydroxyl radical) to detoxify them, as well as performing other critical activities in the cell. So, GSH is probably the most important antioxidant present in cells. A decreased in non-enzymatic antioxidant such as GSH, vitamin C and vitamin E was observed in tissues of alcohol treated rats. The decreased level of GSH in alcoholic disease may due to scavenging of free radicals and inhibition of the synthesis and increased rates of turnover (Lieber, 1997). Methanolic extract of *S. sahendica* had a potent increasing effect on GSH level and vitamin C and E contents on liver and kidney tissues compared to alcohol treated rats.

The enzymatic antioxidant defense system including superoxide dismutases (SODs), catalase, and glutathione peroxidase which can decompose superoxide and hydrogen peroxide in the cells are the main defense against oxidative injuries. SODs catalyze the rapid removal of superoxide radical and based on literature a decrease in SOD activity was observed in liver of alcohol treated rats. Catalase present in the peroxisomes of nearly all aerobic cells, serves to protect the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of

free radicals. Glutathione peroxidase (GPx) catalyzes the reduction of hydroperoxides, including hydrogen peroxides, by reduced glutathione and functions to protect the cell from oxidative damage (Wilce and Parker, 1994). The detoxification of 4-hydroxynonenal is compromised when GST level is reduced. Thus, ethanol or its metabolic products might specifically target GST isoenzymes and the reduction in enzyme activity or expression may contribute to ethanol hepatotoxicity (Alin et al., 1985). CYP2E1 could be the sole catalyst of fetal ethanol oxidation produces 1-hydroxy ethyl radicals, which have been shown to inactivate several proteins including antioxidant enzyme system (Epstein, 1996). In consistent with these reports, our results also showed that decreased activities of SOD, CAT, GPx and GST in tissues on the chronic alcohol treatment in rats. Administration of methanolic extract of *S. sahendica* to alcoholic rats restored the activities of enzymatic antioxidant in liver and kidney. The results presented in this study, suggested that the efficiency of any plant extract as drug is essentially dependent on its capacity of either reducing the harmful effects or in maintaining the normal physiology of cells and tissues, which have been attributed by toxins. The hepatoprotective activity of methanolic extract of *Salvia sahendica* may be due its antioxidative potential which was shown by significant quenching impact on extent of lipid peroxidation and free radical scavenging along with enhancement of antioxidant defense system in alcoholic rat liver and kidney tissues. To elucidate the exact mechanism of this modulator effect further studies regarding the structure elucidation of the active compound(s) and evaluation of their biological activities are essential.

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REFERENCES

- Aebi H (1984). Catalase *in vitro*. In: Methods in Enzymology, Vol 105. (Packer L, ed.) Academic Press, New York, pp. 121-126.
- Alin P, Danielson HU, Mannervik B (1985). 4-Hydroxyalk-2-enals are substrates for glutathione transferase. FEBS Lett. 179: 267-270.
- Arteel GE (2000). Oxidants and antioxidants in alcohol-induced liver disease. Gastroenterol. 124: 778-790.
- Aruoma OI, Kaur H, Halliwell B (1991). Oxygen free radicals and human diseases. J. Roy. Soc. Health. 111: 172-177.
- Block G (1991). Vitamin C and cancer prevention: The epidemiologic evidence. Am. J. Clin. Nutr. 53: 270S-282S.
- Blais MS (1958). Antioxidant determination by the use of a stable free radical. Nature 181: 1199-1200.
- Chen SY, Sulik KK (1996). Free radicals and ethanol-induced cytotoxicity in neural crest cells. Alcohol. Clin. Exp. Res. 20: 1071-1076.
- Collins MA, Zou JY, Neafsey EJ (1998). Brain damage due to episodic alcohol exposure *in vivo* and *in vitro*: Furosemide neuroprotection implicates edema-based mechanism. The FASEB. J. 12: 221-230.
- Desai ID (1984). Vitamin E analysis method for animal tissue. Methods Enzymol. 105: 138-143.
- Ellman GL(1959). Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82: 70-77.
- Emerit J, Edeas M, Bricaire F (2004). Neurodegenerative diseases and oxidative stress. Biomed. Pharmacother. 58: 39-46.
- Epstein M (1996). Renal sodium handling in liver disease. Epstein M (eds) The Kidney in liver disease, Hanley and Belfus Publication, Philadelphia, pp. 1-31.
- Folch J, Less M, Solane SGH (1957). A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.
- Freitas VD, Silva PD, Leite C, Andrade J, Barbosa MMP (2004). Flavonoids from grape seed prevent increased alcohol-induced neuronal lipofuscin formation. Alcohol Alcohol. 39: 303-311.
- Goldberg DM, Watts C (1965). Serum enzyme changes as evidence of liver reaction to oral alcohol. Gastroenterol. 49: 256-261.
- Gutteridge JM (1994). Biological origin of free radicals and mechanisms of antioxidant protection. Chem. Biol. Interact. 91: 133-140.
- Heaton MB, Mitchell JJ, Paiva M (2000). Amelioration of ethanol-induced neurotoxicity in the neonatal rat central nervous system by antioxidant therapy. Alcohol. Clin. Exp. Res. 24: 512-518.
- Hoak JB, Pastorino JG (2002). Ethanol, oxidative stress and cytokine induced liver injury. Alcohol 27: 63-68.
- Kakkar P, Das B, Viswanathan PN (1984). A modified spectrophotometric assay of superoxide dismutase. Indian. J. Biochem. Biophys. 21: 130-132.
- Kintzios SE (2000). In Sage The genus *Salvia*; Kintzios SE(eds) Harwood Academic Publishers, New York, pp. ix-xii.
- Kumar DS, Vasudevan DM (2007). Alcohol-induced oxidative stress. Life. Sci. 3: 177-187.
- Lieber CS (2000). Alcohol: its metabolism and interaction with nutrients. Annu. Rev. Nutr. 20: 395-430.
- Lieber CS (1997). Ethanol metabolism, cirrhosis and alcoholism. Clin. Chim. Acta 257: 59-84.
- Lieber CS (1997). Role of oxidative stress and antioxidant therapy in alcoholic and nonalcoholic liver diseases. Adv. Pharmacol. 38: 601-628.
- Lotfipour F, Samiee M, Nazemiyeh H (2007). Evaluation of the antibacterial activity of *Salvia sahendica* and *Phlomis caucasica*. Pharmaceutical sciences, Journal of Faculty of Pharmacy, Tabriz University of Medical Sciences 1: 29-34.
- Mahakunakorn P, Tohda M, Murakami Y, Matsumoto K, Watanabe H (2007). Antioxidant and free radical-scavenging activity of Choto-san and its related constituents. Biol. Pharmaceut. Bull. 27: 38-46.
- Maksimovic Z, Malencić ND, Kovacević N (2005). Polyphenol contents and antioxidant activity of Maydis stigma extracts. Bioresour. Technol. 96: 873-877.
- Marino MD, Aksenov MY, Kelly SJ (2004). Vitamin E protects against alcohol-induced cell loss and oxidative stress in the neonatal rat hippocampus. Int. J. Dev. Neurosci. 22: 363-377.
- Mozafarian V (1996). A dictionary of Iranian plant names (Latin English Persian) Farhang Moaser Publication, Tehran, pp. 477-479.
- Nordman R (1994). Alcohol and antioxidant system. Alcohol. Alcohol 29: 513-522.
- Nordmann R, Ribiere C, Rouach H (1992). Implications of free radical mechanisms in ethanol induced cellular injury, Free Radic. Biol. Med. 12: 219-232.
- Omaye ST, Turbull TP, Sauberlich HC(1979). Selected methods for determination of ascorbic acid in cells, tissues and fluids. Methods Enzymol. 6: 3-11.
- Osawa T, Kato Y (2005). Protective role of antioxidative food factors in oxidative stress caused by hyperglycemia. Ann. NY. Acad. Sci. 1043: 440-451.
- Pari L, Suresh A (2008). Effect of grape (*Vitis vinifera* L.) leaf extract on alcohol induced oxidative stress in rats. Food. Chem. Toxicol. 46: 1627-1634.
- Rice-Evans CA, Miller NG, Paganga G (1996). Structure-antioxidant activity relationship of flavonoids and phenolic acids. Free Radic. Biol. Med. 20: 933-956.
- Rosalki SB, Rau D(1972). Serum gamma-glutamyl transpeptidase activity in alcoholism. Clin. Chim. Acta. 39: 41-47.
- Sailaja R, Setty OH (2005). Protective effect of *Phyllanthus fraternus*

- against allyl alcohol-induced oxidative stress in liver mitochondria. *J. Ethnopharm.* 105: 201-209.
- Salehi P, Sonboli A, Ebrahimi SN, Yousefzadi M (2007). Antibacterial and antioxidant activities of the essential oils and various extracts of *Salvia sahendica* in different phenological stages. *Chem. Nat. Comp.* 43: 328-330.
- Sergent O, Griffon B, Cillard P, Cillard J (2001). Alcohol oxidative. *Pathol. Bio.* 27: 689-695.
- Sillanauke P (1996). Laboratory markers of alcohol abuse. *Alcohol* 31: 613-616.
- Silva BA, Ferreres F, Malva JO, Dias ACP (2005). Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. *Food. Chem.* 90: 157-167.
- Slinkard J, Singleton VL (1977). Total phenol analysis: automation and comparison with manual methods. *Am.J. Enol. Viticult.* 28: 49-55.
- Wang ZJ, Luo DH (2007). Antioxidant activities of different fractions of polysaccharide purified from *Gynostemma pentaphyllum* Makino. *Carbohydr. Polym* 68: 54-58.
- Wilce MC, Parker MW (1994). Structure and function of glutathione S-transferases. *Biochim. Biophys. Acta.* 1205:1-18.
- Wu D, Cederbaum I (2003). Alcohol, Oxidative Stress, and Free Radical Damage. *Alcohol. Res. Health.* 27: 277-284.
- Yin M, Wheeler MD, Kono H, Bradford BU, Gallucci RM, Luster MI, Thurman RG (1999). Essential role of tumor necrosis factor in alcohol-induced liver injury. *Gastroenterol.* 117: 942-952.
- Zhishen J, Mengcheng T, Jianming W (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food. Chem.* 64: 555-559.
- Zupkó I, Hohmann J, Rédei D, Falkay G, Janicsák G, Máthé I (2001). Antioxidant activity of leaves of *Salvia* species in enzyme-dependent and enzyme-independent systems of lipid peroxidation and their phenolic constituents. *Planta. Med.* 67:366-368.