Research Article

Mahgoub Mohamed Ahmed*

Hepatoprotective role of Solenostemma argel growing in Egypt on ethanol induced oxidative damage in rats
Mısırda büyüyen Solenostemma argel’in sıçanlarda etanole bağlı oksidatif hasara karşı karaciğer korumasındaki rolü

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Abstract

Objective: The objective of the current study is to investigate the protective effect of Solenostemma argel (S. argel) treatment on ethanol (EtOH)-induced hepatotoxicity in rat liver.

Methods: Forty adult male albino rats were divided into four groups as control, S. argel (100 mg/kg), EtOH (3 g/kg) and S. argel + EtOH groups. S. argel was given 1 h prior to EtOH by oral gavage for 28 days.

Results: The results showed that, administration of EtOH caused a significant decrease (p < 0.05) in serum total protein and albumin, whereas ALT and AST and lipid peroxidation (LPO) were increased following EtOH treatment. S. argel treatment significantly (p < 0.05) ameliorated the previous parameters. Protein carbonyl, reduced glutathione and LPO levels were significantly (p < 0.05) increased along with glutathione-S-transferase activity was decreased (as markers of oxidative stress) in EtOH treated rat liver. Previous oxidative stress was attenuated significantly by S. argel treatment. Moreover, in EtOH group, tumor necrosis factor-α (TNF-α) and nitric oxide (NO) contents and cytochrome P450 2E1 activity were significantly increased in liver tissues showing oxidative organ damage. Co-administration of S. argel with EtOH significantly reversed the inflammation in rat livers.

Conclusion: S. argel had a hepatoprotective role against EtOH-induce oxidative stress and inflammation in rat liver.

Keywords: Ethanol; S. argel; Oxidative stress; CYTP2E1; Hepatoprotective; Rats.

Özet

Amaç: Solenostemma argel (S. argel) tedavisinin, sıçanlarda etanole (EtOH) bağlı karaciğer toksisitesine karşı koruyucu etkinliğinin araştırılması.

Metod: Kırk adet yetişkin erkek albino sıçan dört gruba ayrıldı: kontrol, S. argel (100 mg/kg), EtOH (3 g/kg) ve S. Argel + EtOH grupları. S. argel, EtOH uygulamasından bir saat önce 28 gün boyunca ağızdan beslenmiştir.

Bulgular: Veriler EtOH uygulamasının serum toplam protein (TP) oranında ve albümün (Alb) oranında bir azalmaya (p < 0.05) sebep olduğunu gösterdi, bunun yansısı EtOH uygulaması sonucunda ALT ve AST ve lipid peroksidasyonunda (LPO) bir artış gözlandı. S. argel uygulaması bu parametrelerin ağaçlamasını (p < 0.05) bir şekilde iyileşmesine sebep oldu. EtOH e maruz bırakılan sıçanların karaciğerlerinde (oksidatif stres markörü olarak) glutation-S-transferaz (GST) aktivite azalmasının yansıısı, protein karbonil (PC), indirgenmiş glutation (GSH) ve LPO seviyeleri anlamlı bir şekilde artmıştır (p < 0.05). S. argel tedavisi gözenlenen bu oksidatif stres anlamlı bir şekilde azaltmıştır. Hatta EtOH gurubu karaciğer dokusunda, oksidatif doku hasarı gösteren, tümör nekrosis faktör-α (TNF-α) ve nitric oksit (NO) seviyeleri ve cytochrom P450 2E1 (CYTP2E1) aktivitesi anlamlı bir şekilde artmıştır. S. argel ve EtOH’un birlikte uygulanması, sıçan karaciğerinde inflamasyonun anlamlı bir şekilde geri çevrilmesine yol açmıştır.
S. argel is a plant known for its insecticidal effect [10], antibacterial and antioxidant activity [11]. Furthermore, S. argel has anti-diabetic [12], anti-ulcerogenic [13], anticancer activity [14], antioxidant and hypoglycemic effect [15]. Also, pregnane glycosides isolated from this plant were reported to reduce cell proliferation in a dose-dependent manner [16]. No scientific report is available regarding hepatoprotective potential of S. argel leaves, to the best of our knowledge. Therefore, the present study was conducted to evaluate the hepatoprotective effects of S. argel treatment on the ethanol-induced hepatotoxicity model. Moreover, we explored the possible mechanism underlying the intervention on ethanol toxicity with S. argel.

Materials and methods

Chemicals

Ethanol, glutathione (GSH), thiobarbituric acid (TBA), 2,2-dinitrophenyl hydrazine (DNPH) and guanidine hydrochloride were procured from Sigma Chemical Co. (St. Louis, MO, USA). Griess reagents were purchased from Merk-Schuchardt Chemical Company (Hohenbrunn, Germany), with purity of 99%. All other chemicals were of analytical grades.

Plant extracts

Solenostemma argel purchased from Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University was dried, powdered before extraction. The powdered leaf material (100 g) was extracted by percolation with 70% ethanol. The extract was filtered, concentrated under vacuum and freeze-dried. The ethanol extract (70%) was used for further study.

Estimation of in vitro antioxidant activity of S. argel

In vitro antioxidant of S. argel was evaluated by the following three methods.

1. DPPH radical scavenging activity

Radical scavenging activity of the S. argel was estimated by Chen et al. [17] using DPPH* method. The ability of S. argel to scavenge DPPH was calculated.
using the following equation: radical scavenging activity \( \% = [1 - (\text{absorbance of sample/absorbance of control})] \times 100 \). The control contains 2 mL of DPPH\(^*\) solution mixed with 2 mL of ethanol. All tests were done in triplicate and the results were presented as means ± SD.

2. Microsomal lipid peroxidation

Microsomal lipid peroxidation method was used to estimate the inhibition of free radical-induced peroxidation of rat liver microsomes by \( S. \text{argel} \).

3. Preparation of microsomes

Rats were killed by cervical decapitation, livers were removed and the liver microsomes were prepared according to the method of Chang and Waxman [18]. Microsomal protein content was determined by the method of Lowry et al. [19].

4. \( \text{Fe}^{2+}/\text{ascorbate} \) model system

The reaction mixture contained 1 mg microsomal protein/mL, 1.6 mM ascorbic acid, and the \( S. \text{argel} \) or phosphate buffer (for control reaction). Lipid peroxidation products were measured according to the method of Ohkawa et al. [20]. The inhibitory effect of \( S. \text{argel} \) was calculated according to the following equation: \( %I = [1 - (\text{absorbance of sample/absorbance of control})] \times 100 \). All tests were done in triplicate and the results were presented as means ± SD.

5. Metal chelating activity

The chelating of ferrous ions by the \( S. \text{argel} \) was estimated by the method of Dinis et al. [21]. The percentage of inhibition of ferrozine-\( \text{Fe}^{2+} \) complex formation was calculated using the following formula: \( %I = [1 - (\text{absorbance of sample/absorbance of control})] \times 100 \). All tests were done in triplicate and the results were presented as means ± SD.

**Total phenolic, total flavonoid and total tannins contents of \( S. \text{argel} \)**

Total phenolic, total flavonoid and total tannins contents in the \( S. \text{argel} \) leaves ethanol extract were determined according to Singleton and Rossi [22], Kim et al. [23] and Burns [24], respectively.

**Animals and experimental design**

Male albino rats (160±30 g) from the laboratory stock colony of National Organization for Drug Control and Research (NODCAR) were used in the present study. The animals were kept under normal environmental conditions for 2 weeks before the initiation of the experiment. The animals were allowed free access to water and fed on a standard diet. The local ethics committee of NODCAR approved study protocols.

Rats were divided into four equal groups (n = 10) and treatment was given as follows:

1. **Control Group** received 0.5 mL saline and 0.5 mL corn oil orally by gavage once a day, for 28 days.
2. **\( S. \text{argel} \) Group**, received 100 mg/kg \( S. \text{argel} \) in corn oil orally by gavage once a day, for 28 days.
3. **Ethanol Group** received 3 g/kg in normal saline, orally by gavage once a day, for 28 days [25].
4. **\( S. \text{argel} \)+Ethanol Group** received 3 g/kg ethanol 1 h after treatment of \( S. \text{argel} \) (100 mg/kg), orally by gavage once a day, for 28 days.

Blood samples were collected and kept at room temperature for 1 h, then centrifuged at 3000 rpm/30 min and the separated serum was used for estimation of AST and ALT activities and TP and Alb content using Reactivos GPL Kits, Barcelona, España. LPO contents were determined according to Ohkawa et al. [20].

**Preparation of livers homogenates**

Rats were sacrificed by cervical dislocation to obtain livers then washed with cooled saline (0.9%). Livers were homogenized in ice-cold 1.15% KCl with a Potter-Elvehjem glass homogenizer to prepare 10% w/v homogenate. The homogenates were centrifuged at 10,000 × g for 20 min at 4°C (Cooling centrifuge, Sigma-3K30, Germany) to obtain liver supernatants.

**Biochemical determination in liver tissues**

**Determination of Nitric oxide (NO) level**

Nitrite was estimated using Greiss reagent according to Montgomery and Dymock [26].

**Determination of protein carbonyls (PC)**

Levels of protein carbonyls (PC) were determined according to Levine et al. [27].

**Determination of glutathione-S-transferases (GST) activity**

The activity of GST was estimated using the method of Habig et al. [28].
Determination of lipid peroxidation level (LPO)

Lipid peroxidation, as TBARS, was determined according to the thiobarbituric acid reaction described by Ohkawa et al. [20].

Determination of GSH level

GSH levels were assayed in tissue homogenates according to the method of Ellman [29].

Isolation of liver microsomes

Microsomes were isolated using the procedure described by Chang and Waxman [18].

CYP2E1 activity measurement

CYP2E1 activity was measured in liver microsomal fractions as described by Chang et al. [30]. The concentration of p-nitroacetone, the pink-yellow product, is determined from the extinction coefficient 9.53 M⁻¹ cm⁻¹ and CYP2E1 activity was expressed as nm/min/mg protein.

Determination of protein content

Protein content in the homogenate and microsomes fractions was estimated by the method of Lowry et al. [19].

Determination of TNF-α level

Level of TNF-α in rat liver was quantified using ELISA kits according to the manufacturer’s instructions and guidelines.

Statistical analysis

The values were expressed as the mean ± SD for the 10 rats in each group. Differences between groups were assessed by one way analysis of variance (ANOVA) using the statistical package for social sciences (SPSS) software package for Windows (version 13.0). Post hoc testing was performed for intergroup comparisons using the least significant difference (LSD) test. A value corresponding to p < 0.05 was considered statistically significant.

Results

Total phenolic, total flavonoid and total tannins contents of S. argel

The total phenolic, total flavonoid and total tannins contents of the S. argel leaves extract were 95.7 ± 5.7 mg gallic acid/g extract (dry wt.), 23.6 ± 3.1 mg quercetin/g extract (dry wt.) and 21.5 ± 3.8 mg quercetin/g extract (dry wt.), respectively (Table 1).

Antioxidant activity of S. argel

The present study showed that S. argel had antioxidant activity on DPPH• radicals, microsomal lipid peroxidation and the iron-chelating ability and this activity was in a dose-dependent manner (Table 2).

Effects of S. argel on activities of AST and ALT and levels of TP, Alb and LPO in serum

The activities of ALT and AST, as indication of hepatic injuries, and LPO levels were significantly increased in the EtOH-treated group when compared with those of the control group (p < 0.05). S. argel treatment decreased their levels significantly (p < 0.05) compared with EtOH-treated group (Table 3). TP and Alb levels were significantly decreased in the EtOH-treated group compared with those of the control group. On the other hand, S. argel treatment significantly (p < 0.05) improved TP and Alb levels in the S. argel + Ethanol group when compared with the EtOH group (Table 3).

Effect of S. argel on oxidative stress markers

Oxidative stress refers to enhanced generation of reactive oxygen species/reactive nitrogen species and/or

| Contents                      | S. argel extract       |
|-------------------------------|------------------------|
| Total Phenolics, GAE mg/g dw  | 95.7 ± 5.7             |
| Total Flavonoids, QE mg/g dw  | 23.6 ± 3.1             |
| Total Tannins, QE mg/g dw     | 21.5 ± 3.8             |

Data are expressed as mean ± SD of three replicates; GAE, Gallic acid equivalent; QE, Quercetin equivalent.
depletion of antioxidant defense system causing an imbalance between pro-oxidants and antioxidants. Oxidative stress has been demonstrated to be a key mechanism in EtOH induced cell injuries. Ethanol caused intracellular accumulation of LPO and PC levels and a significant decrease in GSH level and GST activity (Table 4, p < 0.05), suggesting oxidative liver damage. *S. argel* markedly inhibited the excessive generation of LPO and PC and the consumption of GSH in rats treated with EtOH.

**Effect of *S. argel* on inflammation markers**

Table 5 showed that NO and TNF-α levels markedly increased in EtOH group. *S. argel* significantly (p < 0.05)
improved this effect in rats treated with EtOH in comparison with EtOH group.

**Effect of S. argel on CYP2E1 activity**

As shown in Table 5, CYP2E1 activity (the rate of oxidation of \(\rho\)-nitrocatechol to \(\rho\)-nitrophenol) was significantly increased in rats treated with ethanol compared to control group. However, co-administration of *S. argel* plus ethanol significantly reduced CYP2E1 activity.

**Discussion**

It is well known that heavy consumption of alcohol is associated with liver damage. In the present study, we evaluated the protective effect of *S. argel* against ethanol-induced hepatotoxicity. The results showed that *S. argel* leaves extract contains significant concentrations of polyphenolics, tannins and polyflavonoids contents. High total phenolic content of *S. argel* leaves indicate that these leaves could be used as a good and cheap source of bioactive constituents. Moreover, *S. argel* leaves extract had in vitro antioxidant activity through inhibiting microsomal lipid peroxidation, scavenging DPPH radical and chelating metal ions in a dose dependent manner (Tables 1 and 2). Flavonoids have been reported to be responsible for antioxidant activities of plants through their scavenging or chelating activity [31]. In this study, *S. argel* leaves extract showed hepatoprotective effect that could be attributed to the free radical scavenging activity of the *S. argel* extract due to the activity of these phytochemicals.

The liver plays a key role in the detoxification and elimination of various harmful agents that can enter the organism through environmental or occupational exposure [32]. The liver exposure a variety of hepatotoxins, such as excessive alcohol intake, heavy metals and organic and inorganic solvents, resulting in excessive generation of free radicals which cause hepatotoxic lesions including acute hepatitis, cirrhosis, portal fibrosis and hepatic carcinoma [33].

In our study, administration of ethanol caused significant increase in AST and ALT activities and decreased total protein. The elevation of AST and ALT activities due to hepatocytes damage caused by the ethanol where the leakage of cell membrane participated in the accumulation of these enzymes into the plasma [34]. *S. argel* reduced the level of these enzyme markers, which could be attributed to its polyphenolic and flavonoid contents. Therefore, treatment of *S. argel* is helping in preventing liver damage. The polyphenolic compounds are beneficial scavengers of superoxide, hydroxyl, peroxyl and peroxynitrite radicals, they chelate redox-active metals and they can protect cell membranes against oxidative attack [35].

The importance of oxidative stress in the development of alcoholic liver disease has long been appreciated. If the antioxidant defense systems impaired, the oxidative stress may produce lipid peroxidation, protein carbonyl formation, and antioxidant enzymes inactivation [36]. In the present work, the levels of PC and LPO in ethanol treated rats increased, whereas GSH content and GST activity decreased. These results are in agreement with other studies demonstrated that ethanol significantly increased LPO level and decreased GSH content in the liver and kidney of ethanol treated rats [37].

Ethanol administration induces hepatic oxidative stress due to increased generation of reactive oxygen species (ROS) and/or reduced antioxidant capacity [38]. ROS increase lipid, protein and DNA peroxidation results in hepatocyte injury [39]. Free radicals can also lead to the formation of protein/protein cross-linkages, oxidation of protein backbone resulting in protein fragmentation and modification of amino acid side chains, which includes oxidation of sulfhydryl moieties and formation of protein carbonyls [40]. The present results showed that co-administration of *S. argel* with ethanol markedly diminished the levels of PC and LPO along with enhanced GSH content and GST activity in liver. This suggests that *S. argel* might be helpful to reverse the oxidative stress damage caused by ethanol, which might be attributing to the antioxidant activity and scavenging of ROS.

TNF-\(\alpha\) and IL-6 are known to be important cytokines linked to hepatocyte damage induced by chronic alcohol consumption [41]. High production of NO reacts with superoxide anion to produce peroxynitrite which is a toxic radical to tissues [42] and capable eliciting lipid peroxidation and cellular damage [43]. In addition, High NO production may be the cause of inflammatory reaction [44]. Our study exerted that ethanol increased NO and TNF-\(\alpha\) levels in liver rats after 4 weeks in comparison with the control group. These results are in agreement with Yang et al. [37] who showed that the levels of inflammatory factors such as TNF-\(\alpha\) and IL-6 were significantly increased by ethanol. In this study, co-administration of *S. argel* with ethanol protected the liver TNF-\(\alpha\) and NO. Therefore, *S. argel* treatment is helping in attenuating inflammation caused by ethanol. Some flavonoids inhibit NO production in response to inflammatory stimuli [45].
Genistein, a flavonoid, blocked the increase in mRNA of IL-1b, IL-6 and TNF-α produced by lipopolysaccharide-stimulated monocytes [46].

CYP2E1 is a key member of CYP450 superfamily and plays an important role in metabolizing low-molecular hydrophobic chemicals [47]. CYP2E1 can convert molecular oxygen to highly reactive compounds, including superoxide anion radical, singlet oxygen, hydrogen peroxide and hydroxyl radical [48]. Our results demonstrated that CYP2E1 activity was elevated by ethanol and this effect is in agreement with Lieber [49, 50].

Many important substrates including ethanol, carbon tetrachloride, and acetaminophen were metabolized to more toxic products by CYP2E1 [47, 49]. CYP2E1 can oxidize ethanol, and generate reactive products from ethanol oxidation, for example acetaldehyde and the 1-hydroxyethyl radical, and can generate ROS, such as the superoxide anion radical and $\text{H}_2\text{O}_2$ [51]. Yang et al. [52] suggested that CYP2E1 is the major contributor to ethanol induced oxidative stress; since CYP2E1 is elevated by alcohol and CYP2E1 catalyzed reactions can generate ROS. Therefore, the induction of CYP2E1 may be partly responsible for the toxicity of ethanol. Co-administration of $\text{S. argel}$ with ethanol diminished CYP2E1 activity in liver tissues. Due to the presence of phytochemicals such as tannins, flavonoids and polyphenols in $\text{S. argel}$ leaves, it may reduce the activity of CYP2E1.

The results of the present study showed that co-administration of $\text{S. argel}$ with ethanol decreased the LPO and increased GSH content along with increase GST in the liver tissues of rats. Furthermore, the protective effect of $\text{S. argel}$ against ethanol-induced hepatotoxicity demonstrated by the significant reduction of serum AST and ALT as well as significant decrease of PC, TNF-α, NO and CYP2E1 in liver tissues. Also, $\text{S. argel}$ may protect the liver from oxidative damages induced by ethanol through antioxidative effects [11].

In conclusion, we have shown that the $\text{S. argel}$ extract had potent hepatoprotective effects against ethanol-induced hepatotoxicity in rats. This extract inhibited the hepatic damage accompanied by decreased activity of serum liver enzymes. Treatment with $\text{S. argel}$ extract resulted in restoration of GSH and GST, the antioxidant defense system, which was impaired by ethanol exposure, and suppressed LPO and PC. Furthermore, the protective effect of $\text{S. argel}$ against ethanol-induced hepatotoxicity demonstrated by the significant reduction of TNF-α, NO and CYP2E1 in liver tissue. Further studies should be done to develop $\text{S. argel}$ as a new and promising dietary supplement for protection against ethanol-induced liver damage.

**Conflict of interest:** The author has no conflict of interest.

**References**

1. Albano E, French SW, Ingelman-Sundberg M. Hydroxyethyl radicals in ethanol hepatotoxicity. Front Biosci 1999;4:D533–D540.
2. Tuma DJ, Casey CA. Dangerous byproducts of alcohol breakdown-focus on adducts. Alcohol Res Health 2003;27:285–90.
3. Lu Y, Cederbaum A. CYP2E1 and oxidative liver injury by alcohol. Free Radic Biol Med 2008;44:723–738.
4. Hirano T, Homma Y, Kasai H. Formation of 8-Hydroxyguanine in DNA by Aging and Oxidative Stress. In: Cutler RG, Packer L, Bertran J, Mori A, editors. Oxidative Stress and Aging. Basel, Switzerland: Birkhauser Verlag, 1995:69–76.
5. Wu D, Cederbaum Al. Alcohol, oxidative stress, and free radical damage. Alcohol Res Health 2003;27:277–284.
6. Mutlu-Turkoglu U, Dogru-Abbasoglu S, Aykac-Toker G, Mirsal H, Beyazurek M, Uysal M. Increased lipid and protein oxidation and DNA damage in patients with chronic alcoholism. J Lab Clin Med 2000;136:287–291.
7. Barry RE, McGivan JD. Acetaldehyde alone may initiate hepatocellular damage in acute alcoholic liver disease. Gut 1985;26:1065–1069.
8. Yermilov V, Rubio J, Becchi M, Friesen MD, Pignatelli B, Ohshima H. Formation of 8-nitroguanine by the reaction of guanine with peroxynitrite in vitro. Carcinogenesis 1995;16:2045–2050.
9. Park JH, Kim Y, Kim SH. Green tea extract (Camellia sinensis) fermented by Lactobacillus fermentum attenuates alcohol-induced liver damage. Biosci Biotechnol Biochem 2012;76:2294–3000.
10. Awad KT, Khalid OA, Tagelsir IM, Sidahmed O. Argel (Solenostemma argel Del. Hayenne) applications for control of the date palm green scale insect (Asterolicanium phoenicis Rao) and yield enhancement. ARPN J Agric Biol Sci 2012;7:6.
11. Shafek RE, Michael HN. Antibacterial and antioxidant activity of two new kaempferol glycosides isolated from Solenostemma argel stem extract. Asian J Plant Sci 2012;11:143–147.
12. Trojan-Rodrigues M, Alves TL, Soarer GL, Ritter MR. Plants used as anti diabetics in popular medicine in Rio Grande do Sul, southern Brazil. J Ethnopharmacol 2012;139:155–163.
13. Mohammed AE, Mohammed O, Shadda SA, Hamad AM. Antilucreogenic activity of the crude methanolic extract of Solenostemma argel Hyne. J Pharm Biomed Sci 2014;4:1084–1089.
14. Hanafi N, Mansour S. Antitumor efficacy of Solenostemma Argel and/or γ-irradiation against ehrlich carcinoma. J Biol Sci 2010;10:468–479.
15. Taj Al-Deen A, Al-Naqeb G. Hypoglycemic effect and in vitro antioxidant activity of methanolic extract from Argel (Solenostemma Argel) plant. Int J Herbal Med 2014;2:128–131.
16. Plaza A, Perrone A, Balestieri M, Felice F, Balestrieric C. New unusual pregnane glycosides with anti-proliferative activity from Solenostemma argel. Steroids 2005;70:594–603.
17. Chen Y, Wang M, Rosen RT, Ho CT. 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging active components from Polygonum multiflorum Thunb. J Agric Food Chem 1999;47:2226–2228.
18. Chang TK, Waxman DJ. Enzymatic analysis of cDNA-expressed human CYP1A1, CYP1A2, and CYP1B1 with 7-ethoxresorufin as a substrate. In: Phillips IR, Shephard EA, editors. Methods in molecular biology: cytochrome P450 Protocols. Totowa, NJ: Humana Press Inc., 1998;107:109–109.

19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol Reagent. J Biol Chem 1951;193:269–275.

20. Ohkawa H, Ohnishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;9:351–358.

21. Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivates (acetoaminophen, salicylate and 5-aminoosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 1994;315:161–169.

22. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144–158.

23. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem 2003;81:321–326.

24. Burns RE. Methods for estimation of tannin in grain sorghum. J Agrom 1971;63:511–512.

25. Pourbaksh H, Taghiabadi E, Abnous KH, Timchek Hariri A, Hosseini SM, Hosseinzadeh H. Effect of Nigella sativa fixed oil on ethanol toxicity in rats. Iran J Basic Med Sci 2014;17:1020–1031.

26. Montgomery H, Dymock J. The determination of nitrite in water. Analyst 1961;86:414–417.

27. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990;186:464–478.

28. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130–7139.

29. Ellman GL. Tissue sulphhydryl groups. Arch Biochem Biophys 1959;82:70–77.

30. Chang TK, Crespi CL, Waxman DJ. Spectrophotometric analysis of human cyp 2E1-catalyzed p-nitrophenol hydroxylation. In: Phillips IR, Shephard EA, editors. Methods in molecular biology: cytochrome P450 Protocols. Totowa, NJ: Humana Press, 1998;107:147–152.

31. Das NP, Pereira TA. Effects of flavonoids on thermal autooxidation of palm oil: structure activity relationship. J Am Chem Soc 1990;67:255–258.

32. Vander AJ, Sherman JH, Luciano DS, editors. Nonimmune metabolism of foreign chemicals. Human physiology: the mechanisms of body function. Toronto: Mc Graw Hill, 1994:738–740.

33. Nakagiri R, Hashizume E, Kayahashi S, Sakai Y, Kamiya T. Suppression by Hydrangea Dulcis Folium of D-galactosamine-induced liver injury in vitroand in vivo. Biosci Biotechnol Biochem 2003;67:2641–2643.

34. Baldi E, Burra P, Plebani M, Salvagnini M. Serum malondialdehyde and mitochondrial aspartate aminotransferase activity as markers of chronic alcohol intake and alcoholic liver disease. Ital J Gastroenterol 1993;25:429–432.

35. Aldini G, Carini M, Piccoli A, Rossoni G, Facino RM. Procoynidians from grape seeds protect endothelial cells from peroxynitrite damage and enhance endothelium-dependent relaxation in human artery: new evidences for cardio-protection. Life Sci 2003;73:2883–2898.

36. Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement and significance. Am J Clin Nutr 1993;57:7155–7255.

37. Yang HY, Lin HS, Chao JC, Chien YW, Peng HC, Chen JR. Effects of soy protein on alcoholic liver disease in rats undergoing ethanol withdrawal. J Nut Biochem 2012;23:679–684.

38. Chambers RE, Stross P, Barry RE, Whitcher JT. Serum amyloid A protein compared with C-reactive protein, alpha 1-antichymotrypsin and alpha 1-acid glycoprotein as a monitor of inflammatory bowel disease. Eur J Clin Invest 1987;17:460–467.

39. Kurose I, Higuchi H, Kato S, Miura S, Watanabe N, Kamegaya Y, et al. Oxidative stress on mitochondria and cell membrane of cultured rat hepatocytes and perfused liver exposed to ethanol. Gastroenterology 1997;112:1331–43.

40. Sathish V, Vimal V, Ebenezar KK, Devaki T. Synergistic effect of nicorandil and amlodipine on mitochondrial function during isoprostane-induced myocardial infarction in rats. J Pharm Pharmacol 2001;54:133–137.

41. Mcvicker BL, Tuma DJ, Kharbanda KK, Kubik JL, Casey CA. Effect of chronic ethanol administration on the in vitro production of proinflammatory cytokines by rat Kupffer cells in the presence of apoptotic cells. Alcohol Clin Exp Res 2007;31:122–129.

42. Yagmurca M, Bas O, Mollaglu H, Sahin O, Nacar A, Karaman O, et al. Protective effects of erdosteine on doxorubicin-induced hepatotoxicity in rats. Arch Med Res 2007;38:380–385.

43. Rubbo H, Rado R, Trujillo M, Telleri R, Kalyanaraman B, Barnes S, et al. Nitric oxide regulation of superoxide and peroxinitrite-dependent lipid peroxidation. J Biol Chem 1994;269:26066–26075.

44. Ahmed R, Abdella EM. Modulatory effects of rosemary leaves aqueous extract on doxorubicin-induced histological lesions, apoptosis and oxidative stress in mice. Iran J Cancer Prev 2010;13:1–22.

45. Raso GM, Meli R, Di Carlo G, Pacilio M, Di Carlo R. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A.1. Life Sci 2001;68:921–31.

46. Geng JY, Zhang B, Lotz M. Protein tyrosine kinase activation is required for lipopolysaccharide induction of cytokines in human blood monocytes. J Immunol 1993;251:6692–700.

47. Guengerich F, Kim D, Iwazki M. Role ofhuman cytochrome P450IIIE1 in the oxidation of many low molecular weight cancer suspects. Chem Res Toxicol 1991;4:168–179.

48. Caradonna F. Cytochrome P450 2E1 variable number tandem repeat polymorphisms and health risks: a genotype-phenotype study in cancers associated with drinking and/or smoking. Mol Med Rep 2012;6:416–420.

49. Lieber CS. Cytochrome P-450: its physiological and pathological role. Physiol Rev 1997;77:517–544.

50. Lieber CS. Microsomal ethanol-oxidizing system (MEOS): the first 30 years (1968–1998)-a review. Alcohol Clin Exp Res 1990;14:291–319.

51. Koop DR. Oxidative and reductive metabolism by cytochrome P450 2E1. FASEB J 1992;6:724–730.

52. Yang L, Wu D, Cederbaum AI. CYP2E1, oxidative stress and MAPK signaling pathways in alcohol-induced hepatotoxicity. J Biochem Pharmacol Res 2014;2:74–90.