Research Article

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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 (CYTP2E1) 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 etkisini araştırılmış.

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* uygulaması 28 gün boyunca ağızdan beslenmiştir.

Bulgular: Veriler EtOH uygulamasının serum toplam protein (TP) oranında ve albümin (Alb) oranında bir azalmaya (p < 0.05) sebep olduğunu gösterdi, bunun yansısı *S. argel* uygulaması sonucunda ALT ve AST ve lipid peroksidadyonunda (LPO) bir artış gözledi. *S. argel* uygulaması bu parametrelerin anlamlı (< 0.05) bir şekilde iyi-ileşmesine sebep oldu. EtOH e maruz bırakılan sıçanların karaciğerlerinde (oksidatif stres marşörü olarak) glutatyon-S-transferaz (GST) aktivite azalmasının yansırsa, protein karbonil (PC), indirgenmiş glutatyon (GSH) ve LPO seviyeleri anlamlı bir şekilde artmıştır (p < 0.05). *S. argel* tedavisi gösteren bu oksidatif stres anlamlı bir şekilde azaltmıştır. Hatta EtOH gurubu karaciğer dokusunda, oksidatif doku hasarı gösteren, tümör nekrosis faktör-α (TNF-α) ve nitric okside (NO) seviyeleri ve cytochrom P450 2E1 (CYTP2E1) aktivitesi anlamlı bir şekilde artmıştır. *S. argel* ve EtOH’ün birlikte uygulanması, sıçan karaciğerinde inflamasyonun anlamlı bir şekilde geri çevrilmesini yol açmıştır.

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Sonuç: S. argel sıçan karaciğerinde EtOH‘e bağlı oksidatif stres ve infalamasyon a karşı karaciğer koruyucu bir rol sahiptir.

Anahtar kelimeler: Etanol; S. argel; Oksidatif stres; CYTP2E1; Karaciğer koruyucu; Sıçan.

Introduction

It is well known that heavy consumption of alcohol is associated with liver damage. Biochemical signs of hepatotoxicity and oxidative damage were displayed in experimental animals exposed to two-carbon alcohol. These signs suggested a possible role of free radicals in causing some of the toxic effects of alcohol [1]. About 80% of ingested alcohol is metabolized to the cytotoxic acetaldehyde by the enzyme alcohol dehydrogenase in the liver. It is well known aldehyde oxidase or xanthine oxidase oxidizes acetaldehyde to acetate, giving rise to reactive oxygen species (ROS) via cytochrome P450 2E1 (CYP 2E1) [2].

CYP2E1 is one of the major P450 forms both in human and rat liver. Cytochrome P450 2E1, induced by alcohol plays a critical role in ROS generation. CYP2E1 metabolizes a variety of substances including multiple drugs, polyunsaturated fatty acids, ethanol, acetalaminophen and most organic solvents [3]. After chronic ethanol feeding CYP2E1 expression and activity are higher compared with pair-fed mice consuming a control diet on a pair-feeding regimen [3]. Production of superoxide anions and hydroxyl radicals were increased after ethanol consumption. These radicals react rapidly with biological materials, causing oxidative damage in living organisms [4]. Excessive alcohol consumption enhances hepatic oxidative stress due to increased generation of reactive oxygen species and reduced antioxidant capacity [5].

Lipid, protein and DNA peroxidation was increased due to oxidative stress. ROS from xenobiotics, including ethanol, cause oxidative DNA damage through single-strand breaks [6]. Oxidative stress increases lipid peroxidation, which can cause membranes damage of cells and organelles leading to the release of reactive aldehydes with potent pro-inflammatory and pro-fibrotic properties [7]. It is well known NO, a ubiquitous cellular mediator of physiological and pathological processes, is produced by endothelial, neuronal and inducible nitric oxide synthase. NO with superoxide anion to generate peroxynitrite, which reacts with protein, lipids, and DNA leading to oxidative stress [8]. Development of new substances that have hepatoprotective effects against ethanol-induced liver toxicity is a prevailing strategy for preventing the progression of alcoholic liver impairment [9]. Natural products are one of the best ways to minimize ethanol hepatotoxicity.

Solenostemma argel belongs to the Asclepiadaceous family had insecticidal effect [10], antibacterial and antioxidant activity [11]. Furthermore, S. argel had 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 \(=\left[1-\left(\text{absorbance of sample}/\text{absorbance of control}\right)\right] \times 100.\) 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 \(S\) 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. Fe\(^{2+}\)/ascorbate model system

The reaction mixture contained 1 mg microsomal protein/mL, 1.6 mM ascorbic acid, and the \(S.\) 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 \(S.\) argel was calculated according to the following equation: \(\%I = [1-\left(\text{absorbance of sample}/\text{absorbance of control}\right)] \times 100.\) 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 \(S.\) argel was estimated by the method of Dinis et al. [21]. The percentage of inhibition of ferrozine-Fe\(^{2+}\) complex formation was calculated using the following formula: \(\%I = [1-\left(\text{absorbance of sample}/\text{absorbance of control}\right)] \times 100.\) All tests were done in triplicate and the results were presented as means ± SD.

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

Total phenolic, total flavonoid and total tannins contents in the \(S.\) 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. \(S.\) argel Group, received 100 mg/kg \(S.\) 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. \(S.\) argel + Ethanol Group received 3 g/kg ethanol 1 h after treatment of \(S.\) 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 \(\times\) 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-nitrophenol, the pink-yellow product, is determined from the extinction coefficient 9.53 mM$^{-1}$ 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

<table>
<thead>
<tr>
<th>Contents</th>
<th>S. argel extract</th>
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<tbody>
<tr>
<td>Total Phenolics, GAE mg/g dw</td>
<td>95.7 ± 5.7</td>
</tr>
<tr>
<td>Total Flavonoids, QE mg/g dw</td>
<td>23.6 ± 3.1</td>
</tr>
<tr>
<td>Total Tannins, QE mg/g dw</td>
<td>21.5 ± 3.8</td>
</tr>
</tbody>
</table>

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 p-nitrophenol to p-nitrocatechol) 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 sulphhydryl 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-α 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-α 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-α and IL-6 were significantly increased by ethanol. In this study, co-administration of *S. argel* with ethanol protected the liver TNF-α 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-1β, 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 H₂O₂ [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 S. argel with ethanol diminished CYP2E1 activity in liver tissues. Due to the presence of phytochemicals such as tannins, flavonoids and polyphenols in S. argel leaves, it may reduce the activity of CYP2E1.

The results of the present study showed that co-administration of 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 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, S. argel may protect the liver from oxidative damages induced by ethanol through anti-oxidative effects [11].

In conclusion, we have shown that the 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 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 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 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**


