

Research Paper:

The Toxicity Evaluation of *Salvia rhytidea* and *Glycine Max* Extracts versus the Biochemical and Histological Indices of Liver and Kidney Functions in Mice



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ABSTRACT

Background: The safety of the use of medicinal plants is a general challenge among consumers. To improve the use, it is necessary to provide complete profiles of the natural medications for quality control and the therapeutic and toxicity effects. This study was conducted to evaluate the structural and functional toxicity of the methanolic extracts of *Salvia rhytidea* and *Glycine max* plants in mice.

Methods: After determining the LD₅₀, NMRI mice with mean weight of 25-30 g were treated intraperitoneally with 100, 200 and 400 mg/kg, and orally with 800 mg/kg of the extracts for 7 consecutive days. After the last treatment, the serum samples were prepared and used for the biochemical assays. The liver and left kidney were removed from the animals and fixed in 10% formalin for histopathological examinations.

Results: The results indicated that the biochemical parameters of liver and kidneys were not significantly different among the experimental and control groups (P>0.05). Mild degenerative changes in the liver and kidneys were observed at the IP dose of 400 mg/kg and oral dose of 800 mg/kg of both extracts.

Conclusion: The use of these plants' extracts did not induce severe toxicity in the short-term; however, caution should be exercised with the long-term use.

Keywords: *Salvia rhytidea*, *Glycine max*, Hepatotoxicity, Nephrotoxicity, Histopathological study

Introduction

Medicinal plants have been widely used for therapeutic purposes especially in developing countries mostly because of accessibility, effectiveness and high acceptance by the populations [1]. Most people who use medicinal plants

to meet their needs are unaware of their adverse side effects and mistakenly believe that all medicinal plants are completely free of toxicity. Also due to the antioxidant properties of medicinal plants [2, 3], people in various nations have found these natural sources useful in the treatment or prevention of many diseases. However, there is evidence to suggest that severe adverse effects for medicinal plants exist. Examples include hyperten-

sion due to *Ephedra*, hepatotoxicity due to pyrrolizidine alkaloids, coltsfoot plant, comfrey, and acute renal failure due to the use of chamomile, aloe and *Glycyrrhiza glabra*, leading to excess cortisol release [4, 5]. Thus, further studies are warranted on the toxicity of medicinal plants to safeguard against the toxic risks [6]. Liver and kidneys are the organs mostly prone to injury from many drugs due to their specific physiological and anatomical structure, and their vital role in metabolism and detoxification processes [7].

Seeds from *Glycine max* L. (*G. max*), known as soybeans from Leguminosae family, are rich sources of various nutrients and phytochemicals. This plant offers health promotion effects and decreases the risk of cancer and osteoporosis, while protecting against cardiovascular disease [8]. Soybean-based diets reduce the blood levels of total cholesterol, LDL cholesterol and triglycerides in humans [9]. Also, consuming soybean is believed to improve learning impairment [10, 11]. Further, *Salvia rhytidea* Benth (*S. rhytidea*) is one of the *Salvia* species which has been widely available in Kerman province in southeastern Iran. The branches and leaves of this plant are known to have antifungal, anti-cholinesterase, anti-leishmania and anti-herpes properties [12-15]. The efficacy of the plant on the serum glucose and alpha amylase inhibition supports its anti-diabetic role in traditional medicine in Iran [16, 17]. Also, various species of *Salvia* have been used for memory enhancement in traditional medicine in Europe [18].

We have previously reported the antioxidant and anti-cholinesterase properties of the methanolic extracts of *G. max* and *S. rhytidea* [13, 19]. As an extension to that work, we conducted this study to further investigate the toxicity of the extracts of these plants on the liver, kidneys and blood parameters of mice. Interestingly, our preliminary findings suggest that the extracts did not induce functional or pathological alterations in the mice liver and kidneys.

Materials and Methods

Plant materials: The *S. rhytidea* aerial parts were collected from Bidkhoon area in Kerman province and soybean was grown in the botanical gardens of the Faculty of Pharmacy, Kerman University of Medical Sciences. The plants were air-dried in the shade and kept in a dark and cold room until the experiments were conducted. The materials from both plants were authenticated by an expert in the Department of Pharmacognosy, where a certificate was issued for the two plant samples, KF1249 and KF1251, respectively.

Extraction process: A sample of *S. rhytidea* and soybean (200 g) each were extracted with 70% methanol by percolation method. The extracts were evaporated under vacuum and dried in an oven at 40°C. The extracts were used combined with 10% dimethylsulfoxide (DMSO), dissolved in normal saline (v/v) and 0.5% CMC (w/v), respectively.

Total Flavonoid Content: The Total Flavonoid Contents (TFC) of the plants were determined as established by previous studies [20, 21]. Briefly, genistein and rutin were used as standards for the TFC determination, using thin layer chromatography fingerprinting. Five serial concentrations were prepared from rutin and genistein separately (25, 50, 100, 200 or 300 ppm). A volume of 2ml of the stock solution was mixed with 2 mL of 2% aluminum chloride and was incubated for 30 min at room temperature. The absorbance of the solutions was read against the blank at 247nm and 381.3 nm for rutin and genistein concentrations, respectively, by a spectrophotometer (Perkin, Elmer, Germany). The total flavonoid content of the plant was calculated from the slope of calibration curve and expressed as gram of rutin or genistein equivalents in 100 g of dried plant extract. All experiments were carried out in triplicate.

Acute toxicity: Eleven groups of six animals each, weighing 25±3g each were treated with varying doses of the extract of *S. rhytidea* or soybean at doses of 250, 500, 1000 or 2000 mg/kg. The animals were observed for 24 hours and the morbidity and mortality were monitored for up to 24 hours [22].

Toxicity studies: A total of 66 adult male NMARI mice weighing 25-30 grams each were randomly divided into the control or experimental groups. They were kept at the animal house in plastic cages under standard laboratory conditions of 12 hours of light and dark cycles at a room temperature of 25±2°C with free access to food and water. The experimental protocol was approved by the university committee for animal care. Animals were acclimatized for a week before the experiments began.

Experimental groups: The animals in groups 1 to 11 received plant extract (IP for 7 consecutive days) as shown in Table 1. The study protocol was approved by the Ethics Committee of Kerman University of Medical Sciences (Registration ID: IR. KMU. REC.1391.533).

Sample collection and pathological studies: The animals were fasted after the last extract administration and sacrificed under ether anesthesia. The blood samples were collected by cardiac puncture and allowed

to stand for one hour, then centrifuged at 4000 rpm for 10 min. The serum samples were separated, transferred to micro-tubes and used for blood biochemical assays. For pathological examinations, the liver and left kidney were removed and immediately washed in normal saline, dried with filter paper and weighed accurately. The organs were fixed in 10% formalin for the histopathological examinations.

Statistical analyses: We performed statistical analyses of the data (ANOVA), using SPSS software, v. 15. The statistical differences at a $P \leq 0.05$ were considered significant. The data were expressed as Mean \pm SD (SE).

Results

The estimated total flavonoid contents of *S. rhytidea* and soybean extracts were 1.23% (rutin equivalent flavonoid/g of dried *S. rhytidea* extract) and 1.16% (genistin equivalent flavonoid/g of dried soybean extract), respectively.

Acute toxicity studies: No mortality was recorded up to 2 g/kg for both extracts in any experimental group of rats. Injection of higher doses in this study was not feasible due to the extract solubility problem.

Changes in body and organ weights: No significant changes were observed in body weight and that of the liver and left kidney of the animals treated with *S. rhytidea* or soybean extract compared to those of the control group (data not shown; $P > 0.05$).

Blood parameters: The data representing liver enzymes, such as ALT, AST, and ALP showed no significant differences in the mice administered with *S. rhytidea* (doses: 100, 200 or 400 mg/kg) compared to those for the control group. Varying doses of soybean extract administered IP caused no significant changes in the above parameters ($P > 0.05$). The creatinine and BUN values in mice treated with *S. rhytidea* or soybean were not significantly different from those of the control group ($P > 0.05$) (Table 2).

Table 1. Treatment protocol implemented in the animal groups

Groups	Drug Regimen	Route
1, 2, 3	<i>S. rhytidea</i> extract (100, 200 or 400 mg/kg, 7 consecutive days)	IP
4, 5, 6	Soybean extract (100, 200 or 400 mg/kg, 7 consecutive days)	IP
7	10% DMSO in normal saline	IP
8	0.5% CMC in normal saline	IP
9	<i>S. rhytidea</i> extract (800 mg/kg, 7 consecutive days)	Oral
10	Soybean extract (800 mg/kg, 7 consecutive days)	Oral
11	Normal diet without any added drug	Oral

Table 2. The serum levels of liver and kidney indices in the animals treated with varying doses of *S. rhytidea* and soybean extracts compared to the controls

Serum Level ($\mu\text{g/mL}$)	Mean \pm SD								
	Control	<i>S. rhytidea</i> Dose (mg/kg)				Soybean Dose (mg/kg)			
		100	200	400	800 (oral)	100	200	400	800 (oral)
Urea	44.8 \pm 6.35	39.1 \pm 4.52	46.75 \pm 2.63	35.09 \pm 3.14	*58.83 \pm 5.64	35.5 \pm 2.71	36.5 \pm 4.39	39.6 \pm 2.33	*63.67 \pm 4.60
Creatinine	0.40 \pm 0.03	0.59 \pm 0.05	*0.65 \pm 0.04	0.23 \pm 0.03	0.45 \pm 0.03	0.42 \pm 0.02	0.45 \pm 0.03	0.45 \pm 0.03	0.77 \pm 0.17
AST	268 \pm 12.5	421 \pm 40.7	*545 \pm 54.6	397.67 \pm 60.8	299.8 \pm 46.4	298.5 \pm 24.4	321.8 \pm 33.6	359.62 \pm 16.0	248.83 \pm 30.1
ALT	90.5 \pm 21.4	112.57 \pm 29.8	118.83 \pm 18.2	124.50 \pm 19.0	126.00 \pm 34.3	86.00 \pm 4.5	102.40 \pm 12.2	120.00 \pm 11.2	125.67 \pm 17.7
ALP	159.75 \pm 4.9	172.57 \pm 29.0	171.83 \pm 30.8	190.60 \pm 48.4	289.50 \pm 35.7	213.83 \pm 15.8	292.2 \pm 30.9	144.29 \pm 15.2	*275.67 \pm 14.2

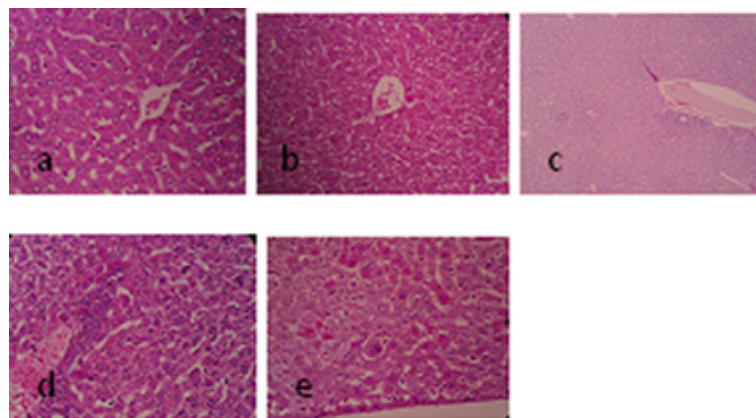


Figure 1. Histopathological micrographs of liver tissue samples from animals treated with varying doses of *Salvia rhytidea* extract compared to the controls

a: control group; b: 100 mg/kg *S. rhytidea*; c: 200 mg/kg *S. rhytidea*; d: 400 mg/kg *S. rhytidea*; and e: 800 mg/kg *S. rhytidea* (oral).

Microscopic examinations: The microscopic examinations of the liver and kidney samples revealed no pathological and tissue lesions in mice treated with *S. rhytidea* extract at 100 or 200 mg/kg. However, some mild cellular lesions were observed, such as small vacuoles in the cytoplasm of hepatocytes. Also, infiltration of lymphocytes and plasma cells was observed within the portal and pre-portal areas plus thickening of liver capsule at the extract dose of 400 mg/kg. Similarly, the oral dose of 800 mg/kg of *S. rhytidea* caused vacuolization in the hepatocytes' cytoplasm, especially around the central vein. There was mild infiltration of mononuclear inflammatory cells into the hepatic parenchyma (Figure 1).

The histological examinations of the kidney tissue samples from the mice treated with *S. rhytidea* extract revealed no pathological alterations at any of the doses of 100, 200 or 400 mg/kg (IP). However, the animals

were affected by the oral dose of this extract at 800 mg/kg, causing hyperemia and degeneration of proximal kidney lobules. Also, mild glomerular atrophy and dilation of urinary spaces were observed (Figure 2).

The soybean extract at a dose of 400 mg/kg exhibited vacuolization of hepatocytes and mild infiltration of mononuclear inflammatory cells within the portal vein area. This extract caused mild degeneration of hepatocytes and focal infiltration of mononuclear inflammatory cells at an oral dose of 800 mg/kg. The histological features of liver appeared normal at the extract doses of 100 and 200 mg/kg (Figure 3). The soybean extract also caused mild degenerative changes in proximal lobules and dilation of urinary spaces at 400 mg/kg (IP) and 800 mg/kg (oral). No microscopic lesions in the tissue samples were detected in the mice treated with the extract dose of 100 or 200 mg/kg (Figure 4).

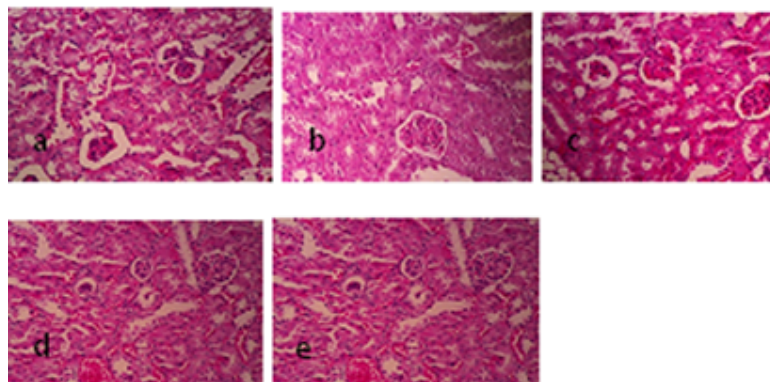


Figure 2. Histopathological micrographs of the kidney tissue samples from animals treated with varying doses of *Salvia rhytidea* extract compared to the controls

a: control group; b: 100 mg/kg *S. rhytidea*; c: 200 mg/kg *S. rhytidea*; d: 400 mg/kg *S. rhytidea*; e: 800 mg/kg *S. rhytidea* (oral).

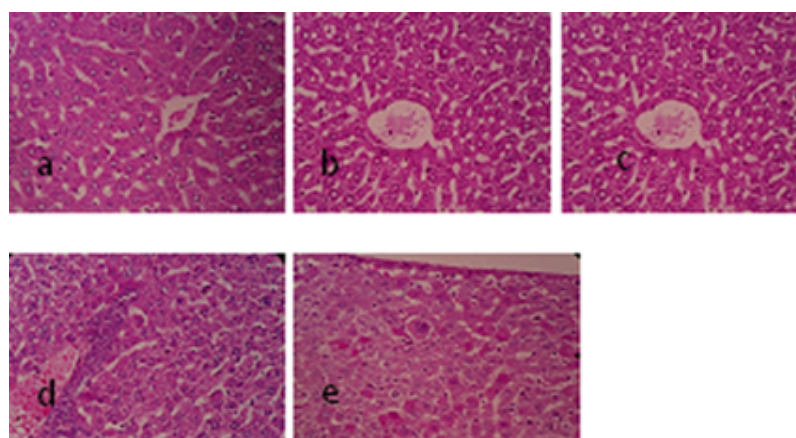


Figure 3. Histopathological micrographs of the liver tissue samples from animals treated with varying doses of soybean extract compared to the control group

a: control group; b: 100 mg/kg soybean; c: 200 mg/kg soybean; d: 400 mg/kg soybean; e: 800 mg/kg soybean (oral).

Discussion

Prior evidence: Evaluation of the acute toxicity and determination of LD_{50} are the primary steps in the toxicological evaluation of unknown compounds, especially those derived from medicinal plants. The *S. rhytidea* and soybean extracts induced no mortality up to 2 g/kg of the mice's body weight. The evaluation of higher doses of the extracts was not feasible due to the solubility issue. We have previously reported the acute toxicity of other plant extracts and essential oils along with their safety margins [22-24]. We believe that the beneficial aspects are largely attributable to the antioxidant effects [13, 25-28].

Liver and kidneys are vital organs of the body, playing important roles in the metabolic and detoxification

processes. Among other vital functions, liver neutralizes toxins, and kidneys are responsible for maintaining the body's homeostasis through excretion of toxic substances [29]. Oxidative stresses induce hepatic damages by inhibiting various cellular functions and promoting free radical generation. These can lead to various hepatic disorders, such as alcoholic liver diseases, cirrhosis and chronic hepatitis. In addition, there is evidence to suggest that in chronic kidney disease, the renal energy loss and uremia are associated with abnormal oxidative stresses, causing inflammatory processes and renal damages [30, 31].

Safety of the extracts: In this study, two plant extracts from *S. rhytidea* and soybean were tested in mice, but caused no mortality up to 2 g/kg of the animals.

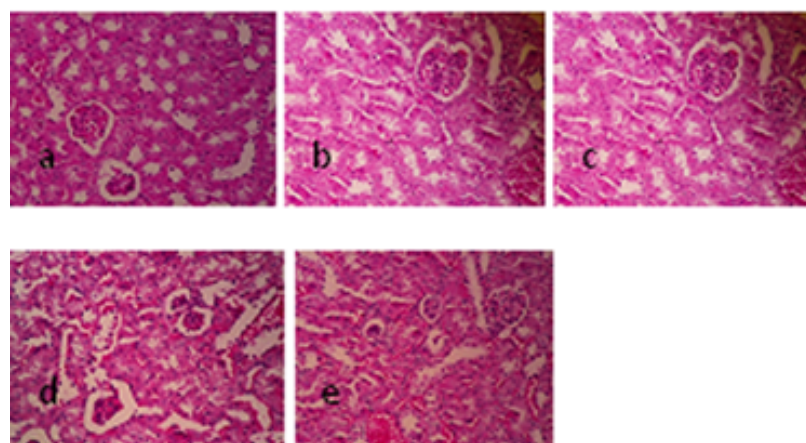


Figure 4. Histopathological micrographs of the kidney tissue samples from animals treated with varying doses of soybean extract compared to the controls

a: control group; b: 100 mg/kg soybean; c: 200 mg/kg soybean; d: 400 mg/kg soybean; e: 800 mg/kg soybean (oral).

None of the oral or IP doses of *S. rhytidea* and soybean extracts at the tested doses induced significant changes in the serum enzymes' levels compared to those for the control group (Table 2). Increases in the ALT and AST values, indicative of disturbance in the integrity of cell membranes, would represent damages to the hepatocytes [32, 33]. The value of ALP was not significantly different in animals treated with varying doses of *S. rhytidea* and soybean extracts. However, as observed through microscopic examinations, *S. rhytidea* extract at doses of 400 mg/kg (IP) and 800 mg/kg (oral) caused small cellular lesions, such as vacuolization of the cytoplasm in the hepatocytes and inflammatory cells infiltration in the liver tissue. Soybean also caused mild degenerative changes in the hepatocytes at doses of 400 mg/kg (IP) and 800 mg/kg (oral). These findings indicated that the toxic effects of soybean extract were mostly structural rather than functional. These changes were not considerable but might become more pronounced with the chronic use of either extract, the study of which was not the focus of this study.

Effects on liver function: The serum levels of ALT and AST did not change significantly, ruling out major hepatocellular damages. The kidney parameters, such as BUN and creatinine, also exhibited no significant alterations in the mice treated with either *S. rhytidea* or soybean extract compared to those observed for the control group. Increases in creatinine is indicative of impaired renal function [34], which did not develop following the administration of the extracts in this study. Results from our microscopic examinations demonstrated that the soybean extract at dose of 400 mg/kg (IP) and 800 mg/kg (oral) caused only mild degeneration of proximal lobules and dilation of urinary spaces in the kidneys. Obviously, a panel of biomarkers was needed to do more accurate assessments of liver and kidney safety margins. However, there are several similar reports for other species of *S. rhytidea* and soybean. For instance, in a study on human subjects, normal drinking water was replaced with *Salvia officinalis* tea for 14 days and the results indicated no pathological changes in the liver enzymes of the participants compared to those of the control group [35]. In another study, three doses of *S. officinalis* increased the serum levels of albumin, creatinine and proteins but significantly decreased the liver enzymes levels [36]. Also in a clinical trial, soy supplementation significantly caused a decrease in the ALT values compared to the controls (casein group), consisting of patients with chronic hepatitis C [37].

Effects on kidney function: There are controversies about the efficacy of the soybean protein in patients

with chronic kidney disease. In a study based on soy protein, decreases were observed in the kidney cyst volumes and the fluid contents. The serum creatinine levels were normalized by the plant too [38]. In another study, *Salvia miltiorrhiza* (danshen), a different species of *Salvia*, the serum urea nitrogen level, malondialdehyde and creatinine were significantly reduced in iron-overload animals that received this plant [39]. This plant also increased the activity of both superoxide dismutase and glutathione peroxidase. The pathological changes in the kidneys were ameliorated by *S. miltiorrhiza* based on the histopathological findings [39]. To our knowledge, this is the first report on both the hepatotoxicity and nephrotoxicity of these two extracts. Elucidation of the significance of the findings reported in the current study awaits future research.

Limitations of the study: This preliminary study was limited to using a small number of animals due to the ethical consideration and to minimize animal suffering. Also, we could not test a panel of biomarkers due to limited laboratory facilities. The biomarkers panel would enable us to present a more accurate assessment of the bio-safety aspects of *S. rhytidea* and soybean extracts in relation to the liver and kidney functions.

Conclusions

The findings of this study suggest that there were no functional disorders caused in the liver and kidneys, and no signs of pathological and structural changes observed in the animals treated with either *S. rhytidea* or soybean extract. These effects may be attributed to the antioxidant properties of the extracts. However, this assertion needs to be verified by future studies to accurately characterize the probable toxicity on the liver and kidneys, which may be the case particularly with the chronic use of the extracts.

Ethical Considerations

Compliance with ethical guidelines

The protocol of this study was approved by the University's Committee on Research Ethics and Standards.

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Author's contributions

All authors were equally contributed in preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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