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ORIGINAL ARTICLE

Antiaflatoxigenic Effect of *Salvia officinalis* L. Extract on Liver Damage in Adult Male Rats

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ABSTRACT

Different parts of the Salvia officinalis L. are used to treat liver disorders, traditionally. The aim of the study is to evaluate the protective effect of Salvia officinalis against Aspergillus parasiticus aflatoxin induced hepatotoxicity in rats. In present study, groups included intact, poisoned control and experimental animals. Animals were poisoned by intraperitoneally injection of aflatoxin at dose 450 µg/kg body weight once in each week for 8 weeks. Sage hydro-methanolic extract was administrated at doses 25, 50, 100 and 150 mg/kg orally for 8 weeks. After 8 weeks, animals were anesthetized by ether and liver sampling was done. Liver sections were prepared by using a rotary microtome and stained with haematoxylin and eosin dye, which was mounted in a neutral deparaffinated xylene medium for microscopic observations. Histophatological study showed that treatment of sage extract attenuate liver damage in poisoned rats in compared control group. This study demonstrates the hepatoprotective activity of Salvia officinalis and thus scientifically supports the usage of this plant for treatment of liver disorders.

Keywords: *Aflatoxin, Aspergillus parasiticus, Sage, Salvia officinalis, Hepatotoxicity, Rat*

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INTRODUCTION

Aflatoxins are secondary toxic fungal metabolites produced by *Aspergillus flavus* and *A. parasiticus*. There are four naturally occurring aflatoxins, the most toxic being aflatoxin B1 (AFB1). Aflatoxins are not only contaminate our food stuffs, but also are found in edible tissues, milk and eggs after consumption of contaminated feed by farm animals [1].

AFB1 is well known to be potent mutagenic, carcinogenic, teratogenic, immunosuppressive and also inhibit several metabolic systems, causing liver, kidney and heart damage [2]. These toxins have been incriminated as the cause of high mortality in livestock and some cases of death in human being [3].

The use of synthetic chemicals as antimicrobials has greatly contributed to management of such losses, but indiscriminate application of chemicals has led to a number of ecological and medical problems due to residual toxicity, carcinogenicity, teratogenicity, homoral imbalance, spermatotoxicity, etc [4]. Natural products and their active principles as sources for new drug discovery and treatment of diseases have attracted attention in recent years. Herbs and spices are generally considered safe and proved to be effective against various human ailments. Their medicinal use has been gradually increasing in developed countries. So, natural substances that can prevent AFB1 to toxicity would be helpful to human and animal health with minimal cost in foods and feed. Traditional medicinal plants were applied by some researchers for their antifungal, antiaflatoxigenic and antioxidant activity [5,6].

Globally, plant based drugs are widely and successfully used in the treatment of liver disorders. The genus *Salvia* L. (Lamiaceae) comprises about 900 species, spread throughout the world, some of which with

great economic value since they are used as spices and flavouring agents by perfumery and cosmetic industries [7]. *Salvia officinalis* (sage, garden sage, or common sage) is a perennial, evergreen subshrub, with woody stems, grayish leaves, and blue to purplish flowers. It is native to the Mediterranean region, being currently cultivated in various countries [8].

The predominant medicinally valuable metabolites of sage are monoterpenes (e.g., α- and β-thujone, 1, 8-cineole, camphor), diterpenes (e.g., carnosic acid) triterpenes (oleanoic and ursolic acids), and phenolic compounds like rosmarinic acid [9].

Salvia sp. has also been used for a long time in folk medicine as medication against fever, rheumatism, perspiration, sexual debility, and in the treatment of chronic bronchitis, as well as mental and nervous diseases [8]. Sage leaves and its essential oil possess carminative, antispasmodic, antiseptic, astringent, antioxidant and antihidrotic properties [9,10].

There is no report about hepatoprotective effect of sage extract against *Aspergillus parasiticus* aflatoxin-induced liver damage in male rats. So, in the present study, we evaluated the protective effect of methanolic extract of *Salvia officinalis* L. against aflatoxin induced hepatotoxicity in rats.

MATERIALS AND METHODS

Sage leaves (*Salvia officinalis* L.) were purchased from Karaj in June 2014, identified by department of botany of Science and Research Branch, Islamic Azad University (Voucher number: 037420, Director: Dr. Ali Mazooji). The plant was cleaned, shed dried at 25°C, and the dried leaves of the plant were ground with a blender, and the powder was kept in nylon bags in a deep freezer until the time of experiments. Dried and ground leaves (about 100 g) were submitted to extraction with 300 ml methanol (80%) in a soxhlet apparatus for 48 h. After extraction, the solvent was filtered and then evaporated by rotavapor. The obtained hydro-methanolic extract was stored at -20 °C until being used.

In this study, male Wistar rats weighing 200–250 g were housed in clean cages with temperature (22–24 1C), 12-h light/12-h dark cycle and relative air humidity 40–60%. Rats had continuous access to food and to tap water. Permission for the study was obtained from the Pastour institute, Tehran, IRAN.

In the present experiment, 48 rats (40 poisoned, 8 intact rats) were used. The rats were divided into six groups. Group 1: Normal control rats (intact) were administrated 0.5 ml of saline as aflatoxin vehicle every week, interperitoneally. Group 2: Control rats were administrated 0.5 ml of aflatoxin at dose 450 μg/kg every week, interperitoneally. Groups 3–6: rats were co-administrated 0.5 ml of aflatoxin every week, interperitoneally and sage methanolic extract orally at doses 25, 50, 100 and 150 mg/kg body wt. daily for 8 weeks.

After 8 weeks of treatment, the animals were weighted and anesthetized by ether. Their livers were removed; weighted and liver coefficients were measured as liver weight divided to body weight for each animal.

For qualitative analysis of liver histology, the tissue samples were fixed for 48 h in 10% formalin-saline and dehydrated by passing successfully in different mixtures of ethyl alcohol–water, cleaned in xylene and embedded in paraffin. Sections of the tissue were prepared by using a rotary microtome and stained with haematoxylin and eosin dye, which was mounted in a neutral deparaffinated xylene medium for microscopic observations. Each damage is given one score according to Ishak's procedure (11) and final score of each specimen is sum of scores.

All the data were expressed as mean ± S.E.M. Statistical analysis was carried out using one-way ANOVA followed by Tukey post hoc test. The criterion for statistical significance was $p<0.05$.

RESULTS AND DISCUSSION

The present results showed i.p. treatment of aflatoxin at dose 450 microg/kg increased liver coefficient in poisoned rats in comparison with control rats ($p<0.001$). Oral treatment of sage extract at doses 25, 50, 100 and 150 mg/kg decreased liver coefficient in treated rats ($p<0.001$) (Fig. 1).

Also, the results showed aflatoxin increased liver damages in poisoned rats in compared to healthy rats ($p<0.05$). Administration of sage extract attenuated damages of liver tissue in treated rats in comparison with control poisoned rats ($p<0.05$) (Fig. 2).

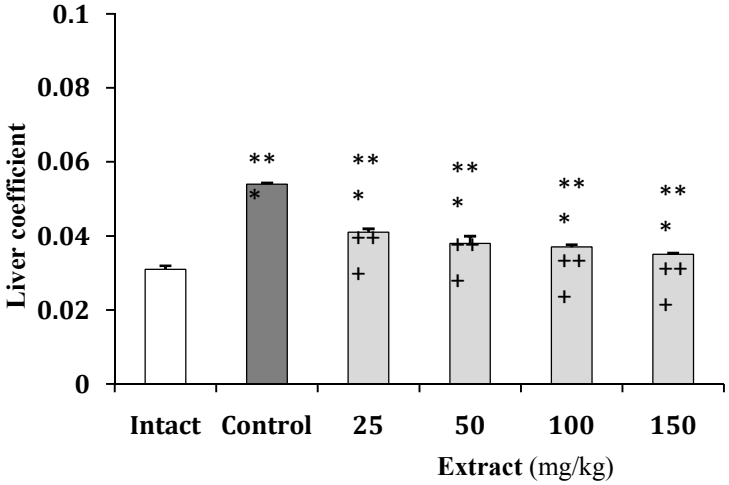


Fig. 1. Effect of hydro-methanolic extract of *Salvia officinalis* at dose 25, 50, 100 and 150 mg/kg on liver coefficient in poisoned rats with aflatoxin. Each column represents mean ± SEM for 8 rats. Control group was administrated water as vehicle of extract. ***p<0.001 different from intact group. +++p<0.001 different from control group.

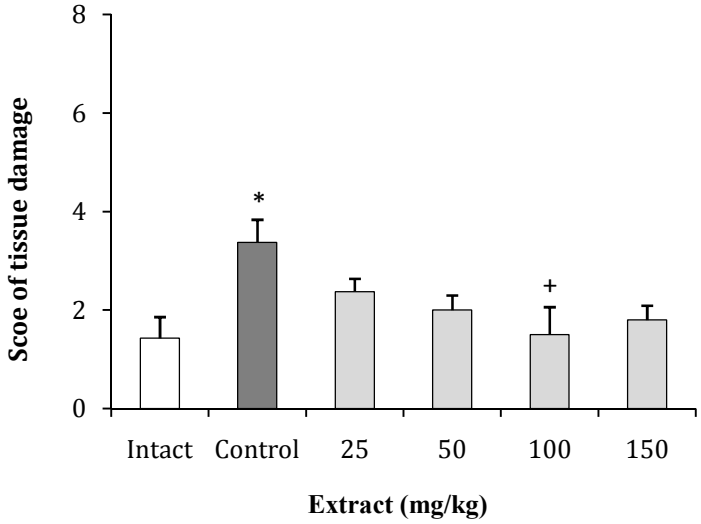


Fig. 2. Effect of hydro-methanolic extract of *Salvia officinalis* at dose 25, 50, 100 and 150 mg/kg on liver damages in poisoned rats with aflatoxin. Each column represents mean ± SEM for 8 rats. Control group was administrated water as vehicle of extract. *p<0.05 different from intact group. +p<0.05 different from control group.

The results showed injection of aflatoxin at dose 450 microg/kg increased necrosis of liver tissues in poisoned rats in compared to healthy rats (p<0.05). Administration of sage extract at doses 25, 50, 100 and 150 mg/kg decreased necrosis of tissues in treated rats in comparison with control poisoned rats (p<0.05) (Fig. 3) (Pictures 1,2).

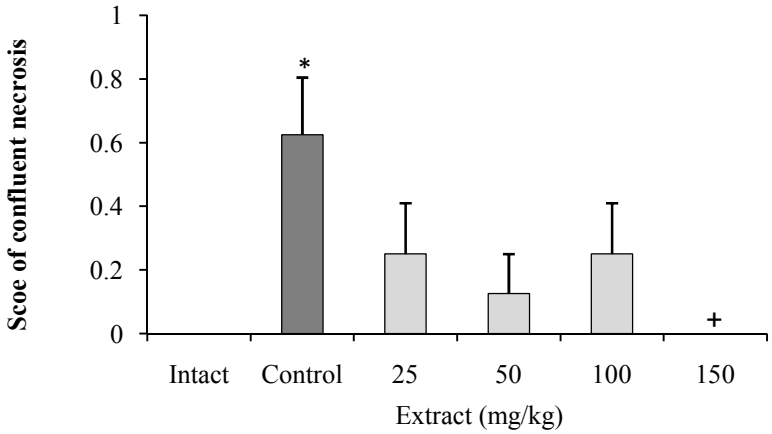
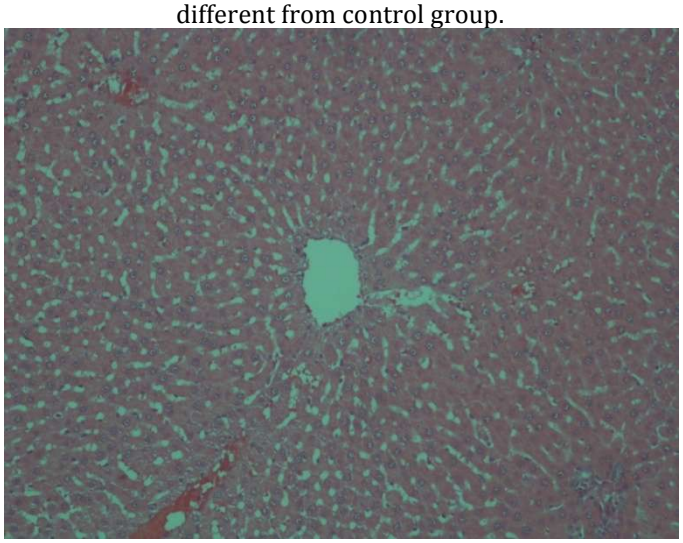
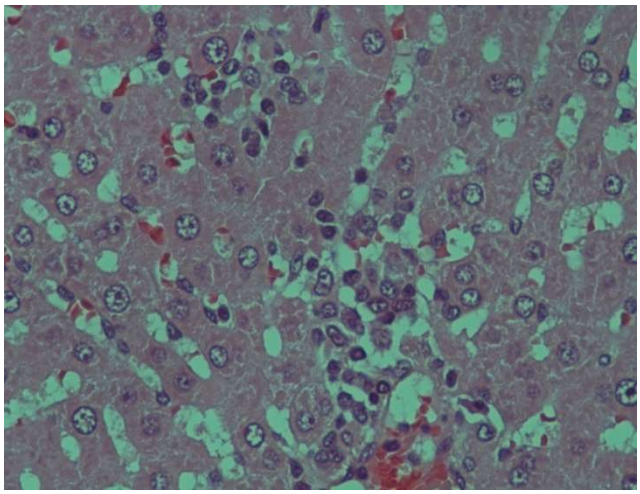


Fig. 3. Effect of hydro-methanolic extract of *Salvia officinalis* at dose 25, 50, 100 and 150 mg/kg on necrosis of liver tissues in poisoned rats with aflatoxin. Each column represents mean ± SEM for 8 rats. Control group was administrated water as vehicle of extract. *p<0.05 different from intact group. +p<0.05 different from control group.



Picture 1 – Histological sections of liver in healthy rats. Samples were stained with Hematoxylin-Eosin, ×100.



Picture 2 – Histopathological sections of necrosis of liver tissue in poisoned rats. Samples were stained with Hematoxylin-Eosin, ×400.

Fungal infections may discolour grains, change its chemical and nutritional characteristics, reduce germination and most importantly, contaminate it with mycotoxins, such as aflatoxins which are highly toxic to man and animals [12]. *Aspergillus parasiticus* is one of the major storage fungi found regularly in important cereals cultivated in the world, which produces aflatoxins such as aflatoxin B1, B2, G1, G2 [13]. Aflatoxin is predominantly perceived as an agent promoting liver cancers, although lung cancer is also a risk among workers handling contaminated grain [14]. The risk of cancers due to exposure to the various forms of aflatoxin is well established [15] and is based on the cumulative lifetime dose. The International Cancer Research Institute identifies aflatoxin as a Class 1 carcinogen, resulting in the regulation of this toxin to very low concentrations in traded commodities [16].

The leaves extract of sage (*Salvia officinalis* L.) have been documented to have wide range of biological effects [9].

The present results showed weight of liver in rats with liver damage by aflatoxin was more than normal rats. So, their liver coefficients were higher than control group. Treatment of sage extract decreased liver coefficient in treated animal and improved liver inflammation. The therapeutic effect of sage extract may be to have antioxidant activity and removed reactive oxygen species [9].

In conclusion, the hydro-methanolic extract of *Salvia officinalis* could effectively improve liver injury in poisoned animals. The protective effect of sage extract may be attributed due to the reduced lipid peroxidation and improved defence of the hepatocytes against the reactive oxygen species. Therefore the study scientifically supports the usage of this plant in various traditional medicines for treatment of liver disorders.

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