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RESEARCH ARTICLE

HEPATOPROTECTIVE EFFECT OF SAGE (SALVIA OFFICINALIS L.) LEAVES HYDRO-METHANOLIC EXTRACT AGAINST ASPERGILLUS PARASITICUS AFLATOXIN-INDUCED LIVER DAMAGE IN MALE RATS

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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. Various biochemical parameters like serum alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (AP) and total protein (TP) levels were determined. The treatment of aflatoxin at dose 450 µg/kg increased serum ALT, AST and AP levels, while decreased total protein levels in contaminated rats in comparison to control normal rats. Treatment of sage extract at doses 25, 50, 100 and 150 mg/kg body weight decreased the raised serum AST, ALT and AP levels and increased serum total protein level in treated rats in comparison to control rats. This study demonstrated the hepatoprotective activity of *Salvia officinalis* and thus scientifically supports the usage of this plant for treatment of liver disorders.

Key words: Aflatoxin, *Aspergillus parasiticus,* Sage, *Salvia officinalis*, Hepatotoxicity, Hepatic enzymes.

INTRODUCTION

Liver is the organ for metabolism and detoxification of various components entering into the body. It is involved in wide range of functions and hence it is exposed to toxic substances and drugs absorbed from the intestine.

Aflatoxins are a group of closely related compounds with small differences in chemical composition (Cullen and Newberne, 1993). Aflatoxins were first isolated about 40 years ago after outbreaks of disease and death in turkeys (Blount, 1961) and of cancer in rainbow trout (Rucker *et al* 2002) fed on rations formulated from peanut and cottonseed meals. The toxins are produced as secondary metabolites by *Aspergillus flavus* and *Aspergillus parasiticus* fungi. The fungi responsible are ubiquitous and can affect many of the developing-country dietary staples of rice, corn, cassava, nuts, peanuts, chillies, and spices (Rucker *et al* 2002).

Aflatoxicosis is the poisoning that results from ingesting aflatoxins. Two forms of aflatoxicosis have been identified: the first is acute severe intoxication, which results in direct liver damage and subsequent illness or death, and the second is chronic subsymptomatic exposure. The symptoms of severe aflatoxicosis include

hemorrhagic necrosis of the liver, bile duct proliferation, edema, and lethargy. Aflatoxin B_1 is the most prevalent form and also the most potent of these toxins (Cullen and Newberne, 1993).

Globally, plant based drugs like *Silybum marianum* (Luper, 1998), *Picrorhiza kurroa* (Chander *et al* 1992), *Phyllanthus emblica* (Gulati *et al* 1995) etc. 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 (Longaray Delamare *et al* 2007). *Salvia officinalis* (sage, garden sage, or common sage) is a perennial, evergreen subshrub, with woody stems, greyish leaves, and blue to purplish flowers. It is native to the Mediterranean region, being currently cultivated in various countries (Raal *et al* 2007).

Since ancient times, plants are well known for their pharmacological potential (Jain *et al* 2011; Jenny *et al* 2012; Deb *et al* 2013). 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 (Cuvelier *et al* 1994).

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 (Raal *et al* 2007). Sage leaves and its essential oil possess carminative, antispasmodic, antiseptic, astringent, antioxidant and antihidrotic properties (Cuvelier *et al* 1994; Kamatou *et al* 2005).

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

Plant material

Sage leaves (*Salvia officinalis* L.) were purchased from Karaj in June 2014, identified by Department of Botany, 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 (**Figure 1**).

Fig. 1. Leaves of *Salvia officinalis* L. (Sage)

Animal

In this study, male Wistar rats weighing 200–250 g were housed in clean cages with temperature (22–24°C), 12h light/12h dark cycle and relative air humidity 40–60%. Rats had continuous access to food and tap water. Permission for the study was obtained from the Pastour institute, Tehran, IRAN. In the present experiment, 48 rats (40 contaminated, 8 intact rats) divided into six groups were used. To group 1, 0.5 ml of saline as aflatoxin vehicle was administered intraperitoneally to normal control rats (intact) every week. To group 2, 0.5 ml of aflatoxin at dose 450 μg/kg was administered intraperitoneally to control rats every week. To groups 3-6, 0.5 ml of aflatoxin intraperitoneally and sage methanolic extract orally at doses 25, 50, 100 and 150 mg/kg body wt were co administered daily for 8 weeks.

Biochemical measurements

After 8 weeks of treatment, weight of each rat was measured. Then, the animals were anesthetized by ether and blood samples were drawn from heart. Serum total protein, aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase levels were determined by kit (Parsazmoon, Iran). Also, their livers removed and weighed.

Then, liver coefficients were measured as liver weight divided to body weight for each animal.

Statistical analysis

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

There were significant elevations in serum ALT ($p<0.001$), AST ($p<0.001$) and AP ($p<0.001$) levels in contaminated rats, while significant attenuation in serum total protein level in the contaminated control rats in comparison with control normal rats ($p<0.001$).

The present results showed that treatment of sage leaves extract decreased serum ALT ($p<0.001$), AST ($p<0.001$) and AP ($p<0.001$), while increased serum total protein level ($p<0.001$) in treated contaminated rats in comparison to control rats. Also, treatment of aflatoxin increased liver coefficient in contaminated control rats ($p<0.001$) and sage extract decreased liver coefficient in treated rats in comparison to contaminated control rats ($p<0.001$) (**Table 1**).

Table 1. Effect of sage extract on liver coefficient and serum parameters in *Aspergillus parasiticus* aflatoxin-induced liver damage in male rats.

Group parameter	Intact	Control	Extract (mg/kg)			
			25	50	100	150
AST (U/l)	161±3	196±1***	191±2*** +++	185±1*** +++	180±1*** +++	172±1*** +++
ALT (U/l)	79±4	108±1 ***	101±1 *** +++	95±1*** +++	93±1*** +++	90±2*** +++
AP (U/l)	190±4	230±1***	221±1*** +++	211±3*** +++	201±1*** +++	199±0.3*** +++
Total protein (g/dl)	7.8±0.056	6.5±0.045***	6.8±0.03*** +++	7.2±0.07*** +++	7.2±0.02*** +++	7.4±0.03*** +++
Liver coefficient	0.031±0.001	0.054±0.0004***	0.041±0.001*** +++	0.038±0.002*** +++	0.037±0.0007*** +++	0.035±0.0004*** +++

***p<0.001 difference from intact group. +++p<0.001 difference from control group.

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 (Paster *et al* 1993). *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 (Paster, 1995).

Aflatoxin is predominantly perceived as an agent promoting liver cancers, although lung cancer is also a risk among workers handling contaminated grain (Kelly *et al* 1997). The risk of cancers due to exposure to the various forms of aflatoxin is well established (Gorelick *et al* 1993) 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 (Henry *et al* 1999). The leaves extract of sage (*Salvia officinalis* L.) have been documented to have wide range of biological effects (Cuvelier *et al* 1994). The present results showed that 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 hepatic cells consist of higher concentrations of AST and ALT in cytoplasm and AST in particular exists in mitochondria (Wells, 1988). Due to the damage caused to hepatic cells, the leakage of plasma (Zimmerman and Seef, 1970) caused an increased levels of hepatospecific enzymes in serum. The elevated serum enzyme levels like AST and ALT are indicative of cellular leakage and functional integrity of cell membrane in liver (Drotman and Lawhorn, 1978). The hepatoprotective index of a drug can be evaluated by its capability to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been induced by a hepatotoxin. The measurement of serum AST, ALT and ALP levels serves as a means for the indirect assessment of condition of liver.

Hepatic enzymes included ALT, AST and AP in serum increased in rats with liver injury which

are markers of liver damage. Administration of *Salvia officinalis* extract attenuated serum ALT, AST and AP levels, significantly.

The total protein including albumin levels depressed in hepatotoxic conditions due to defective protein biosynthesis in liver (Clawson, 1989). The aflatoxin causes disruption and dissociation of polyribosomes on endoplasmic reticulum and thereby reducing the biosynthesis of protein.

The pre-treatment of sage extract well restored the proteins synthesis by protecting the polyribosomes. Serum total protein level decreased in rats with liver damage, because of liver dysfunction. Treatment of sage elevated total protein level in treated animal. The therapeutic effect of sage extract also showed the antioxidant activity and removed reactive oxygen species (Cuvelier *et al* 1994).

CONCLUSION

The hydro-methanolic extract of *Salvia officinalis* could effectively control serum AST, ALT, AP and total protein levels and increased the protein levels in the protective studies. The protective effect of sage extract may be attributed to the reduced lipid peroxidation and improved defence of the hepatocytes against the reactive oxygen species. Therefore, the study scientifically supports the usage of plant in traditional medicines for liver disorders.

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