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The effect of *Satureja bachtiarica* on IL-6 and TNF-α in rat treated with Thioacetamide

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ABSTRACT

Background & Aim: Acute liver damage, inflammation and oxidative stress are underlying tissue necrosis and development of ciroces. The aim of this study was to evaluate the effect of *Satureja bakhtiarica* extract on acute liver disease induced by the thioacetamide and TNF-α and IL-6 cytokines level.

Experimental: In experimental study, 36 Wistar rats were selected and divided into 6 groups of 6. The amount of 0.03g thioacetamide dissolved in 1ml of distilled water and injected intraperitoneally to all mice except of control group twice a week and for a period of three weeks. Negative control group received only thioacetamide and the other group received 8mg/kg sibilin by gavage in addition to thioacetamide. After injection of thioacetamide, the experimental groups were treated with doses of 5, 10 and 20 mg/ml extracts of *S. bakhtiarica*for two weeks. Peripheral blood samples were taken from rat hearts. Then TNF-α and IL-6 cytokines levels were measured by Elisa method. Histopathological changes of liver also were examined. Data analysis was performed with SPSS ver. 20.

Results: The results showed a significant difference in TNF-α and IL-6 cytokines between groups ($p<0.001$). Mean concentration of TNF-α and IL-6 in the groups treated with the doses of 5($P = 0.002$, $P <0.001$), 10 ($P= 0.010$, $P <0.001$) and 20 mg/ ml ($P <0.001$, $P <0.001$) of *S. bakhtiaricaca* significantly decreased compared with group treated with thioacetamide. Histopathological results also supported dose dependent protective effect of *S. bakhtiaricaca*. *S. bakhtiaricaca*extract has anti-inflammatory properties that can reduce the toxicity of thiouzamide by reducing the levels of TNF and IL-6 cytokines as pro-inflammatory cytokines.

Recommended applications/industries: Regarding that the use of herbal medicines to treat many diseases are on the rise, hydroalcholic extract of Satureja bachtiarica due to having antioxidant properties can be used to treat liver disease with drug therapy.

1.Introduction

Liver diseases and hepatotoxicity are one of the main causes of morbidity and mortality in people of all ages. Reports of many studies indicated global epidemiology of hepatitis B and hepatitis C in people either acute or chronically infected (Olthof *et al*., 2016; Al-Attar *et al*., 2015).

The hepatotoxin thioacetamide (TAA) was frequently used in different doses to induce liver damage (Al-Attar, 2012; Madani *et al*., 2006). Several studies have demonstrated that a single dose of this hepatotoxic agent could induce centrilobular hepatic necroses and chronic administration cause to cirrhosis (Aydin *et al.*, 2010; Kantah *et al.*,2011). Liver damage is associated with proinflammatory marker changes as well as histopathological alteration. Thioacetamide metabolites leading to production of thioacetamide S-oxide that contribute in oxidative stress reaction (Kim *et al.*, 2014).

The correlation between inflammation and oxidative stress in the course of liver injury is indisputable. Tumor necrosis factor (TNF) is a pleiotropic cytokine that conjunction with interleukin (IL)-6 involved in inflammation and acute-phase response in liver (Bohm *et al.*, 2010). It is suggested these cytoines have antioxidant gene expression ability. There are abundant published reports of critical role of TNFa and IL-6 both in animal models and in vitro systems of hepatotoxicity (Fachini-Queiroz *et al.*, 2012; Abe *et al*., 2003). For example TNF-a or IL-6 gene knocked out mice had partially ability to regenerate liver and after liver damage had increased mortality rates (Bohm *et al.*, 2010).

In recent decades, due to decreasing of patient satisfaction from the side effect of synthetic drugs, high costs general trend now is to use the Herbal products for medicinal application in their natural available form (Hamzawy *et al.*, 2012). In recent studies has paid special attention to the protective effects of antioxidants by natural origin against poisoning caused by chemical agents (Kose *et al.*, 2012). Phenolic compounds, best-known antioxidants, are a group of phytochemicals which protective effects were attributed to many of them (Amarowicz *et al.*, 2008).

Satureja bakhtiarica is endemic plant belongs to the family Lamiaceae. It is noted to analgetic and anti-infection effect of *S. bakhtiarica* in traditional medicine. This plant has anti-inflammatory as well as antimicrobial and antioxidant potential (Moeina *et al.*, 2012). The numbers of 20 composition of the *S. bakhtiaricaca* extract have been identified in Fars province. The main component of this extract is carvacrol and flavonoids. Flavonoids have been indicated to have antioxidant, cytoprotective, and anti-inflammatory effect (Cavar *et al*., 2008).

Thus, the aim of this study was to investigation the effects of *S. bakhtiarica* extract on IL-6 and TNF-α in rat treated with thioacetamide.

2. Materials and Methods

2.1. Satureja bakhtiarica leaf extraction

Aerial parts of *S. bakhtiarica* plant were collected from Chaharmahal and akhtiari Province mountainous and dried in the room with the airflow, away from direct sunlight, temperature and humidity. Then dried parts were powdered by electric mill. The resulting powder was mixed with ethanol and filtered after 5 hours. The obtained extract was dried by using rotary evaporator. Selected doses of extract (5, 10 and 20mg/dl) were prepared by dissolving the extracts in a suitable solvent (distilled water).

2.2. Animals

Three-month old male Wistar rats ranging from 300± 20 g in weight were obtained from the animal laboratory facilities at Isfahan University of Medical Science. Islamic Azad University of Shiraz Ethics Committee approved the animal protocols. All experimental animals were housed in standard environmental conditions in cages and maintained at an ambient temperature of 25 ± 2 °C and received 12 hours of light and dark daily.

2.3. Experimental design

A total of thirty-six rats were randomly divided into six experimental groups, six of rats each. The experimental groups were treated as follows:

I. Rats of group 1 were served as controls and intraperitoneally injected with saline solution (0.9% NaCl), twice weekly for 3 weeks.
II. Rats of group 2 were given 100 mg/kg body weight of TAA (Sigma–Aldrich Corp., St. Louis, MO, USA) by intraperitoneal injection, twice weekly for 3 weeks.
III. Rats of group 3 were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented with Silibinin in the 8 mg/kg body weight/day for 3 weeks.
IV. Rats of group 4 were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented with *S. bakhtiarica* leaves extract at a dose of 5 mg/kg body weight/day for 3 weeks.

V. Rats of group 5 were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented with *S. bakhtiarica* leaves extract at a dose of 10 mg/ kg body weight/day for 3 weeks.

VI. Rats of group 6 were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented with *S. bakhtiarica* leaves extract at a dose of 20 mg/kg body weight/day for 3 weeks.

2.4. Blood serum analyses

After 48 hours of the last rat's gavage, the experimental animals were fasted for 12 h; water was not restricted, and then anaesthetized with intraperitoneal injection of ketamine and Vazaylazyn. Blood samples were taken from the mice hearts. The collected blood was centrifuged at the 4000 rpm for 20 minutes and serum was separated. TNF-α and IL-6 cytokines (Eastbiopharm company) were measured by ELISA kits.

2.5. Histopathological examinations

After blood sampling, rats were dissected and the liver tissues were preserved in 10% buffered formalin immediately after removal from the animals, embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 4mm thickness and stained with hematoxylin and eosin. All liver sections were examined using a light microscope and photographed.

2.6. Statistical analysis

The data were analyzed using the SPSS version 20.0. Each value is expressed as mean± standard deviation (S.D.) and values were analyzed using two-way analysis of variance (ANOVA) to determine differences between the mean values of experimental groups. P-values of less than 0.05 were considered as significant

3. Results and discussion

3.1. Blood serum analyses

The mean concentration of TNF-α significantly increased (p<0.001) in TAA group as well as IL-6 compare with control group (P<0.001). A significant decrease was found in TNF-α at doses of 5, 10 and 20 mg/kg of *S. bakhtiarica* extract (for all concentration, P<0.001) compare to TAA group (Figure 1).

Moreover, reduction in the level of serum IL-6 were observed in rats treated with TAA plus *S. bakhtiarica* extract at a dose of 5 (79.50±1.87 mg/kg, P=0.010), 10(76.33±3.44 mg/kg, P<0.001) and 20 (74.16± 2.63 mg/kg, P<0.001) compared with TAA rats (Figure2).

The concentrations of TNF and IL-6 cytokines in rats treated with silibinin +TAA were 18.15±1.47 and 78.50±2.58,) respectively, which showed significant difference compared with rats treated with thioacetamide (P<0.001) (Table1).

Table1. Comparison of mean±SD of TNF and IL-6 cytokines in tested groups.

Treatments	TNF	IL-6
20 mg/kg extract+TAA	14.78±1.11*	74.16±2.63**
10 mg/kg extract+TAA	16.53±1.86**	76.33±3.44**
5 mg/kg extract+TAA	18.20±1.86**	79.50±1.87***
Silibinin	18.15±1.47**	78.50±2.58**
TAA	23.20±1.02*	84.83±1.47*
Control	14.23±1.69	73.33±2.42

*=(P<0.001) statistically difference with control group.
**= (P<0.001) statistically difference with TAA group.
***=(P=0.010) statistically difference with TAA group

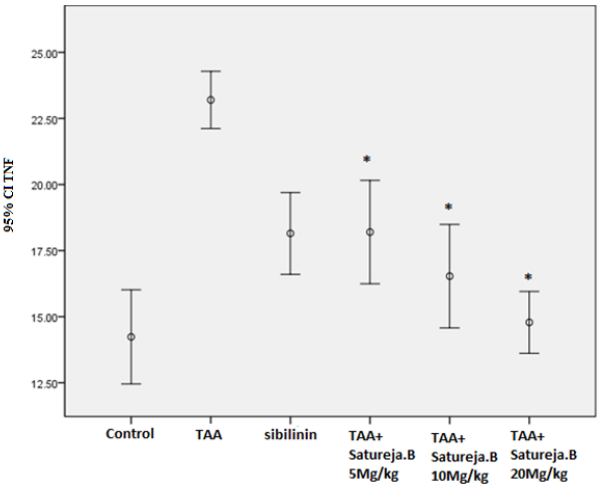

Figure 1. The mean serum levels of TNF in the studied groups.* Statistically difference with TAA group (P<0.001).

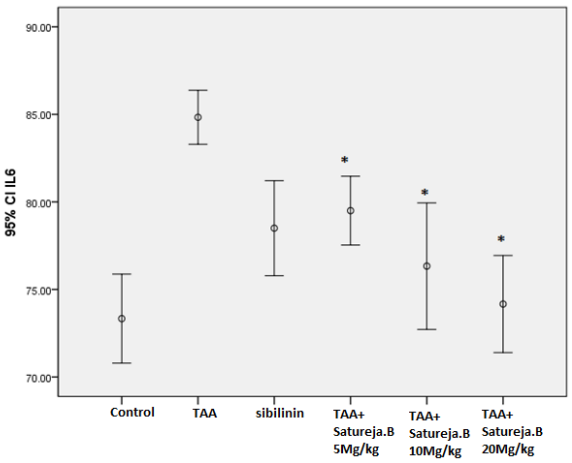

Figure 2. The mean serum levels of IL-6 in the studied groups.* Statistically difference with TAA group (P<0.001).

3.2. Histopathological examination of the livers

Light microscopic picture indicated a normal structure of the liver in the control group. In the rats treated with only TAA, there was a marked space dilation of sinusoid vacuolar, degenerative changes of hepatocytes and an increase in the volume of the nucleus of hepatocytes. Nevertheless, a severe congestion of central venous and the presence of mild connective tissue were observed in the group treated with *S. bakhtiarica* extract at lower concentration. By increasing in extract dosage, some evidences of connective tissue in the portal, the vein congestion of portal area, sinusoid mild hyperemia, changes in the structure of liver lobules and disruptive hepatocytes which was more acquainted with vesicular nuclei were found. According to Figure 3, necrosis and cytoplasmolisis, degenerative changes of around central hepatocytes and central venous dilatation can be observed in group that received TAA+sibilin.

Results of this study indicated hepato protective effect of *S. bakhtiarica* extract in toxocity induced by TAA. Additionally, TAA has pathologic effect on liver cells. Adverse effect of TAA was demonstrated in degenerative changes of hepatocytes and an increase in the volume of the nucleus of hepatocytes after 3 weeks.

The results of present study are in agreement with Kim *et al.* (2000) who showed the administration of thioacetamide produce thioacetamide –s oxide by cytochrome P 450 enzyme in liver microsomes. S-Oxide makes an oxidative stress and damage to liver cells and eventually necrosis and apoptosis of these cells. Thioacetamide metabolites, thioacetamide S-oxide, attack membrane proteins and lipids; then change the cell permanently and increase intracellular calcium concentrations through increasing the volume of nucleus.

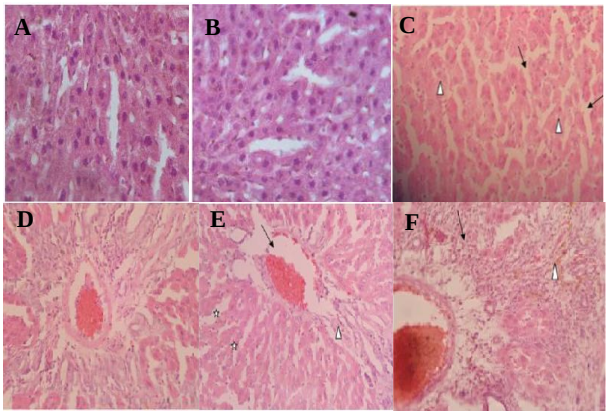

Figure 3. Photomicrographs of liver sections in each group. (A) Control, (B) sibilin, (C) TAA, (D) TAA +5 mg/kg of *S. bakhtiarica* extract, (E) TAA +10 mg/kg of *S. bakhtiarica* extract, (F) TAA+20 mg/kg of *S. bakhtiarica* extract (×200).

Our result indicated treatment with *S. bakhtiarica* extract led to significantly decrease in TNF-α, IL-6 serum levels compared with the control group and TAA group (positive control). These results were confirmed by Nafees *et al.* (2013) and Bozkurt et al. (2014). They used TAA (300 mg/kg) to induction liver damage and reported that Caroacrol pretreated was able to reduce oxidative stress in the rats by affecting inflammation, apoptosis and enzyme system.

Wolde and collageous (2010) reported that the extract of Ethiopian Satureja had antioxidant effect which was attributed to flavenoids.

In addition, it has been reported that flavonoids extracted from *S. bakhtiarica* can prevent hemorrhagic cystitis via suppression of oxidative stress (Cavar*et al.*, 2008). Therefore, it seems that strong antioxidant activity of *S. bakhtiarica* is the main mechanism to decrease inflamatory cytokines. Results of recent studies have suggested other mechanisms to inhibition of proinflammatory cytokines by flavonoids. These mechanisms included prevention of histamine release in the mast cells (Gehane *et al.*, 2011), elevation of cAMP and GAMP, inhibition property of phosphodiesterase, decreasing leukocyte infiltration and lipid peroxidation (Scoula *et al.*, 2005; Arabbi *et al.*, 2004).

4. Conclusion

It can be concluded that the *S. bakhtiarica* extract has liver protective effect against exotoxins. *S. bakhtiarica* cultivated in south of Iran has anti-oxidant potential as well as other different Satureja species. It is recommended to conduct further studies on the effects on immune system and other chemical toxins.

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