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Investigating the effect of *Astragalus hamosus* extract on hepatotoxicity caused by thioacetamide in rats

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ABSTRACT

Background & Aim: Thioacetamide (TAA) is a potent hepatotoxin that destroys liver cells. The use of plants is increasing day by day due to their protective effect against diseases such as cancer. The purpose of this study was to investigate the effect of the *Astragalus hamosus* extract on liver toxicity induced by TAA.

Experimental: 32 rats were randomly divided into four groups of eight. The first group only received food and water rations. The second group received only TAA (50 mg/kg). The third group received both TAA and *A. hamosus* extract at a dose of 200 mg/kg. The fourth group received the extract at a dose of 400 mg/kg along with TAA. To investigate and compare liver function in different groups, serum transaminases including alanine aminotransferase alanine transaminase (ALT), aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), gamma-glutamyl transferase (GGT), and malondialdehyde (MDA) were measured. Then the samples were examined histopathologically.

Results: The best effect was observed with a dose of 400 mg of *A. hamosus* extract which significantly reduced SGPT and SGOT levels ($P<0.05$). Additionally, the extract at a dose of 400 mg significantly reduced ALP, MDA levels, which were increased due to TAA consumption ($P<0.05$). Histopathological analysis of liver tissue in the groups showed that liver cells in the TAA group exhibited central venous hyperemia and necrosis. However, liver cells were healthy in the group receiving the extract at a dose of 400 mg. This study showed that the effect of the extract was dose-dependent, and the most significant effect was observed with a dose of 400 mg of the extract.

Recommended applications/industries: The present findings showed that the *A. hamosus* extract, probably due to its antioxidant properties, will modulate liver enzymes and improve the tissue structure of the liver in hepatotoxicity.

1. Introduction

The risk of exposure to toxic chemicals with acute and long-term adverse health effects has increased worldwide. A large amount of chemicals is annually released into the environment. Some chemicals undoubtedly have positive use, but many are noxious and their harm to the environment overshadows their benefit to humanity (Wang *et al.*, 2018). The liver is one of the vital organs of the body that regulates many physiological activities and plays an important role in eliminating the pathogenic effects of most drugs and other exogenous compounds, thereby making it an important target for toxicity. Toxic agents can react with the main cellular components and induce almost all liver lesions. Not surprisingly, liver protective

measures against toxic liver damage are among the major clinical treatment challenges (Iqubal, *et al.*, 2016). Any disturbance in liver function is associated with a series of disorders that can cause irreparable damage to this organ. Agents such as oxidative stress, free radicals, alcohol, chemicals, viruses, and drugs can damage the liver tissue. Studies on effective protection involve knowledge of the mechanisms leading to liver damage. A variety of chemical toxins are used as model substances in causing toxicity, in testing liver mechanisms under in-vivo and in-vitro conditions (Rosser *et al.*, 2009; Domenicali *et al.*, 2009).

Thioacetamide (TAA) is an organosulfur compound (C_2H_5NS) widely used in animal studies as a hepatotoxin and carcinogen. It is a white crystalline solid substance that is soluble in water and alcohol and can be used as a source of sulfide ions in the synthesis of organic and inorganic compounds (rubber chemicals, metallurgy, pesticides, and drugs), leather processing, textile and paper industries, laboratories and as a stabilizer of motor fuels (Hajovsky *et al.*, 2012). It can damage various organs such as the liver, lungs, intestines, kidneys, spleen, and pancreas. It is quickly metabolized by cytochrome P450 and flavin-containing monooxygenase (FMOs) into reactive metabolites (Thioacetamide-S-oxide and reactive oxygen). Production of reactive oxygen species (ROS) is followed by lipid peroxidation and reduction of glutathione and SH-thiol groups. These factors, together with disturbances in calcium homeostasis and an increase in the intracellular concentration of Ca^{++}, lead to the activation of several mechanisms related to cell damage or cell proliferation. Through oxidation processes, TAA causes oxidative stress in liver cells, ultimately leading to liver necrosis and disrupting the synthesis of protein, RNA, DNA, and gamma-glutamyl transpeptidase (GGT) (Lin *et al.*, 2019).

Free radicals and antioxidants are normal components of the body's functioning. However, when their balance is disrupted, it leads to oxidative stress. This can harm various tissues and contribute to the development of diseases. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced during regular cell activities, playing important roles in cell processes including cell death and apoptosis prevention. When free radicals outnumber antioxidants, they can damage fat tissue, DNA, and proteins. It is impossible to completely inhibit free radicals and oxidative stress. Nevertheless, steps can be taken to minimize their impact. The most effective approach is to boost antioxidant levels and decrease free radical formation. One method is to obtain enough antioxidants through the diet (Danen *et al.*, 2019; Vali *et al.*, 2020).

Numerous plants utilized in traditional medicine for treating various physical and mental ailments are known for their abundance of antioxidant, anti-inflammatory, antibacterial, and disinfectant properties (Boudker *et al.*, 2020). *A. hamosus* stands out as one of these invaluable plants."

A. hamosus is an edible plant belonging to the Fabaceae family and the Papilionaceae subfamily. Its fruit was commonly used as an anti-epileptic drug in traditional Iranian medicine. This plant reduces capillary permeability, acts as a blood thinner, and prevents blood clotting. It is anti-varicose, expectorant, urinary tract disinfectant, anticonvulsant, anti-asthma, and anti-bronchitis; treats dysentery and intestinal edema; and prevents joint swelling (Al-Snafi *et al.*, 2015).

In Iran, it is also known as melilotus, yellow sweet clover, basing, basieh, shades, yellow clover, and qeisar plant. Its flowers have antispasmodic, chest-tightness-relieving, pain-relieving, and antiseptic properties (Wolf *et al.*, 2003)

It removes the smell of urine and increases its secretions. It also disinfects the urinary tract and is used to treat bloody diarrhea, intestinal inflammation, and rheumatism. Since it is a pain reliever and hypnotic, it is used in nervous irritations, types of neuralgia, nervous coughs, and intestinal gas and to treat bronchial flu and swelling of the uvulitis and eye conjunctivitis. Its boiled leaves and flowers are good for asthma and flatulence, pertussis, gall bladder secretions, liver failure, and skin diseases. Its pollen is sprinkled on the circumcision of children for disinfection in the south of Algiers. It also prevents meat from spoiling (Vali *et al.*, 2020; Boudker *et al.*, 2020, Newman *et al.*, 2003).

This study aimed to investigate the effect of hydroalcoholic extract of *A. hamosus* on liver toxicity induced by TAA.

2. Materials and Methods

2.1. Animals

In this study, 32 male rats with a certain weight range were purchased from the Laboratory Animal Breeding Center of Islamic Azad University of Shahrekord, Iran. They were kept under standard temperature conditions of 25 to 30 ºC and lighting conditions of 12 h of light and 12 h of darkness per day. They were in favorable conditions in terms of access to hygienic water and food and a non-contaminated environment by the principles included in the ethical guidelines for the use of animals in research.

2.2. Grouping

The animals were randomly divided into 4 groups of eight rats. Liver damage was induced in groups 2, 3, and 4 by intraperitoneal injection of TAA (50 mg/kg as a solution in normal saline at an interval of 72 h, and one rat was selected from each group after 48 h from the last injection. The liver damage was confirmed by taking blood from the rat's tail and testing liver enzymes. Group 1 consisted of healthy rats. This group received only water and food ration (pellet). Group 2 was injected intraperitoneally with TAA at a dose of 50 mg/kg as a solution in normal saline and at an interval of 72 h. For groups 3 and 4, the procedures were the same as for the second group and together with TAA, they also received the hydroalcoholic extract of *A. hamosus* at a dose of 200 mg/kg and 400 mg/kg orally, respectively, for 21 days.

2.3. Hydroalcoholic extract preparation

The maceration method was used to prepare the hydroalcoholic extract of the plant. *A. hamous* plant was purchased from the reliable medicinal plant supply centers in Chaharmahal and Bakhtiari provinces, Iran. The dried fruit was used in this study for extraction according to Mohammadpour Zehab *et al.* (2016) procedure.

2.4. Preparation of blood samples

Initially, the rats were anesthetized and subsequently positioned on the dissection table. Blood sampling was carried out using a 5 mL syringe. The needle was carefully inserted into the left area of the midline, adjacent to the xiphisternum and beneath the sternum.

The blood-containing tubes were then left at normal ambient temperature for 30 min to allow complete clotting. Following this, we subjected the tubes to centrifugation at 3000 rpm for 10 min to achieve serum separation. Subsequently, a sampler and sampling head were employed to extract the serum, which was then transferred into a microtube. The collected serum was stored at -20°C for subsequent analysis of serum biochemical parameters.

2.5. Valuation and comparison of liver function

To assess and compare liver function in distinct groups, the research team employed an auto-analyzer device (Ideal Tec.600.IRAN) to measure serum transaminases, including ALT, AST, ALP, GGT, and MDA.

2.6. Histological analysis

Following the blood collection phase, liver samples were dissected for histological examination through the following procedure:

2.6.1. Sample dissection

A longitudinal incision was made on the abdominal surface. The liver was carefully removed using dissecting forceps. Each liver sample was placed in individual sampling containers containing 10% formalin. This step aimed to stabilize the tissue and prepare samples for subsequent procedures, including tissue sectioning.

2.6.2. Tissue preparation

After formalin preservation, tissue sections were prepared for analysis. Hematoxylin-eosin (H&E) staining was applied to visualize the tissue architecture. The stained tissue sections underwent examination under an optical microscope by a pathologist to assess structural and cellular changes. This method facilitated a comprehensive histological evaluation of liver tissue to identify any pathological alterations or abnormalities in the various experimental groups.

2.7. Statistical analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA) followed by Tukey tests at a probability level of 5% ($P<0.05$). Data were expressed as the mean ± standard error (SD). Statistical

Package for Social Scientists (SPSS 25.0) was employed for the analysis.

3. Results and discussion

Analysis of Table 1 and Fig. 1 revealed noteworthy statistical differences in SGPT and SGOT levels among the various test groups (P-value<0.05). Notably, the 400-mg dosage of *A. hamosus* extract demonstrated the most promising impact by effectively reducing the elevated SGOT and SGPT levels induced by TAA usage.

Moreover, ALP, GGT, and MDA levels also exhibited significant variations among the tested groups (P-value<0.05). The data presented in Table 1 and Fig 1 underscored the substantial effectiveness of the 400-mg dose of *A. hamosus* extract. This particular dose significantly reduced elevated levels of GGT, MDA, and ALP, which previously increased due to TAA administration.

The liver, a vital organ, contributes extensively to drug detoxification, the removal of waste products, and the regulation of various metabolic processes. Its multifaceted functions include the production of blood clotting agents, storage of glycogen, and active participation in sugar and fat metabolism. Additionally, the liver plays a crucial role in fat absorption and acts as a defense against microbes and toxins absorbed through food (Sepehrinezhad *et al.*, 2021; Khosravi *et al.*, 2013).

Liver enzymes, specifically AST, ALT, and ALP, serve as reliable markers for assessing liver damage and necrosis. These enzymes, concentrated in the liver, exhibit a sharp increase following hepatocyte damage, drug-related necrosis, toxins, ischemia, and hepatitis (Zhou *et al.*, 2021). Thioacetamide (TAA), a potent liver toxin metabolized by the cytochrome P450 detoxification system, acts similarly to substances like acetaminophen, antibiotics, ethanol, and carbon tetrachloride, leading to liver cell destruction and cirrhosis (Kim *et al.*, 2000; Alavi *et al.*, 2010).

Studies indicate that plant extracts rich in phenolic compounds and flavonoids offer protective effects against oxidative stress. Phenolic compounds, encompassing vitamins, pigments, and flavonoids, possess anti-mutation and anti-cancer properties. The antioxidant activity of these compounds stems from their reducing power and chemical structure, enabling them to neutralize free radicals and inhibit oxidation reactions (Shun *et al.*, 2003). Consequently, the phenolic compounds and flavonoids in *A. hamosus* extract may reduce oxidative stress, alleviating side effects caused by TAA consumption.

TAA, with its crystalline structure and water solubility, undergoes metabolism by the cytochrome P450 system. While microsomal enzymes oxidize and neutralize TAA's toxicity, elevated concentrations can denature proteins and peroxidize lipids, causing an imbalance in liver enzymes and tissue destruction. Previous studies support the correlation between TAA-induced liver damage, increased free radical production, and cell membrane damage (Lin *et al.*, 2000). The present study confirms these findings, highlighting the significant increase in ALP and MDA due to TAA, which *A. hamosus* effectively moderated.

Sazegar *et al.* (2018) explored the protective effects of *Satureja bachtiarica* and *Thymus daenensis* Celak. extracts on TAA-induced liver fibrosis in rats. Their results align with the current study, emphasizing the dose-dependent protective effects of antioxidant plants. Notably, the *A. hamosus* extract exhibited a significant decrease in SGPT and SGOT levels following TAA-induced elevation, with the 400-mg dose showed the most pronounced effects.

Further validation comes from Mohammadpour *et al.* (2016), who investigated the effects of *Prosopis farcta* hydro-alcoholic seed extract on thioacetamide-induced acute liver toxicity in rats. Their findings, consistent with the present study, emphasize the significant reduction in ALT, AST, and ALP levels and improved tissue health due to the antioxidant properties of the extract.

Akbari *et al.* (2020) investigation of *Tamarix dioica* flower's effect on thioacetamide-induced changes in rats, corroborate the protective impact of plant extracts. Histopathological analysis emphasizes the ability of the extract to reduce liver tissue destruction.

The antioxidant properties *of A. hamosus* are due to its flavonoid compounds, which, as demonstrated by various studies, play a crucial role in protecting liver cells. Flavonoids increase the activity of antioxidant enzymes and reduce membrane lipid peroxidation. Additionally, the presence of unsaturated fatty acids in *A. hamosus* contributes to maintaining the structural

integrity of liver cell membranes (Urfi *et al.*, 2018; Rahman *et al.*, 2011; Sehrawat *et al.*, 2006).

Table 1. Comparison of the mean serum concentrations of liver enzymes following the administration of different amounts of *A. hamosus*.

Groups	No	SGPT	SGOT	ALP	MDA	GGT
Control	8	51.94±2.43[a]	99.00±9.68[a]	208.50±8.21[a]	3.19±0.41[a]	3.81±0.52[a]
Thioacetamide	8	63.50±2.14[b]	147.19±5.54[b]	445.06±49.54[b]	6.02±0.41[b]	6.98±0.69[b]
***Astragalus hamosus* (200 mg)**	8	61.00±1.07[b]	144.06±5.51[b]	423.88±47.32[b]	5.51±0.54[b]	6.67±0.67[b]
***Astragalus hamosus* (400 mg)**	8	53.44±2.64[a]	101.88±8.57[a]	230.19±36.12[a]	3.39±0.48[a]	4.16±0.59[a]

Groups with different letters within a column have statistically significant differences (P<0.05).

1. Histopathological analysis of liver tissue in different groups

The histopathological analysis of the liver tissue in the groups showed that the liver cells showed necrosis in the TAA-intoxicated group. Central vein hyperemia and bleeding were also observed. In the group treated with 200 mg of extract, the degeneration of the liver hepatocytes and the increased space of the sinusoids were also observed. The liver cells were in healthy condition in the group treated with a dose of 400 mg of the extract, and only a slight increase in the space of the sinusoids was observed (Figures 1 a-d).

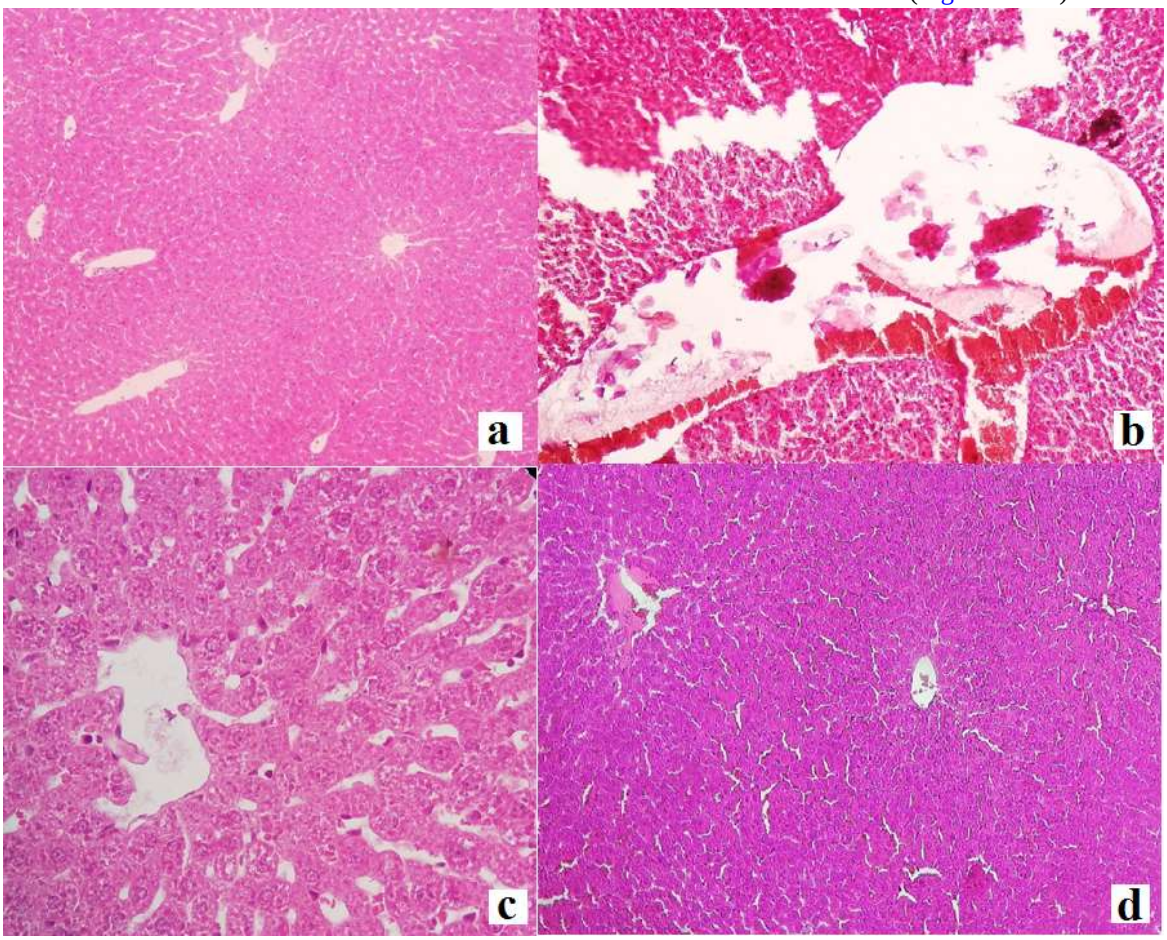

Fig 1. a: Control group: liver tissue and its cells are healthy **b**: Thioacetamide Group: necrosis of liver cells and hyperemia of the central vein and its severe expansion - necrosis of the cells around the central vein can be observed **c**: Group receiving the extract at a dose of 200 mg. Healthy liver cells and a slight increase in the space of the sinusoids as well as a slight degeneration of the cells can be observed **d**: Group receiving the extract at a dose of 400 mg - healthy liver cells and a slight increase in the space of the sinusoids can be observed (H&E staining, 100x magnification).

4. Conclusion

The results obtained in the present study indicated that the extract of the plant *A. hamosus* was rich in antioxidant properties and could reduce the side effects caused by the consumption of thioacetamide. These properties are both in the form of modulation of liver enzymes and in the form of repair of liver tissue. It is noteworthy that the effective dose of the *A. hamosus* extract to benefit from its antioxidant properties, as determined in the present study, was 400 mg. In general, the findings obtained in the present study indicated that the simultaneous consumption of *A. hamosus* extract with thioacetamide, probably due to the antioxidant properties of *A. hamosus*, caused modulation of liver enzymes and improvement of liver tissue structure.

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