TRADITIONAL AND INTEGRATIVE MEDICINE

Trad Integr Med, Volume 11, Issue 2, Spring 2026

Original Research

Study on the Liver-Protecting Properties of *Ajuga chamaecistus* ssp. *tomentella* against Acetaminophen-Induced Liver Damage in Rats

Nona Sadat Mousavi Ayatollahi[1], Seyede Nargess Sadati Lamardi[2], Omid Sabzevari[3], Niloofar Nazari[4], Hamid Reza Monsef Esfehani[5], Mahdi Vazirian[5]*

[1]School of Pharmacy, International Campus, Tehran University of Medical Sciences (IC-TUMS), Tehran, Iran
[2]Department of Traditional Pharmacy, School of Persian Medicine، Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Toxicology and Pharmacology, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[4]Department of Pharmacognosy and Traditional Pharmacy, School of Pharmacy, Baqiyatallah University of Medical Sciences, Tehran, Iran
[5]Department of Pharmacognosy, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Received: 8 Oct 2025 **Revised:** 7 Feb 2026 **Accepted:** 8 Feb 2026

Abstract

Ajuga species have traditionally been used in Persian medicine to treat conditions such as joint pain, gout, and jaundice. *Ajuga chamaecistus* ssp. *tomentella*, known locally as "komafitoos," is one of the five native species in Iran. This study evaluated the antioxidant and hepatoprotective effects of the aerial part extract of *A. chamaecistus* ssp. *tomentella* against acetaminophen-induced liver toxicity in rats. Thirty-six rats were divided into six groups, including a control group receiving normal saline and others were administered with acetaminophen (500 mg/kg) for seven days. The treatment groups received hydro-ethanolic extracts of the plant at doses of 200, 400, and 800 mg/kg by gavage; while silymarin (100 mg/kg) was used as a positive control. Biochemical analyses on day eight measured liver function markers (alanine transaminase, aspartate transaminase, alkaline phosphatase , bilirubin, total protein, and albumin) and oxidative stress indicators (glutathione and malondialdehyde). The extract significantly reduced liver enzyme levels and improved lipid profiles by lowering cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein concentrations compared to the acetaminophen-only group. The 200 mg/kg dose showed the strongest protective effects, preventing glutathione depletion and tissue lipid peroxidation, with histological findings confirming minimal hepatic damage comparable to silymarin. These results suggest that *A. chamaecistus* ssp. *tomentella* extract at 200 mg/kg exhibits potent hepatoprotective and lipid-lowering properties against acetaminophen-induced liver injury.

Keywords: *Ajuga chamaecistus;* Hepatoprotective; Antioxidant; Acetaminophen; Persian Medicine

http://doi.org/10.18502/tim.v11i2.22054

Citation: Mousavi Ayatollahi NS, Sadati Lamardi SN, Sabzevari O, Nazari N, Monsef Esfehani HR, Vazirian M**. Study on the Liver-Protecting Properties of *Ajuga chamaecistus* ssp. *tomentella* against Acetaminophen-Induced Liver Damage in Rats.** Trad Integr Med 2026;11(2): 162-169. http://doi.org/10.18502/tim.v11i2.22054

*Corresponding Author:Mahdi Vazirian
Department of Pharmacognosy, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
Email: mahdivazirian.tums@gmail.com

Introduction

Ajuga, belonging to the Lamiaceae family and also known as ground pine or bugleweed, is a flowering annual plant with over a hundred species, including fifty subspecies and varieties spread across Europe, Africa, Southeast Australia, and parts of Asia [1]. *A. chamaecistus* is a shrub and perennial plant endemic to Iran. It possesses medicinal properties such as being an anti-jaundice, diuretic, appetite stimulant, tonic, and antipyretic [2,3]. The metabolites isolated from this plant exhibit effects such as hypoglycemic, anti-inflammatory, anti-malarial, antineoplastic, antibacterial, antifungal, analgesic, and liver protective [4,5]. Studies have identified compounds like flavonoids, iridoids, phytoecdysteroids, neo-clerodane diterpenoids and withanolides in *Ajuga*. In a previous study by Sadati et al., compounds like 20-hydroxyecdysone, cyasterone, ajugalactone, makisterone A, and 24-dehydroprecyasterone (phytoecdysteroids), 8-acetylharpagide (iridoid), cis- and trans-melilotoside, lavandulifolioside, leonoside B, and martynoside (phenylethanoid glycosides) were identified and isolated from this subspecies [6].

The liver is crucial for the body's physiological functions, handling metabolism, detoxification, secretion, and storage of various substances. Liver diseases can result from toxic chemicals (like carbon tetrachloride, antibiotics, chemotherapy drugs, and peroxidized oils), autoimmune disorders, alcohol abuse, and infections such as cirrhosis, hepatitis, and hepatosis. In Iran, gastrointestinal diseases are a leading cause of death, accounting for about 10%. Many of these deaths are due to various types of hepatitis, fibrosis, liver cirrhosis, and liver failure. However, there are still gaps in medical treatments for conditions like cirrhosis, fatty liver, and chronic hepatitis. Thus, discovering effective medications and treatments with minimal side effects remains a priority in medical science for these diseases [7, 8]. Given the significant impact of liver diseases on health, the critical role of medications like acetaminophen and anti-tuberculosis drugs in causing liver cell dysfunction, and the need for new medicinal compounds, this study was conducted to investigate the free radical inhibition and liver-protective effects of *A. chamaecistus* in a rat model of acetaminophen-induced liver toxicity.

Materials and Methods

Plant material

In June 2017, aerial parts of *A. chamaecistus* Ging. ssp. *tomentella* (Boiss.) Rech. f. were gathered from the eastern regions of Tehran, Iran. These parts were then dried appropriately and authenticated by Professor G. Amin. A voucher specimen (THE-6697) was deposited in the herbarium at the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Hydroalcoholic extraction

The thoroughly dried and ground aerial parts of *A. chamaecistus* Ging. ssp. *tomentella* (Boiss.) Rech. f. (100 grams) were extracted three times with 80% ethanol (3×1200 ml) at room temperature. The solvent was then evaporated using a rotary evaporator and a vacuum oven to produce a dark brown extract.

Determining DPPH radical scavenging activity

The hydroalcoholic extract was assessed for its free radical scavenging activities using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, as described by Brand Williams et al. (1995), with some modifications based on a prior study [8,9].

Based on the initial investigation, we prepared total extract concentrations of 100, 500 and 1000 µg/ml. A DPPH concentration of 400 µg/ml was prepared in methanol and stored as a total DPPH solution. Each time the DPPH solution was diluted at a 10:1 ratio for sample absorption readings, this working solution was used for testing. For the test using a stable DPPH radical, three test tubes were prepared for each concentration. Each tube received 2 ml of DPPH and 1 ml of the sample. The control tube contained the highest extract concentration, 2 ml of methanol, and a blank sample, which included 2 ml of DPPH and 1 ml of methanol. After 30 minutes, the absorbance was measured at 517 nm. All tests were performed in triplicate. The percentage of radical scavenging activity of the sample was calculated using the following equation:

$$\text{Inhibition\%} = [(A0 - As)/A0] \times 100$$

where A0 is the absorbance of the control and As is the absorbance of the sample.

The half maximal inhibitory concentration (IC50), indicating the concentration of the sample (mg/ml) required to scavenge 50% of DPPH, was calculated from the plotted graph of scavenging activity versus the concentration of the extract using linear regression analysis.

Animals

Drugs and Chemicals

Silymarin and Acetaminophen was purchased from Sigma (chemical St. Louis, MO, The USA) and the assay Elisa kits were purchased from Zellbio© GmbH (Germany) Padginteb co.

Ethical considerations

This study was approved by the ethics committee of Tehran University of Medical Sciences (TUMS), Tehran, Iran (IR.TUMS.PSRC.REC.1395.1166).

Animals for hepatoprotective study

Male rats weighing 200 ± 12 g were sourced from the School of Pharmacy at Tehran University of Medical Sciences and kept in groups of six under constant conditions (24±1°C) with a 12-h light-dark cycle. The rats

were given free access to tap water and standard laboratory mouse food and were allowed a week to acclimate to the laboratory before the experiments began.

Following a previous study by Sadati et al. (2018), which determined the LD50 of the total plant extract to be more than 5500 mg/kg, total extracts at concentrations of 100, 200, and 500 mg/ml were prepared in DMSO solvent (10% in distilled water) for liver protection testing. Thirty-six rats were randomly divided into six groups for injections and gavage treatments (2 ml). From the first to the seventh day, groups one to five received acetaminophen (500 mg/kg) as a hepatotoxic agent via gavage. After one hour, the second group received 2 ml of silymarin (100 mg/kg), a known hepatoprotective agent, dissolved in 2 ml of ethanol due to low water solubility, then administered with 10% DMSO in distilled water up to 84 ml. The third to fifth groups received extracts at doses of 200, 400, and 800 mg/kg, respectively. The control group, consisting of six rats, received no toxin or medication and were fed normal saline.

On the seventh day, after anesthetizing the animals with xylazine (5 mg/kg) and ketamine (100 mg/kg), blood samples were taken from the heart to measure liver-related serum parameters including triglycerides, cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). To prepare samples for histopathology, half of each animal's liver was fixed in 10% formalin for 10 days (replacing the formalin after 24 hours). Tissue sections were then prepared, and the effects of the extract on rat liver tissue were examined.

Glutathione (GSH) and malondialdehyde (MDA) assay

The other half of the liver samples was designated for the analysis of tissue GSH and MDA levels and stored in a freezer at approximately -80°C. To measure GSH, 100 µl of the supernatant from tissues previously homogenized with normal saline were placed in 96-well plates. Then, 200 µl of DTMB solution (5,5-dithiol-2-nitrobenzoic acid) was added, and its absorption was measured at 412 nm using an ELISA Reader. For the blank sample, 100 µl of distilled water and 200 µl of DTMB solution were used. The GSH content in the samples was determined in µg/ml by comparing it to standard GSH concentration vs. absorption curves. The protein concentration was measured using the Bradford method, which was expressed in mg of protein per ml. By combining these values, the GSH amount was calculated in µg/mg of protein. To draw a standard GSH curve, a storage concentration (1 mg/ml) was prepared and diluted to 25, 50, 100, and 200 µg/ml to measure reduced glutathione. The reduction of DTMB with GSH in an alkaline medium forms a yellow compound, directly related to the GSH concentration in the sample.

For MDA measurement, 0.2 g of liver tissue was homogenized in 0.5 ml of 0.1% trichloroacetic acid (TCA), and the extract was centrifuged at 6000 g for 5 min. Four ml of 20% TCA was added to 1 ml of supernatant containing 0.5% thiobarbituric acid and incubated in a hot water bath at 95°C for 30 minutes. The mixture was then quickly cooled in an ice bath and centrifuged at 6000 *g* for 10 minutes. The supernatant's absorbance was measured at 532 nm, with non-specific absorption subtracted. MDA concentration was calculated using a correction factor (µ mol^{-1} cm^{-1}) of 0.155 and expressed in µg/g of tissue. For the calibration of pure MDA standards, concentrations of 0.5 to 128 µg/ml were prepared in distilled water, and 180 µg of the reaction mixture was added to the samples. These were incubated in a hot water bath at 95-98°C for an hour, cooled, and 150 µg of N-butanol was added. The micro-tubes were centrifuged at 5000 rpm for a minute, and the absorbance of the supernatant (onion skin pink) was read using a fluorimeter at Excitation = 530.25 and Emission = 575.15.

Statistical analysis

The statistical analysis was performed using One-way Analysis of Variance (ANOVA) with SigmaPlot version 12.0. Significance was determined with P values less than 0.05.

Results

DPPH radical scavenging activity

In this study, the DPPH scavenging activity of the total 80% ethanol extract at concentrations of 100, 500, and 1000 µg/ml was assessed. The IC50 value of the total extract was determined to be 368.72 µg/ml.

Hepatoprotection test

Figure 1 illustrates the variables studied in blood plasma for the liver protection test of *A. chamaecistus* ssp. *tomentella*. The measured values of GSH and MDA are presented in Figure 2. Statistical analysis indicates significant differences between the groups treated with various concentrations of *Ajuga* and silymarin compared to the control group. A comparison of all groups shows that aspartate transaminase (AST) and alanine transaminase (ALT) enzyme levels differ significantly between the treated groups. The worst results for AST and ALT tests were observed in the acetaminophen treatment group, followed by the 800 and 400 mg/kg *Ajuga* treatment groups. In contrast, the 200 mg/kg *Ajuga* and silymarin treatment groups exhibited the lowest serum activity of these enzymes. This suggests that silymarin is more effective in preventing hepatocyte damage than the 400 and 800 mg/kg *Ajuga* extract. The same trend was observed with alkaline phosphatase, where silymarin had a much greater effect on preventing the increase of this enzyme. Although the silymarin treatment group showed a much smaller difference in total serum protein compared to other groups, the increase in lactate dehydrogenase (LDH) levels in this group suggests a different type of damage to hepatocyte cells. It should be noted that no significant difference in

LDH levels was observed between the acetaminophen and control groups, indicating that the acetaminophen dose used did not significantly affect LDH levels, while silymarin increased LDH levels over a short period. Further studies are needed to investigate this effect.

Animals treated with *Ajuga* extract had significantly lower blood cholesterol, triglyceride, and LDL levels compared to those treated with acetaminophen and silymarin. These findings suggest that *Ajuga* helps prevent an increase in major blood lipid concentrations. Additionally, blood lipid levels in the acetaminophen treatment group were lower than in the silymarin treatment group, indicating a more pronounced effect of silymarin on increasing blood lipid levels. However, this effect was temporary and is expected to decrease and stabilize over time.

There were no significant differences in total bilirubin values among the groups compared to the control group. Measurements of tissue factors showed that in the acetaminophen-receiving groups, glutathione levels decreased sharply and MDA levels increased significantly. Both the 200 mg/kg dose and silymarin significantly affected GSH reduction and MDA increase, while the 400 and 800 mg/kg doses of *Ajuga* extract did not show much reversal. Therefore, the optimal concentration of *Ajuga* for maintaining GSH is 200 mg/kg. Additionally, the best concentration for reducing inflammatory markers like MDA is the lowest concentration, which is 200 mg/kg.

Histological results

Histology results indicate that *Ajuga* administration at concentrations of 200 and 400 mg/kg and silymarin can reduce inflammatory reactions compared to 800 mg/kg and acetaminophen treatment groups, thereby decreasing the incidence of bleeding and the severity of inflammatory cell accumulation. The least damage was observed in the 200 mg/kg treatment group, while the liver damage in the 800 mg/kg group was greater than in the 400 mg/kg group. These findings suggest that a 200 mg/kg concentration of the plant extract is less toxic than higher concentrations and helps prevent damage caused by inflammatory factors or liver damage (Figure 3).

Discussion

This study investigated the liver-protective, antioxidant, and blood lipid-lowering effects of *Ajuga chamaesictus* subsp. *tomentella* in the presence of acetaminophen, and compared them with silymarin. Our findings reveal that in the acetaminophen group and at *Ajuga* concentrations of 400 and 800 mg/kg, GSH levels significantly decreased and MDA levels increased. However, *Ajuga* at 200 mg/kg and silymarin were the most effective in preventing the increase of MDA and the reduction of GSH in the presence of acetaminophen toxicity. Chemical analyses of *Ajuga* species have identified the presence of various flavonoids, tannins, terpenes, steroids, and phytoecdysoids [10]. Flavonoids inhibit free radicals and, similar to tannins and triterpenes, possess antioxidant properties [11]. Recent studies have discovered and documented multiple phenolic and steroidal compounds in the examined plant [3]. Previous studies have indicated that phytoecdysteroids exhibit strong antioxidant activity against free radicals.

[12]. Additionally, the extract contained 2.58% 20-hydroxyecdysone (0.46% in the dried plant), which is higher than in other plants with phytoecdyteroids [13].

Bouderbala et al. studied rats with hypercholesterolemia treated with *Ajuga iva* aqueous extract. They discovered that the plant's extract exhibited the highest antioxidant power for red blood cells. Additionally, the rats treated with the *Ajuga* extract showed reduced levels of MDA and LDL compared to the control group, along with increased activity of the superoxide dismutase enzyme, which is involved in the antioxidant activity of red blood cells and tissues [14].

In our study, a 400 mg/kg concentration of *Ajuga* hydroalcoholic extract showed the most significant effect on reducing total cholesterol levels, as observed by Bouderbala et al [15]. In another study on *Ajuga iva*, rats with hypercholesterolemia were given 5, 10, or 15 mg of *Ajuga* per kG for 15 days. After the treatment period, total cholesterol levels in the groups treated with 5 and 10 mg/kg were reduced by 1.2-1.4 units, respectively. The levels of MDA, a reactive agent with thiobarbituric acid, decreased by 2.3, 2.9, and 3 units in the groups treated with 5, 10, and 15 mg/kg, respectively. Additionally, the activity of superoxide dismutase and glutathione reductase increased in all treated groups compared to the control group. The researchers concluded that consuming the plant extract significantly reduced cholesterol and oxidative stress caused by hypercholesterolemia, suggesting it could be used as a preventive medicine for hypercholesterolemia.

The current study has an advantage over previous ones as it also investigated liver enzymes, LDH, and total protein values, providing a clearer view of liver toxicity. The inflammatory condition of rat liver tissue was reported, better reflecting the histological effect of the studied concentrations. Different concentrations of *Ajuga* in the present study had less protective effect on liver enzymes compared to silymarin. However, *Ajuga* performed better in reducing lipids, and administering 200 mg/kg of extract normalized liver tissue changes due to acetaminophen better than the silymarin group.

Chenni et al. (2007) studied the hypolipidemic effect of *Ajuga iva* aqueous extract on rats with hypercholesterolemia. They reported that the plant's aqueous extract significantly lowered total cholesterol and VLDL levels compared to the control group; while increasing HDL levels. Triglyceride levels also decreased compared to the control group. MDA levels in plasma and kidney tissue were significantly reduced, but no significant difference was observed in adipose tissue. Glutathione reductase ac-

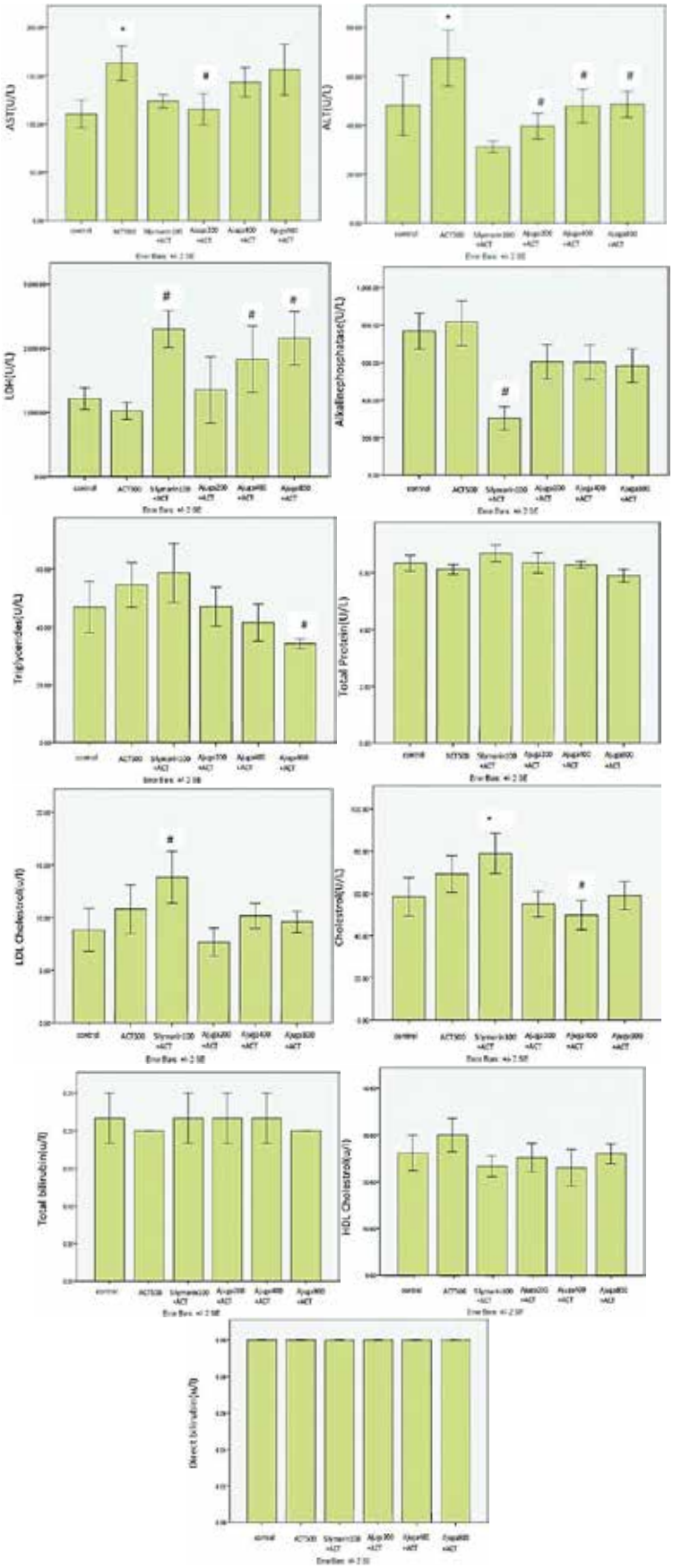

Figure 1. Plasma index charts examining the effect of the total extract of *Ajuga chamaecistus* ssp. *tomentella* on liver damage induced by acetaminophen compared to the control group.
* Significant difference compared to the control group ($p<0.05$).
# Significant difference compared to the acetaminophen group ($p<0.05$).

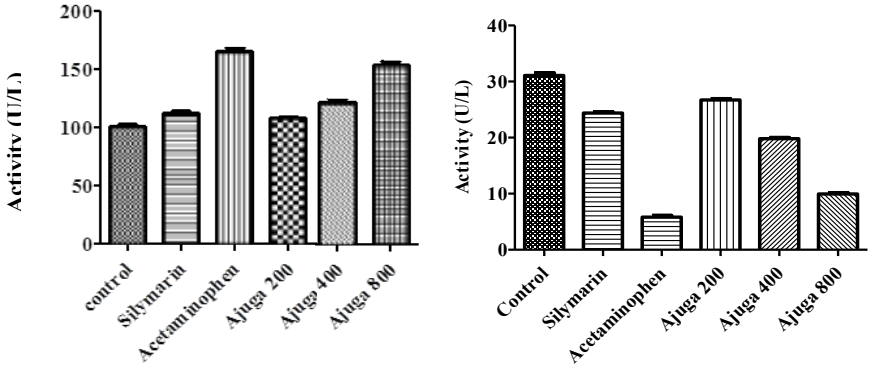

Figure 2. Variables indicating liver damage in liver tissue caused by the administration of *A. chamaecistus* ssp. *tomentella* extract.
*** Significant difference compared to the control group ($p<0.05$).

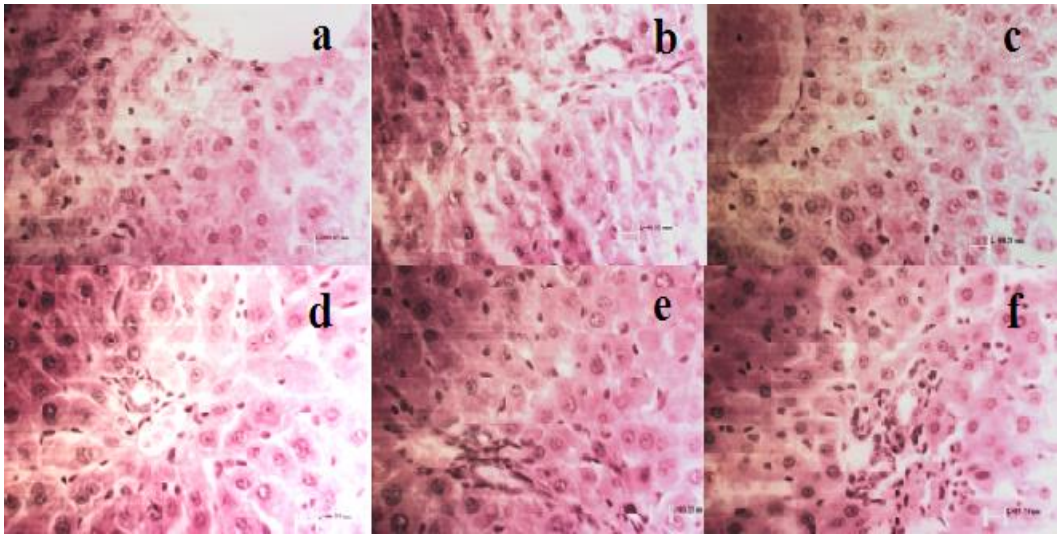

Figure 3. Mouse liver tissue with 400x magnification,
- a: Control group
- b: Acetaminophen toxicity induction group (500 mg/kg)
- c: Acetaminophen + 200 mg/kg extract group
- d: Acetaminophen + 400 mg/kg extract group
- e: Acetaminophen + 800 mg/kg extract group
- f: Acetaminophen + 100 mg/kg silymarin group (positive control)

tivity decreased in adipose tissue but increased in liver and kidney tissues compared to the control group. The researchers concluded that *Ajuga* aqueous extract significantly reduced triglyceride and cholesterol levels, improved antioxidant status by reducing lipid oxidation in plasma and tissue, and increased antioxidant enzyme activity in hypercholesterolemic rats. Their results also indicated that *Ajuga* reduces intestinal cholesterol absorption [16]. The researchers' findings on total triglycerides and cholesterol were corroborated in our study. Notably, triglyceride levels in our study were dose-dependently lower than in the control group. Regarding GSH and MDA, our results align with those of the researchers, with the best outcomes in our study observed at the lowest *Ajuga* concentration. It is important to note that we used a hydro-alcoholic extract; while the researchers used an aqueous extract. For the effects of 200 mg/kg *Ajuga* extract on GSH and MDA, silymarin (100 mg/kg) had a similar effect. Thus, the 200 mg/kg Ajuga extract concentration can be comparable to silymarin, with *Ajuga* being more effective at lowering cholesterol and triglycerides, and silymarin better protecting liver enzymes.

El-Hilaly et al. demonstrated that *Ajuga iva* aqueous extract lowered fat, triglycerides, and total cholesterol in normal and diabetic rats, highlighting its importance due to its hypoglycemic properties [17]. A previous study by Sadati et al. investigated the antioxidant effects, radical inhibition, and total phenol content of aqueous and meth-

anol extracts of *A. chamaecistus* ssp. *tomentella* using FRAP, DPPH, and Folin-Ciocalteu methods, along with the blood sugar-lowering effects of this plant. Diabetic mice induced with Streptozotocin (STZ) were divided into groups and treated with aqueous and methanol extracts (200, 400, and 800 mg/kg), metformin (500 mg/kg), and a negative control group. The results showed that the *n*-butanol extract had the highest total phenol content and antioxidant power, as well as the most significant radical inhibition activity in diabetic mice. Additionally, repeated oral administration of all doses of the extract significantly reduced plasma glucose levels after 3, 14, and 28 days [18].

Administering 200 mg/kg of *Ajuga* hydroalcoholic extract can prevent liver tissue damage and fibrosis in rats poisoned with acetaminophen. The anti-inflammatory effects of *Ajuga* have been documented in numerous studies.

In a prior study, the analgesic and anti-inflammatory activities of various extracts of *A. chamaecistus* ssp. *tomentella* were examined using formalin in mice. The results showed that oral administration of aqueous and hydroethanolic extracts (at a dose of 400 mg/kg) exhibited significant analgesic activity during the chronic stage. These findings support the traditional use of this plant for treating joint pain and gout, likely due to the presence of compounds with an iridoid structure, such as 8-acetyl-harpagide [5]. This compound is recognized for its analgesic and anti-inflammatory properties, as highlighted in a previous study [6,19].

Hsieh et al. have demonstrated that *Ajuga bracteosa* exhibits anti-inflammatory effects in fibrous liver model mice. Because fibrosis results from immune reactions and inflammation, scientists seek compounds that can halt or mitigate these processes in living organisms when necessary. Using chloroform extract of *Ajuga*, Hsieh et al. successfully inhibited intracellular inflammatory processes in liver cell lines (cell culture models). They also employed a laboratory animal model to show that the extract could reduce carbon tetrachloride-induced fibrosis in mice. Histopathological analysis and aminotransferase enzyme status were improved in mice treated with *Ajuga*. Furthermore, they investigated the expression of TNF-α, CD14, lipopolysaccharide-linked proteins in Gram-negative bacteria, alpha-1 collagen, and actin, finding that *Ajuga* extract inhibited the expression of these genes. All this evidence points to the anti-inflammatory and anti-fibrotic effects of *Ajuga* extract [20].

The results of the current study indicated that the fat profile was elevated in mice treated with silymarin. Additionally, there was no significant difference in LDH levels between the acetaminophen and control groups, suggesting that the used dose of acetaminophen had no significant impact on LDH levels. However, silymarin caused an increase in LDH levels over a short period. Further investigation is needed to understand the reason for this effect.

Conclusion

This study found that a 200 mg/kg concentration of *Ajuga* hydro-alcoholic extract effectively lowers blood fats without significantly affecting liver enzyme levels, compared to the 400 and 800 mg/kg concentrations. Additionally, the 200 mg/kg concentration is more advantageous than silymarin, as it also reduces the elevated liver enzymes caused by acetaminophen. Therefore, both Ajuga and silymarin can reduce fat and liver enzymes under inflammatory conditions, with silymarin primarily protecting liver enzymes. The importance of the 200 mg/kg *Ajuga* extract concentration was further highlighted by its protective effect on liver parenchyma, as observed in the histopathological analysis.

Funding

This publication and work is based on studies sponsored and funded by the Vice Chancellor for Research (Project No. 31789), Tehran University of Medical Sciences, Tehran, Iran.

Conflict of Interests

The authors declare that there are no conflicts of interest and have read and understood the Conflict-of-Interest Policy from the Journal.

Acknowledgements

None.

References

[1] A MV. dictionary of Iranian plant names. 2007, Farhang-e-moaser; Tehran.

[2] Ardekani MS, Khanavi M, Taheri P, Samadi N, Safaripour E, et al. The essential oil composition of ajuga chamaecistus Ging. subsp. tomentella Rech. f. J Essent Oil Bear Plants 2010;13:45-51.

[3] Sadati N, Ostad S, Karimian Z, Ardekani MS, Akbarzadeh T, et al. Phytochemical study and in vitro cytotoxic effect of Ajuga chamaecistus ssp. tomentella. Asian J Chem 2012;24:71-82.

[4] Hamden K, Ayadi F, Jamoussi K, Masmoudi H, Elfeki A. Therapeutic effect of phytoecdysteroids rich extract from Ajuga iva on alloxan induced diabetic rats liver, kidney and pancreas. Biofactors 2008;33:165-175.

[5] Khanavi M, Davoodipoor AM, Sadati SN, Ardekani MRS, Sharifzadeh M. Antinociceptive effect of some extracts from Ajuga chamaecistus Ging. ssp. tomentella (Boiss.) Rech. f. aerial parts. DARU 2014;22:56.

[6] Sadati N, Jenett-Siems K, Siems K, Ardekania MRS, Hadjiakhoondi A, et al. Major constituents and cytotoxic effects of Ajuga chamaecistus ssp. tomentella. *Zeitschrift für Naturforschung C.* 2012;67:275-281.

[7] Hamed IHKA. Determining normal liver stiffness in khartoum state population by ultrasonography. 2016, Sudan University of Science and Technology.

[8] Brand-Williams W, Cuvelier M-E, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 1995;28:25-30.

[9] Tofighi Z, Es-haghi A, Asl MM, Tajic AR, Navai MS, et al. Investigation of chemical keys for relationship between plants and their unifloral honeys by hydrodistillation and SPME and biological activities of honeys. Eur Food Res Technol 2014;238:665-673.

[10] Luan F, Han K, Li M, Zhang T, Liu D, et al. Ethnomedicinal uses, phytochemistry, pharmacology, and toxicology of species from the Genus Ajuga L.: a systematic review. Am J Chin Med 2019; 47:959-1003.

[11] Karak P. Biological activities of flavonoids: an overview. Int J Pharm Sci Res 2019;10:1567-1574.

[12] Das N, Mishra SK, Bishayee A, Ali ES, Bishayee A. The phytochemical, biological, and medicinal attributes of phytoecdysteroids: An updated review. Acta Pharm Sin B 2020.

[13] Sadati SN, Jahantab S, Ziyarati P, Khanavi M, Shams-Ardekani MR. Quantification of 20-hydroxyecdysone, a Major Phytoecdysteroid, in Ajuga Chamaecistus ssp. Tomentella Using High-Performance Liquid Chromatography. *Trad Integr Med* 2016;1:96-100.

[14] Bouderbala S, Lamri-Senhadji M, Prost J, Lacaille-Dubois M, Bouchenak M. Changes in antioxidant defense status in hypercholesterolemic rats treated with Ajuga iva. Phytomedicine 2008;15:453-461.

[15] Bouderbala S, Prost J, Lacaille-Dubois MA, Bouchenak M. Iridoid extracts from Ajuga iva increase the antioxidant enzyme activities in red blood cells of rats fed a cholesterol-rich diet. Nutr Res 2010;30:358-365.

[16] Chenni A, Yahia DA, Boukortt F, Prost J, Lacaille-Dubois M, et al. Effect of aqueous extract of Ajuga iva supplementation on plasma lipid profile and tissue antioxidant status in rats fed a high-cholesterol diet. J Ethnopharmacol 2007;109:207-213.

[17] El-Hilaly J, Tahraoui A, Israili ZH, Lyoussi B. Hypolipidemic effects of acute and sub-chronic administration of an aqueous extract of Ajuga iva L. whole plant in normal and diabetic rats. J Ethnopharmacol 2006;105: 441-448.

[18] Sadati Lamardi SN, Majidi Z, Alipour C, Farajzadeh S, Gohari A, et al. Antioxidant potential, hypoglycemic effect and safety of Ajuga chamaecistus Ging. ssp. tomentella (Boiss.) Rech. f. aerial parts. *Res J Pharmacogn* 2018;5:53-63.

[19] Ramírez-Cisneros MÁ, Rios MY, Aguilar-Guadarrama AB, Rao PP, Aburto-Amar R, et al. In vitro COX-1 and COX-2 enzyme inhibitory activities of iridoids from Penstemon barbatus, Castilleja tenuiflora, Cresentia alata and Vitex mollis. *Bioorg Med Chem Lett* 2015;25:4505-4508.

[20] Hsieh W-T, Liu Y-T, Lin W-C. Anti-inflammatory properties of Ajuga bracteosa in vivo and in vitro study and their effects on mouse model of liver fibrosis. J Ethnopharmacol 2011;135:116-125.