



Comparing the Effects of Sulfasalazine and Shilajit on Liver Damage Caused by Acetic Acid-Induced Ulcerative Colitis in Male Rats

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Article Type	ABSTRACT
Research Paper	Background and Objective: Liver damage is one of the common complications after ulcerative colitis. The aim of this study is to compare liver protective effects of sulfasalazine and shilajit after ulcerative colitis. Methods: In this experimental intervention study, 49 male Wistar rats weighing 200-250 grams were used in seven groups of 7: sham, ulcerative colitis, oral solution, oral Shilajit, sulfasalazine, rectal solution, and rectal Shilajit. To induce ulcerative colitis, after anesthetizing the animal, 2 cc of acetic acid (4%) was used by rectal administration. Four days after the development of colitis, Shilajit was administered for four days at a dose of 250 mg/kg by gavage and rectal methods. In each group, the levels of liver enzymes (SGPT, SGOT, ALP), direct bilirubin, total, and serum albumin were evaluated eight days after the induction of ulcerative colitis. Findings: Sulfasalazine decreased total bilirubin (0.5967 ± 0.04) and SGOT (113.3 ± 12.7). Administering Shilajit rectally reduced direct bilirubin (0.10 ± 0.02), albumin (3.17 ± 0.2), SGOT (156.2 ± 12.7) and administering Shilajit by gavage reduced SGOT (125.0 ± 12.7), direct bilirubin (0.094 ± 0.02), SGOT (125.0 ± 12.7) and SGPT (93.8 ± 7.5). Conclusion: The results of the study showed that oral administration of Shilajit has more protective effects on liver damage caused by ulcerative colitis than rectal Shilajit, and this result is comparable to sulfasalazine. Keywords: <i>Ulcerative Colitis, Liver, Shilajit, Sulfasalazine.</i>

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Introduction

Inflammatory Bowel Disease (IBD) is an idiopathic and chronic intestinal inflammation and is one of the multifactorial intestinal abnormalities. Ulcerative Colitis and Crohn's disease are among these types of diseases and are characterized by intestinal inflammation (1). Inflammation and increased oxidative stress are indicators of damage to different parts of the body (2). Epidemiological studies show a very high prevalence of these diseases in developed countries, especially Iran (3, 4).

Hepatobiliary abnormalities are among the most common extraintestinal manifestations in inflammatory bowel diseases (5). Anatomically speaking, the gastrointestinal tract and the hepatobiliary system are closely related. This characteristic makes the liver and biliary system the direct target of damage during colon inflammation in inflammatory bowel diseases (6). Ulcerative colitis can be associated with increased intestinal permeability. Therefore, this process may provide suitable conditions for the transfer of intestinal flora to the liver (7).

The increase in bacterial transmission is related to the increase in the level of lipopolysaccharides, which causes inflammation and tissue damage (8). It has been reported that infection, inflammation, and tissue damage cause changes in the profile of lipid metabolism, including hypertriglyceridemia, lipolysis, and reduction of fatty acid oxidation in the liver (9). It is also stated in some reports that ulcerative colitis leads to inflammatory responses and fibrosis in the liver (10). Since colitis is a multifactorial disease, it has several treatment methods. Among others, we can mention sulfasalazine, corticosteroids, anti-inflammatory drugs, antibiotics, anti-tumor necrosis factor drugs and immune system suppressors (11). Sulfasalazine is used in the treatment of inflammatory bowel diseases, including ulcerative colitis and Crohn's disease (12, 13). Sulfasalazine does not cure ulcerative colitis, but it can reduce the number of attacks (12). The main activity of sulfasalazine drug comes from the product of its metabolism; 5-amino salicylic acid (mesalamine), which is released in the large intestine and inhibits the production of leukotriene and lipooxygenase (13).

Medicinal plants and natural substances have been used for the treatment of human diseases for many years. On the other hand, due to the low side effects of herbal medicines and the variety of effective compounds in plants, there is a great tendency to use medicinal plants (14) and the use of these compounds has been recommended by the World Health Organization. Shilajit (mummy) is a natural brown substance that is deposited in the crevices of rocks and caves in the form of a gum-like substance. In the past, this substance was used orally and topically for broken limbs, sprained joints, contusions and ruptures of nerves and muscles (15). In a study by Alimahdi et al., it was proven that Shilajit effectively improves ulcerative colitis by reducing inflammatory cytokines (3).

Serum albumin is a predictor of postoperative outcomes in non-cardiac surgeries, and hypoalbuminemia is seen in about one-fifth of patients with IBD. Even in the early stages of the disease, about 80% of hospitalized patients with ulcerative colitis and 50% of patients with Crohn's disease struggle with hypoalbuminemia (16). Therefore, considering that ulcerative colitis is actually a complex disorder that can involve many tissues, including the liver, the purpose of this study is to investigate the effect of Shilajit on biochemical parameters of the liver, serum albumin and compare it with sulfasalazine (the main drug for the treatment of ulcerative colitis) on liver damage caused by acetic acid-induced ulcerative colitis in male rats.

Methods

In this experimental study, 49 male Wistar Bauzen rats, 200-250 g, were used after approval by the ethics committee of Kerman University of Medical Sciences with ethics code IR.KMU.REC.1401.470. All animals were kept at a temperature of 22°C with free access to food and water. Animals were randomly divided into the following groups:

- 1. Sham group:** healthy male rats that do not develop colitis.
- 2. Ulcerative colitis group:** male rats in which colitis was induced by injecting 1.5 mm of acetic acid into the rectum, but did not receive any medication (3).
- 3. Rectal Shilajit solvent group (Veh-IA):** male rats that received rectal Shilajit solvent (saline) equal to the volume of the drug for 4 days after the development of colitis (3).
- 4. Oral Shilajit solvent group (Veh-G):** male rats that were administered Shilajit solvent (saline) equal to the volume of the drug by gavage for 4 days after the development of colitis (3).
- 5. Rectal Shilajit group (SHil-I):** male rats that were administered rectal Shilajit at a dose of 250 mg/kg for 4 days after the development of colitis (3).
- 6. Oral Shilajit group (SHil-G):** male rats that were administered Shilajit at a dose of 250 mg/kg by gavage for 4 days after the development of colitis (3).
- 7. Sulfasalazine (Sulfa) group:** male rats that were given sulfasalazine at a dose of 250 mg/kg as a standard treatment for 4 days by gavage (3).

It should be noted that in all treated groups, shilajit and sulfasalazine were given at 4:00 pm in order to maintain the same time of use.

Preparation method of Shilajit: Shilajit was prepared from Sardoiye region, Jiroft city. Pure samples of this substance were prepared based on its solubility in water. First, 100 grams of crushed samples were poured into 500 milliliters of water and shaken for 12 hours at room temperature. Then, the resulting solution was filtered through a 0.45 mm filter in order to separate the insoluble impurities, and then the solution was dried in vacuum distillation. Then, Shilajit aqueous extract with a concentration of 250 mg/ml was prepared for use (17).

Sulfasalazine preparation method: Sulfasalazine tablets were prepared from a pharmacy and then powdered in a mortar and dissolved in solvent with a dose of 250 mg/kg of the same volume of Shilajit. For the freshness of the drug, it was prepared at the time of administration (18).

Colitis induction method: In order to induce colitis, rats were starved for 36 hours with free access to water to empty their intestines (3). Then, under mild anesthesia with ketamine/xylazine, 1.5-2 ml of 4% acetic acid was injected into the colon of the animal through the rectum through a plastic tube with an inner diameter of 2.5 mm and a length of 8 cm. Then, the animals were kept in individual cages for 24 hours. The wound in the animal was usually created one hour after the operation and was completed within 3-5 days. The day after the operation was considered as the first day of the wound, and from the fourth day, the medication continued for 4 days (19).

Gavage method: Shilajit, sulfasalazine and their solvent were prepared in separate vials and administered in equal volumes of 1 ml with a needle suitable for gavage, but the dosage of the drugs was maintained (3).

The method of measuring liver biochemical parameters: ALT, AST, GGT, Albumin and serum bilirubin parameters were also measured using Pars Azmoun kit and by chemical colorimetric method (20).

Statistical analyzes were performed using SPSS 24. One-way ANOVA test was used to compare the quantitative variables between the tested groups if the assumptions of normal data distribution were observed. The final results were reported as Mean±SEM and $p < 0.05$ was considered significant.

Results

Changes in serum albumin: The serum albumin level in the sham group was 3.38 ± 0.2 pg/mL, which decreased to 2.36 ± 0.2 pg/mL after induction of colitis ($p < 0.001$). Treatment with oral Shilajit 3.3 ± 0.2 and rectal Shilajit 3.17 ± 0.2 increased the amount of albumin. These values did not show significant difference in sulfasalazine group 3.4 ± 0.2 compared to Shilajit group (Figure 1).

Serum ALP changes: The serum ALP value in the sham group was 643.0 ± 0.78 pg/mL, which increased to 1000 ± 0.78 pg/mL after the induction of colitis. Treatment with oral Shilajit reduced ALP to 683.7 ± 0.7 pg/mL. These values in rectal Shilajit group 878.5 ± 0.7 pg/mL against its solvent and sulfasalazine 691.8 ± 0.7 pg/mL compared to rectal Shilajit showed no significant difference (Figure 2).

Changes in serum total bilirubin: The total serum bilirubin in the sham group was 0.45 ± 0.04 pg/mL, which increased to 0.80 ± 0.04 pg/mL after the induction of colitis ($p < 0.001$). Treatment with sulfasalazine reduced total bilirubin to 0.59 ± 0.04 pg/mL compared to Shilajit ($p < 0.01$). These values in oral Shilajit group 0.67 ± 0.04 pg/mL and rectal Shilajit 0.79 ± 0.04 pg/mL did not show significant difference compared to their solvent (Figure 3).

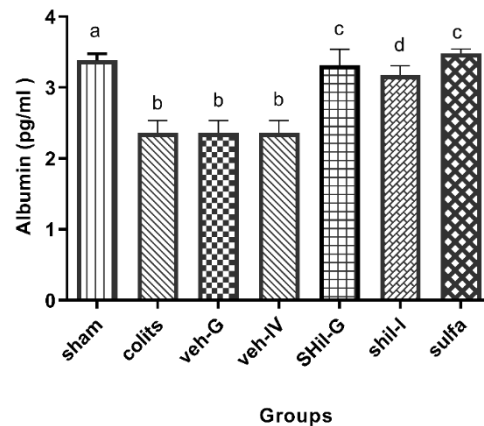


Figure 1. Comparison of mean serum albumin (pg/ml) in different study groups (n=7). The findings are reported based on Mean $\pm$ SEM. Lower case letters in each column indicate significant differences.

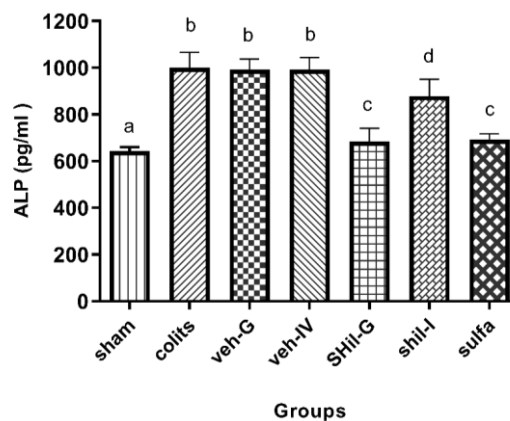


Figure 2. Comparison of serum ALP value (pg/ml) in different study groups (n=7). The findings are reported based on Mean $\pm$ SEM. Lower case letters in each column indicate significant differences.

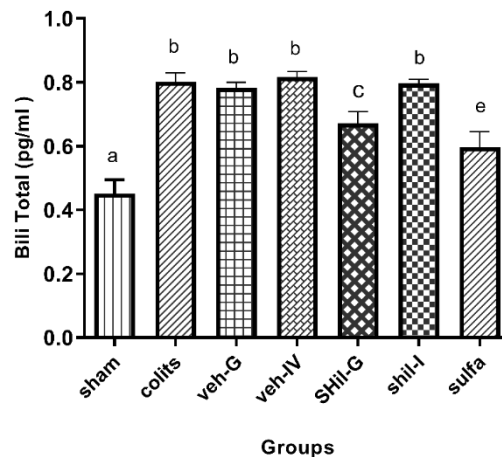


Figure 3. Comparison of total serum bilirubin (pg/ml) in different study groups (n=7). The findings are reported based on Mean±SEM. Lower case letters in each column indicate significant differences.

Changes in direct bilirubin: The serum level of direct bilirubin in the sham group was 0.07 ± 0.02 pg/mL, which increased to 0.26 ± 0.02 pg/mL after the induction of colitis ($p < 0.001$). Treatment with oral Shilajit 0.094 ± 0.02 pg/mL and rectal Shilajit 0.10 ± 0.02 pg/mL reduced direct bilirubin ($p < 0.001$). This value did not show a significant difference in the sulfasalazine group (0.07 ± 0.02 pg/mL) compared to the Shilajit group (Figure 4).

Serum SGOT changes: The serum SGOT value in the sham group was 123.3 ± 12.7 pg/mL, which increased to 215.8 ± 12.7 pg/mL after induction of colitis ($p < 0.001$). Treatment with oral Shilajit 125.5 ± 12.7 pg/mL ($p < 0.001$) and rectal Shilajit 156.2 ± 12.7 pg/mL ($p < 0.05$) decreased the amount of this index. This value in the sulfasalazine group was 113.3 ± 12.7 pg/mL compared to the rectal Shilajit group ($p < 0.05$) (Figure 5).

Serum SGPT changes: The serum SGPT value in the sham group was 70.8 ± 7.5 pg/mL, which increased to 130.8 ± 7.5 pg/mL after the induction of colitis ($p < 0.001$). Treatment with oral Shilajit reduced this index by 93.80 ± 7.5 pg/mL ($p < 0.01$). This value was not significant in Shilajit rectal group 112.8 ± 7.5 pg/mL compared to its solvent and Sulfasalazine 91.25 ± 7.5 pg/mL compared to Shilajit (Figure 6).

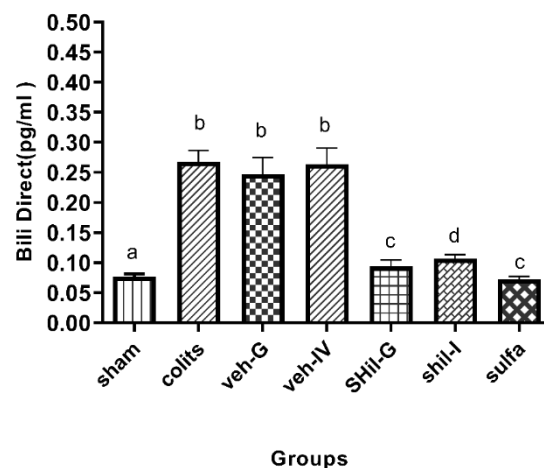


Figure 4. Comparison of serum direct bilirubin (pg/ml) in different study groups (n=7). The findings are reported based on Mean±SEM. Lower case letters in each column indicate significant differences.

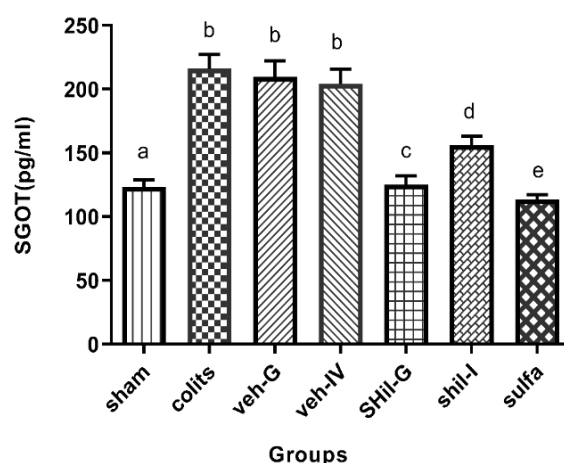


Figure 5. Comparison of serum SGOT value (pg/ml) in different study groups (n=7). The findings are reported based on Mean±SEM. Lower case letters in each column indicate significant differences.

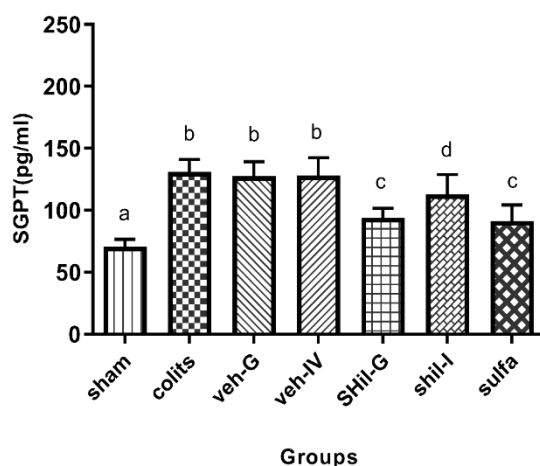


Figure 6. Comparison of serum SGPT value (pg/ml) in different study groups (n=7). The findings are reported based on Mean±SEM. Lower case letters in each column indicate significant differences.

Discussion

In the present study, it was shown that the use of Shilajit both by gavage and rectal administration reduces the liver damage caused after ulcerative colitis by reducing liver enzymes, direct and total bilirubin and increasing serum albumin. Although receiving Shilajit by gavage method showed more protective effects than the group receiving Shilajit by rectal method in a way that receiving Shilajit by gavage method was able to bring the level of liver enzymes closer to their normal values in the sham group, which is probably due to the better absorption of Shilajit's active ingredients and the effects it probably has on intestinal secretions indirectly or due to its direct effect on the liver before the systemic effects of Shilajit in this method. In this study, the healing effects of Shilajit are comparable with sulfasalazine, which is a common drug in the treatment of colitis. Sulfasalazine can improve mild to moderate clinical symptoms in patients with Crohn's disease (21). This drug is associated with side effects, and patients who suffer from inflammation of the small intestine or have undergone surgery benefit less from this drug (22). Therefore, a

natural and safe alternative should be provided for this drug and its side effects. The anti-inflammatory effects of Shilajit in colon disease are related to the presence of dibenzo- α -pyrones along with humic acid and fulvic acid in its physiological structure (23, 24). In addition, several reports have proven the antioxidant effects of Shilajit, including Khaksari et al. who showed that this substance reduces oxidant factors in brain trauma (25). Joukar et al. reported that Shilajit reduces lipid peroxidation and increases antioxidant capacity in experimental myocarditis injury (26). Shilajit can also increase the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) in the striatum and frontal cortex of rats (27). In agreement with the present study, the hepatoprotective effects of Shilajit in alcohol-induced liver damage have been reported through the modulation of oxidative stress indicators (MDA, GSH, SOD) (28). Probably, the anti-inflammatory effect of Shilajit is exerted through the effect on antioxidant factors, except for the change in the amounts of cytokines (29). Furthermore, Jambi et al. showed that Shilajit extract can improve non-alcoholic fatty liver disease in male rats by reducing inflammation and modulating serum levels of IL-1 β , TNF- α , IL-10, and adipokine (30). In addition, bacteria or PAMPs have been reported to be one of the causes of colitis (31). The possibility that Shilajit with its antimicrobial property has reduced inflammation should also be considered. Studies have shown that Shilajit reduces sensitivity and inflammation by changing the shape and function of macrophages and increasing the resistance of mast cells to antibodies (32, 33).

In general, the results of the present study showed that the hepatoprotective effects of Shilajit in rats with ulcerative colitis caused by acetic acid are applied through the reduction of liver enzymes (SGPT, SGOT, ALP), direct and total bilirubin, and the increase of serum albumin. This study showed that oral administration of Shilajit has more protective effects on colitis-induced liver damage than rectal Shilajit. These findings may be a new therapeutic strategy for the treatment and prevention of liver damage caused by ulcerative colitis.

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References

1. Tegtmeyer D, Seidl M, Gerner P, Baumann U, Klemann C. Inflammatory bowel disease caused by primary immunodeficiencies-Clinical presentations, review of literature, and proposal of a rational diagnostic algorithm. *Pediatr Allergy Immunol*. 2017;28(5):412-29.
2. Amirkhosravi L, Khaksari M, Sheibani V, Shahrokhi N, Ebrahimi MN, Amiresmaili S, et al. Improved spatial memory, neurobehavioral outcomes, and neuroprotective effect after progesterone administration in ovariectomized rats with traumatic brain injury: Role of RU486 progesterone receptor antagonist. *Iran J Basic Med Sci*. 2021;24(3):349-59.
3. Alimahdi F, Shahrokhi N, Khaksari Hadad M, Asadikaram G, Abolhasani M. Effect of shilajit on the levels of pro-inflammatory and anti inflammatory cytokines in ulcerative colitis induced by acetic acid in male rats. *J Med Plants*. 2020;19(75):78-91. [In Persian]
4. Barnes EL, Loftus EV, Kappelman MD. Effects of Race and Ethnicity on Diagnosis and Management of Inflammatory Bowel Diseases. *Gastroenterology*. 2021;160(3):677-89.
5. Ionescu VA, Gheorghe G, Varlas VN, Stanescu AM, Diaconu CC. Hepatobiliary Impairments in Patients with Inflammatory Bowel Diseases: The Current Approach. *Gastroenterol. Insights*. 2023;14(1):13-26.
6. Jena G, Trivedi PP, Sandala B. Oxidative stress in ulcerative colitis: an old concept but a new concern. *Free Radic Res*. 2012;46(11):1339-45.
7. Scalavino V, Piccinno E, Bianco G, Schena N, Armentano R, Giannelli G, et al. The Increase of miR-195-5p Reduces Intestinal Permeability in Ulcerative Colitis, Modulating Tight Junctions' Expression. *Int J Mol Sci*. 2022;23(10):5840.
8. Maes M, Kubera M, Leunis JC, Berk M, Geffard M, Bosmans E. In depression, bacterial translocation may drive inflammatory responses, oxidative and nitrosative stress (O&NS), and autoimmune responses directed against O&NS-damaged neoepitopes. *Acta Psychiatr Scand*. 2013;127(5):344-54.
9. Juárez-Hernández E, Chávez-Tapia NC, Uribe M, Barbero-Becerra VJ. Role of bioactive fatty acids in nonalcoholic fatty liver disease. *Nutr J*. 2016;15(1):72.
10. Trivedi PP, Jena GB. Ulcerative colitis-induced hepatic damage in mice: studies on inflammation, fibrosis, oxidative DNA damage and GST-P expression. *Chem Biol Interact*. 2013;201(1-3):19-30.
11. Eltantawy N, Kassem AB, Elzayyadi IA, Elberry AA, Salah LM, Abdelrahim ME. Azathioprine and biological treatment genetic association with clinical response and toxicity in inflammatory bowel disease patients. *Bull Pharm Sci Assiut Univ*. 2023;46(1):305-30.
12. Cai C, Lu J, Lai L, Song D, Shen J, Tong J, et al. Drug therapy and monitoring for inflammatory bowel disease: a multinational questionnaire investigation in Asia. *Intest Res*. 2022;20(2):213-23.
13. Mikami Y, Tsunoda J, Suzuki S, Mizushima I, Kiyohara H, Kanai T. Significance of 5-Aminosalicylic Acid Intolerance in the Clinical Management of Ulcerative Colitis. *Digestion*. 2023;104(1):58-65.
14. Keshavarzi Z, Amiresmaili S, Shahrokhi N, Bibak B, Shakeri F. Neuroprotective effects of auraptene following traumatic brain injury in male rats: The role of oxidative stress. *Brain Res Bull*. 2021;177:203-9.
15. Ghezelbash B, Shahrokhi N, Khaksari M, Asadikaram G, Shahrokhi M, Shirazpour S. Protective Roles of Shilajit in Modulating Resistin, Adiponectin, and Cytokines in Rats with Non-alcoholic Fatty Liver Disease. *Chin J Integr Med*. 2022;28(6):531-7.

- 16.Heimann TM, Swaminathan S, Greenstein AJ, Steinhagen RM. Incidence and Factors Correlating With Incisional Hernia Following Open Bowel Resection in Patients With Inflammatory Bowel Disease: A Review of 1000 Patients. *Ann Surg.* 2018;267(3):532-6.
- 17.Ghaaazi Firozsalari F, Shahrokhi N, Khaksari Hadad M, Asadikaram G, Atashbar J. Effect of Shilajit on the Levels of Pro-inflammatory and Anti-inflammation Cytokines in Hepatic Injury in Male Rats. *J Mazandaran Univ Med Sci.* 2018;28(159):1-13. [In Persian]
- 18.Ran ZH, Chen C, Xiao SD. Epigallocatechin-3-gallate ameliorates rats colitis induced by acetic acid. *Biomed Pharmacother.* 2008;62(3):189-96.
- 19.Keshavarzi Z, Ashekar A, Vatanchian M, Abbaspour A, Bibak B, Behnamfar M, et al. The effect of woody endocarpium of walnut alcoholic extract on acetic acid-induced ulcerative colitis in rats. *Braz J Pharm Sci.* 2022;58:e19520.
- 20.Khodir AE, Said E. Nifuroxazide attenuates experimentally-induced hepatic encephalopathy and the associated hyperammonemia and cJNK/caspase-8/TRAIL activation in rats. *Life Sci.* 2020;252:117610.
- 21.Seyedian SS, Nokhostin F, Malamir MD. A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease. *J Med Life.* 2019;12(2):113-22.
- 22.Coward S, Kuenzig ME, Hazlewood G, Clement F, McBrien K, Holmes R, et al. Comparative Effectiveness of Mesalamine, Sulfasalazine, Corticosteroids, and Budesonide for the Induction of Remission in Crohn's Disease: A Bayesian Network Meta-analysis. *Inflamm Bowel Dis.* 2017;23(3):461-72.
- 23.Van Rensburg CE. The Antiinflammatory Properties of Humic Substances: A Mini Review. *Phytother Res.* 2015;29(6):791-5.
- 24.Shahrokhi N, Keshavarzi Z, Khaksari M. Ulcer healing activity of Mumijo aqueous extract against acetic acid induced gastric ulcer in rats. *J Pharm Bioallied Sci.* 2015;7(1):56-9.
- 25.Khaksari M, Mahmmodi R, Shahrokhi N, Shabani M, Joukar S, Aqapour M. The Effects of Shilajit on Brain Edema, Intracranial Pressure and Neurologic Outcomes following the Traumatic Brain Injury in Rat. *Iran J Basic Med Sci.* 2013;16(7):858-64.
- 26.Joukar S, Najafipour H, Dabiri S, Sheibani M, Sharokhi N. Cardioprotective effect of mumie (shilajit) on experimentally induced myocardial injury. *Cardiovasc Toxicol.* 2014;14(3):214-21.
- 27.Wilson E, Rajamanickam GV, Dubey GP, Klose P, Musial F, Saha FJ, et al. Review on shilajit used in traditional Indian medicine. *J Ethnopharmacol.* 2011;136(1):1-9.
- 28.Yadav SC, Govindasamy J, Ramnani R. Antioxidant and hepatoprotective activity of shilajit (asphaltum punjabinum) against alcohol induced liver injury in wistar rats. *Int J Ayur Pharma Res.* 2020;8(6):1-8.
- 29.Stohs SJ. Safety and efficacy of shilajit (mumie, moomiyo). *Phytother Res.* 2014;28(4):475-9.
- 30.Jambi EJ, Abdulaziz Alshubaily F. Shilajit potentiates the effect of chemotherapeutic drugs and mitigates metastasis induced liver and kidney damages in osteosarcoma rats. *Saudi J Biol Sci.* 2022;29(9):103393.
- 31.Erridge C, Duncan SH, Bereswill S, Heimesaat MM. The induction of colitis and ileitis in mice is associated with marked increases in intestinal concentrations of stimulants of TLRs 2, 4, and 5. *PLoS One.* 2010;5(2):e9125.
- 32.Rahmani Barouji S, Saber A, Torbati M, Fazljou SMB, Yari Khosroushahi A. Health Beneficial Effects of Moomiaii in Traditional Medicine. *Galen Med J.* 2020;9:e1743.

33.Lawley S, Gupta RC, Goad JT, Canerdy TD, Kalidindi SR. Anti-Inflammatory and Anti-Arthritic Efficacy and Safety of Purified Shilajit in Moderately Arthritic Dogs. J Vet Sci Anim Husb. 2013;1(3):302.