SUJMS
Sana'a University Journal of Medical Sciences

Original Article

Anti-Ulcerogenic Effects of Shilajit on Gastric Ulcer in Rats

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Abstract

Problem statement: To evaluate the effects and mechanisms of action involved in anti- ulcer activity of different native shilajit samples which were collected in the mountain region of Yemen (Al-Jouf and Rayma), Russia (Tien-Shan) and India (Kumoan). Stomach ulcers were induced in rats by oro-gastric ingestion of ethanol/HCl. Pre- treatment with ranitidine

(100 mg kg -1, p.o.) and shilajit samples (600 mg kg -1, p.o.) occurred for 14 days before the ulcer induction. Al-Jouf and Indian shilajit samples inhibit both ulcer score and lesion area by greater percentages than either ranitidine or other samples. Some of studied shilajit samples have anti- ulcer against induced gastric ulcer mightily due to combined mechanisms of shilajit's constituents, including hypolipidemic, antioxidant, anti-inflammatory, anti-stress, anti-anxiety, regenerative, repairing and healing mechanisms.

Key words: Anti-oxidant, ranitidine, anti-ulcer, fulvic acid, shilajit

INTRODUCTION:

Shilajit is a herbo-mineral, marine animal origin-dead/fossil invertebrates, semi-hard brownish black resin formed through long-term humification of several plant types, mainly bryophytes such as Euphorbia and Trifolium (clover) plants and lichen, present in the vicinity of shilajit-exuding rocks. This plant type of shilajit called Mumie-asil to being distinguished from other types (petroleum mumie and mumie- kiem). It has common names; vegetable asphalt, mineral pitch, mountain sweat, mountain oil, rock juice. The name ''mumie, hajar-musa or mumia'' was devised by the Arabs and in ancient Egypt, this wonderful resin was used for embalming mummies (1,2,3,4).

It is found in specific mountain regions of the world at altitudes between 0.6 and 5 km on the walls of caves, embedded in rocks or as rock exudates and can exceed 500 kg in weight; inKashmir, Afghanistan, Nepal, Bhutan, Pakistan, China, Tibet, Yemen, Asian parts of Russia and neighboring areas (5,4). Its samples from different regions of the world have similar physical properties and qualitative chemical composition but they differ in the ratio of individual components (6).

Shilajit humus consists of organic matter (60-80%), mineral matter (20-40%) and 5% trace elements. The main chemical components of shilajit are humic acid, fulvic acid, benzoic acid, benzoates andhigh concentration of vitamin A, B, C esters (7,1). Modern chemical analyses identified six new compounds named as shilajityl acetate, shilajitol, shilacatechol, shilaxanthone, shilanthranil and naphsilajitone along with pyrocatechol and their stereostructures (2).

It traditionally used for obstinate diseases including; tuberculosis, cervical lymphadenitis, diabetes mellitus-type I, digestive disorders, cough, obesity, hemorrhoids, Jaundice, poison begotten distempers and internal tumors (8).

Mucosal injury may occur when noxious factors "overwhelm" an intact mucosal defense or when the mucosal defense is somehow impaired (9). Ethanol induced gastric damage has been soon to be associated with depletion of gastric mucus breaking of the mucosal barrier, back diffusion of acid, increased gastric mucosal

permeability, leads to increasing leak of hydrogen ion from the lumen,decrease in the transmucosal electrical potential difference, changes in the mucosal blood flow, destruction of micro-vascular and nonvascular type of cells, mast cell degranulation, neutrophill mediated mucosal injury (release of oxygen free radicals, proteases and lysosomal enzymes, digestion of proteins and lipid peroxidation in cell membrane) and depletion of certain oxygen free radical scavenger (10).

Peptic ulcer being one of the most uncontrolled gastrointestinal disorders representing a major health hazards in terms of morbidity and mortality (11,12). Common antiulcer drugs include H2 receptor antagonists, proton pump inhibitors or cytoprotective agents such as sucralfate. Patients used these drugs suffering severe side effects likearrythmias, gynaecomastia, enterochromaffin like cell hyperplasia and hematopoietic changes (12).

The present study aimed to compare among shilajit samples of different native origin {Yemen (Al-Jouf and Rayma), Russia (Tien-Shan) and India (Kumoan)} in: gastro-protective activity against ethanol/HCl induced gastric ulcer in rat.

MATERIALS AND METHODS

Chemicals, solutions and equipments: Ranitidine powder as hydrochloride (Shiba Pharma, ceuticals and chemicals Mgf. Ltd. Yemen); ferrous sulfate (FeSO4); Carboxy-Methylcellulose 0.5%CMC) ;sodiu chloride (WAKO Pure Chemical Industries, Osaka, Japan); (Life Technologies Japan Ltd., Tokyo, Japan). Absolute ethanol (©Merk KGaA, Darmstadt, Germany); Company China); Hematoxylin/eosin, xylene and formalin; Mueller Hinton agar (Oxoid CM337, Basingstoke, Hampshire, UK). Drugs and chemicals were dissolved in analytical grade buffers and solvents and prepared upon usage. Stomach tube; Microtome, Rotary evaporator; mortar and pestle; wattmanfilterpaper; Petri-dishes; autoclave; Hiscotron homogenizer; electric balance (Sartorius AG, Gottingen, Germany, BP310S); centrifuge (HERNIE Z 400, Wehingen); UV/Visible spectrophotometer (Shimadzu); micropipette of different size ; deep freeze.Preparation and purification of shilajit samples: Shilajit was collected in the mountain region of Yemen (Al-Jouf and Rayma), Russia (Tien-Shan) and India (Kumoan). Purification of shilajit samples was done according to the principle of the solubility in distilled water as described by(13). All samples of shilajit were milled to very small pieces, then weighing 100 gm of each sample and transferred to the volumetric flasks, then, 500 mL of distilled water were added and flasks were putted on a shaker for 10 h at room temperature. After that, sample solutions were purified by filtration through 0.45-mm filter to remove the insoluble impurities. The extract was dried; 100 mL of each sample was placed in Rotary evaporator and evaporating the solvent by reducing the pressure to avoid the decomposition of shilajit constituents and purified samples were attained. For the biological experiments, the dried shilajit extract was suspended in a balanced salt solution and filtrated through a 0.2-mm filter.

Antiulcer activity:

Experimental animals: 72 healthy Waster Albino rats and 130 Swiss Albino mice of both sexes were obtained from Central Animal House of the Science College in Sana'a University. The animals were housed and fed with standard pellet diet and water ad libitum. Animals were allowed to acclimatize in the local laboratory for 1week prior to the experimentation. They were housed group wise in polypropylene cage (17 11 6) and kept underm controlled environmentalconditions (temperature: 22 2°C, humidity: 50-55%, natural light/dark cycle).

Coprophagy was prevented by keeping the animals in cages with grating as the floor. The protocol of the study was following the local institutional animal ethical committee, for the care and use of laboratory animals.

Experimental design: 72 Wister albino rat (weighing between 200-250 g) and 130 Swiss albino mice (weighing between 25-35 g) of either sex were used for antiulcer and toxicity studies respectively. Mice were divided into 13

groups of 10 animals (5 males and 5 females) and rats into 6 groups of 12 animals (6 males and 6 females). Fresh conventional antiulcer drug (Ranitidine) and shilajit solutions were prepared fresh at the time of administration. All animals were deprived of food at least 36 hr before start of either acute oral toxicity or intra-gastric ulcer induction experiment but allowed free access of water and returned to diet just after beginning of the toxicity experiment while killed later 4 h after intra-gastric induction experiment. All experiments were performed during light phase between 9.30 AM-3.30 PM.

Group designing for acute oral toxicity study: Toxicity study was undertaken on 130 adult Swiss albino mice using 300, 600 and 900 mg kg -1 of different 4 shilajit samples suspended in 1% Carboxy Methyl Cellulose (CMC) which administered (p.o.) daily for 10 days (13 group; 10 mice for each, saline group, 3 groups for each shilajit sample) and animals receiving saline 0.9% served as control. The signs and symptoms associated with the shilajit administration were observed at 0, 30, 60, 120, 180 and 240 min after and then once a day for the next 10 days. At the end of the period, the number of survivors were recorded and the acute toxicological effect was estimated through the method described by(14) to find out the safe dose.

Group designing for ethanol/HCL induced ulcer models: 72 rats were divided into six groups (12 rats for each). Saline group; animals received only one dose of saline (1mL of 0.9% saline 200g -1), Ranitidine group; animals received one dose of Ranitidine (100 mg kg -1, suspended in 1 mL of 1% CMC) and Shilajit groups; animals received one dose of Russian, Indian, Al-Jouf, Rayma shilajit samples respectively (600 mg kg -1, suspended in 1 mL of 1% CMC) per os dialy for 14 days and until 1 hour before ulcerogenic procedure. On day 14, rats were fasted (except for water and treatments) for 36 h before oro-gastric intubation (on day 15) of (0.1 mL 20g -1) of a HCl/ethanol mixture, containing 150 mM HCl in 98% ethanol (15) for each rat 1 hr after respective dosing.

Morphological examination of stomach: Stomachs were removed, placed on ice-cold normal saline, opened along the greater curvature, washed with normal saline and observed for the severity of the ulcers by measuring both gastric lesion Ulcer Index (ULI) and Ulcer Score (US). Ulcers of the gastric mucosa appear as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach. The mucosal layer of stomach was observed under magnifying lens and was checked for ulcers, hemorrhagic areas perforations. The length and width of the ulcer (mm) were measured by a planimeter (10×10 mm2). The ulcerated area was measured by counting the number of small squares, 2×2 mm, covering the length and width of each ulcer band. The sum of the areas of all lesions for each stomach was applied in the calculation of ULI. The ulcer index was determined as ULI = 10/X Where X= Total area of stomach mucosa / Total ulcerate area) (16).

Evaluation of gastric mucosa for the presence of gastric ulcer using ulcer score; the severity of hemorrhagic section in the acid secreting glandular mucosa was done on a scale of 0-5 score [0= No erosion, 1= Normal, 2= Hyperemia or thinness, 3= Superficial mucosal erosion, 4 = Deep ulcer or transmascular necrosis and 5= Perfusion] as described by(17).

Histological examination of stomach: Stomach portion of each rat was used for histological examination. Gastric tissue samples were fixed in neutral buffered formalin for 24 h. Stomach sections (three for each group) were dehydrated with graded ethanol, passed through xylene and embedded in paraffin. The three paraffin sections (of 5 µm in thickness of each pretreated group were stained with Hematoxylin/Eosin (HE) staining and the slides were viewed under a light microscope equipped for photography (18).

Table 1: Comparison of both ulcer score and gastric lesion index among different studied groups in percentage of inhibitions

Treatment (one dose daily for 14 day) (in mm)	Ulcer score (0-5)	Percentager of Inhibition	Gastric lesion index	Percentager of Inhibition
Saline (1mL 200g^{-1} day^{-1})	4.08+0.23	---	23.50±0.74	---
Ranitidine (100 mg Kg^{-1}day^{-1})	1.33±0.36$^{***.}$	67.43	2.08±0.26***	91.15
Russian (600 mg Kg^{-1}day^{-1})	1.92±0.23$^{***.\bullet}$	53.07	6.42±0.92$^{***.\bullet\bullet}$	72.72
Indian (600 mg Kg^{-1}day^{-1})	1.17±0.27$^{***.}$	71.44	3.00±0.44***	87.23
Al-Jouf (600 mg Kg^{-1}day^{-1})	0.42±0.15$^{***.\bullet}$	89.81	1.83±0.24***	92.21
Rayma (600 mg Kg^{-1} day^{-1})	1.33±0.22$^{***.}$	67.35	4.67±0.66$^{***.\bullet\bullet}$	80.17

The statistical data were expressed as Mean S.E.M., number =12 rat for each group. Ulcer Score represented as follow; 0= no erosions, 1= 1-3 small erosion, 2= more than 3 small erosion or one large erosion, 3= one large erosion and more than 3 small erosion, 4= 3-4 large erosion and 5= any very large erosion or ulcer perfusion. or = $p<0.05$; or = $p<0.01$;or = $p<0.001$. = compare among different groups vs. saline group while= compare among different groups vs. ranitidine group Morphological findings of stomach: In the present study oral administration of ethanol/HCl produced severe ulceration. All shilajit and ranitidine treated groups were inhibit both ulcer score and gastric lesion index in various percentages when compared to saline pretreated group. In particular, Al-Joufs hilajit pretreated group showed a greater inhibition of both ulcer score and gastric lesion index than ranitidine pretreated group as shown in Table 1 and Fig. 1A and 3B…respectively.

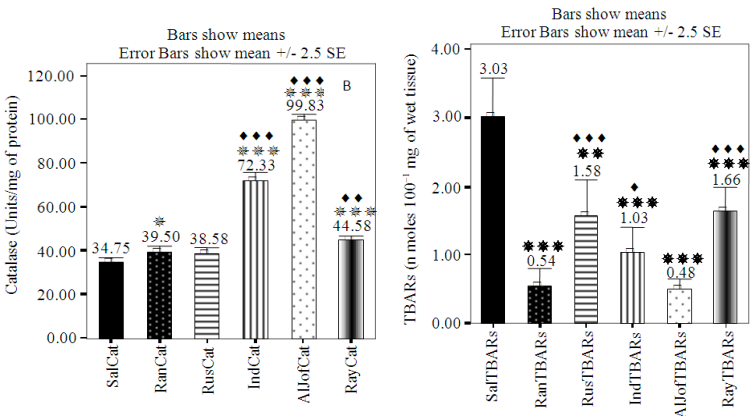
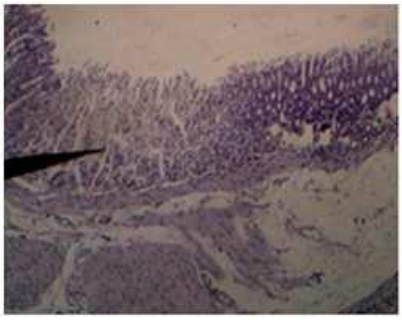
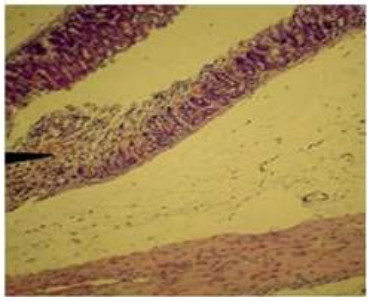
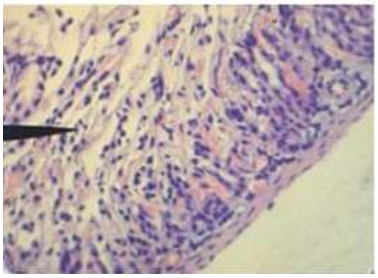


Fig. 1: Effects of shilajit samples (600 mg kg -1) and ranitidine (100 mg kg -1) pretreatment on both gastric ulcer score (A) and lesion index (B) in rats with ethanol/HCl-induced gastric ulcer. It showed significant decrease in both ulcer score and lesion index with studied treatments than saline treated group, especially in Al-Jouf shilajit treated groups







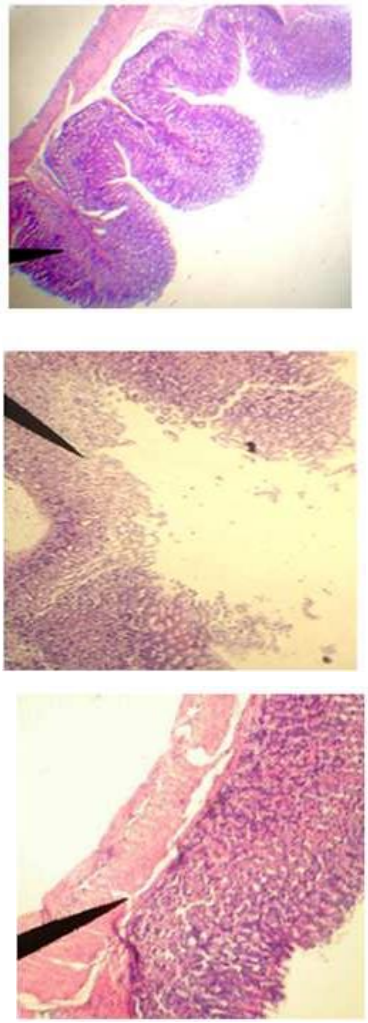


Fig. 2: Histological section of gastric mucosa in a rat pre-treated; (A) with saline (1 mL 200g -1): there is mucosal lesion, severe disruption to the surface epithelium, deep hemorrhagic erosions and edema of the sub-mucosa layer with leucocyte infiltration, (B) with Russian shilajit (600 mg kg -1): there is severe disruption to the surface epithelium, deep hemorrhagic erosions and edema of the sub-mucosa layer with leucocyte infiltration, (C) with Rayma shilajit (600 mg kg -1): there is severe disruption to the surface epithelium, hemorrhage, degenerative changes, (D) with Ranitidine (100 mg kg -1): there is mild disruption to the surface epithelium mucosa, immune response, with no edema and no leucocytes infiltration of the sub-mucosal layer, (E) with Indian shilajit (600 mg kg -1): there is a disruption and superficial hemorrhagic erosions to the surface epithelium, no edema, no immune response and no leucocytes infiltration of the normally intact sub- mucosal layer and (F) with Al-Jouf shilajit (600 mg kg -1) there is a normal gastric layer with no disruption to the surface epithelium, no edema, no immune response and no leucocytes infiltration of the sub-mucosal layer (H and E stain)

DISCUSSION

Shilajit is generally considered safe in moderate doses and is readily available in the United States both as a stand-alone product and in the traditional formula. It has also compounded in many patent medicines from India (19). It is useful in cases of gastrointestinal tract (20), digestive disorders, wound healing and stomach ulcer (11).

Toxicity study: Only shilajit doses of 900 mg kg-1 slightly increase body temperature of the mice and 4 mice dead of total 40 mice for that dose of different shilajit samples and no acute toxicity or macroscopic changes in daily body or organ weights were observed using shilajit at dose 600 mg kg -1. The current results in agreement with(2) whom reported that shilajit may be utilized safely in clinical practice because shilajit is reported to be quite safe up to a dose of 3g kg -1 (p.o) and 1g kg -1 (intraperitoneal injection) in mice (24 h mortality).

Antiulcer activity: The gastric mucosa is constantly exposed to potentially noxious stimuli of endogenous (acid, pepsin, bile) and exogenous (alcohol, drugs) origin. It is commonly believed that the tolerance of the gastric mucosa to damage, originates from continuously operating defensive mechanisms, whichinclude mucosal blood flow, mucus and bicarbonate secretion and gastric mucosal potential difference. It is generally accepted that it results from an imbalance between aggressive factors (such as acid, pepsin) and the maintenance of the mucosal integrity through the endogenous defense mechanism (21). Generally, shilajit has an expected broad biochemical and pharmacological activities due to its contents of fulvic acid and other antioxidant materials. Fulvic Acids (FA)

has been taken orally as a therapy for gastritis, stomach ulcers and colitis (1,4).

Morphological and histological findings of stomach: Peptic ulcer occurs due to imbalance between offensive (acid-pepsin secretion. H.pylori, bile, increased free radicals and decreased antioxidants) versus impaired mucosal resistance (mucus, bicarbonate secretion, prostaglandins, blood flow and the process of restitution and regeneration after cellular injury) (11,12).

The current study revealed that oro-gastric ethanol/HCl intubation for only one day resulted in production of gastric mucous membrane erosions evidenced macroscopically by significant rise in erosion scores and microscopically by areas of superficial and deep hemorrhagic erosions, necrosis of gastric mucous membrane in saline pretreated group. This is in accordance with the other studies using ethanol- induction of peptic ulcer (22).

Both ulcer score and gastric lesion index data showed that both Al-Jouf and Indian shilajit pretreated samples had a greater inhibition % on both factors than either Russian or Rayma shilajit pretreated samples when compared to either Ranitidine or saline pretreated groups. We assume as mentioned above that this difference in activity of shilajit samples was due to a difference in their constituent %, pH values and geographical origin of each sample.The presence of polyphenols compounds such as fulvic acids, 4-methoxy-6-carbomethoxybi-phenyl, tirucallane-type triterpenoids and benzoic acid in shilajit samples have antioxidant activity, cellular repairing and regeneration, played very important role in decrease acid pepsin secretion, cell shedding, gastric ulcer index, tendency to increase mucin secretion and carbohydrate/protein ratio which have very important role as anti-oxidant effect and anti-inflammatory as reported by(7,23,11,24,25,26,2) so they stated its use in gastric ulcer and wound healing.

In context, anti-anxiety activity and anti-stress effects of shilajit has a role in healing of gastric ulcer as stated by (19) whom indicate that shilajit has significant anxiolytic and anti-stress activity as reported by). Due to bacteriostatic and anti- inflammatory action, shilajit extract facilitates the process of wound cleaning from necrotic tissues, granulation and epithelization and decreases the period of wound healing (27,28). Shilajit is truly a remarkable substance with a long history of human usage for healing and should be subjected to further investigations (19).

CONCLUSION

Then, we could report that all studied shilajit samples in varying degrees have antiulcer, regenerative and repairing effects on ethanol/HCl induced ulcer in rats. The antiulcer effect of shilajit especially Al-Jouf and Indian shilajit samples might be due combined biochemical effects including antioxidant, antimicrobial, anti-inflammatory, anti- stress, anti-anxiety, healing and regenerative effects. However, further investigations are required for its exact mechanism of action before many of shilajit actions can be affirmed.

ACKNOWLEDGEMENT

We would like to thank technician staff in histology department of Medicine College, Central animal house in Science College and Animal ethical committee, of Sana'a University, Yemen for their help during the experiments.

Funding: There is no any funding for the current study.

Conflict of interest: The authors declare that there are none conflict of interest.

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