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Original Article

EFFECT OF SHILAJIT ON EXPERIMENTAL MODELS OF INFLAMMATORY BOWEL DISEASE IN RATS

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ABSTRACT

Objective: Inflammatory bowel disease (IBD) is a chronic condition of the intestine with unknown etiology involving multiple immunes, genetic and environmental factors. Oxidative stress is believed to be a key factor in the pathogenesis and perpetuation of the mucosal damage in IBD. The present study is to elucidate the effects of *shilajit* extract on the extent and severity of enterocolitis induced by subcutaneous administration of Indomethacin (7.5 mg/kg b. w) in Wistar rats.

Methods: Study comprised of 6 groups (n=6), normal vehicle control, indomethacin-induced (7.5 mg/kg, 2days), *shilajit* alone 50 mg/kg b. w, *shilajit* treated groups (25 and 50 mg/kg, p. o) and sulfasalazine treated (100 mg/kg, p. o) groups. Drug treatment continued for 11 d and on 12th d scarification was done. The colonic mucosal injury was assessed by macroscopic scoring, biochemical (LDH, MPO, GSH and LPO) tests were performed.

Results: Pretreatment with *shilajit* showed a decrease in macroscopic scores, LDH, MPO, LPO and elevation levels of GSH as compared to the indomethacin-treated group.

Conclusion: The present study suggests that the protective effect of *shilajit* in indomethacin-induced enterocolitis might be attributed to its scavenging effect on oxygen-derived free radicals and may be beneficial in patients with inflammatory bowel disease.

Keywords: Inflammatory bowel disease, *Shilajit*, Indomethacin, Subcutaneous

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic recurrent disease characterized by intestinal mucosal inflammation and includes ulcerative colitis (UC) and Crohn's disease (CD). The pathogenesis of IBD is a multifactorial process [1]. UC and CD are the two major phenotypes. These diseases have a great impact on the quality of life of the affected persons and their families. UC is characterized by confluent mucosal inflammation of the colon starting at the anal verge and extending proximally for a variable extent (*e. g.,* proctitis, left-sided colitis, or pancolitis). CD, by contrast, is characterized by transmural inflammation of any part of the gastrointestinal tract but, most commonly in the area adjacent to the ileocecal valve. Medical therapy for IBD is challenging because no unique abnormality has been identified. For many years Glucocorticoids and Sulfasalazine (SLZ) were the mainstays of medical therapy for IBD2. Infliximab, a monoclonal antibody against TNF-α has been proved highly effective in the clinical management of both forms of IBD. Considering oxidative stress is one of the factors in IBD, antioxidants could be likely to provide relief [2].

Shilajit is a herbo-mineral drug, blackish-brown exudation, obtained as a mineral resin or as a plant fossil composed of humus and organic plant material that has been compressed by layers of rock mixed with microbial metabolites [3]. It is found in the serene surroundings of Himalayas. The common names are vegetable asphalt, mineral pitch, mountain sweat, mountain oil, rock juice. *Shilajit* is an important drug of the ancient Hindu *materia medica* and is extensively used by the Hindu physicians to treat a variety of diseases. It is said to be efficacious against phthisis, chronic bronchitis, asthma, digestive troubles, sexual and bladder calculi, dropsy, nervous diseases, leprosy, diabetes, and fracture of bones. It is also used in parasitic diseases of the skin and as an antiphlogistic. The biologically important classes of compounds of *shilajit* include dibenzo-alpha pyrones, phospholipids, triterpenes and phenolic acids of low molecular weight, humins and humic acids, fulvic acids which are known as "carrier molecules" and trace elements (Fe, Ca, Cu, Zn, Mg, Mn, Mo, P) [4].

Shilajit has been reported for its anti-ulcerogenic and anti-inflammatory, antioxidant, antidiabetic, memory enhancement, anxiolytic, antistress, immunomodulatory and anti-allergic activity [5]. Although the majority of natural drugs are derived from plant and animal origins, a few of them, obtained from mineral sources, like *shilajit,* are of paramount significance as pharmaceutical aids. No scientific data regarding the activity of *shilajit* on IBD is available. Hence, the present study was designed to evaluate the effect of *shilajit* on experimental models of IBD in rats. The objective of the study is to investigate the effect of *shilajit* for treating IBD in rats by using indomethacin-induced enterocolitis by estimating macroscopic sores and enzyme markers such as lactate dehydrogenase (LDH), myeloperoxidase (MPO), lipid peroxidase (LPO), glutathione (GSH) and compare effect of shilajit with that of standard drug i.e. Sulfasalazine (SLZ) in the above model.

MATERIALS AND METHODS

Albino Wistar Rats of either sex weighing 150-200 g were used for the present study. The animals were collected from animal house. The animals were maintained under controlled conditions of temperature (22±2 °C), humidity (50±5%) and 12h light-dark cycles. All the animals were acclimatized for seven ds before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. Animals were habituated to laboratory conditions for 48 h prior to the experimental protocol to minimise if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Sree Siddaganga College of Pharmacy, Tumkur, Karnataka. Approval No. SSCPT, IAEC, Clear 110/2011-12 dated 30/11/2011, according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Preparation of suspension

Shilajit was dissolved in distilled water and SLZ was suspended in distilled water using sodium carboxymethyl cellulose (sodium CMC, 0.3%). Both these suspensions were administrated orally to the animals with the help of an intragastric catheter.

Experimental methods

The male Wistar rats (200-250 gm) were selected and randomized in to following groups [6]. Small intestinal inflammation was induced by two subcutaneous injections of Indo (7.5 mg/kg, s. c) spaced 24h apart. The study comprised of six different groups each consisting of six animals as follows:

Group I: Normal control animals. (Vehicle control)

Group II: Animals received only Indo (7.5 mg/kg, s. c.) on two consecutive ds. (Positive control)

Group III: Received pretreatment with *shilajit* (50 mg/kg bw. p. o) till 11th d.

Group IV: Received pretreatment with *shilajit* (25 mg/kg bw. p. o), followed by Induction with Indo (7.5 mg/kg, s. c) on 8th and 9th d of treatment.

Group IV: Received pretreatment with *shilajit* (50 mg/kg bw. p. o), followed by induction with Indo (7.5 mg/kg, s. c) on 8th and 9th d of treatment.

Group V: Received SLZ (100 mg/kg b.w., p. o) for 7 d and followed by induction with Indo (7.5 mg/kg, s. c) on 8th and 9th d of treatment.

Drug treatment will be continued till 11th d. On the 12th d after treatment, animals were anaesthetized with ether and blood was collected by retro-orbital puncture. Later, the animals were sacrificed by cervical dislocation and dissected open to remove the ileum portion. Ileum was flushed gently with saline and cut open. Inflammation was assessed based on the macroscopic scores [7] and quantification of inflammation was done by the assay of serum Lactate Dehydrogenase [8] (LDH) and tissue Myeloperoxidase activity [9] (MPO), GSH [10] and LPO [11].

RESULTS AND DISCUSSION

Table 1: Effect of *Shilajit* pre-treatment on macroscopic score, LDH and MPO in Indo induced enterocolitis in rats

Treatment	Macroscopy (Scoring of ulcers)	LDH (U/l)	MPO activity (U/g)
Normal Control	1.360±0.0678	1869±43.95	1.670±0.1640
Indomethacin Alone (7.5 mg/kg, s. c)	7.225±0.2153###	2444±41.17###	8.390±0.2313###
Shilajit Alone (50 mg/kg, p. o)	1.900±0.0948	1864±44.87	4.778±0.4489
Indo+Shilajit (25 mg/kg, p. o)	6.060±0.4020*	2175±71.89*	6.433±0.4326*
Indo+Shilajit (50 mg/kg, p. o)	3.975±0.1019***	1806±12.73***	3.630±0.2812***
Indo+SLZ (100 mg/kg, p. o)	3.325±0.1392 ***	1756±40.90***	2.715±0.2360 ***

Values are expressed as mean±SEM, n=6. Values of Macroscopic Score are expressed as Points, while LDH is expressed as units/liter and MPO activity as U/g. ### p<0.001 significantly different from normal control group. * p<0.05, ** p<0.01 and ***p<0.001 significantly different from Indo group. One-way ANOVA followed by Tukey's post test

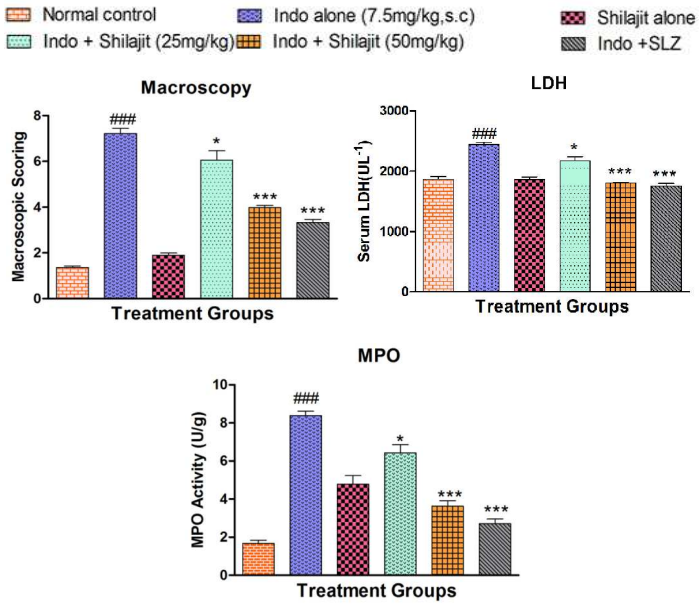

Fig. 1: Effect of *Shilajit* and SLZ on Macroscopic score, LDH and MPO results in rats treated with Indo. Each bar represents mean±SEM, n=6. ### p<0.001 significantly different from normal group. * p<0.05 ** p<0.01 and *** p<0.001 significantly different from Indo treated group

Table 2: Effect of *Shilajit* and SLZ on small intestine levels of GSH and LPO content in rats treated with Indomethacin

Treatment	Glutathione (µmol/mg of protein)	Tissue LPO (nmol/g of protein)
Normal Control	403.0±30.57	0.2793±0.0169
Indo Alone (7.5 mg/kg, s. c)	141.5±6.118 ###	0.6830±0.0419###
Shilajit Alone (50 mg/kg, p. o)	386.6±18.85	0.3103±0.0271
Indo+Shilajit (25 mg/kg, p. o)	304.3±11.21*	0.5130±0.0459*
Indo+Shilajit (50 mg/kg, p. o)	415.7±22.94***	0.4253±0.0348***
Indo+SLZ (100 mg/kg, p. o)	490.2±61.45***	0.4025±0.0110 ***

Values are expressed as mean±SEM, n=6. ### p<0.001 significantly different from NC group. * p<0.05, ** p<0.01 and *** p<0.001 significantly different from Indo group. One-way ANOVA followed by Tukeys post test

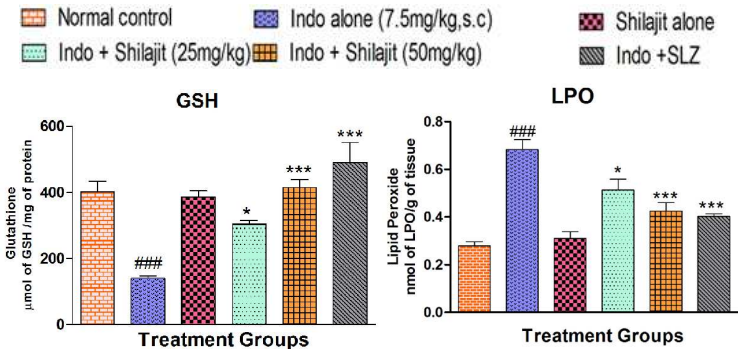

Fig. 2: Effect of *Shilajit* and SLZ on GSH and LPO results in rats treated with Indo. Each bar represents mean±SEM, n=6. ### p<0.001 significantly different from normal group. * p<0.05, ** p<0.01 and *** p<0.001 significantly different from Indo treated group

RESULTS AND DISCUSSION

The *shilajit* and SLZ treated groups showed lower scores compared to Indo alone treated group. An elevation of LDH in serum indicates a shift towards anaerobiosis resulting in the enhanced production of lactic acid. In the present study, serum LDH levels were significantly elevated due to Indo administration compared to normal animals. Pre-treatment with *shilajit* and SLZ inhibited the elevation of serum LDH level.

MPO is a biochemical marker of neutrophil infiltration in the damaged tissue. In Indo alone treated animals, the MPO activity was significantly elevated. This increase in MPO activity was substantially attenuated in rats pre-treated with *shilajit* and SLZ. (table 1 and fig. 1)

Antioxidants constitute the foremost defence system that limits the toxicity associated with free radicals. The equilibrium between antioxidants and free radicals is an important process for the effective removal of oxidative stress in intracellular organelles. However, in pathological conditions like enterocolitis, the generation of ROS, lipid peroxidation can dramatically upset this balance with an increased demand on the antioxidant defence system. Present study shows Indo causes increased levels in lipid peroxidation while GSH levels decreased. Pre-treatment of rats with *shilajit* significantly afforded protection against Indo induced increase of intestinal MDA contents. While the antioxidant power of cell such as GSH content was significantly preserved. (table 2 and fig. 2) The protective effect of *shilajit* is associated with its antioxidant properties, as it acts as ROS scavenger and an inhibitor of lipid peroxidation.

CONCLUSION

In conclusion, the present study demonstrates that *shilajit* possess protective effects against Indomethacin-induced enterocolitis and the results may be comparable to that of Sulfasalazine. This protective effect may, at least in part, due to its anti-inflammatory and/or antioxidant actions.

CONFLICT OF INTERESTS

Declare none

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