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Research Article

Effect of shilajit on acetic acid induced inflammatory bowel disease in rats

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ABSTRACT

One of the chronic conditions of intestines is Inflammatory bowel disease (IBD).The etiology of IBD is unknown but involves multiple immune, genetic and environmental factors. Mucosal damage in IBD is mainly due to oxidative stress. The main goal is to study the effects of *shilajit* extract on the ulcerative colitis induced by intrarectal acetic acid administration (2ml of 4% v/v) in Wistar rats. Study comprised of 6 groups (n=6), normal vehicle control, acetic acid induced (2ml of 4% v/v, on eighth day), *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 days and on 12th day scarification was done. The mucosal injury was assessed by macroscopic scoring, biochemical (LDH, MPO, GSH, LPO) tests were performed. Pre-treatment with *shilajit* showed a decrease in macroscopic scores, LDH, MPO, LPO and elevation levels of GSH when compared to acetic acid group. The present study says that, the protective effect of *shilajit* in ulcerative colitis induced by acetic acid might be attributed to its neutralising effect on oxygen derived free radicals and may be more useful for patients suffering from inflammatory bowel disease.

Keywords: Acetic acid; inflammatory bowel disease; *shilajit;* Intra rectal.

INTRODUCTION

One of the recurrent chronic disease of intestine is inflammatory bowel disease (IBD) and is characterized by intestinal mucosal inflammation which includes both ulcerative colitis (UC) and Crohn's disease (CD). The pathogenesis of IBD is a multifactorial process (Scaldaferri F, Fiocchi C., 2007). UC and CD are considered as major phenotypes. Appearance of confluent mucosal inflammation at the anal verge of colon is the characteristic feature of UC and extends proximally to an extent (*e.g.,* proctitis, left-sided colitis, or pancolitis). On contrary, CD's characteristic feature is appearance of transmural inflammation of any part of the gastrointestinal tract commonly in the adjacent area to the ileocecal valve. Medical therapy is challenging for IBD, because no unique treatment has been identified. For long years Glucocorticoids and Sulfasalazine (SLZ) served the purpose of medical therapy for IBD. Infliximab which is a monoclonal antibody effective against TNF-α was proved to be highly effective in the management of both forms of IBD. Considering one of the factors in IBD is oxidative stress, antioxidants could be likely to provide relief (Hanan HH, Azza EM., 2007).

Shilajit is a herbo-mineral drug, blackish brown exudation, obtained as a mineral resin or as a plant fossil consists of organic plant material and humus which was compressed by layers of rock mixed with metabolites of microbes (Ghosal S et al., 1991 & Nadkarni KM., 1976) . It is found in the serene surroundings of Himalayas. The common names are mineral pitch, vegetable asphalt, mountain oil, mountain sweat, rock juice. *Shilajit* is one of the most important drugs of the ancient Hindu book *materia medica* and is widely used by the Hindu physicians to treat wide variety of diseases. It is said to be effective against chronic bronchitis, phthisis, digestive troubles, asthma, sexual & bladder calculi, nervous diseases, dropsy, diabetes, leprosy, and bone fractures. It is also used as an antiphlogistic and in skin parasitic diseases. The important classes of compounds of *shilajit* include phospholipids, dibenzo-alpha pyrones, humins and humic acids, triterpenes and phenolic acids of low molecular weight, fulvic acids which are known as "carrier molecules" and trace elements such as (Ca, Fe, Mg, Zn, Cu, Mo, Mn, P) (Ghosal S.,1990).

Shilajit has been reported for its anti-ulcerogenic and anti-inflammatory, antioxidant, anti-diabetic, memory enhancement, anxiolytic, anti-stress, immunomodulatory and anti-allergic activity (Agarwal SP et al., 2007). *Shilajit* is obtained from mineral sources and has got a greater significance as pharmaceutical aids (Kokate CK et al., 2008). No scientific data was available regarding the activity of *shilajit* on IBD. Hence, the present study was to evaluate the effect of *shilajit* on experimental models of IBD in rats.

OBJECTIVE

The main objective of the study is to investigate the effect of *shilajit* for treating IBD in rats by using acetic acid induced ulcerative colitis by estimating

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Table 1: Effect of Shilajit pre-treatment on Colon weight/length, spleen weight & macroscopic score in AA induced UC in rats

Treatment	Colon weight to length ratio	Spleen weight (g)	Macroscopic score
Normal Control	0.0710 ± 0.0035	1.103 ± 0.1441	0.500 ± 0.2887
Acetic acid Alone (4% v/v)	0.1863 ± 0.0030 ###	2.079± 0.0350 ###	8.548 ± 0.2244 ###
A.A + Shilajit (25 mg/kg, p.o)	0.1818± 0.0061*	0.6200± 0.0932**	7.443± 0.2794**
AA + Shilajit (50 mg/kg, p.o)	0.1498± 0.0157***	0.3700± 0.0997***	3.638± 0.1754***
AA + SLZ (100 mg/kg, p.o)	0.1444± 0.0263 ***	0.3775± 0.0843***	2.763± 0.2426 ***

Values are expressed as mean±SEM, n=6. Values of Macroscopic Score, Spleen weight and Colon weight/length (mg/cm). ### p< 0.001 significantly different from NC group. * p<0.05, ** p< 0.01 and ***p< 0.001 significantly different from AA group. One-way ANOVA which is followed by Dunnet's test.

Table 2: Effect of Shilajit pre-treatment on LDH, MPO, GSH and LPO in UC induced by AA in rats.

Treatment	LDH (U/L)	MPO activity (U/g)	Tissue LPO (nmol /g of protein)	Glutathione (µmol /mg of protein)
Normal Control	848.1± 41.26	1.753± 0.1925	4.323± 0.3120	1349±47.86
Acetic acid Alone (4% v/v)	1138± 27.99###	6.363± 0.4373###	9.000± 0.3342###	962.3±29.78###
A.A + Shilajit (25 mg/kg, p.o)	957.0± 37.28*	5.115± 0.1960*	6.920± 0.2331**	1151±19.29*
AA + Shilajit (50 mg/kg, p.o)	801.3± 15.85***	2.768± 0.1722***	3.998± 0.2612***	1253±49.31***
AA + SLZ (100 mg/kg, p.o)	785.4± 42.97***	1.913± 0.2004***	2.825± 0.3935***	1317±32.37***

Values are expressed as mean±SEM, n=6. Values of LDH are expressed as units/liter, MPO activity expressed as U/g, LPO expressed as nmol /g of protein & GSH as µmol /mg of protein. ### p< 0.001 significantly different from NC group. * p<0.05, ** p< 0.01 and ***p< 0.001 significantly different from AA group. One-way ANOVA which is followed by Tukeys post-test.

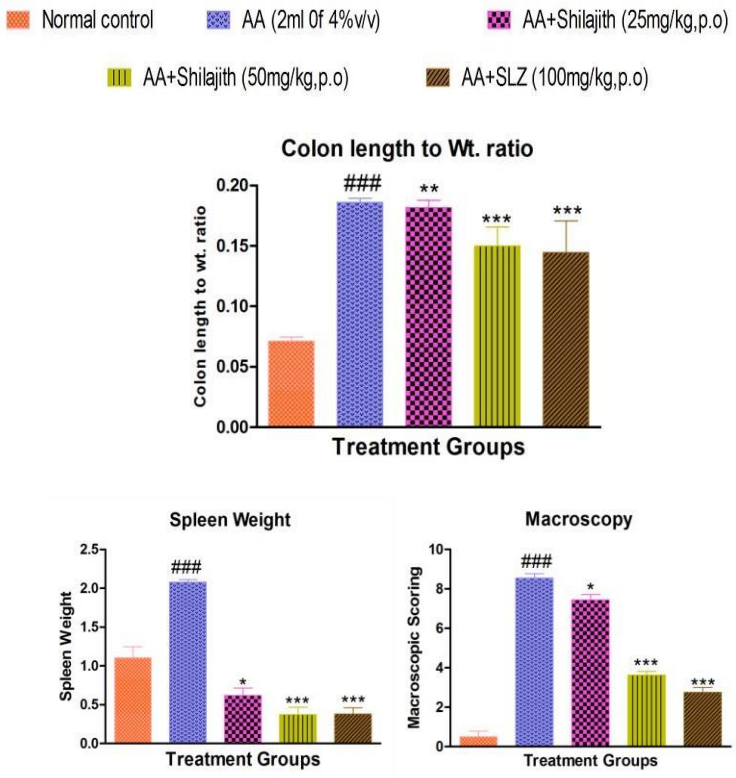

Figure 1: Effect of Shilajit and SLZ on Colon weight/length, Spleen weight and Macroscopic score results in rats treated with AA. Each bar represents mean±SEM, n=6. ### p<0.001 significantly different from normal group. * p<0.05, ** p<0.01 and *** p<0.001 significantly different from AA treated group.

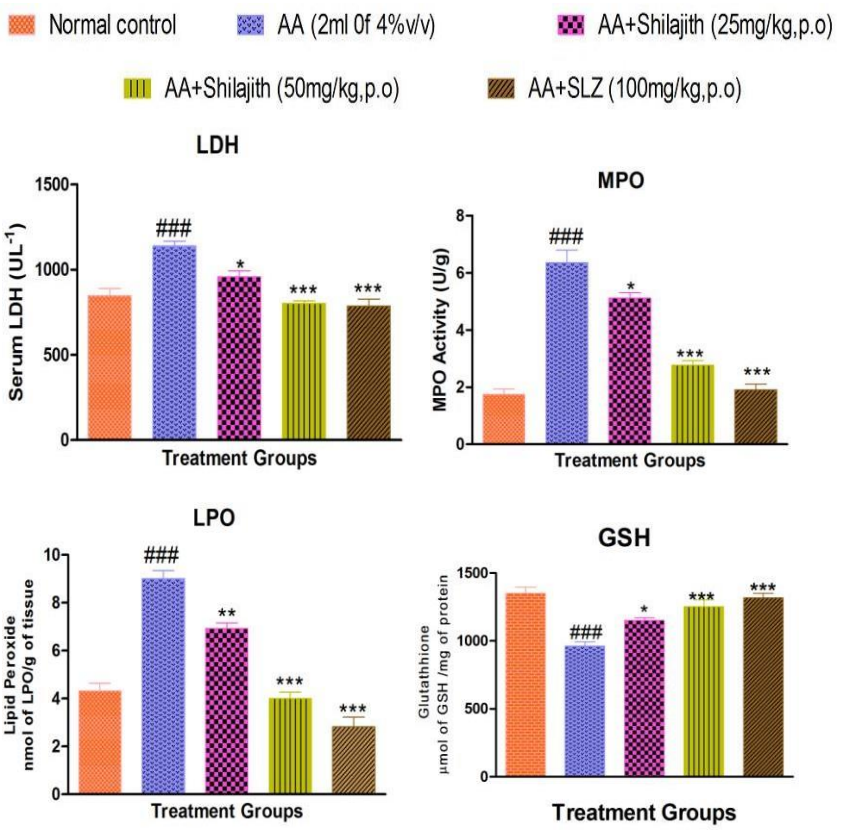

Figure 2: Effect of Shilajit and SLZ on LDH, MPO, GSH & LPO results in rats treated with AA. Each bar represents mean ± SEM, n=6. ### p<0.001 significantly different from normal group. * p<0.05, ** p<0.01 and *** p<0.001 significantly different from AA treated group.

macroscopic sores, weight loss, and enzyme markers such as glutathione (GSH), lipid peroxidase (LPO), lactate dehydrogenase (LDH), myeloperoxidase (MPO), 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 ± 2^0C), humidity (50 ± 5%) and 12h light-dark cycles. All the animals were acclimatized for seven days 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 experimental protocol to minimize 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 and 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 sodium carboxy methyl cellulose (sodium CMC, 0.3%) using distilled water. These suspension was administrated orally to the animals with the help of an intra gastric catheter.

Experimental study design

Male Wistar rats (200-250 g) were used for the study. Rats were anaesthetized by using ether followed a 24 h fast, and then a polyurethane canal of medical grade is used for enteral feeding & was inserted into the anus and the tip advanced to 8 cm proximal to the anus verge. 2 ml of AA (4% v/v in 0.9% saline) was instilled into the colon through the cannula for 30 seconds, after which fluid was withdrawn (Elson CO et al., 1995 & Millar AD et.al., 1996).

Study comprised of five different groups with 6 animals each & as follows:

Group I: Vehicle control group

Group II: AA control group, received 2ml of 4% v/v AA intrarectally on the 8th day (Positive control)

Group III: *shilajit* (25 mg/kg) at low dose treated animals, received pretreatment with 25 mg/kg of *shilajit,* p.o and 2ml of 4% AA, intrarectally on the 8th day. Drug treatment was continued till the 11th day.

Group IV: *shilajit* (50 mg/kg) at high dose treated animals, received pretreatment with 50 mg/kg of *shilajit,* p.o and 2ml of 4% AA, intrarectally on the 8th day. Drug treatment was continued till the 11th day.

Group V: SLZ (100mg/kg) treated group, received pretreatment with 100 mg/kg of SLZ, p.o and 2ml of 4% AA, intrarectally on the 8th day. Drug treatmentwas continued till the 11th day.

After 72 h of AA treatment, animals were anaesthetized by using ether and blood was collected by puncturing retro orbitals. By following cervical dislocation method, animals were sacrificed and dissected for colon removal. Saline was used to flush colon and weighed. The lowest part of distal colon (2cm) was cut and used for colonic contractility studies. The remaining portion of colon was opened by longitudinal section cut and scored for inflammation based on the macroscopic features. The assay of serum LDH and tissue MPO was done to quantify inflammation (Gwlvan G, Saltman P., 1990).

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 an increased anaerobiosis which results in the elevated production of lactic acid. In the present study, serum LDH levels were significantly elevated due to AA administration compared to normal animals. Pre-treated groups with *shilajit* and SLZ inhibited the elevation of serum LDH level.

The major biochemical marker in damaged tissue during neutrophil infiltration is MPO. The MPO activity was increased in acetic acid alone treated animals. This increase in MPO activity was decreased in rats pretreated with *shilajit* and SLZ. (Table.1 & Fig.1)

Antioxidants play a major role in defense system that scavenges the toxicity which is associated with free radicals. The equilibrium between free radicals and anti-oxidants is an major process in intracellular organelles for the removal of oxidative stress. But, in pathological conditions like ulcerative colitis, the generation of ROS, lipid peroxidation can destroy this balance with an increasing demand on the antioxidant defense system. Present study shows, Acetic acid causes increased levels in lipid peroxidation while GSH levels decreased. Pre-treatment of rats with *shilajit* significantly afforded protection against AA induced increase of intestinal MDA contents. While antioxidant power of cell such as GSH content was significantly preserved. (Table.2 & Fig.2) The protective effect of *shilajit* is majorly due to its antioxidant properties, as it acts as ROS scavenger and an inhibitor of lipid peroxidation.

CONCLUSION

The present study shows that *shilajit* possess protective effects against ulcerative colitis induced by Acetic acid and the results may be comparable to that of Sulfasalazine. Therefore the protective effects of *Shilajit* may be due to its anti-inflammatory activity and anti-oxidant actions.

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