

SHILAJIT – BIOACTIVE CHEMICAL CONSTITUENTS

Shilajit is a blackish brown exudation found in the serene surroundings of Himalayas. It is also found in most of the sedimentary rocks especially in Afghanistan, Bhutan, China, Nepal, Pakistan, USSR, Tibet as well in Norway, where they are gathered from steep rock faces at attitudes between 1000 and 5000 m.

In Ayurveda, Shilajit is classified as a ‘rasayan’ (meaning rejuvenator and immunomodulator in Sanskrit) and as a ‘medhya rasayan’ [rejuvenator of ‘medha’ (intellect)]. Shilajit is believed to slow down the process of aging by rejuvenation and immunomodulation¹.

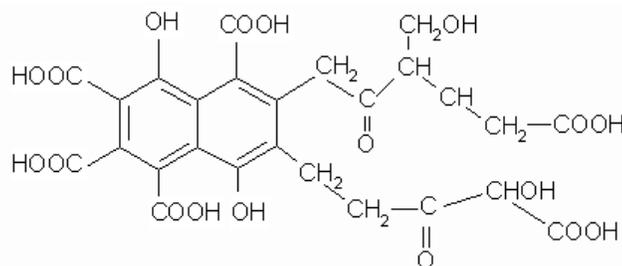
Until the mid 80’s, Shilajit was variously described as an inorganic mineral, a bitumen, an asphalt, a mineral resin, a plant fossil exposed by elevation of the Himalayas, and so forth.

Focused research has now shown that Shilajit is essentially constituted of fresh and modified remnants of **humus**- the characteristic organic constituent of soils.

SHILAJIT – BIOACTIVE CHEMICAL CONSTITUENTS:

The biologically important classes of compounds of shilajit include^{2, 3}:

- Dibenzo-alpha pyrones, phospholipids, triterpenes and phenolic acids of low molecular weight
- Fulvic acids: “carrier molecules”
- Humins and humic acids
- Trace elements (Fe, Ca, Cu, Zn, Mg, Mn, Mo, P)



Model structure of fulvic acid

The low MW bioactive organic compounds, e.g. oxygenated dibenzo- α - pyrones (or equivalent biphenyl carboxylates) are the major entities. The medium MW fulvic acids (FA), act as carrier molecules to the bioactive substances during their systemic transport. The trace elements contribute to the healthful properties. Differences in the biological effects of native shilajit can be attributed to qualitative and quantitative variations of both bioactive organic compounds and the fulvic acids in Shilajit samples from different locations.

Characterization of the biologically active compounds in Shilajit:

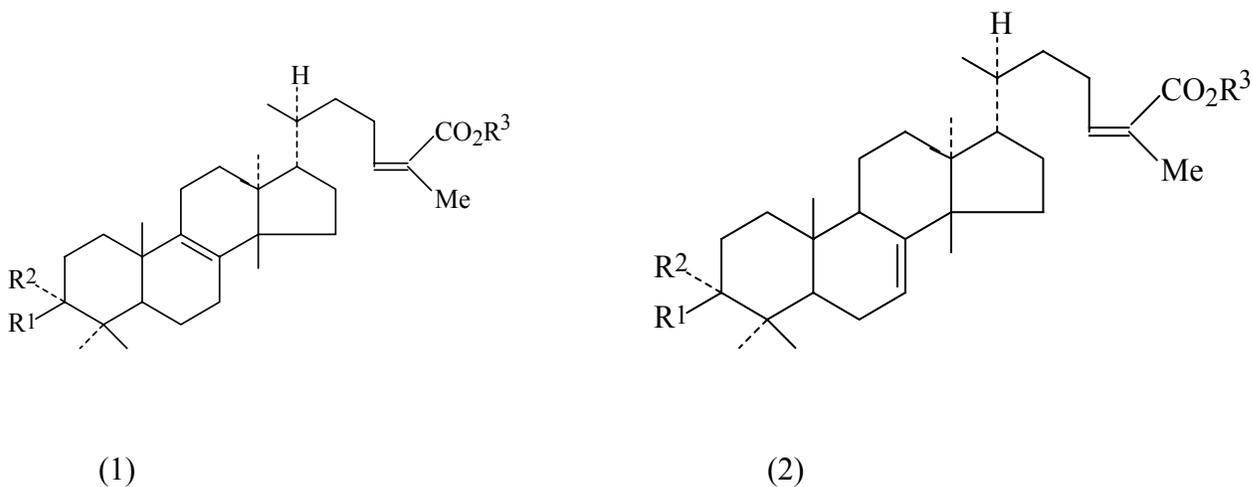
Among the low molecular weight compounds, are the dibenzo- α -pyrones and biphenylcarboxylates:

- 3,4', 5-trimethoxybiphenyl ($C_{15}H_{16}O_3$)
- methyl 4'-methoxybiphenyl-2-carboxylate ($C_{15}H_{14}O_3$)
- methyl 2', 4' -dimethoxybiphenyl-2-carboxylate ($C_{16}H_{16}O_4$)

The organic compounds in shilajit can be broadly grouped into humic and non-humic substances. The non-humic substances are the low molecular weight organic compounds discussed above. They can be characterized by the chemical and spectroscopic methods. The humic substances, however, do not exhibit any specific physical and chemical characteristics like sharp m.p., consistent elemental composition, well-defined spectra etc. Humic substances are produced by interaction of plants, algae, mosses and microorganisms.

After separation of the low MW organic compounds, the remaining mass of shilajit (80-85%) consists of a mixture of high MW humic substances- fulvic acids (FA), humic acids (HA) and residual humic acids (RHA). Humic (HA) and fulvic acids (FA) are metal-organic complexes of soil humus, which contain nitrogen, oxygen and sulphur as heteroelements in their molecules⁴. FA and HA are usually separated by

pH-gradient extraction followed by charcoal chromatography as polydispersed mixtures of amorphous substances. In the isolation of the chemical components of Shilajit, retained low molecular weight organic compounds are removed from the humic acids by exhaustive solvent extraction. The humic acids are then hydrolyzed by boiling with water. Extraction of the hydrolysate with solvents of graded polarity lead to the separation of C₁₆-C₃₀ fatty acids, p-hydroxy – and 2, 5-dihydroxybenzoic acids, the triterpenic acids (1 and 2) and the conjugated dihydroxydibenzo- α -pyrones ⁵.



BIOLOGICAL POTENTIAL OF SHILAJIT IN INTEGRATED MEDICINES:

The biological effects of Shilajit are ascribable to two distinct classes of compounds viz. oxygenated di-benzo- α pyrones and the fulvic acids.

The following pharmacological actions have been observed consistently in various biological models.

1. Anti-ulcerogenic / Anti-stress – Adaptogenic Activity:

Shilajit was found to possess anti-ulcerogenic effects by its ability to decrease gastric acid secretion and peptic output and was also found to be effective in restrain stress models. The adrenocortical response to stress appears to be a common mechanism for the anti-stress / adaptogenic activity. Shilajit treatment produced decreased ulcerogenicity in 4 hr pylorus ligated

rats. This finding lends credence to the suggested use of shilajit for peptic ulcers⁶. Shilajit increased the carbohydrate/protein ratio and decreased gastric ulcer index, indicating an increased mucus barrier. These results substantiate the use of shilajit in peptic ulcer⁷. Some active constituents isolated from shilajit are Fulvic acid and 4/-methoxy 6-carbomethoxy bi phenyl. These active constituents were found to have ulcer protective effect as a result a per se decrease in acid-pepsin secretion and cell shedding⁸. They studied the effects of the Shilajit constituents, fulvic acids (FA) and 4' -methoxy-6-carbomethoxybiphenyl (MCB) against gastric ulcers induced by restraint stress and aspirin in pylorus ligated albino rats as well as in cysteamine-induced duodenal ulcers in rats. Both FA and MCB were effective and decreased the incidence of duodenal ulcers in the experimental model.

For the first part of the study, to determine the efficacy of Shilajit constituents on the development of restraint stress and aspirin-induced ulcers, albino rats were divided into groups 8 groups of 7-12 animals each.

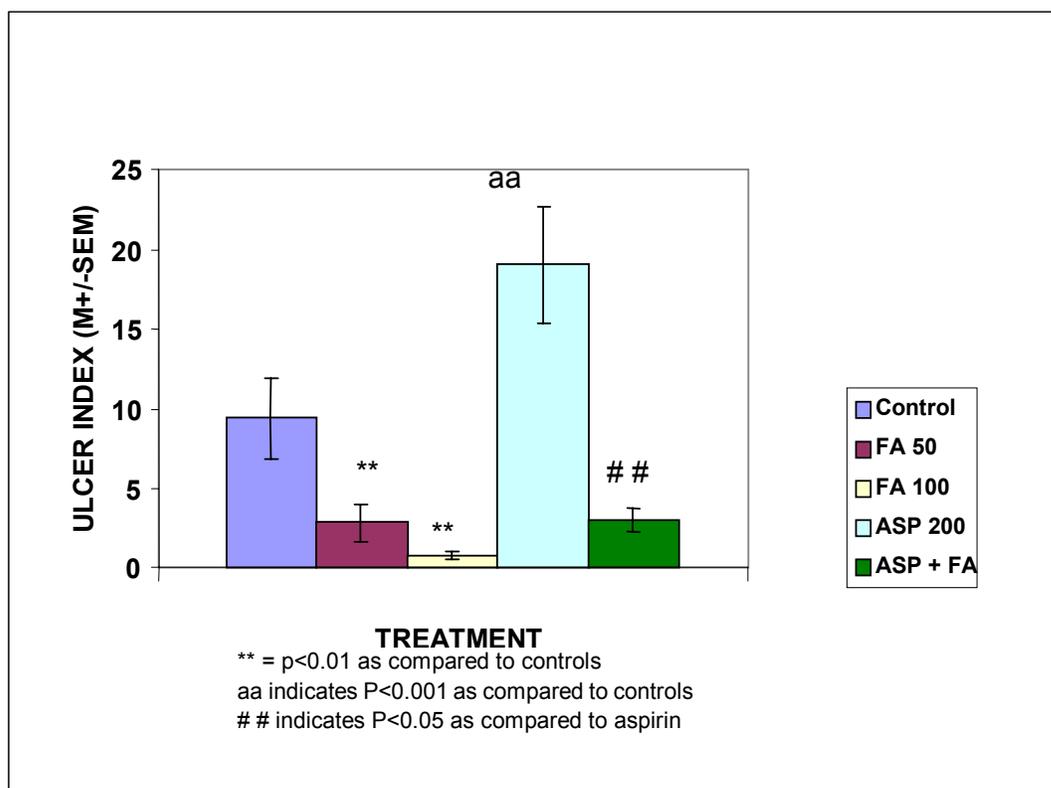
- Group 1 was given saline (control group).
- Group II received FA at 50 mg/kg level twice daily for 6 weeks.
- Group III received 100 mg/kg FA twice daily for 6 weeks.
- Group IV received MCB 100 mg/kg twice daily for 6 weeks
- Group V received FA+MCB (25 + 25 mg/kg) once daily for four weeks
- Group VI received aspirin (ASP, an ulcerogenic agent) 200 mg/kg once daily for 3 weeks
- Group VII received ASP + ACB (200 mg/kg once daily for 3 weeks + 50 mg/kg once daily for four weeks).
- Group VIII received Aspirin +FA (200 mg/kg once daily for 3 weeks + 50 mg/kg once daily for four weeks)

In the second part of the study, FA (50 mg/kg) or MCB (100 mg/kg) was administered twice daily for 5 days. The animals were fasted overnight and then treated with 30 mg/kg cysteamine subcutaneously.

Both FA and MCB isolated from Shilajit significantly decreased the restraint-stress ulcer index in pylorus ligated albino rats as compared to the control and the aspirin-treated groups, FA being more

effective (Fig. 1). FA+MCB also retained the efficacy in the duodenal ulcers experimental model (Fig. 2).

In the first part of the study, evaluation of the results with MCB revealed that the compound alone and in the presence of aspirin decreased the volumes of gastric secretion and the acid and peptic output significantly, as compared to the control as well as the aspirin treated groups. MCB had practically no effect on the protein content of the gastric juice, but it reversed the adverse effects of aspirin. MCB had a favorable effect on the total carbohydrate: protein ratio in the gastric juice, indicating that it stimulates the secretion of mucus.



(The numbers in the legend refer to the dose in mg/kg body weight)

Fig. 1 : Anti-ulcerogenic effects of Shilajit constituents against restraint stress and aspirin induced ulcers in rats.

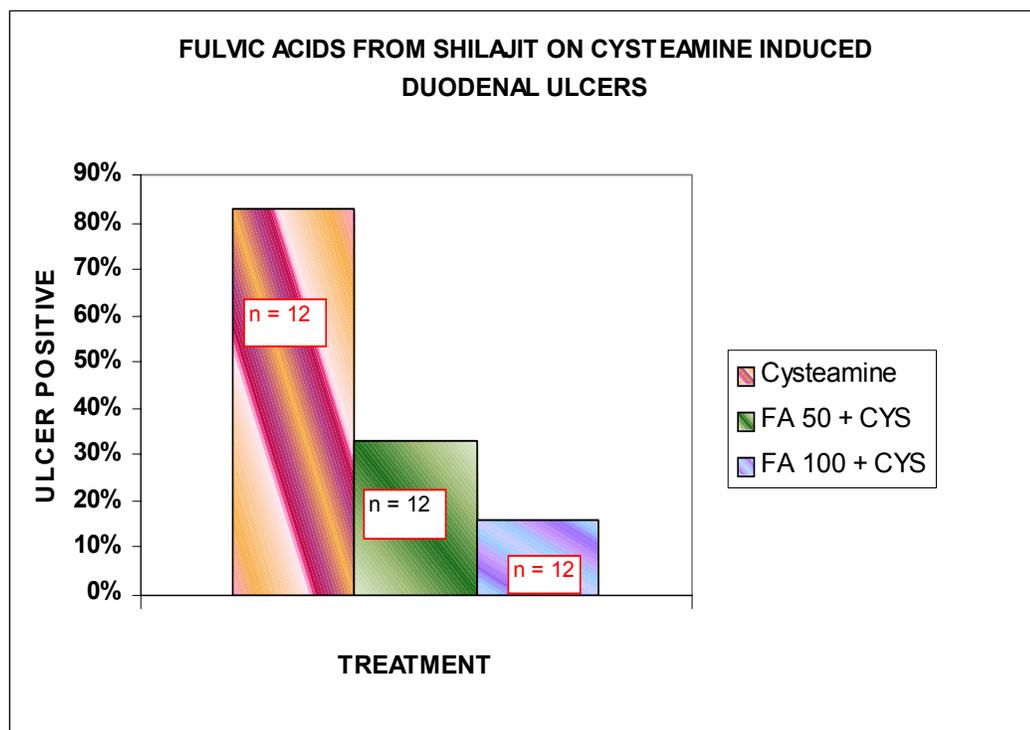


Fig. 2: Effect of fulvic acids on cysteamine induced duodenal ulcers in rats

2. Immunomodulator :

Ghosal et al.⁹ also investigated the immunomodulatory potential of shilajit constituents. The screening was done on three crucial parameters, viz.

1. elicitation and activation of peritoneal macrophages,
2. their effect on the lysosomal marker enzyme (acid phosphatase),
3. effects on tumor cells.

In all the selected immunological parameters, FA and MCB showed significant immunostimulatory effects. This makes shilajit a useful agent as promoter of non-specific immunological defense. Shilajit treatment not only induced morphological and morphometric changes on the peritoneal macrophages, it also dose dependently augments the phagocytic activity. This was validated in another study by the same authors, wherein they studied the effects of processed Shilajit on mouse peritoneal macrophages¹⁰. In this study, the dose and time-dependent effects of processed Shilajit (SJP) on the structure and functions of mouse peritoneal macrophages was evaluated. 0.025 to 900 mcg per mouse intraperitoneally for

different periods of time upto several hours. SJP (300-900 mcg) produced morphological changes in the adherant cells in the peritoneum in a dose-dependent manner. The results on cell size are shown in Fig. 3. The shape of the macrophage cell body appeared to be heterogeneous and the cells were found to be constituents of an intricate network. There were round and elongated cells and the axes of extension increased progressively with time (Fig. 4)

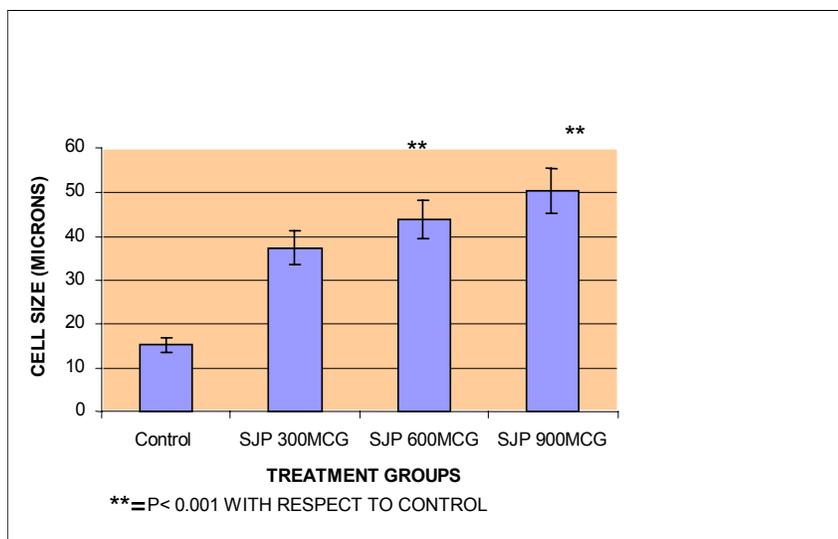


Fig. 3: Effects of processed Shilajit on morphological changes in mouse peritoneal macrophages.

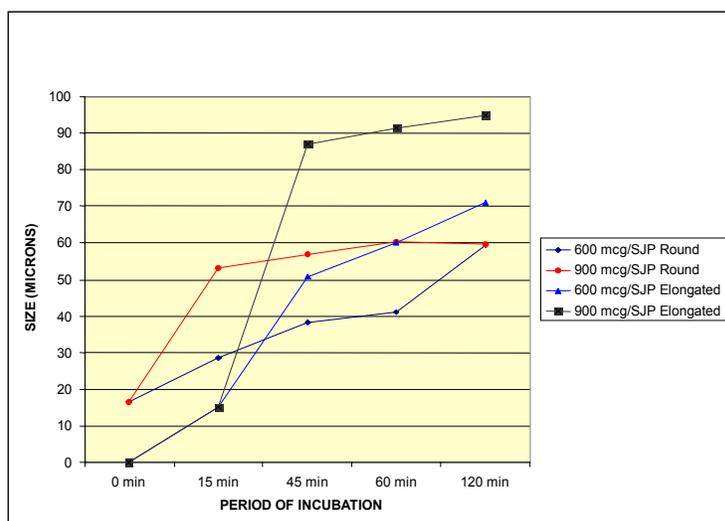


Fig. 4: Dose and time dependent morphometric changes of mouse peritoneal macrophages induced by processed Shilajit

The dose and time dependent effects exhibited by SJP (Fig. 4) lend support to the postulate made by earlier researchers that the immunological response could be due to a direct interaction with the target cells or through secretory-type cells¹¹. The phagocytic index depended on the function of the individual activated macrophage and not upon the number of macrophages present. A significant observation from this study was that higher doses of SJP (7.5 to 15 mcg) produced “greedy” macrophages that were subjected to lysis and disintegration. This observation is significant in that it indicates that the dose and duration of administration of Shilajit should be carefully configured to avoid impairment in the immunological response of the users. The dose dependent effect of systemic exposure to Shilajit on the phagocytic activity is shown in Fig 5.

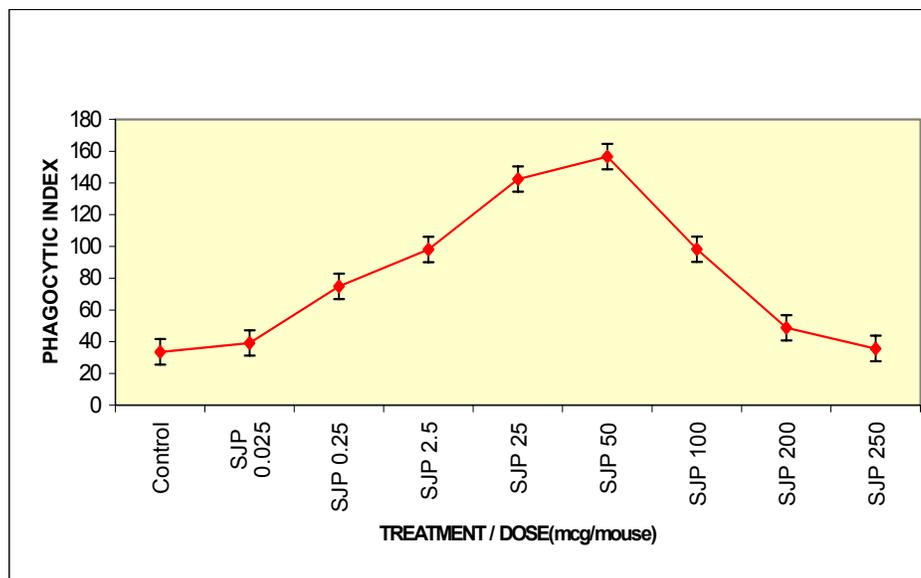


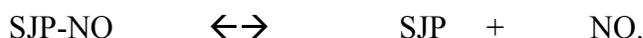
Fig. 5: Effect of systemic exposure to Shilajit on phagocytic activity

3. Antioxidant Activity :

Shilajit was found to be a strong regulator of enzymic and non enzymic anti oxidant activity. It is a powerful radical captodative agent of NO and hydroxy radical generated from Fenton reaction. Shilajit is known to mimic the actions of the systemic antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). These actions are believed to be due to the presence of iron-containing quinone-semiquinone-hydroquinone complex structures in the core of Shilajit. The regenerative cycle of antiradical-antioxidant effects of

processed shilajit (SJP) on reactive oxygen species (ROS) and nitric oxide (NO) and the attendant H₂O₂ cleaving effect is well-researched¹². SJP containing fulvic acids and DBP provided complete protection against hydroxyl radical induced polymerization of MMA (methylmethacrylate) and was shown to be a reversible nitric oxide- captodative agent.

The observed absorption and desorption is through the reaction



This is a distinctly remarkable phenomenon that is probably mediated by the iron-nitrosyl complex.

SJP (20 and 50 mg/kg/day, i.p., for 21 days) induced a dose-related increase in superoxide dismutase (SOD) (Fig. 6), catalase (CAT) (Fig. 7) and glutathione peroxidase (GPx) (Fig. 8), activities in frontal cortex and striatum in experimental animals (rats). The numbers in the legend sections of these figures correspond to the dose level in mg given once daily for 7, 14, 21 days. The results presented are for the 21 day treatment¹³.

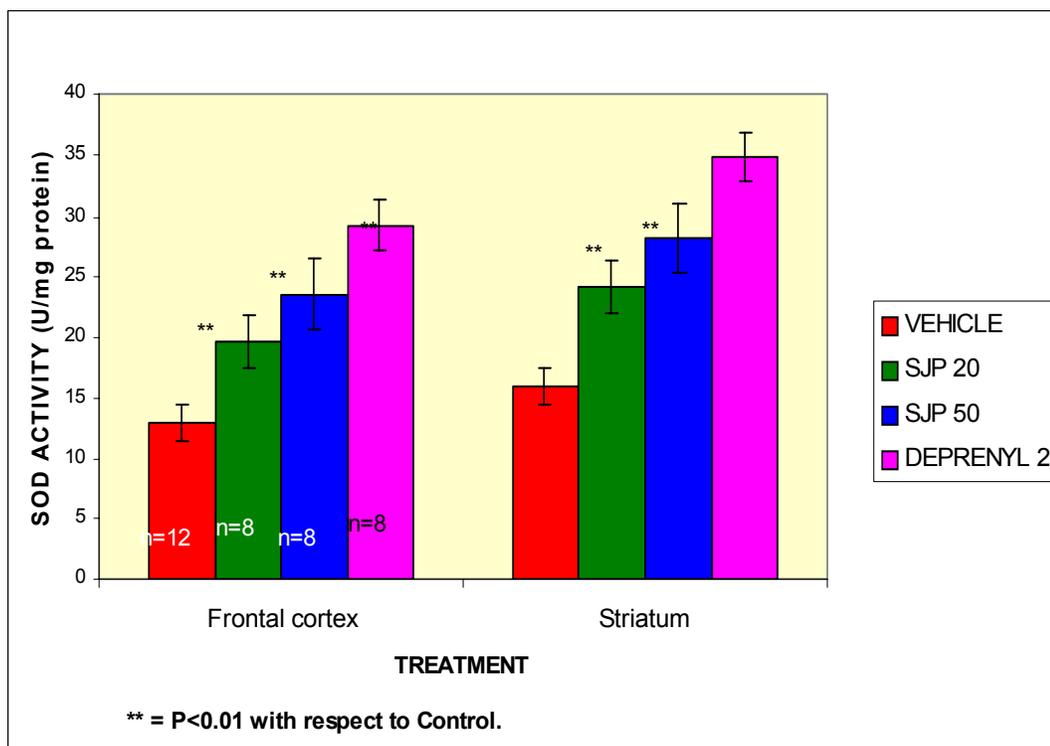


Fig. 6: Effect of shilajit administration on superoxide dismutase activity in the brain in rats

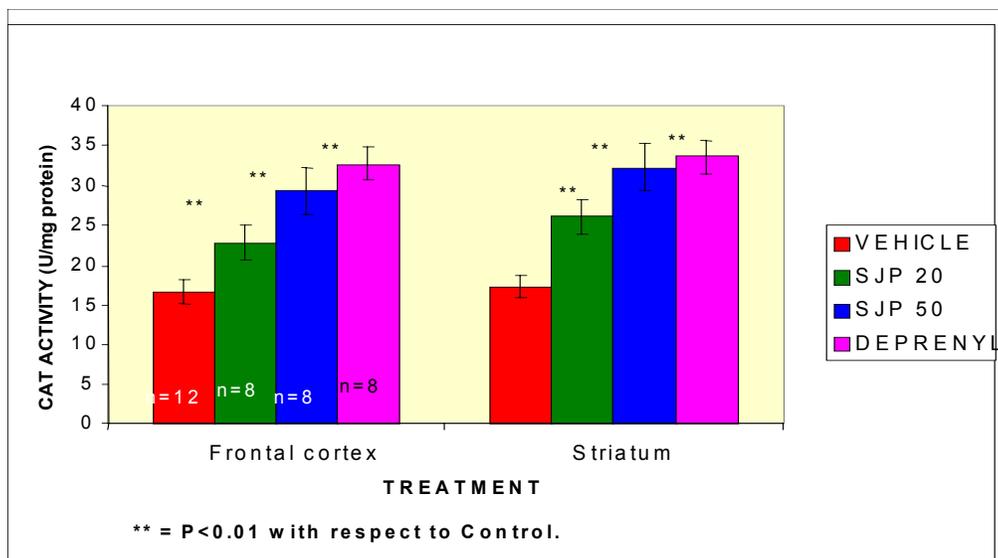


Fig. 7: Effect of shilajit administration on catalase activity in the brain in rats

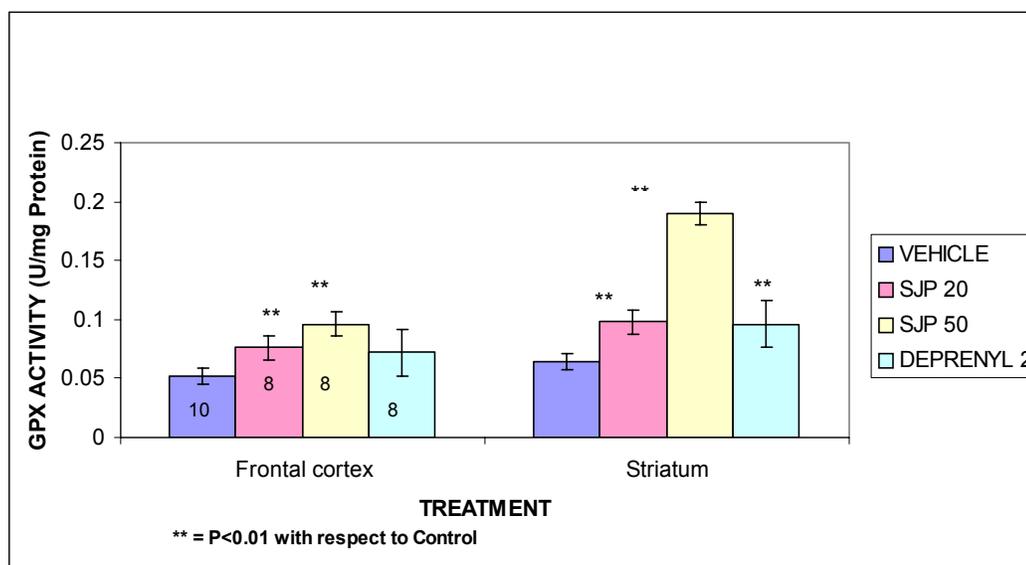


Fig. 8: Effect of shilajit administration on glutathione peroxidase activity in the brain in rats

The effectiveness of Shilajit was comparable to that of (-) deprenyl (2mg/kg/day, i.p. x 21 days) with respect to SOD and CAT levels and better than (-) deprenyl for the GPx levels. The radical scavenging activity of SOD should be followed by the actions of CAT and GPx in order to

remove the hydrogen peroxide generated by SOD, which is a toxic metabolite. Thus Shilajit provides comprehensive antioxidant support by increasing the effectiveness of all three antioxidant enzymes. Additionally, the authors reported that unlike (-) deprenyl, Shilajit is not a monoamine oxidase inhibitor.

4. Analgesic activity:

Aqueous suspension of an authentic sample of shilajit was found to have significant analgesic activity in albino rats. Observed analgesic activity of shilajit probably justifies its use in different painful conditions⁶. In Swiss mice, the concomitant administration of proceeded shilajit with morphine, from day 6 to day 10, resulted in a significant inhibition of the development of tolerance to morphine induced analgesia¹⁴.

5. Anti-inflammatory activity:

Aqueous suspension of an authentic sample of shilajit was found to have significant anti-inflammatory activity in albino rats. This research supports the use of shilajit in Ayurvedic medicine for rheumatism⁶. Shilajit was found to have significant anti-inflammatory effect in carrageenan-induced acute pedal oedema, granuloma pouch and adjuvant-induced arthritis in rats. These results substantiate the use of shilajit in inflammation⁷.

6. Nutritive Tonic:

The effect of shilajit was investigated on the body weight of young rats for a period of one month. The body weight of the rats was found to be significantly greater in the rats taking shilajit compared with a control group. Researchers suggest a better utilization of food as a cause of the weight gain¹⁵.

7. Blood sugar lowering effects of Shilajit:

A formulation containing processed Shilajit along with *Withania somnifera*, *Tinospora cordifolia*, *Eclipta alba*, *Ocimum sanctum*, *Picrorrhiza kurroa* was orally administered at the level of 50 and

100 mg/kg, to male rats once daily for 28 days along with streptozotocin (STZ, 45 mg/kg, s.c x 2days, an agent that induces diabetes). The formulation attenuated the hyperglycemic response of STZ in a dose related manner, as observed by assessing the superoxide dismutase (SOD) activity of pancreatic islet cells on days 7, 14, 21 and 28. Although the formulation did not reduce blood sugar levels as such, a dose- related decrease in STZ induced hyperglycaemia and attenuation of STZ induced decrease in islet SOD activity was observed. The authors concluded that the results indicate that the earlier reported anti-hyperglycaemic effect of the formulation may be due to free radical scavenging activity of the ingredients in the pancreatic islet cells, The hyperglycaemic activity of STZ is believed to be due to a decrease in islet SOD activity leading to the accumulation of degenerative oxidative free radicals in islet beta-cells¹⁶.

8. Shilajit modulates neurochemicals:

Shilajit (25 and 50 mg/kg, intraperitoneal) administration to rats was found to modulate the brain monoamines¹⁷. Processed Shilajit (SJP) augments the levels of Dopamine (DA) and Norepinephrine (NE) and their metabolism in various regions of the brain including the striatum. Furthermore, the treatment decreases serotonin (5HT) and its metabolism in the frontal cortex. These neurochemical changes substantiate the observed behavioral effects of shilajit in animal models, such as anxiolytic activity and nootropic activity these actions are attributable to decreased 5HT levels. Fig. 9 depicts the percentage change in the levels of various neurotransmitters on Shilajit administration¹⁸.

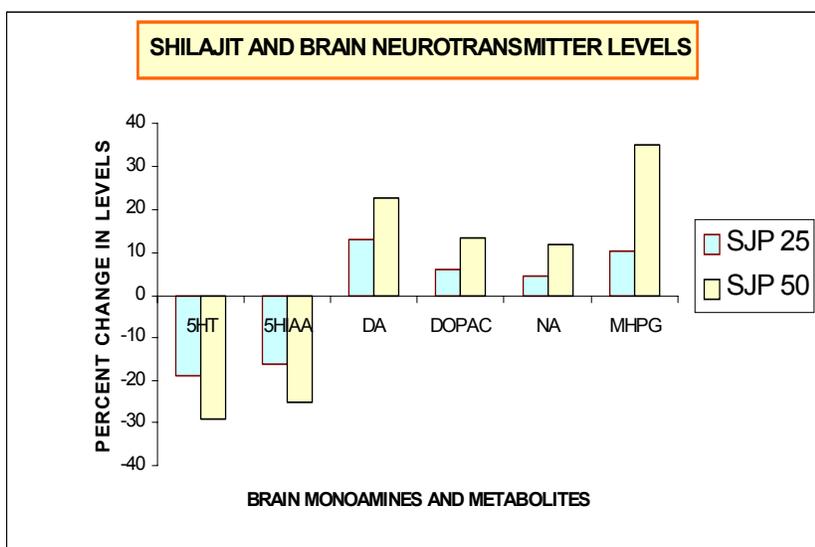


Fig. 9: Effects of Shilajit on brain neurochemicals

Additionally, the systemic administration of shilajit differentially affected the cholinergic nerves in the basal fore brain nuclei including medial septum and the vertical limb of the diagonal band, when subjected to autoradiographic studies and histochemical analysis the treatment did not affect either GABA_A and benzodiazepine receptor binding nor NMDA and AMPA glutamate receptor subtypes in any of the cortical or subcortical regions studied. The findings validate the use of Shilajit as a nootropic especially during aging ¹⁹

Short-term memory is more dependent on the neurotransmitter dopamine, whereas long-term memory is more dependent on the neurotransmitter acetylcholine.

Medications which increase the amount of acetylcholine in the brain, improve memory function in patients with Alzheimer's disease. The effects of Shilajit on acetylcholinesterase, the enzyme that reduces acetylcholine levels is shown in Fig. 10.

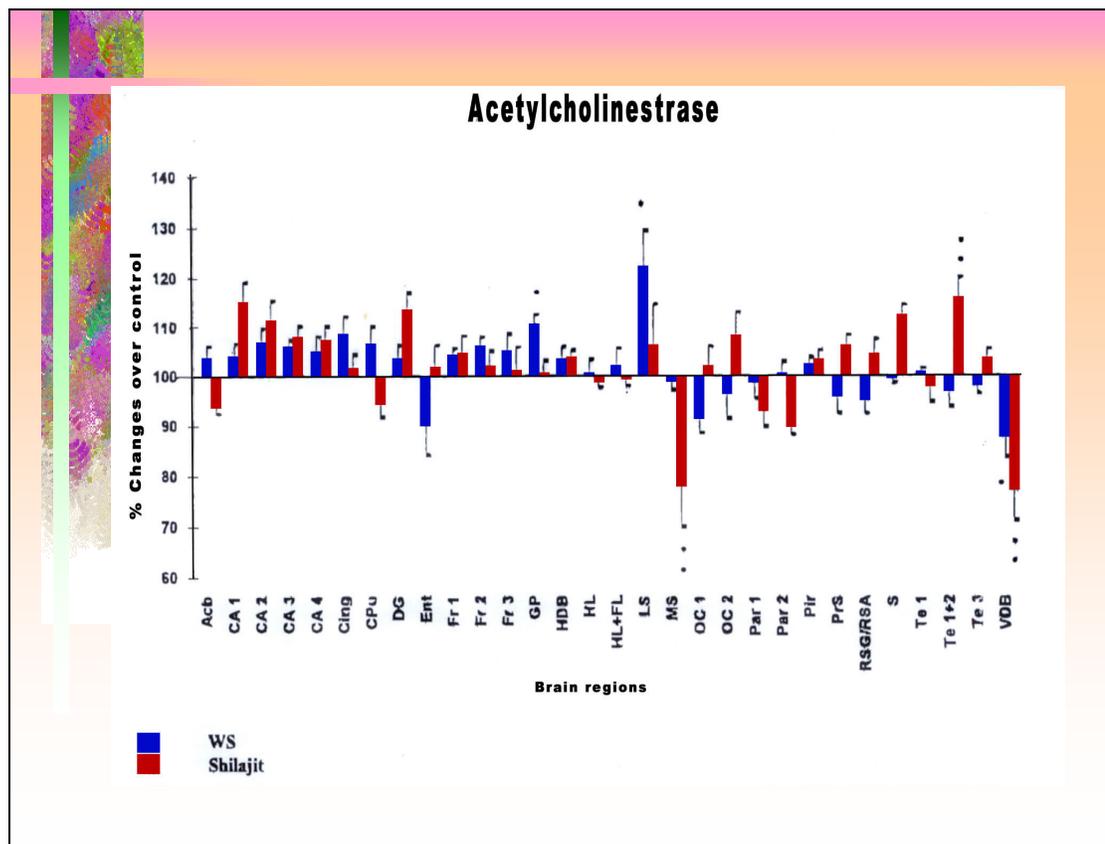


Fig. 10: Effects of Shilajit and Withania somnifera (WS) on acetylcholinesterase activity in various regions of the brain

9. Aphrodisiac/Reproductive Health support:

Shilajit has been used as a rejuvenator and an adaptogen for thousands of years, in one form or another, as part of traditional systems of medicine in a number of countries²⁰.

10. Antiallergic action:

Shilajit treatment also stabilizes mast cells and prevents its degranulation. The effects of Shilajit and its constituents, the fulvic acids (FA), 4'-methoxy-6-carboxyphenylmethyl (MCB) and 3, 8-dihydroxy-dibenzo-a-pyrone (DDP) were studied for protective effects against mast cell degranulation²¹. Mast cells are pivotal in the allergic response type I or the anaphylactic type - a rapidly progressing chain reaction that causes the allergic response. Mast cells are ubiquitous and are found around blood vessels in the connective tissue, in the lining of the gut and importantly in the lining of the upper and lower respiratory tract. These are large mononuclear cells heavily granulated, with granules containing a host of pharmacologically active substances. The allergen (antigen) enters into the human body through the respiratory tract, skin and/or gastrointestinal Tract (GIT). After the exposure to antigens, antibodies directed against specific antigens. (i.e., IgE, immunoglobulin E) are formed and are fixed to their respective receptors on the surface of the mast cells. This process is called sensitization of mast cells. During the second exposure to antigens, the antigens react with these antibodies at the cell surface. This event leads to a series of biochemical reactions. These migrate to the periphery in the secretory expulsion of the mast cell granules containing active substances (vasoactive amines and chemolytic amines) causing allergy symptoms. This process is called "mast cell degranulation". Shilajit or its combined active constituents were found to offer significant protection against experimental mass cell degranulation induced by allergens (Fig. 11). Shilajit or its combined constituents produced a dose-dependent inhibition of spasms in the sensitized guinea pig ileum, induced by antigens (Fig. 12).

Fig. 11: Effects of Shilajit and its constituents *in vitro* against antigen-induced degranulation of sensitized mast cells

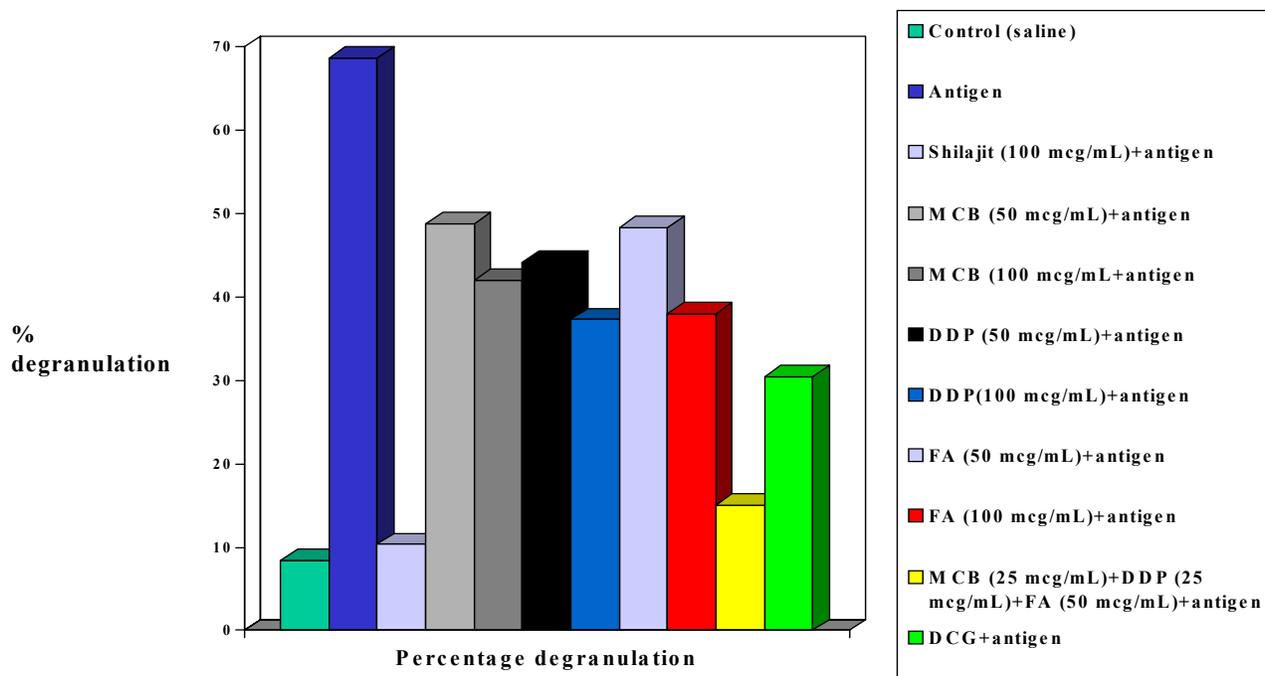
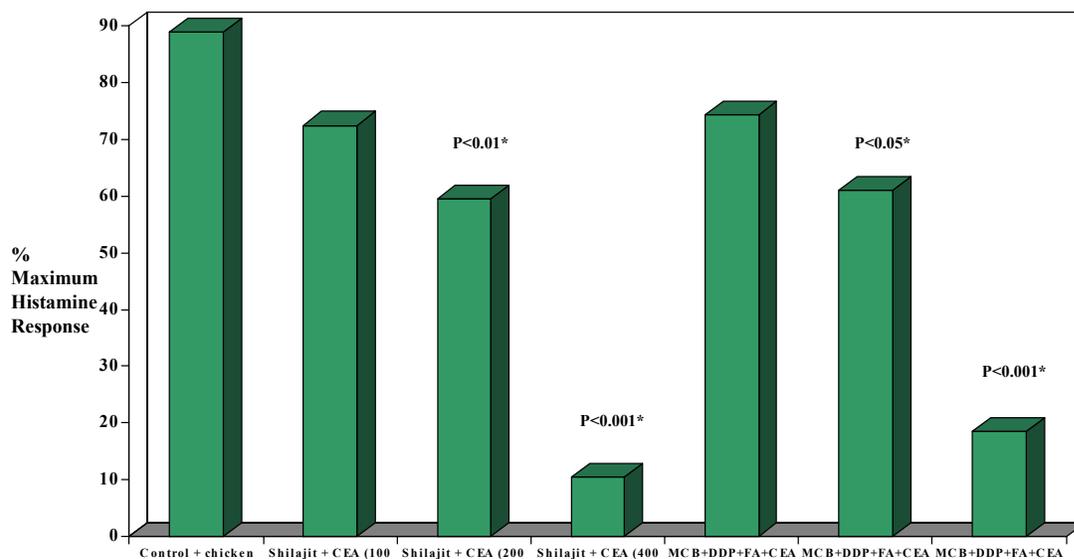


Fig. 12: The effects of Shilajit and its constituents against active anaphylaxis against guinea pigs



Thus Shilajit treatment in experimental models augments the lytic potential of macrophages without increasing the dead tumor cells. Shilajit is further postulated to assist in normal physiological functions by acting as a biocatalyst. The trace elements present in shilajit are likely to be of importance in this action. Major amounts of nutrient metals from shilajit have been found to be bioavailable²².

10. **Cognition Enhancer²³:**

Shilajit was found to augment learning acquisition and memory retrieval in the battery of validated animal models while native Shilajit was found to exhibit inconsistent response. These findings also suggested the role of Shilajit in facilitating communication between immune and the central nervous systems. Further this cognition enhancing property was located to the dibenzo-alpha-pyrones and fulvic acids. Shilajit was also found to be effective in animal models of Alzheimer's disease. This nootropic activity was due to its ability to enhance the acetyl choline levels and muscarinic cholinergic receptors binding activities coupled with decreased serotonergic activity in the hippocampus and frontal cortex.

In a battery of tests, shilajit has been found to augment learning acquisition as well as short and long-term memory (retention) in rats ^{11, 24, 25}. A positive effect of shilajit is postulated to be mediated by facilitating communication between the immune and the central nervous systems.

To test the effects of Shilajit administration on learning and memory, rats were subjected to two sets of tests²⁶:

1. The active avoidance learning and re-learning test, in which rats were exposed to a conditioning stimulus followed by electric shock. Avoidance response was measured by how quickly the rats moved to the unelectrified chamber, to avoid the electric shock. The rats were administered the test compound (either processed Shilajit (SJP), unprocessed native Shilajit (SJN) or the extract containing Fulvic acids and dibenzo- α - pyrones (FAA+DBP) at doses of 5, 10, 25 or 50 mg/kg body weight.

2. The elevated plus-maze test for learning and memory: Here the rats were individually placed at the end of one arm that faced away from a central platform. The time taken by the rats to move from the open arm to either of two enclosed arms at the platform was measured.
3. Electroconvulsive shock(to induce amnesia) was administered to the test animals and they were subjected to the plus maze test
4. An open field behavior test to assess anxiety symptoms and behavior.

Fig. 13 - 16 provides the results of these tests. It is observed that there was significant shortening in the number of trials for active avoidance learning and memory (as measured by relearning capability), in rats treated with processed Shilajit or the extract. It was also observed that higher doses of unprocessed Shilajit reversed the learning process, further validating the need for purification of Shilajit. Processed Shilajit was found to be more effective than the isolated active principles.

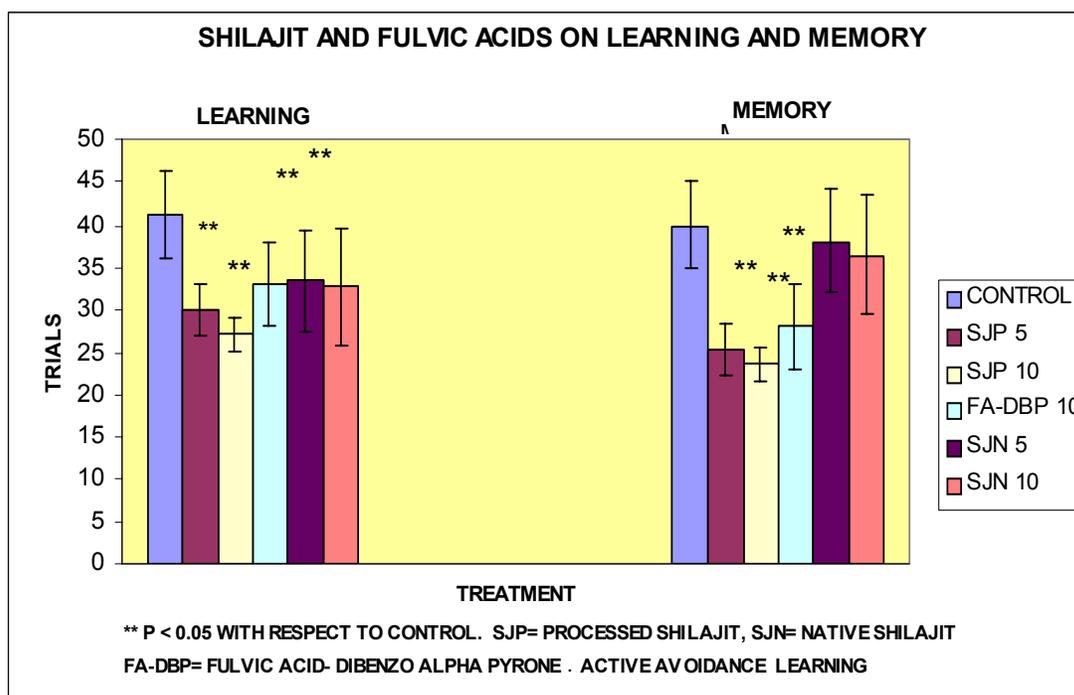


Fig. 13: Effects of Shilajit and its active constituents on active learning in rats

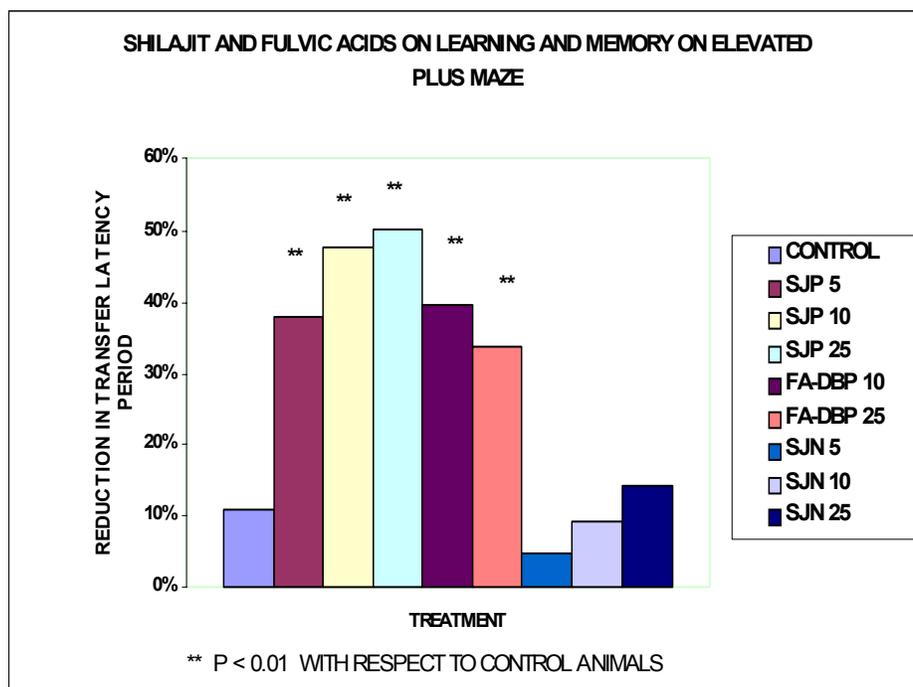


Fig. 14: Effect of Shilajit and the isolated active principles on learning and memory as measured by the elevated plus maze test in rats.

Similar results were observed in the plus maze test and by rats subjected to electric shock as well as animals that were not subjected to electric shock.

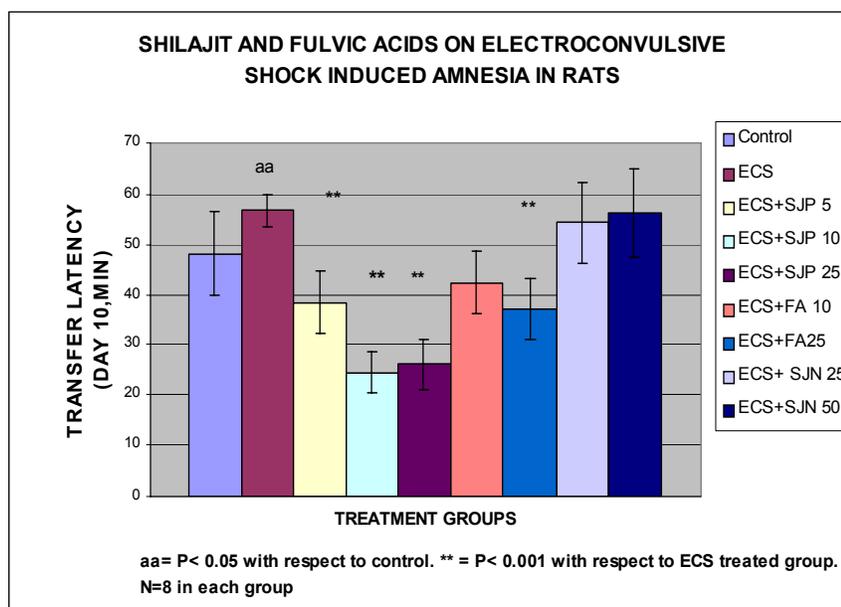


Fig. 15: Effect of Shilajit and fulvic acids on electroconvulsive shock induced amnesia in rats

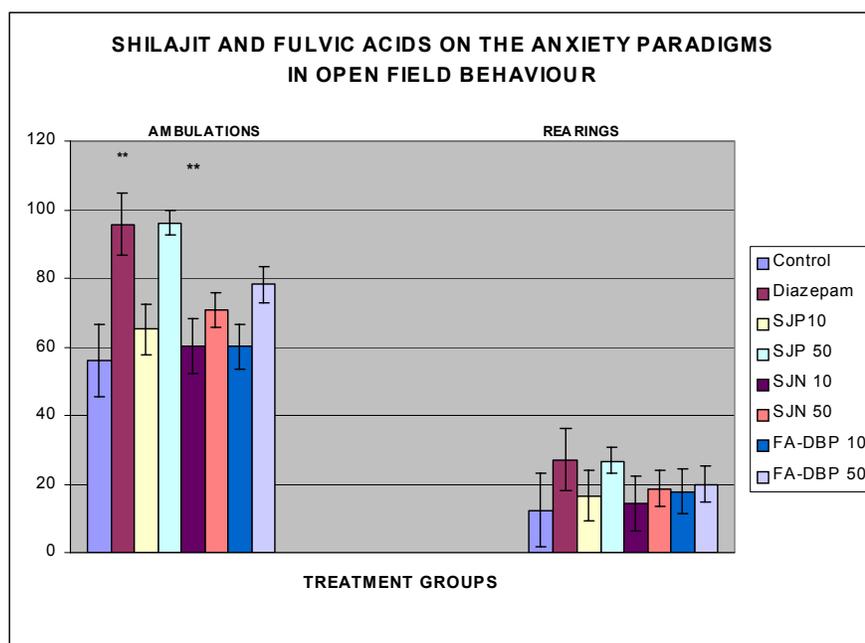


Fig. 16: Effect of Shilajit and isolated active principles on anxiety paradigms in open field behavior in rats.

In the open field behavior test, processed Shilajit and the isolated active constituents showed significant efficacy in diminishing anxiety symptoms. However, native Shilajit was not very effective, probably on account of the free radical contamination in such material, further validating the need for purification.

Conclusion:

Shilajit can be used in antioxidant and anti-aging formulations and to act as a delivery system for other therapeutic agents in mixed formulations.

These applications advocated in the ancient Ayurvedic texts, have been validated by recent research into the chemistry and biological actions of this ancient panacea.

Shilajit has been used historically for general physical strengthening, anti-aging, blood sugar stabilization, libido, injury healing, enhanced brain functioning potency, support immune system, arthritis management, hypertension and obesity.

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