

ORIGINAL RESEARCH ARTICLE

Anxiolytic and Antidepressant Activity of Processed *Shilajatu*

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ABSTRACT

Context: *Shilajatu*, pale-brown to blackish-brown exudation, of variable consistency, from layers of rocks in many mountain ranges of the world.

Aim: To Assess anxiolytic and antidepressant activity of *Shilajatu*.

Materials and methods: Anxiolytic and antidepressant activity of *Shilajatu* was assessed on Open Field Exploratory Behavior, Elevated plus Maze and Behavioral Despair model in dose of 108 mg/kg body weight.

Results: Ambulation, Rearing and grooming time were significantly increased in animals treated with water purified *Shilajatu*, *Guduchi* and *triphala bhavit shilajatu* in Open Field Exploratory Behavior model and animals treated with *guduchi kwatha bhavita shilajatu* showed significantly Immobility and have more entry in open as well as closed arm in Elevated plus Maze and Behavioral Despair model respectively.

Conclusion: water purified *Shilajatu*, *Guduchi* and *triphala bhavit shilajatu* possess significant anxiolytic and antidepressant activity.

Key words: *Shilajatu*, antidepressant, anxiolytic.

INTRODUCTION

Psychopharmacology is the study of drug-induced changes in mood, sensation, thinking, and behavior [1]. It encompasses a wide range of substances with various types of psychoactive properties. These drugs interact at particular target sites or receptors found in the nervous system to induce widespread changes in physiological or psychological functions. Drugs crosses the blood-brain barrier and has an effect on behavior, mood or cognition are researched for their physicochemical properties, physical side effects, and psychological side effects [2] therefore the effects are evaluated in experimental animals. Stress-induced brain changes in animal models of depression may validly represent the brain changes in depressed humans, and antidepressant-induced changes in animal models may validly represent antidepressant mechanisms in depressed humans.

Shilajatu has been used for thousands of years, in one form or another, under the indigenous systems of medicine. It is a pale-brown to blackish-brown exudation, of variable consistency, from layers of rocks in many mountain ranges of the world,

especially the Himalayan ranges of the Indian subcontinent [3]. It is bitter in taste and smells like pungent cow's stale urine [4]. It has been ascribed a number of pharmacological activities and has been used for ages as a rejuvenator and for treating a number of disease conditions [5] like genitourinary disorder, epilepsy, nervous disorder, and chronic bronchitis.

MATERIAL AND METHODS

Pharmaceutical study

Crude *Shilajatu* was procured from Hans Ayurvedic Pharmacy Premnagar Ashram, Haridwar (Uttarakhand) and identified by expert's member of *Rasa Shastra Department*, Faculty of *Ayurveda*, IMS, Banaras Hindu University. From this crude sample, *shilajatu* was extracted by two method i.e. Water extraction and *Gomutra* extraction. Water extracted *shilajatu* was further subjected to seven times of *bhavana* process with *guduchi* and *triphala kwatha*. In this way *guduchi* and *triphala kwath bhavita shilajatu* was prepared. Both *bhavita shilajatu* along with extracted *shilajatu* were used in our experimental study [6].

Animals

Adults Charles Foster albino rats of either sex (150-200gm) were used for this study. Animals were procured from the Central Animal House IMS, BHU. They were housed in groups of 6 in colony cages at ambient temperature of $25 \pm 2^{\circ}$ C and 50-60% relative humidity with 12 h light/dark cycle. They had free access to pellet chow (Brook Bond, Lipton, India) and water. Animals were exposed only once to every experiment. The experiments were performed after approval from the Institutional Ethical committee and principle of laboratory animal care (NIH) publication No. 86-23. Received 1985) guidelines were followed throughout.

Drug treatment

Total thirty six animals were taken in our study. These animals were divided randomly irrespective of sexes in to six groups namely group A: Control (Vehicle), B: Standard, C: Water purified *Shilajatu*, D: *Triphala kwath bhavita Shilajatu*, E: *Guduchi kwath bhavita Shilajatu* and F: *Gomutra shodhit Shilajatu*.

Statistical Analysis

The data, expressed as Mean \pm SD, was subjected to Kruskal-Wallis one way analysis of variance (ANOVA). Inter group comparisons were made by Mann-Whitney-U-test (two tailed) for only those responses, which yielded significant treatment effects in the ANOVA test. $P < 0.05$ was considered statistically significant.

Anxiety:**Open Field Exploratory Behavior Test**

An open field apparatus similar to that of Bronstein and modified by Jaiswal was used to study the open field exploratory behavior in rats. It was made of plywood and consists of a square (61x 61cm) with high walls (61x61 cm). The entire apparatus was painted black except for 6mm white lines that divided the floor into 16 squares. The entire room except the open field was kept dark during the experiment. The open field was lighted by a 60 w bulb focusing on to the field from a height of about 100cm from the floor. Each animal was centrally placed in the apparatus for 5 min and ambulations, Immobility, rearing,

grooming times along with number of faecal pellets excreted during the period were noted.^[7,8]

Elevated plus Maze Test:

The plus maze consist of two opposite open arms, (50 x 10cm) crossed with two opposite enclosed arms of the same dimension with walls 40cm high. The arms were connected with a central square (10 x10 cm) to give the apparatus a plus-sign appearance. The maze was kept elevated 50 cm above the floor in a dimly lit room. The rats were placed individually on the central square of plus maze facing an enclosed arm. The time spent and the number of entries made by the rats during the next 5 min, on the open and enclosed arm was recorded. An arm entry was defined when all four limbs of the rat were on the arm^[9].

Depression**Behavioral Despair Test**

The rat was placed in cylinder (45 x 20cm) containing 38 cm water ($25 \pm 2^{\circ}$ C), so that the rat could not touch the bottom of the cylinder with its hind limb or tail. rats also could not climb over the edge of the chamber. Two swim sessions were conducted, an initial 15 minutes pre test, followed by the 5 minutes test 24hrs later. Drugs were administered after pre test. The period of immobility (remained floating in water without struggling and making only those movements necessary to keep its head above water) during 5 minutes test period was noted^[10].

RESULTS

Open Field Exploratory Behavior Test Ambulation, Rearing and grooming time were increased significantly in Group B, C,D, and E as compared with control group animals. Immobility time was decreased significantly in group B and Group E as compared to control group is summarized in (Table 1) and animals of group B and E have more entry in open as well as closed arm in comparison to control group is presented in (Table 2).

In behavioral despair test, period of immobility was significantly decreased in B, D, E and F group animals as compared to control group animals are summarized in (Table 3).

Table 1: Showing Effect of purified and processed *Shilajatu* on open- field exploratory behavior test in rats

Group	Ambulation	Immobility Period (sec)	Rearing	Grooming	Faecal pellet
A	43.16 \pm 6.46	70.34 \pm 16.40	10.5 \pm 2.42	3.16 \pm 1.47	4.83 \pm 1.6
B	68.66 \pm 1.86 ^{***}	21.50 \pm 19.27 ^{**}	20.66 \pm 4.76 ^{***}	11.00 \pm 2.00 ^{***}	5.16 \pm 1.47
C	57.5 \pm 2.42 ^{***,###}	39.33 \pm 20.22	16.16 \pm 1.72 [*]	7.33 \pm 0.82 ^{***,##}	5.33 \pm 1.21
D	59.16 \pm 1.60 ^{***,##}	68.84 \pm 24.26 [#]	16.5 \pm 1.64 ^{**}	6.5 \pm 1.22 ^{***,###}	4.16 \pm 0.98

E	67.33 ± 1.63 ^{***}	30.5 ± 18.60 [*]	20.83 ± 2.31 ^{***}	9.00 ± 1.09 ^{***}	4.5 ± 1.04
F	48.66 ± 5.39 ^{###}	36.44 ± 18.22	14.16 ± 2.56 ^{##}	6.83 ± 1.47 ^{**,###}	4 ± 1.26

A: Control, B: Piracetam (Standard), C: Water purified *shilajatu*, D: *Triphala kwath bhavita Shilajatu*, E: *Guduchi kwath bhavita Shilajatu*, F: *Gomutra shodhit Shilajatu*, n – Six animals in each group; Values are mean ±S.D, **p* <0.05, ***p* <0.01, ****p* < 0.001 compared to control, #*p* < 0.05, ##*p* < 0.01, ###*p* < 0.001 showed decrease significance level as compare to standard group

Table 2: Showing Effect of purified and processed *Shilajatu*, on elevated plus maze test in rats

Groups	No. of Entries	
	In open arm	Enclosed arm
A	10.66±1.21	34 ± 3.68
B	17.83±1 ^{***}	18.83 ± 1.47 ^{***}
C	10.66±1.86 ^{###}	23.16 ± 3.18 ^{***}
D	12.66±3.20 ^{###}	24.16 ± 3.65 ^{***}
E	16.5 ± 1.51 ^{***}	17.16 ± 2.48 ^{**}
F	10.33±1.21 ^{###}	26.16 ± 3.31 ^{**,##}

A: Control, B: Piracetam (Standard), C: Water purified *shilajatu*, D: *Triphala kwath bhavita Shilajatu*, E: *Guduchi kwath bhavita Shilajatu*, F: *Gomutra shodhit Shilajatu*, n – Six animals in each group; Values are mean ±S.D, **p* <0.05, ***p* <0.01, ****p* < 0.001 compared to control, #*p* < 0.05, ##*p* < 0.01, ###*p* < 0.001 showed decrease significance level as compare to standard group

Table 3: Showing Effect of purified and processed *Shilajatu*, on behavioral despair test in rats

Groups	Time (seconds)
A	52.33 ± 5.12
B	37.50 ± 3.50 ^{***}
C	46.50 ± 5.20 ^{##}
D	41.16 ± 3.25 ^{***}
E	34.50 ± 3.61 ^{***}
F	44.66 ± 2.87 ^{*,#}

A: Control, B: Piracetam (Standard), C: Water purified *shilajatu*, D: *Triphala kwath bhavita Shilajatu*, E: *Guduchi kwath bhavita Shilajatu*, F: *Gomutra shodhit Shilajatu*, n – Six animals in each group; Values are mean ±S.D, **p* <0.05, ***p* <0.01, ****p* < 0.001 compared to control, #*p* < 0.05, ##*p* < 0.01, ###*p* < 0.001 showed decrease significance level as compare to standard group

DISCUSSION

In the open field behaviour test, animals express their anxiety and fear by decreasing ambulation, exploration, freezing, rearing, grooming behavior and increase in defecation due to heightened autonomic activity. These behavioral changes are attenuated by classical anxiolytic and augmented by anxiogenic agents. In our study, water *shodhit*, *triphala bhavita*, *guduchi bhavita shilajatu* and Piracetam treated animals showed increased ambulation, grooming and rearing than control group animals and immobility period of *guduchi bhavita shilajatu* and piracetam treated animals was less than control group animals. Water purified *gomutra shodhit* and *triphala kwatha bhavita shilajatu* showed decrease level of significance in ambulation and grooming as compared to piracetam treated animals. This showed that water *shodhit*, *triphala bhavita*, *guduchi bhavita shilajatu* and piracetam decreases

level of anxiety significantly. It was reported that *Shilajatu* modulate the brain Monoamines [11] activity, *triphala* possess antioxidant activities [12] and *Tinospora cordifolia* protected the dopaminergic neurons [13] It may be possible that modulation of brain monoamines by *shilajatu*, antioxidant properties of *triphala* and protective activity of dopaminergic neurons by *guduchi* helping towards decreased level of anxiety.

Elevated plus maze is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the gamma-aminobutyric acid (GABA)-benzodiazepine complex [14]. Rodents have a natural aversion for high and open spaces and prefer closed arms, which have a burrow like ambience and therefore, spend greater amount of time in the closed arm. When exposed to the novel maze alley, the animals experienced an approach-avoidance conflict, which was stronger in the open arms as compared to closed arms. The decrease aversion to the open arms was the result of an anxiolytic effect expressed by an increased number of open arm entries. Animals of piracetam and *guduchi bhavita shilajatu* have increased no. of entry in open arm and less in closed arm. This indicates that animals treated by piracetam and *guduchi bhavita shilajatu* decrease aversion to the open arms showed its anxiolytic effect. piracetam affects both glutaminergic [15] and cholinergic [16] neurotransmission and actions on both of these systems have been proposed for piracetam's behavioural effects. *Shilajatu* was reported to decrease brain 5-hydroxytryptamine turnover in rat, associated with an increase in dopaminergic activity leading to an increase in memory and anxiolytic activity in albino rats [17]. It might be possible that *guduchi bhavita shilajatu* and piracetam have same mechanism of action towards behavioural effect.

Antidepressant effect on forced swimming model of depression provides a rapid and reliable behaviour screening test for anti-depressants. The model is valid for a broad spectrum of antidepressants mainly including tricyclics and Mono-amine oxidase (MAO) inhibitors, which significantly decrease immobility time in forced swimming test (FST) [18]. Immobility is thought to reflect either a failure to persist in escape directed behaviour after persistent stress, or the

development of passive behaviour that disengages the animal from active forms of coping with stressful stimuli ^[19]. *Shilajatu bhavita* with *guduchi* and *triphala*, *gomutra shodhit*, water purified and piracetam showed decreased immobility time, and antidepressant activity because antidepressants reduce the immobility after forced swimming ^[20]. Piracetam potentiated the activating effect of antidepressants ^[21] It may be possible that *Shilajatu* itself as well as *Shilajatu bhavita* with *triphala* and *guduchi* potentiates antidepressant activity similar mechanism to that of piracetam.

CONCLUSION

Water purified *shilajatu*, *triphala* and *guduchi kwath bhavita shilajatu* possess significant anxiolytic activity as well as antidepressant activity.

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