Effect of Shodhita Shilajatu and Rajata Bhasma on Anxiety – A vitro study

Abstract

Background: Shilajatu and Rajata are important mineral drugs having medhya property. There are studies available on effect of shilajatu on diabetes, obesity and anxiety also, Rajata bhasma have proven to increase the memory, concentration. The main objective of the study was to observe the effect of Shodhita Shilajatu and Rajata Bhasma on anxiety in mice.

Methods:

Shodhana of Shilajatu was carried out by suryatapi method/, Shodhana of Rajata was carried out according to classical texts by using taila,takra,gomutra, kanji, kulattha kwatha. Rajata Bhasma was prepared using Kumari swarasa Bhavana as per Ayurveda Prakasha. Organoleptic characteristics, ash value analysis, SEM analysis of Rajata and Shilajatu were carried out according to standards. Anti Anxiety activity was analyzed by using light and dark test, open and closed arm activity test on four group of mice

Results:

Both Rajata and Shilajatu group rats have showed highly spent more time in light chamber 105.3±6.296, 114.8±6.215 respectively and open space against control group with Diazepam (94.0±2.39) and standard groups (30.5±1.50) $p<0.001$/ Locomotor activity was more in Shilajatu and Rajata Bhasma groups (429.2±3.73,437.7±6.31) when compared to Normal saline and Diazepam group (289.3±5.07,374.7±4.83)

Conclusion:

Rajata Bhasma and shuddha Shilajatu improved significantly the time spent in light chamber and locomotor activity when compared to normal saline and Diazepam groups of mice suggesting the good Anti Anxiety effect.

Key words- Anxiety, Rajata Bhasma, Shuddha Shilajatu, light chamber, locomotion

Introduction

Āyurveda, the ancient medical science is an aspect of Vedic lore closely connected to Rigveda and Atharvaveda. Ayurveda proclaims the imbalanced tridoshas `(*vāta, pitta and kapha*, which are the metabolic principles maintaining all the functions in the body) are the causes of diseases. Increase or diminution of doshas causes diseases. Equilibrium of doshas gives health. Two seats of diseases are body and mind. Rajas, and tamas are two doshas of the mind (manas). [1,2,3,4]

The main objective of Ayurveda is to counter act the diseases through Rasoushadhi- minerals and metals which are used for the preparation of medicine, dealt in Rasashastra and Bhaishajya Kalpana. History of the Rasoushadhis is much old as it explained in Atharvaveda also.

Rasashastra deals with the knowledge of alchemical and pharmaceutical processes.viz. samskaras related to mercury, shodhana, marana of different metallic, mineral, substances.References for Rasoushadhi and their therapeutic utilization can be found since the period of Brahatrayis and laghutrayis. [1,2,3,4,5,6]

Shilajatu has been explained in the Brahatrayis. Some of the Rasashastra texts consider it as mahārasa, .Charaka samhita, Ayurveda prakasha and Rasa tarangini explain the therapeutic uses of Śilajatu as Medhya and unmādahara. [1,7,8]

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.

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 like genitourinary disorder, epilepsy, nervous disorder, [9,10]

A study have observed the effect of Shilajatu and Asnadi Ghana vati on Diabetes and showed statistically significant improvement was observed in sign and symptoms as well as on blood sugar level in both groups after the completion of treatment.

Decreasing effect on the recurrent resistance of HIV virus of shilajatu when compared to ART Anti Retroviral Therapy (ART) has been proved. [11,12]

Improving of learning memory in rats have been proved by administering Polyherbal formulation containing Shilajatu. [13]

Rajata, which is included under lohavarga have medhya, rasayana, apasmarahara, smrti vardhaka properties. Rajat Bhasma which has Vata-Shamak, Madhura Vipaka, Kashaya,Amla, Rasa, Sheetala, Snigdha and also Brimhana so it play important role in nervous system. A study have elaborately explained the standard manufacturing procedure of Rajata Bhasma. [14]

Ethanol leaf extract of Ocimum Sanctum in anxiety and depression has been proven effective in animal models. [15]. Ashwagandha and Shilajatu have been proven effective for alcohol withdrawal anxiety. [16]. Clinical efficacy manasamitra vatakam on generalized anxiety disorder has been proven. [17].

A study have demonstrated 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. [18]

Rajata Bhasma and Shankha pushpin syrup have improved remote recent memory, remote memory,attention and concentration. [19]

Methodology

Method of preparation of medicines:

Shodhana of Shilajatu

Ashuddha Shilajatu was powdered and added to warm Triphala Kwatha and was stirred for 20-25 minutes with a spatula.Then Triphala Kwatha containing Ashuddha Shilajatu was continuously stirred till it completely dissolved in Triphala. This was kept undisturbed in sunlight. Next day the supernatant liquid was filtered and stored, whereas residual material was collected separately.

In residual materials again hot water was added and mixed well and was kept undisturbed for another 24 hours. Next day the supernatant liquid was again filtered and residual matter was separated. This process was continued for 7 times and soluble matter from the residue was collected and stored. [6]

Preparation of Rajata Bhasma

First Samanya shodhana was carried put by doing nirvapa in taila,takra, gomutra, kanji, kulattha kwatha. Vishehsa shodhana was carried put by doing nirvapa in Jyotishmati taila**.**

Then 22 gm of Shodhita Rajata Patra was applied with kalka of made of 40gm of Kajjali and Kumari Swaras and dried. After sharava samputa varaha puta was given .(Ayurveda

Prakasha) From the second Puta 10 ml of Kumari Swarasa Bhavana was done. Totally five putas were given . [7]

2.2.Method of screening of anti anxiety acivity:

The experimental procedures were approved by Institutional Animal Ethical Committee (IAEC), SET's College of pharmacy, Dharwad, Karnataka (REG.No.112/1999/CPCSEA). According to prescribed guidelines of committee for the purpose of Control and Supervision of Experiments in Animals (CPCSEA), Government of India

Pre-procedure

Selection of animal species and dosage

Swiss albino mice weighing 25 ± 5 g, of either sex were used in the study. The Inbred colonies of mice were purchased from Venkateshwara enterprises, Bangalore

The animals were randomized into 4 groups of six animals each into control, standard, Rajata Bhasma intervention and Shodhita Shilajatu intervention groups and are housed. In each group the animals were marked with numbers at the tail to permit individual identification.

Rajata Bhasma

The classically advised, normal human adult dose of Rajata Bhasma is one by fourth to one Ratti, which is equal to 31.25mg to 125 mg. This was converted into animal dose based on Paget and Barner's surface area ratio which works out to be

approximately 3.12 mg to 12.05 mg /kg body weight.

i.e. Mice dose / kg body wt. = 0.018 x Human dose x 5

= 0.018 x 1 ratti x 5

= 0.018 x 125mg x 5

Therefore, Mice dose of Rajata Bhasma = 12.05 mg / kg body weight.

For the current study the dose of Rajata Bhasma was selected

as 10mg/kg body weight as per the advice of the expert committee.

Shodhita Shilajatu

The classically advised, normal human adult dose of Shodhita Shilajatu is two to eight Ratti, which is equal to 250 mg to 1gm.

This was converted into animal dose based on Paget and Barner's surface area ratio which works out to be approximately 22.5 mg to 90 mg /kg body weight.

i.e. Mice dose / kg body wt. = 0.018 x Human dose x 5

= 0.018 x 2 ratti x 5

= 0.018 x 250mg x 5

Therefore, Mice dose of Rajata Bhasma = 22.5 mg / kg body weight.

For the current study the dose of Shodhita Shilajatu was selected

as 20mg/kg body weight as per the advice of the expert committee.

Diazepam

The standard the Mice dose of Diazepam = 2mg / kg body weight.

• Preparation of dose

Weighed quantity of Rajata Bhasma /Standard drug was suspended in water using 0.5% tragacanth and administered orally to experimental animals. Suspension of Bhasma was prepared freshly each day. The Bhasma was administered at a constant volume for each animal.

Procedure of data collection

A. Light and dark Chamber test method

Procedure- The apparatus consisted of two 20 cm×10 cm×14 cm plastic boxes: one was dark and the other was transparent. The mice were allowed to move from one box to the other through an open door between the two boxes. A 100W bulb placed 30 cm above the floor of the transparent box was the only light source in the room. A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light and dark box were recorded for 10 min immediately after the mouse stepped into the dark box. The apparatus was cleaned thoroughly between trials.

B. Test for Locomotor Activity using Acto-photometer

Procedure- The locomotor activity of the test animals were recorded for a period

of 10 minutes using Medicraft Actophotometer, Model No. 600-4D (INCO, Ambala, India). The apparatus consists of a square area (30x30x25 cm) with wire mesh bottom, in which animal moves. The actophotometer operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photo cell is cut off by the animal, a count is recorded.

C. Test for Rearing behaviour using Open field exploration test method

The acclimated mice were transported to the test room or test apparatus singly and each mouse was placed in the centre of a chamber. Care was taken to be as distant and unmoving as possible once the test session has started. Sudden motion or noise were avoided. Mice were allowed to freely explore the chamber for the duration of the test session. Each line crossed is scored as one unit of activity and the number or rearing behaviour in the specified time will be noted. For assessing novel environment exploration, 10-min test length was considered. Mice were allowed to freely explore the test arena for the entire session duration

Recording results

The mean values of % of The values of Mean % were recorded by considering-

• Entry in light and dark chamber.

• Time spent in dark and light chamber.

• Locomotor activity counts in the actophotometer.

• Number of rearing in the open field test board

Data analysis

Collected readings were statistically analyzed using ANOVA of variance assuming completely randomized design and least significance method. The results were expressed as mean, ANOVA F ratio and comparison was done with t test. As P

Results

Table 1 Analytical study results of Rajata Bhasma

Sl.No.	**Parameters**	**Result n=3 %w/w Rajata Bhasma**
1	Loss on drying at 105^{o}	0.347%
2	Total ash	91.03
3	Acid insoluble ash	13.93
4	Loss on ignition	0.85
7	pH	5.40

Table 2 -Analytical study results of Shodhita Shilajatu

Sl.No.	**Parameters**	**Result n=3 %w/w Shilajatu**
1	Loss on drying at 105^{o}	8.90%
2	Total ash	15.35%
3	Acid insoluble ash	1.0%

5	Alcohol soluble extractive	20.16
6	Water soluble extractive	63.48
7	pH	6.37

Table 1 and 2 gives analytical tests results. Indicating medicines are prepared as per standard operating procedure.

Table 3 Effect of Shilajatu and RajataBhasma on anti anxiety activity (Time taken in secs)

Treatment	Time spent in light chamber	Time spent in dark chamber	Entries into light chamber	Entries into dark chamber
Normal Saline	34.50±1.500	268.3±3.913	11.67±1.054	19.17±0.7923
Diazepam	94.0±2.394***	182.2±4.053***	25.83±1.138***	6.333±0.4944***
Shilajatu	105.3±6.296***	188.5±3.117***	18.50±0.8466***	9.833±0.8724***
Rajata Bhasma	114.8±6.215***	198.8±6.442***	21.33±0.8433***	10.83±0.6009***

The values are expressed as mean ± SEM (n=6) $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$

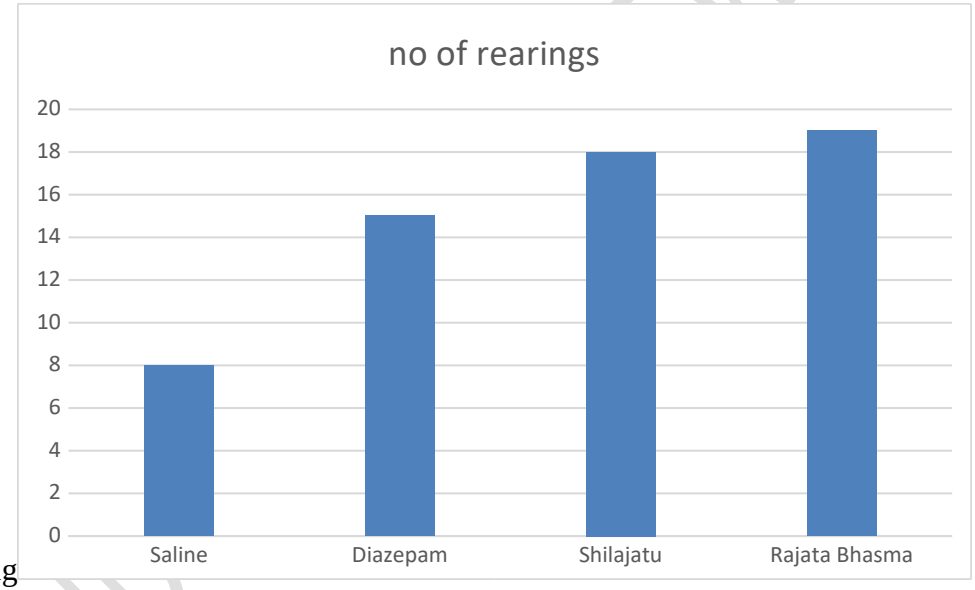
Table 3 gives the values for time spent in light chamber, and dark chamber, of four groups of animals with normal saline, diazepam, Shilajatu, Rajata bhasma. For Shilajatu and Rajata bhasma group it is more than Placebo and standard drug group. While for time in dark chamber it is reduced for Shilajatu and and Rajata bhasma when compared to saline group.

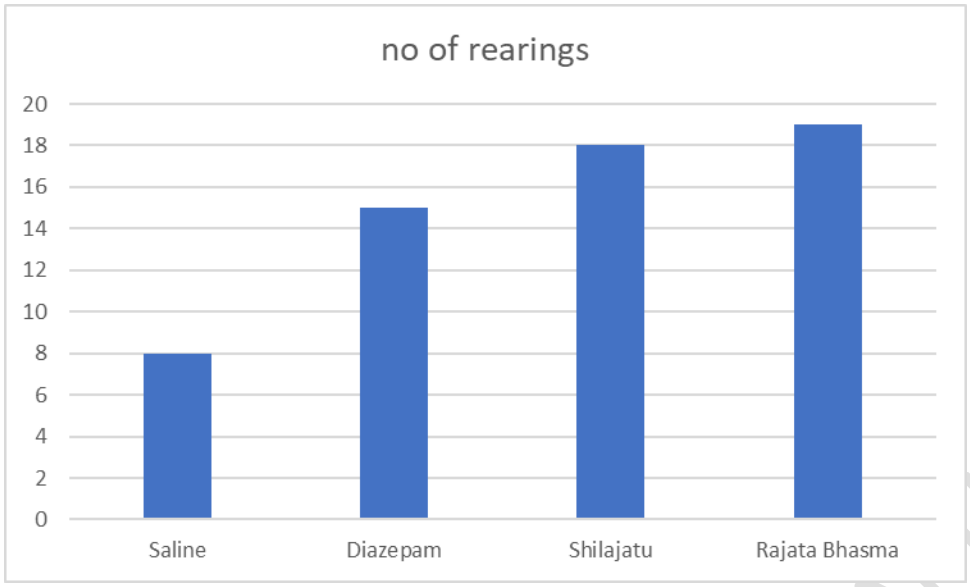
Table 4 Effect of Shilajatu and RajataBhasma on locomotor activity per seconds

Treatment	Locomotor Activity
Normal Saline	289.3±5.077
Diazepam	374.7±4.835***
Shilajatu	429.2±3.736***
RajataBhasma	437.7±6.312***

Table 4 gives the values for locomotor activity of four groups of animals with normal saline, diazepam, Shilajatu, Rajata bhasma. For Shilajatu and Rajata bhasma group it is more than Placebo and standard drug group

Graph 1- Number of rearing





Discussion

In the open field behaviour test, animals express their anxiety and fear by decreasing ambulation, exploration, freezing, rearing, grooming behaviour and increase in defecation due to heightened autonomic activity. These behavioural changes are attenuated by classical anxiolytic and augmented by anxiogenic agents.

In our study, Shodhita shilajatu and Rajata Bhasma treated animals showed increased ambulation, grooming and rearing than control group animals. It was reported that Shilajatu modulate the brain Monoamines $p < 0.001$ showed decreased significance level as compare to standard group activity, It may be possible that modulation of brain monoamines by shilajatu, protective activity of dopaminergic neurons by guduchi helping towards decreased level of anxiety. [20,21]

In the light/dark test, anxiety is generated by the conflict between the tendency to explore and the initial tendency to avoid the unfamiliar and can be evaluated according to the

number of transitions in to and the time spent in the light chamber where in increase in these parameters is considered to reflect anxiolytic-like properties. In the present study results showed that the Rajata Bhasma, (10 mg/kg)increased time spent in the light chamber, suggesting anxiolytic action.

Moreover, anxiolysis was studied by measuring external signs like ambulation, rearing, in open field test (OFT). OFT is used for evaluating the effect of drugs on gross general behaviour and is used to measure the level of nervous excitability when the animals are exposed to a novel environment.

Exploration in a new environment is an essential part of normal behaviour in animals; lower ambulation, exploration and reduction in normal rearing are due to anxiety and fear. However dis inhibitory actions of anxiolytics increase these behavioural activities in new environment by induced suppression of behaviour.

In the present study we have used light and dark test and open field test. And we observed that Shilajatu and Rajata Bhasma group were significantly better than placebo(normal saline) group and standard drug(Diazepam) group in time spent in light chamber and dark chamber. While in entries in light chamber Shilajatu and Rajata Bhasma group were significantly better than normal saline group. But, it was less than diazepam group. And Rajata Bhasma group was better than Shodhita Shilajatu group. And loco-motor activity also significantly increased in Shodhita Shilajatu and Rajata Bhasma group when compared to normal saline group and Diazepam group. All these result suggesting both Rajata Bhasma and Shodhita Shilajatu possesses anxiolytic property.

Possible mechanism of action:

Anxiety and hypno-sedation are principally mediated in the CNS by the $GABA_A$ receptor complex, which is also involved in other physiological functions related to behaviour and in various psychological and neurological disorders such as epilepsy, anxiety,

depression, Parkinson syndrome and Alzheimer's disease. GABA synergic activity in the brain can be increased by three ways, by GABA agonists; barbiturates and benzodiazepines directly increase inhibitory chloride conductance and/or upregulate the effect of synaptically released GABA on the $GABA_A$ receptor respectively. Diverse drugs which are used in various psychological and neurological disorders might modify the GABA system at the level of the synthesis of GABA, induce anxiolysis or hypnosis in animals by potentiating the GABA mediated postsynaptic inhibition through an allosteric modification of GABA receptors, and thirdly by direct increase in chloride conductance or indirectly by potentiating GABA-induced chloride conductance with simultaneous depression of voltage activated Ca^{++} currents like barbiturates· sequence of Ca^{++} channel blockade, Ca^{++} entry into presynaptic nerve terminals is blocked, leading to inhibition of release of excitatory neurotransmitters such as glutamate. This results in net reduction of excitatory synaptic transmission. [22]

Strength of the study is that it is first study to check the effect of Rajata bhasma on anxiety, with control group. As the continuation of the research process further study needs to be carried out to explore the pharmacokinetic and Pharmaco dynamic effects of Shodhita Shilajatu, Rajata Bhasma over the anxiety.Study using the functional MRI and reverse pharmacology methods may help in further exploration of the mode and site of action of Shodhita Shilajatu and Rajata Bhasma. Effect of Shodhita Shilajatu and Rajata Bhasma over there Manasika Vikaras like unmada needs to be explored.

.Conclusion

Both Shilajtu and Ratajabhasma have shown significant effect of reducing anxiety in light –dark test and loco motor activity test when compared to placebo and standard drug . Rajatabhasma was comparatively better than Shilajatu.

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