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Evaluation of Antidepressant activity of Shilajit in experimental animals Sugar Levels In Diabetic Patients

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RESEARCH ARTICLE
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Abstract:

Introduction: Depression is a serious medical (mental) illness that negatively affects how you feel, the way you think and how you act. The presently using drugs can impose a variety of side-effects including cardiac toxicity, hypopiesia, sexual dysfunction, body weight gain, and sleep disorder. During the last decade, there is a growing interest in the therapeutic effects of natural products on mental disorders. Shilajit was investigation for antidepressant activity.

Methods: Antidepressant activity of pure Shilajit was investigated by using Forced swimming test (FST) and Tail suspension test (TST) and Open square test (OST) models. Lead acetate and Imipramine were used as reference standards.

Results: It has been observed from our study that both the MEAS at higher concentration showed significant ($p<0.01$) reduction in immobility in tail suspension and forced swim model of depression comparable to Lead acetate and Imipramine.

Discussion: However further study is needed to understand mechanism of action and to identify active component responsible for antidepressant like activity

Keywords: Shilajit, Fulvic acid, Immobility time, Ambulation frequency, Rearing frequency.

INTRODUCTION:

Depression causes feeling of sadness and/ or loss of interest in activities once enjoyed. It can lead to a variety of emotional and physical problems and can decrease a person's ability to function at work or at home. Major depression is characterized by symptoms like sad mood, loss of interest and pleasure, low energy, worthlessness, guilt, psychomotor retardation or agitation, change in appetite and sleep, melancholia, suicidal thoughts etc.

According to the World Health Organization report, mood disorders are the second leading cause worldwide of disability adjusted life years and the leading cause of years lived with disability in all ages. Each drug used to treat this disorder has a success rate of about 60%. In addition, most therapies require several weeks of treatment before improvement of signs and symptoms is observed and there are numerous side effects caused by antidepressants (Wong and Licinio, 2001). Thus, the high prevalence of depression and the fact that a significant proportion of individuals do not respond well to any currently marketed antidepressants or treatments support the need for new therapeutics to treat depression. Numerous antidepressant compounds are now available, presumably acting via different mechanisms including serotonergic, noradrenergic and/ or dopaminergic systems (Elhwuegi, 2004). Medical plant therapies may be effective alternatives in the treatment of depression, and has progressed significantly in the past decade (Zhang, 2004). Therefore, the present work aimed to evaluate firstly the antidepressant-like effect of the Shilajit in the models predictive of antidepressant action.

MATERIALS AND METHODS:

Materials:

List of chemicals:

1. Shilajit (Patanjali)
2. Lead acetate
3. Imipramine (Sunpharma)

Experimental Animals

Albino Mice and Rats were obtained from Dr. Vithalrao Vikhe Patil Foundation's College of Pharmacy, Ahmednagar. These all animals were stored in standard polypropylene cages and maintained at 270C ± 20C, under 12 hours dark/light cycle. These cages were specially

designed to separate urine and feaces of the animals. The animal experiments were approved by institutional animal ethics committee (IAEC) of Dr. Vithalrao Vikhe Patil Foundation's College Of Pharmacy, Ahmednagar. (Reg. No. 1670/PO/ReBiBt/S/12/CPCSEA)

Antidepressant Activity

Experimental Design for anti-depressant activity:

The rats were divided six groups (n=6). Drugs/ vehicle were administered to the animals 60min prior to study.

Group I: control, administer saline

Group II: Negative control Lead acetate (100 mg/kg orally).

Group III: Lead acetate. (100mg/kg) + Standard Imipramine(100mg/kg)

Group IV: Test I Lead acetate (100mg/kg) + Shilajit (100mg/kg)

Group V: Test II Lead acetate (100mg/kg) + Shilajit (200mg/kg)

Group VI: Test III Lead acetate (100mg/kg)+ Shilajit (400mg/kg)

METHODS:

Open Field Test:

Procedure: Open Field Test was carried out in a square wooden arena (80 cm × 80 cm × 40 cm high) with white smooth polished floor divided by black lines into 16 equal squares. The test was performed under white light in a quiet room. Each of the rats was placed at the same corner square and observed for 5 min. The floor was cleaned after testing each rat. The following parameters were recorded during the 5 min observation period;

a) Latency: time taken by each animal till it starts moving in the arena,

b) Ambulation frequency: number of squares crossed by the animal,

c) Rearing frequency: number of times the animal stood, stretched on its hind limbs with or without forelimb support and

d) The number of fecal pellet.

Forced swim test:

Procedure: The forced swim test was performed according to the method described by Porsolt., et al. where each rat was placed for 5 minutes in a cylindrical water tank (70 cm high, 40 cm diameter) where, water level was about 40 cm and water temperature maintained at 23 - 25°C. The tank was emptied and washed with fresh water, flushed between each rat to remove any traces of urine or faeces. Two sessions was conducted; an initial 15 minutes training session (pre-test session) followed 24 hour later by a 5 minutes test session. During the test session, the immobility time, swimming and climbing times was observed. The total duration of the immobility was measured during the 5 minutes of test session. Upon removal from the water, rats were towel dried and finally returned to the cage. This was carried out on the 6th and 7th day respectively.

Tail suspension test:

Procedure: The rat was suspended individually by its tail on a 10cm horizontal metal rod distanced 50cm above the bench and 120cm from the ground using an adhesive tape, fixed one cm from the tip of the tail. During the first minute the rat was tried to escape from its condition by showing twisting movements. Afterward, rat developed behavioral despair characterized by disturbing response and immobility, an indication for depression state. Drugs categorized as antidepressants are believed to reduce immobility time displayed by mice following vigorous and unsuccessful trials of escape after suspended by the tail. In this experiment, mice were divided into four groups (n=6 for each group). Thirty minutes before experiment, rat was treated with i.p. dose of Shilajit, and other groups and doses are similar as described early in forced swim test. Control group received 10ml/kg normal saline. Each rat was then suspended, upside down, for five minutes. The immobility conduct, "rat hung-down passively and entirely motionless", was observed and duration of immobility (the entire time that the rat displayed no movement) was recorded during testing period using a digital stop watch.

Statistical analysis:

Arithmetic means of the values of readings were calculated for each experiment the result obtained was used for statistical analysis using INTA software. The data obtained from various models of nephrotoxicity in rats experiments were subjected to analysis of variance (ANOVA) followed by Dunnett t-test using INTA software. Value of $p < 0.01$ was considered statistically significant

RESULTS:

1) Open field test:

Effect of SHILAJIT on rats using Open field Test:

Effect of SHILAJIT on rats using Open field Test.

GROUPS	Treatment	Ambulation Frequency (count/5min)	Rearing Frequency (count/5min)	No. of faecal pellets
Group I	Control (Normal saline)	41.50±1.80	31.83±1.74	3.33± 0.55
Group II	Negative control Lead acetate. (100mg/kg)	17.66±1.87###	12.50±2.01###	5.66± 0.80###
Group III	Lead acetate. (100mg/kg) + Standard Imipramine (100mg/kg)	51.50± 2.20***	34.33±1.30***	1.50± 0.34***
Group IV	Test I Lead acetate (100mg/kg) + Shilajit (100mg/kg)	25.16±2.08^{NS}	13.50 ±1.47^{NS}	4.16± 0.87^{NS}
Group V	Test II Lead acetate(100mg/kg) + Shilajit (200mg/kg)	33.66±1.49***	24.16±2.15***	2.33± 0.33***
Group VI	Test III Lead acetate(100mg/kg)+ Shilajit (400mg/kg)	46.00±1.86***	29.00±1.78***	1.83 ± .30***

2) Forced swim test:

Effect of SHILAJIT on Immobility period (Secs) of rats using Forced Swim Test

GROUP	Treatment	IMMOBILITY TIME(Seconds)
Group I	Control (Normal saline)	153.33±2.40
Group II	Std control Imipramine (100mg/kg)	82.16± 2.13***
Group III	Shilajit (100mg/kg)	140.83± 3.08**
Group IV	Shilajit(200mg/kg)	118.66± 2.95***
Group V	Shilajit(400mg/kg)	102.16± 1.53***

3) Tail suspension test:

Effect of SHILAJIT on Immobility Period (Secs) of rats using Tail suspension Test

GROUP	Treatment	IMMOILITY TIME (Seconds)
Group I	Normal control (Normal saline)	253.83 ±2.02
Group II	Std controlImipramine (100mg/kg)	198.66±1.72***
Group III	Shilajit (100mg/kg)	242.50 ± 3.13**
Group IV	Shilajit(200mg/kg)	180.83± 2.50***
Group V	Shilajit(400mg/kg)	164.16 ± 2.01***

DISCUSSION:

Lead is a pervasive and persistent environmental pollutant that can be detected in almost all phases of environment and biological systems. Lead constitutes the most abundant non-essential element in the humans, due to its dispersion in ambient air, many foods, drinking water, and dust. Humans have used lead since ancient times. Lead toxicity or lead poisoning is a medical condition caused by increased levels of the heavy metal lead in the body. Symptoms of lead toxicity include abdominal pain, headache, anemia, irritability and in severe cases seizures, coma and death. Lead is a health hazard to humans if it is inhaled or ingested, interfering with the production of red blood cells it has the potential to disrupt many biological systems, particularly proteins because it forms complexes with important functional chemical groups including carboxyl (-COOH), amine (-NH) and thiol (-SH). Thus, many enzymes are potential targets. Several researches indicated that lead can cause neurological, hematological, gastrointestinal, reproductive, circulatory, and immunological pathologies.

The brain is the magnificent and most complex organ in a vertebrate's body. The brain is found within the head, usually close to the primary sensory organs for such senses as vision, hearing, balance, taste, and smell. It is made up of more than 100 billion nerves that communicate in trillions of connections called synapses. The brain is made up of many specialized areas that work together: The cortex which is the outermost layer of brain cells. Thinking and voluntary movements begin within the cortex. The brain stem is between the medulla spinals and also the remainder of the brain. Basic functions like breathing and sleep are controlled there; the basal ganglia are a cluster of structures in the centre of the brain. The basal ganglia coordinate messages between multiple other brain areas. [4]

The open-field test is used to provide a qualitative and quantitative measurement of exploratory and locomotor activity in rodents. In the open field test, rodents are exposed to an unusual background, where they express fear and anxiety in terms of increased defecation and decreased activities such as rearing, ambulation, and self-grooming along with less activity in the center.

Shilajit at the dose of 100, 200 and 400 mg/kg were used for the in vitro antidepressant activity and the doses were selected based on the acute toxicity studies from the literature. Open field test was performed to induce and confirm depression using lead acetate 100 mg/kg. From the results shown in table no.4, there is a significant decrease in the ambulation frequency, rearing frequency and significant increase in fecal pellets in lead acetate administered group confirming the induction of depression when compared to normal group. Whereas there is a significant increase in the ambulation frequency, rearing frequency and reduction in fecal pellets in animals treated with Imipramine (100mg/kg) Standard group when compared with the negative control group. Also, there is a significant increase ($p<0.001$) in the ambulation frequency, rearing frequency and reduction in fecal pellets in animals treated with Shilajit200 and 400 mg/kg when compared with negative control group that received 100 mg/kg of lead acetate but there is no significant increase in the ambulation frequency, rearing frequency and reduction in fecal pellets in animals treated with Shilajit 100 mg/kg when compared with negative control group as there is no change in the state of depression in animals. Thus, Shilajit 200 and 400 mg/kg showed dose dependent anti-depressant activity when compared to with negative control group.

The forced swim test (FST) was developed by Porsolt, so as to examine the antidepressant activity of drugs. FTS is one of the most widely used tests for screening of antidepressant drugs and its action. Although it works in a subacute condition (30 minutes after drug administration). When rat or mice are subjected to force swim in a limited space with no way to escape then a characteristic immobility develops in them after some time of forced swimming. From our results, shown in table no.5, there is a significant decrease in the immobility period in animals treated with Imipramine (100mg/kg) Standard group when compared with the control group showing its anti-depressant activity. Shilajit at the dose of 100 mg/kg showed very less decrease ($p< 0.01$) in the immobility period in animals when compared with the control group, whereas Shilajit at the dose of 200 and 400 mg/kg showed significant decrease ($p<0.001$) in the immobility period when compared with the control group, showing antidepressant activity in a dose dependent manner.

In the Tail Suspension test, rodents are placed in an inescapable but moderately stressful situation. Lack of escape related behavior is considered immobility. When a rodent is suspended by its tail, the immobility is displayed because of inescapable stress. It reflects behavioral despair. The principle of this test is that suspending rodent upside down leads to characteristic behavior of immobility which resembles to human depression. From our results, shown in table no.6, there is a significant decrease in the immobility period in animals treated with Imipramine (100mg/kg) Standard group when compared with the control group showing its anti-depressant activity. Shilajit at the dose of 100 mg/kg showed very slight decrease ($p< 0.01$) in the immobility period in animals when compared with the control group, whereas Shilajit at the dose of 200 and 400 mg/kg showed significant decrease ($p<0.001$) in the immobility period when compared with the control group, showing antidepressant activity in a dose dependent manner.

The antidepressant effects of Shilajit in lead acetate induced depression may be due to active antistress principles. The active constituent of Shilajit are fulvic acid, humic acid, α- dibenzopyrone etc. Thefulvic acid as the key molecule is one of the most powerful electron donors available. Because it is such a powerful electron donor for the repair of tissues, it greatly enhances the bioavailability of trace minerals and rebalances the electrical imbalance in damaged cells. Shilajit neutralizes toxins free radicals and metals throughout the body and is able to detoxify both simple and complex toxins. As per this view and above results, we assume that the herbo-mineral drug shilajit possess antidepressant activity.

CONCLUSION:

Depression is an important global public health problem due to both its relatively high lifetime prevalence and the significant disability that it causes. Patients with major depression have changes in brain monoamine neurotransmitters, specifically norepinephrine, serotonin, and dopamine.

Stress hormones have a most of negative effects on the human body. Stress induced cortisol actually because the body store fats leading to stubborn weight gain and even obesity. Stress hormones can cause insomnia and poor sleep or the opposite: lethargy and fatigue. Stress can raise blood pressure, as well as cause digestive problems. In short: stress is very bad for the human body. However, Shilajit contains humates including Humic & Fulvic acid which are known to suppress stress hormones within the body allowing us to relax and rest. Both medical studies and anecdotal evidence supports the fact that Fulvic Acid Supplements reduce destructive stress hormones.

From the present study, lead acetate was able to cause depression, and this was determined in the behavioral test i.e. open field test model, where Shilajit 200 and 400 mg/kg showed dose dependent anti-depressant activity reducing the depression induced by lead acetate.In the Open Field Test, our results showed significant increase in Ambulation frequency, rearing frequency and the decrease in number of faecal pellet in wistar rats.This indicates that Shilajit it is almost as effective as Imipramine in reducing the depression produced by lead acetate.

Also, Shilajit was effective in producing significant antidepressant effects in the forced swimming test and tail suspension test in rats, as it is evident from the reduction in the immobility time. In both the models animals were forced to swim in a restricted space from which there was no escape, and will, after periods of agitation, cease attempts to escape and become immobile. It is accepted that immobility is seen in rodents during swimming reflects behaviour despair as seen in human depression and that the antidepressant drugs are able to reduce the immobility time in rats.

Fulvic acid actively takes part in the transportation of nutrients into deep tissues and helps to overcome tiredness, lethargy, and chronic fatigue. It also works effectively as a tonic for cardiac, gastric, and nervous systems, adaptogen and anti-stress agent. Fulvic acid has been shown to discourage age-related cognitive impairment, Alzheimer's disease, jaundice, bronchitis and anemia as well[7]. The presence of minerals, metals and vitamins in Shilajit, have been shown to possess anti-depressant effect. The present study proves the anti-depressant activity of Shilajit in a dose dependent manner. We believe that Shilajit has the potential to be used as an adjuvant in the treatment of depression and other mood disorders.

Shilajit possess anti-stress or adaptogenic properties have often been pointed out by several modern scholars and researchers and have revealed that adaptogens, are the therapeutically interesting antidepressants and anxiolytics like and have stress response desensitizing effects. The activation of 5-HT3 receptor in the brain leads to the release of monoamines like dopamine and serotonin. Since 5-HT3 receptor antagonists delivered central effects equivalent to those of anti-depressants and anxiolytics. So, the anti-depressants activity of Shilajit might involve an action or effects on serotonergic transmission or due to its mixed aminergic potentiating effect.

The study concluded that the Shilajit showed significant antidepressant-like effect in lead acetate induced depression in rats (OFT) and also in models of forced swim test and tail suspension test.

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