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Research Article

EVALUATION OF ANTI-DEPRESSANT POTENTIAL OF MEDICINAL *GHRITA*

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ABSTRACT

This article aims to evaluate the possible antidepressant effect of the *Ghrita* prepared from the combination of these plants and to provide probable scientific explanations for using medicated *Ghrita* (ghee) in Ayurvedic system of medicine. Herbs for *Ghrita* like *Marsilea quadrifolia, Lawsonia inermis, Mimosa pudica, Piper betle* were collected freshly during the month of June. One part *Kalka* (herb bolus), 4 parts pure cow ghee, and 16 parts *Dravadravya* were used to make *Ghrita* (*Swarasa*). Anti-depressant potential of the *Ghrita* was evaluated by forced swimming, tail suspension, locomotor activity, rota-rod test (motor co-ordination), elevated plus maze (EPM) model, and hole board test were used to assess the *Ghrita's* anti-depressant potential. According to the Irwin schedule, *Gritha* medication reduces alertness but has no effect on other parameters, but imipramine treatment reduces responsiveness, alertness, grooming, and writing reflexes to some extent. Following 14 days of therapy with medicinal *Ghrita*, the immobility time in the forced swim test and the tail suspension test were reduced by 29% and 42%, respectively. In the rotarod test, it had no significant effect on muscle gripping ability when compared to control animals, whereas imipramine produced a significant result. It was observed that the medicinal *Gritha* significantly ($p<0.05$) decreases the locomotor activity in actophotometer test and head dips counts in hole board test as compared to control at the end of 14 days study in a 5 minute observation. *Ghrita* increased the number of entries in the elevated plus maze by 50% and the time spent in the open arm by 72%. The results of this investigation suggest the possible antidepressant potential of medicinal *Ghrita*.

INTRODUCTION

Depression is considered as an affective disorder characterized by change in mood, lack of interest in the surroundings, psychomotor retardation and melancholia. The prevalence of depression in general population is estimated to be around approximately 450 million. An estimated 5.8% of men and 9.5% of women experience a depressive episode in their lifetime with suicide being one of the most common outcomes of depression[1]. *Ghrita* is a compound formulation containing butter fat obtained from cow's milk along with other active ingredients from natural origin.

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Ghee (clarified butter) in such preparations act as both, a vehicle for extracting and holding the active therapeutic principles from the drugs and an active ingredient due to its many actions claimed in Ayurveda. [2]

The tested *Ghrita* compound formulation containing ghee along with *Marsilea quadrifolia* having anti-cancer, anti-diabetic, analgesic, anti-diarrhoeal, anti-amnesic and antianxiety[3], *Lawsoniainermis* having analgesic, hypoglycemic, hepatoprotective, immunostimulant, anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antiparasitic, antitrypanosomal, antidermatophytic, antioxidant, tuberculostatic and anticancer properties[4] (Santosh Yadav 2013). *Mimosa pudica* having its effect against leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, asthma, leucoderma, fatigue and blood diseases[5] (Baby Joseph, 2013), *Piper betle* possesses antimicrobial anti-diabetic, gastroprotective, immunomodulatory,

antioxidant, antifertility, hepato-protective, cytotoxicity, radioprotective activity etc. [6]

A number of medicinal plants have been studied for the antidepressant properties. However most of the works have been carried out on these plants are on their extracts or the chemical fractions. In traditional system of medicine generally plant are given as combinations in various formulations as per Ayurveda. In an effort to correlate the ancient knowledge with the modern concepts of research in the pharmacology, we decided to study the effects of *Ghrita* on some neuropharmacological parameters including antidepressant activity in rats.

MATERIAL AND METHOD

Preparation of *Ghrita*

Fresh leaves of *Marsilea quadrifolia, Lawsonia inermis, Mimosa pudica, Piper betle* were collected from rural belt Tadepaligudem in the month of June and ghee was procured from the local dairy farm. The *Ghrita* was prepared by adopting classical method according to need. Fine paste (*Kalka*) of ingredients was prepared and was spread evenly on the inner side of the bottom of a stainless steel container. Cow ghee in proportion of 4 parts to the paste, was slowly spread over the paste without disturbing it. *Dravadravya* or decoction, herb juice (16 parts) was mixed with the cow ghee in the container and thoroughly mixed without disturbing the paste spread at the bottom. The container was then placed on a gas burner and heating was started on a low to moderate flame as per the requirement. The contents were constantly stirred and heating was continued till half of the *Dravadravya* got evaporated, after which heating was discontinued and the contents were left to cool overnight. The heating was then resumed on the next morning observing the same precautions described above till all the water content in *Dravadravya* evaporated from the ghee. The contents were frequently tested for completion of the process and to decide the termination of heating. The heating was stopped as soon as the end point was reached. The container was then removed from the flame and left to cool on its own. The contents were filtered in warm condition through a muslin cloth. The filtrate i.e., *Ghrita* was collected in a clean autoclaved glass bottle and weighed. The prepared *Ghrita* was stored in an airtight umber colour bottle for further use.

Animals: Healthy adult male Swiss albino mice (20-25 g) were used for the study of anxiolytic The animals were housed in a group of six per cage and maintained under standard environmental conditions: 25±2°C temperature, 12:12 h light and dark cycle, and 45-55% relative humidity, with free access to food and water *ad libitum.* Mice were treated with Imipramine (20mg/kg,), medicinal *Ghrita* (0.1ml) P.O. for 14 days. 30 min after treatment with imipramine and 1h after treatment with control and/ or *Ghrita*, the following tests were performed. This study was performed in the animal house (Regd.No:439/01/CPCSEA) at Sri Vasavi Institute of pharmaceutical sciences, Pedatadepalli, Andhra Pradesh, India after approval by IAEC with approval no SVIPS/2018/MPC/002.

Forced Swimming Test[7]

Male Swiss albino mice weighing 20-25 g were used and were divided into 3 groups. Mice were individually forced to swim inside a vertical Flexi-glass cylinder (height: 40cm; diameter: 18cm, containing 15 cm of water maintained at 25°C).Mice placed in the cylinders for the first time was initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2–3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. After 5–6 min immobility reaches a plateau where the mice remain immobile for approximately 80% of the time. After 15 min in the water the mice was removed and allowed to dry in a heated enclosure (32°C) before being returned to their home cages. They were again placed in the cylinder 24 h later and the total duration of immobility was measured during a 5 minuets. Floating behaviour during this 5 min period was found to be reproducible indifferent groups of mice. An animal was judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface

Tail-suspension test[8]

The total duration of immobility induced by tail suspension was measured. Mice both acoustically and visually isolated were suspended 50cm above the floor by adhesive tape placed approximately 1cm from the tip of the tail. The animals behavior were observed for a period of 6 min. The immobility time was measured to assess the antidepressant potential of the test compound.

Locomotor Activity

The spontaneous locomotor activity of animal was recorded individually for 10 min using actophotometer. The locomotor activity (horizontal activity) can be easily measured using an Actophotometer, which operates on photoelectric cells, which are connected in circuits with a counter. When the animals cut off a beam of light falling on the photocell, a count is recorded. Difference in locomotor activity before and after administration of drug was used for assessing the effect.

Rotarod Test (Motor Co-ordination)

The apparatus consists of a horizontal metal rod or metal rod coated with rubber with 3cm diameter attached to a motor with the speed adjusted to 20-25 rpm. The rod is divided into 4 compartments and present at a height to prevent the escape of animals from the trial. Fall of the animal from the

rotating rod result in stopping the timer from which the time of residence is measured. Male Swiss albino mice with a weight between 18 and 25g undergo a pre-test on the apparatus.

Elevated plus maze (EPM) model [9]

The elevated plus-maze comprised two open (30cm×5cm×0.25cm) and two enclosed (30cm×5 cm×15cm) arms that radiated from a central platform (5cm×5cm) to form a plus sign. The maze is preset at height of 50 cm from the ground to prevent the escape of animals during trial. The trial is started by placing an animal on the central platform of the maze facing an open arm. The number of entries into, and the time spent in, each of the two types of arm, are counted during a 5 min test period. The residence of animal in open arm and the time of entry to the open arm serve as the indicator of the potency of the test compound. A mouse is considered to have entered an arm when all four paws are on the arm.

Statistical Analysis

The mean ± S.E.M. values were calculated for each group. The data were analysed using one-way ANOVA followed by student t- test to cheek the significance P<0.05 was considered to be statistically significant

RESULTS

Table 1: Effect of Medicinal *Gritha* on forced swim test: (Immobility Time in sec)

Groups	Number of days				
	B.T	**1**	**4**	**8**	**14**
Control	153±2.6	161.6±3.2	156.6±4.3	163.3±2.5	166.6±1.8
(Normal saline0.1 ml)	135.2±2.2	127.7±1.8 (5%)	118.3±0.9 (13%)	106.8±3.3* (26%)	95.3±0.6* (29%)
Test	132.3±2.6				72.3±1.4* (45%)

The observations are mean ± SEM of 6 animals. * p<0.05 as compared to that of vehicle treated group (One way ANOVA followed by student t- test).

In forced swim test the medicinal *Gritha* produced significantly (p<0.05) decreased in the total duration of immobility as compared to the control. In control animals there were no significant variation in the immobility time throughout the study.

Table 2: Effect of *Gritha* on *Tail* suspension (Immobility Time in sec)

Groups	Number of days				
	B.T	**1**	**4**	**8**	**14**
Control (Normal saline 0.1ml)	160.52±4.3	176.4±3.6	183.2±4.5	173.2±1.7	152±1.4
Test (*Gritha* 0.1ml)	166.66±7.3	150.6±2.4 (9%)	134.32±2.6 (19%)	124±10.4* (25%)	96±0.9*(42%)
Standard (Imipramine 20mg\kg)	165.3±6.4				88.6±1.2*(46%)

The observations are mean ± SEM of 6 animals. * p<0.05 as compared to that of vehicle treated group (One way ANOVA followed by student t- test).

In tail suspension test the animals treated with *Gritha* has shown significant (p<0.05) decreases the immobility time as compared to the controlled animal.

Table 3: Effect of Medicinal *Gritha* on Rota rod: Fall latency

Groups	Number of days				
	B.T	**1**	**4**	**8**	**14**
Control (Normal saline 0.1ml)	150±1.6	168.42±2.8	173.8±4.8	163±3.3	173.8±46
Test (*Gritha* 0.1ml)	136.16±2.5	124.36±1.56 (8.9%)	120.2 ±1.4 (11.7%)	118.76±0.5 (12.7%)	116.7±0.2 (14%)
Standard (imipramie 20mg\kg)	140±1.3				80.6±0.8* (42%)

The observations are mean ± SEM of 6 animals. * p<0.05 as compared to that of vehicle treated group

In current study the administration of medicinal *Gritha* produced non-significant effect on muscle gripping capability as compared to controlled animal which indicates the in-effectiveness of *Gritha* in muscle coordination. Imipramine decreases the muscle coordination significantly.

Table 4: Effect of Medicinal *Gritha* on Locomotor Activity: Number of days

Groups	Number of days				
	B.T	1	4	8	14
Control (Normal saline 0.1ml)	130±12.03	145.3±11.6	129.6±4.5	131.8±7.2	141±3.4
Test (*Gritha* 0.1ml)	240.5±20.6	196.6±12.02 (18%)	171.83±11.6* (28%)	157.4±10.3* (34%)	148.8±9.8* (38%)
Standard (Imipramie 20mg\kg)	140.3±9.6				134.5±9.2* (42%)

The observations are mean ± SEM of 6 animals. * p<0.05 as compared to that of vehicle treated group.

It was observed that the medicinal *Gritha* significantly (p<0.05) decreases the locomotor activity as compared to control.

Table 5: Effect of Medicinal *Gritha* on Hole Board test (No. of head dips)

Groups	Number of days			
	1	4	8	14
Control (Normal saline 0.1ml)	47.2±11.6	42.8±9.6	36±1.5	38±1.9
Test (*Gritha* 0.1ml)	36±3.33	31.6±2.3 (13%)	28.8±1.8* (22%)	23.6±1.4* (36%)
Standard (Imipramine 20mg\kg)	38.6±3.42			19.6±1.2* (49%)

The observations are mean ± SEM of 6 animals. * p<0.05 as compared to that of vehicle treated group (One way ANOVA followed by Dunnett's test).

In current study administration of *Gritha* significantly (p<0.05) decreased head dip counts as compared to control.

Table 6: Effect of Medicinal *Gritha* on Elevated pluse maze: (No. of entries)

Groups	Number of days		
	1	8	14
Control (Normal saline 0.1ml)	23±1.9	22±0.9	21.5±1.3
Test (Gritha 0.1ml)	12±1.5	16±0.4* (25%)	24±0.2* (50%)
Standard (Imipramine20mg\kg)	8.5±1.2		20.6±1.5* (58%)

The observations are mean ± SEM of 6 animals. * p<0.05 as compared to that of vehicle treated group (One way ANOVA followed by Dunnett's test).

In current study administration of *Gritha* significantly (p<0.05) decreased the no. of entries into open arm as compared to control.

Table 7: Times spend in open arms

Groups	Number of days		
	1	8	14
Control (Normal saline(0.5ml)	51.6±4.3	57.2±4.5	59.6±3.5
Test (gritha0.1ml)	57.4±3.33	73.2±5.66* (27%)	88.8±7.6* (72%)
Standard (imipramine 20mg\kg)	52.4±3.6		98.6±6.2* (88)%

The observations are mean ± SEM of 6 animals. * p<0.05 as compared to that of vehicle treated group (One way ANOVA followed by Dunnett's test)

In current study administration of *Gritha* significantly (p<0.05) increased the time spent in open arm as compared to control.

DISSCUSSION

Psychiatric illness often associated with suicide and there are between 10 and 20 million suicide attempts every year. Studies have estimated the prevalence rate of depression varied from 1.7 to 74 per thousand populations and is recognized to be symptomatically, psychologically and biologically heterogeneous. The disorder was characterized by apathy, loss of energy, retardation of thinking and activity, as well as profound feelings of gloominess, despair and suicidal ideation[10]. Over the last years, large number of studies has been published from India addressing epidemiology, demographic and psychosocial risk factor neurobiology, symptomtology, assessment and diagnosis, impact of depression, treatment related issues and prevention of depression in addition to the efficacy and tolerability of various anti-depressants. In spite of a large variety of available antidepressant medications and alternative therapeutic modalities including several forms of psychotherapy (e.g., cognitive behavioral therapy) and several other approaches such as yoga, exercise, and sleep deprivation, depression suffers a huge treatment gap worldwide, whereby large numbers of individuals who require care do not receive treatment. [11]

Brahmi Ghrita described in ancient text i.e., Charak Samhita, Astang Hridya having different compositions differing in their ingredient and

therapeutic claim. In Charak Samhita, this *Ghrita* contain *Brahmi* (*Bacopa monneri*), *Vacha* (*Acorus calamus*), *Shankhapushpi* (*Evolvulus alsinoids*), *Kushtha* (*Saussurea lappa*) and cow's ghee for treatment of *Unmad*, *Apasmar* and *Graha* disorders while Astang Hridaya contain *Brahmi Vyosh*, *Trivrit*, *Danti*, *Shankhapuspi*, *Aragvadh*, *Saptala*, *Vidang* and *Ghrita* indicated for *Apasmara*, *Unmada*, *Vandhyatva*, *Kushtha*, *Vaksvarabhringa*, *Smritiksaya* and *Buddhimandya*. Charka Samhita it is clearly mentioned that physician may add and remove the ingredients of any formulations as per their wisdom in accordance with patients, nature of disease, *Desh, Kala* etc. to avoid any untoward effect on patients. So variation at ingredients level mentioned in different manuscripts of Ayurveda will be accepted accordance with patients, nature of disease, *Desh, Kala* etc.

In preclinical investigations animal models are employed for understating the etiopathology of the disease and effectiveness of the test drugs. Although animal models greatly help our understanding of psychiatric disorders, they do have their own imitations. The distinction between stress-induced depression-like and anxiety-like behaviors is difficult to ascertain, particularly since both types of behaviors respond to anti-depressants. Thus, an important challenge of the field has been to produce along-lasting state of depressive pathology in laboratory animals, which has seldom been achieved.[12]

The forced swimming test in mice is the most widely used tool for assessing antidepressant activity. [13] It is based on the observation that rodents rats and mice develop an immobile posture when placed an inescapable cylinder with water. The immobility is thought to reflect either a failure of persistence in escape-direct behaviour (despair behaviour) or the development of passive behaviour, meaning the loss of the animals ability to cope with stressful stimuli. *Ithas* been shown that stress induces a state of helpless despair condition in animals which is equivalent and claimed to a condition similar to human depression [14]. This attribution of animal's response to the development of depression process can be managed by the treatment with antidepressant medicines. In this study a significant reduction in the immobility was observed following the 14 days oral administration of medicinal *Gritha* accompanied with an increase in swimming time.

Tail suspension leads behavior of despair resulting in reduction of monoamine turn over in some specific part of the rat brain. Stress induced by tail suspension test increases the neither tissue content of nor adrenaline, whereas serotonin is not affected[15]. Typically, the suspended rodents perform immediately escape like behaviours, followed by developing an immobile posture. If antidepressants are given prior the test, the subjects will be engaged in escape–directed behaviors for longer periods of time than after saline treatment. The increase in duration of immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. It is sensitive to acute treatment only and its validity for non-monoamine antidepressant is uncertain. In this study a significant reduction in the immobility was observed following the 14 days oral administration of medicinal *Gritha* which indicate the antidepressant property of it.

The locomotor activity can be easily measured using an Actophotometer, which operates on photoelectric cells with a counter. When the animals cut off a beam of light a count is recorded. Difference in locomotor activity before and after administration of drug was used for assessing the effect.[16] Locomotor activity and muscle coordination area an index of alertness and muscle relaxation. Reduction indicates that it may possess a sedative and skeletal muscle relaxant effect. Decrease in motor activity and muscle relaxation is an indication of CNS depressant. In present study *Gritha* shows the significant decreases the locomotor activity of mice as compared to control which indicates the decrease in the reactivity of the animal to the stressfulness of stimulation. Decrease in motor activity and muscle relaxation is an indication of anti-depressant property of the drug.

Rotarod test is used to evaluate the activity of drugs interfering with motor coordination. In 1956, Dunham and Miya suggested that the skeletal muscle relaxation induced by a test compound could be evaluated by testing observing the retention time and no of ridings on the constantly rotating rod. This forced motor activity has subsequently been used by many investigators.[17] In this study the *Gritha* does not produces any significant alteration in muscle coordination as compared to control which indicates the ineffectiveness of *Gritha* in muscle coordination. The study of imipramine significant $p<0.05$ decreases the muscle coordination

The number of head dips in hole board test gives an indication of decrease in exploratory tendency their by decrease in the reactivity of the animals. In the present study, there was a significant decrease in the number of head dips on treatment with *Gritha* as compared to control, which indicate depression activity[18]. This Decreases in depressive disorder signs like hyper reactivity of the animals can be interfered with the antidepressant potential of the *Ghrita*. The decreased stereotypical movements again reflect the effectiveness of *Gritha* & imipramine in the condition of bipolar depression.

In Elevated plus Maze test (EPM), a recently established model of extreme anxiety in rats which has all four arms exposed and oscillated in the horizontal plane. 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 allay, the animals experienced an approach-avoidance conflict, which was stronger in the open arms as compared to closed arms. The increase aversion to the open arms was the result of an depressant effect expressed by an increased number of open arm entries, and time spent in the elevated plus maze, and the increased time spent on the central platform was another indication of a reduced decision making behavior. Treatment with *Gritha* significantly increases the entries into open arm and significantly increases the time spent in open arm. Further, it also increased the number of entries from closed to open arm which was found to be affected by administration of imipramine.

CONCLUSION

From the above experiment it has been concluded that the test medicinal *Ghrita* possesses a significant anti-depressant potential which can be used as a nutraceutical agent or an adjuvant medicament for improvement of mental health. Further studies have to be carried out to find out the exact mechanism of effects.

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