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ANTI-DEPRESSANT EFFECTS OF *WITHANIA SOMNIFERA* FAT (ASHWAGANDHA GHRUTHA) EXTRACT IN EXPERIMENTAL MICE.

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ABSTRACT

Anti-depressants play a major role in today's life style. There are evidences of the ayurvedic formulation Ashwagandha ghrutha (AGG, fat extract) being effective in various neuro- psychiatric conditions. The anti-depressant activity of *Withania somnifera* (AGG) was studied using 3 models, Behavioral despair tests- Forced swim test (FST), Tail suspension test (TST) and Anti-reserpine test. Effect of different doses of AGG (20, 40mg/kg), Imipramine (15mg/kg) and their combination (10mg/kg each) were studied on behavioral despair tests induced immobility time and reserpine antagonism. WS produced dose dependent decrease in immobility time in chronic studies in FST and TST model, maximum effect being observed with WS 40 mg/kg. On anti-reserpine models, ptosis, catatonia and sedation scores in the standard, test and combination drug groups were significantly different from the control group. The findings support the use of WS as potential adjuvant in depressive disorders.

KEY WORDS

Withania somnifera, Anti -depressant, Forced swim test, Tail suspension test and Anti- reserpine test.

INTRODUCTION

Depression is a heterogeneous disorder that affects a person's mood, physical health and behavior. It is caused not only by changing lifestyle as perceived by the general public but also by some of the allopathic drugs for example, anti-hypertensive drug, reserpine that depletes neuronal storage granules of nor-epinephrine, serotonin and dopamine, causes clinically significant depression in more than 15% of patients[1]. Patients with major depression have symptoms that reflect changes in brain monoamine neurotransmitters, specifically nor-epinephrine, serotonin and dopamine[2]. The prevalence of depression in the general population worldwide is estimated to be about 5%. Among patients, it ranges from 9% in ambulatory medical patients to 30% in hospitalized patients. According to a World Health report about 450 million people suffer from a mental or behavioral disorder, yet only a small proportion of them receive even the basic treatment. Depression accounts for about 12% of the global burden of disease which is expected to rise to 15% by 2020[3].

The major problems of existing allopathic antidepressant drugs include delayed clinical benefit, serious side-effects, and a response rate of less than 50 percent[4]. Commonly used drugs for depression are monoamine oxidase inhibitors and tricyclic antidepressants (TCAs). They increase the synaptic concentration of at least two of three neurotransmitters, namely 5-HT, NE and dopamine (DA). The combined effect of Serotonin selective reuptake inhibitor (SSRI) and serotonin reuptake transporter (SERT) inhibitor increases synaptic concentration of 5-HT and its duration of action. Therefore, identification and validation of plant derived substances for the treatment of various depressive disorders attracts the attention of researchers.

In Ayurveda, the roots of Withania somnifera (Ashwagandha) is believed to possess aphrodisiac, sedative, rejuvenative and life prolonging properties. It is traditionally used to treat the following symptoms and conditions, although there are few scientific studies on the health benefits of Ashwagandha[5, 6]. Although Ashwagandha has been shown to be effective in the treatment of chronic fatigue, nervous exhaustion, memory loss[5, 6] and neurodegenerative disorders [7] only a few studies are there with the evidence of health benefits of Ashwagandha.

Withania somnifera grows as a short shrub (35-75 cm) with a central stem from which branch extend radially in a star- like pattern (stellate) and covered with a dense matte of wooly hairs (tomentose). The flowers are small and green, while the ripe fruit is orange-red and has milk-coagulating properties. The plant also has long brown tuberous roots that are used for medicinal purposes. It is cultivated in many of the drier regions of India such as Manasa, Neemuch, and Jawad tehsils of the Mandsaur District of Madhya Pradesh, Punjab, Sind and Rajasthan[5].

Properties of AGG can be studied with various extracts, however the traditional usage of herbs have shown that its CNS activity will be better when administered along with ghee or honey[8]. Hence, the present study in order to see the effect of Ashwagandha, its grutha (fat) extract was considered. Specific objectives of the study were: a) to evaluate the anti-depressant activity of Ashwagandha, b) to compare the anti depressant activity of

imipramine, Ashwagandha and their combination in experimental mice.

MATERIAL AND METHODS

Preparation of test drug: Ashwagandha roots were obtained from Govt. Ayurveda Medical College, Mysore and authenticated. A mixture of Ashwagandha root paste, ghee and water in the ratio of 1:4:16 was prepared and boiled till the water component evaporated. Ghee portion was then filtered & collected in an air-tight container.
Test dose: A pilot study was conducted with different doses (5 mg/kg, 10 mg/kg, 20 mg/kg, 30mg/kg and 40 mg/kg) to assess the appropriate dose for the study. The anti-depressant activity were observed at the dose of 20 and 40 mg/kg of body weight and hence the same doses were used in the study. In addition we also included a group treated with combination of low doses of test and standard drugs (i.e., 10 mg/kg of AGG and Imipramine each).

Chemicals: Imipramine, Reserpine, Normal saline and other chemicals were of analytical grade.

***Instruments*:** Glass cylinder (25 × 12 × 25 cm^3), Metal lever and Stop watch.

Animals: Swiss albino mice weighing around 25 g – 30 g of either sex were obtained from Central animal facility of JSS Medical College, Mysore. Animals were maintained under standard laboratory conditions at an ambient temperature of 25±1°C. Animals had free access to food and water with a natural light and dark cycle. Animals were acclimatized for at least 5 days before behavioral experiments. The study protocol was approved by Institutional Animal Ethics Committee (IAEC) of the college and the experiments were carried out as per CPCSEA guidelines.
A total of 90 mice were randomized into three groups of 30 each to be utilized for the three models. Further, within each model, animals were randomly allocated to the 5 groups of 6 mice each. Group I - Control group treated with normal saline (0.1ml/10gm), Group II – Standard group treated with 15mg/kg of Imipramine, Group III - T1 treated with 20 mg/kg AGG, Group IV - T2 treated with 40 mg/kg AGG and Group V – T3 treated with 10 mg/kg of AGG and Imipramine each.
Experimental procedures were carried out with 2 dosing schedules. Acute study- The drugs were administered as a single dose one hour prior to the observation. Chronic study- The drugs were administered for 7 days continuously. On 8^{th} day morning outcome observations were obtained. To study the anti-depressant effect of the drugs, three models namely, Behavioral despair test [Forced swim test (FST), Tail suspension test (TST)] and Anti- reserpine test were utilized.

Forced-swim test:
Forced swim test was proposed as a model to test antidepressant activity by Porsolt et al [9]. The method was the same as described by Dhingra and Sharma[10]. Mice were forced to swim individually in glass jar (25 x 12 x 25 cm^3) containing fresh water up to 15 cm height and maintained at 25± 3°C[11]. After an initial period of vigorous activity for two minutes, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the water without struggling and making only minimum movements of its limbs necessary to keep its head above the water. The total duration of immobility was recorded during the next 4 min of the total test duration of 6 minutes after administering the drugs to the respective groups of animals.

Tail-suspension test:
The total duration of immobility following tail suspension was measured according to the method described for evaluating potential antidepressants[12]. Mice were suspended on the edge of a table, 50 cm above the floor with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was

recorded during 6 minutes period in different groups. The animal was considered to be immobile when it did not show any movement of the body and hanged passively.

Reserpine test:

Five groups of animals were reserpinised by administration of reserpine (2.5 mg/kg, i.p.) one hour after the respective drug administration. The acute and chronic effect of Ashwagandha and Imipramine on reserpine induced ptosis, catatonia & sedation were noted on 1st and 8th day respectively[13]. Mice were observed for the presence of ptosis, catatonia & sedation at 1, 2, 3 & 4 hours after reserpine injection and the symptoms were scored depending on severity[14]. *Scoring pattern was as follows- Ptosis score:* Eyes open – 0, Eyes ¼ closed- 1, Eyes ½ closed – 2, Eyes ¾ closed – 3, Eyes closed – 4. *Catatonia score:* Scoring was based on duration of mice remaining in head down position on horizontal aluminium wire of catatonia stand. Score 1: 0-30 sec, Score 2: 30-60 sec, Score 3: ≥ 60 sec. *Sedation score* was assessed using concentric circles of 5, 7 and 9 cm diameter and the distance moved by the mice placed at the centre of the circles. Score 0 - no movement, Score 1- between 5-7 cm, Score 2 - between 7-9cm, Score 3 - moving away from 9 cm.

STATISTICAL ANALYSIS:

The effects of standard and test drugs were estimated by working out mean and standard error of mean (SEM) of duration of immobility in FST & TST (in minutes) and ptosis, catatonia & sedation scores in reserpine antagonism. One-way analysis of variance (ANOVA) was performed to test the significance of differences in mean duration of immobility and mean antagonism of reserpine among the five groups followed by scheffe's post-hoc test. All the tests of significance were interpreted at 5% significance. In order to quantify the effects of the drugs, we calculated the duration of immobility in the drug groups relative to the control group and presented the same diagrammatically.

RESULTS

Effect of AGG on immobility periods in behavioral despair tests: [Table: 1; Figures 1(a), 1(b)]

The anti-depressant effect of Withania somnifera (WS), imipramine and their combination was studied by looking at the changes in the duration of immobility in the two models; namely Forced swim test (FST) and Tail suspension test (TST). We used these models to study both acute as well as chronic effects.

The acute anti-depressant effect: FST and TST models suggested statistically significant anti-depressant effect of imipramine and their combination. The inhibition of FST induced immobility time was highest (20%) for the combination group, followed by 16% for Standard and 6-8% for the test drug in FST model and respectively 12, 11and 5-9% in TST model. There were significant differences between the standard and the combination groups indicating that test drug has some effect when administered with the standard drug.

The chronic anti-depressant effect: The chronic anti-depressant effect was seen in all the groups in both the models with the inhibition being highest in the standard and the combination groups (50-60%). In the test drug group, reduction in duration of immobility was 30-40%. A significant difference was noted between standard and other groups indicating that the effect of test drug was not as much as the standard drug. The higher dose of test drug showed a greater inhibition compared to the lower dose, however the difference was not statistically significant. The combination of standard and test drug showed an inhibition in immobility similar to the standard drug alone.

Table 1

Mean and its standard error (SEM) of duration of immobility (in minutes) induced by forced swim test (FST) and tail suspension test (TST) in acute and chronic studies.

Groups (n=6 in each)		Doses	FST		TST	
			Acute	Chronic	Acute	Chronic
I	Control- NS	0.01ml/gm	212.8± 4.02	195.5± 5.22	200.4± 4.02	190.5± 5.22
II	Imipramine.	15 mg/kg	180.3± 1.54*	82.2 ± 1.4*	178.4± 1.54*	95.5 ± 1.32*
III	T1: AGG	20 mg/kg	200.4± 4.54	135.5± 4.34**	190.5± 3.87	132.3± 4.31**
IV	T2 : AGG	40 mg/kg	195.1± 5.20	112.7± 4.20**	183.3± 5.20*	116.3± 5.20**
V	T3 : AGG & Imipramine	10 mg/kg each	170.4± 1.54**	98.3± 1.37**	176.5± 1.54*	99.6± 1.21*

****: significantly different from control group, **: significantly different from control and standard drug groups.***

Figure 1: Relative (%) quantum duration of immobility in the standard and test drug groups compared to control (100%) in acute and chronic studies of FST model.

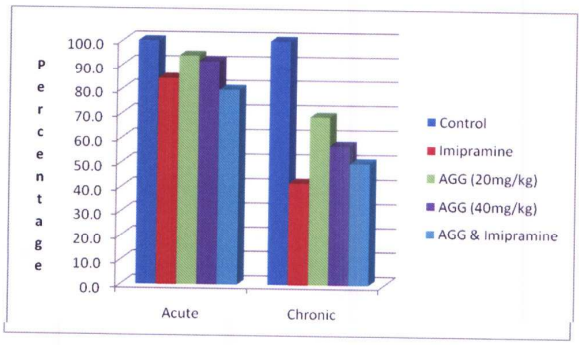
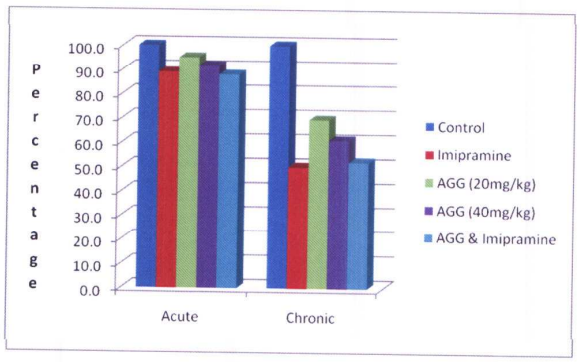


Figure 2: Relative (%) quantum duration of immobility in the standard and test drug groups compared to control (100%) in acute and chronic studies of TST model.



Effect of AGG on reserpine induced ptosis, catatonia and sedation: [Tables-2(a), (b), (c)]

As for as the acute effect is concerned, it was not seen in any of the groups. Therefore only the chronic effect was studied in detail. It was studied by looking at the antagonism scores of ptosis, catatonia and sedation at 1, 2, 3 and 4 hours. *Ptosis*: As for ptosis is concerned scores were significantly different for the standard and the combination groups compared to control group at all the 4 time points. For the test drug however the scores were different only at 2nd and 3rd hour. This indicates that test drug takes longer time to act and the effect lasts for a shorter duration.

Catatonia: All the groups showed significantly lower scores of antagonism at all time periods. This indicates that the test and the standard drugs reveal their effect within 1 hour itself which lasts for atleast 4 hours. The average scores for the standard and the test drugs were reduced upto half that of the control group. In terms of average scores the combination drug seemed to work as good as the standard drug alone. Higher dose of test drug showed higher reduction in antagonism score however, the difference was not statistically significant.

Sedation: Antagonism effect on sedation was seen at all 4 time points for the standard and the combination drug groups. However in case of

test drugs the effect was seen at 2, 3 and 4 hrs. This indicates that the test drug effect perhaps takes longer duration to start but lasts for atleast 4 hrs.

Table 2(a)

Mean and its standard error (SEM) of antagonism score of reserpine induced ptosis at different time intervals in a chronic study.

Groups (n=6 in each)		**Doses**	**Ptosis** (mean ± SEM)			
			1 hr	2 hr	3 hr	4 hr
I	Control -NS	0.01ml/gm	2.0±0.00	3.2±0.32	3.0±0.25	3.3±0.05
II	Imipramine.	15 mg/kg	1.2±0.10*	1.2±0.10*	1.3±0.21*	2.4±0.04*
III	T1 –AGG	20 mg/kg	2.4±0.13	2.2±0.16*	2.6 ±0.21	3.2±0.02
IV	T2- AGG	40 mg/kg	2.1±0.11	1.4±0.12*	1.6±0.30*	3.1±0.21
V	T3- AGG + Imipramine	10 mg/kg each	1.3±0.23*	1.7±0.20*	1.93±0.25*	2.2±0.23*

AGG- Ashwagandha ghrutha, *: significantly different from control group (p < 0.05).

Table 2(b)

Mean and its standard error (SEM) of antagonism of reserpine induced catatonia at different time intervals in a chronic study.

Groups (n=6 in each)		**Doses**	**Catatonia** (mean ± SEM)			
			1 hr	2 hr	3 hr	4 hr
I	Control - NS	0.01ml/gm	2.8±0.16	2.8±0.16	3.2±0.05	3.5±0.24
II	Imipramine .	15 mg/kg	1.3±0.12*	1.2±0.63*	1.4±0.93*	2.1±0.32*
III	T1 –AGG	20 mg/kg	1.6±0.21*	1.9±0.25*	2.3±0.34*	2.7±0.34
IV	T2- AGG	40 mg/kg	1.3±0.23*	1.5±0.24*	1.9±0.21*	2.5±0.23*
V	T3- AGG + Imipramine	10 mg/kg each	1.1±0.11*	1.2±0.34*	1.5±0.23*	2.1±0.43*

AGG- Ashwagandha ghrutha, *: significantly different from control group (p < 0.05).

Table 2(c)
Mean and its standard error (SEM) of antagonism of reserpine induced sedation at different time intervals in a chronic study.

Groups (n=6 in each)		Doses	Sedation (mean ± SEM)			
			1 hr	2 hr	3 hr	4 hr
I	Control -NS	0.01ml/gm	1.8±0.46	2.6±0.21	3.3±0.24	3.2±0.12
II	Imipramine.	15 mg/kg	0.1±0.34*	0.3±0.12*	1.5±0.10*	2.2±0.11*
III	T1 –AGG	20 mg/kg	0.9±0.41	0.9±0.22*	1.9±0.40*	2.4±0.23*
IV	T2- AGG	40 mg/kg	0.8±0.40	0.7±0.41*	1.9±22*	2.3±0.25*
V	T3- AGG + Imipramine	10 mg/kg each	0.3±0.20*	0.3±0.12*	1.7±0.10*	2.2±0.11*

AGG- Ashwagandha ghrutha, *: significantly different from control group (p < 0.05).

DISCUSSION

The introduction of drugs like amitriptyline, fluvoxamine, imipramine, citalopram, venlafaxine and others have revolutionized the treatment of depression. The amazing efficacy of imipramine and fluoxetine in these depressive disorders has paved the way for the introduction and use of newer anti-depressant agents. However, the safety factor in respect of both the imipramine and fluoxetine anti-depressant drugs has been rather intriguing and hence a definite need is visualized for the introduction of safer anti-depressant drugs having no troublesome adverse effects. There are several studies on ethanolic, methanolic and aqueous root extracts of Ashwagandha but studies on the fat (Grutha) root extract are sparse hence, the present study has been undertaken.

The major biochemical constituents of Ashwaganda root are steroidal alkaloids and steroidal lactones in a class of constituents called withanolides[15]. About 12 alkaloids, 35 withanolides, and several sitoindosides from this plant have been isolated and studied. A sitoindoside is a withanolide containing a glucose molecule at carbon 27. Much of Ashwaganda's pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D.

FST and TST models of depression are widely used to screen newer antidepressant drugs [9, 10,11,12,16]. These tests are quite sensitive and relatively specific to all major classes of antidepressant drugs including tricyclics, selective serotonin reuptake inhibitors, monoamine oxidase (MAO) inhibitors and atypicals[11, 12, 17]. Imipramine is a pre-synaptic uptake inhibitor of both nor-adrenaline as well as serotonin[18]. Since catecholamines and 5-HT have been implicated in the aetiology of depression, the positive effect of these drugs in FST and TST seems to be due to increased availability of these neurotransmitters at the postsynaptic receptor sites following their re-uptake inhibition. In FST, mice were forced to swim in a restricted space, which induced a characteristic behavior of immobility. In TST, mice were suspended by their tip of the tail from a metal rod which also induced a state of immobility in animals like that in FST. This immobility reflects a state of despair in animals and is claimed to reproduce a condition similar to depression in humans[12, 19]. It has been argued

that the TST is less stressful than FST and has greater pharmacological sensitivity[20]. Animals after anti-depressant treatment struggle more even in desperate situation, and they spend less time with immobility. In the present study, AGG in the dose of 20 and 40 mg/kg produced significant dose-dependent antidepressant-like effect in behaviour despair tests (FST and TST), as they reduced the immobility time. Also, the decrease in produced by combination group (WS & imipramine 10mg/kg each) was comparable to that produced by the standard imipramine (15 mg/kg). These results suggest that WS enhances the effect of imipramine on stress induced immobility time.

Reserpine is a vesicular re-uptake blocker, which depletes catecholamines or lowers nor-adrenaline turnover in the brain to produce a depression like syndrome in animals[21]. Reserpinised mice (2.5 mg/kg) were observed for ptosis, catatonia & sedation at 1, 2, 3 & 4 hours & scored on a scale from 0-3. WS (40 mg/kg) and imipramine (15mg/kg) significantly reduced reserpine induced ptosis, catatonia and sedation. Moreover, combination consisting of sub-therapeutic doses of WS and imipramine (10 mg/kg each) also produced significant reserpine antagonism in mice. Since reserpine induced depressive state is found to be significantly reversed by WS, it is tempting to suggest the involvement of biogenic amines in its antidepressant action.

In an earlier study, WS traditional extract 250 mg/kg in a solvent of water, honey & ghee and methanolic extract of WS showed significant anti-depressant and memory enhancing effects in stressed rats[22]. In another study, Ashwagandha root extract administered orally to rats once daily for five days exhibited an antidepressant effect comparable to the Standard imipramine in the forced swim-induced “behavioural despair” and “learned helplessness” tests[23]. In another research, treatment with the single dose of WS root extract in various dose ranges of 25, 37.5, 50, 100 and 200 mg/kg i.p and a combination of WS (37.5 mg/kg, i.p.) with that of either imipramine (2.5 mg/kg, i.p.) or fluoxetine (2.5 mg/kg, i.p.) produced significant decrease in the MIT in FST. Also, single dose of WS (100 mg/kg) and its combination group significantly reduced MIT in reserpinised mice suggesting that, anti-depressant effect of imipramine as well as fluoxetine were enhanced by concomitant administration of WS[24].

CONCLUSION

A number of studies on WS, or its major active principles, have shown an antioxidant, adaptogen, anxiolytic, antidepressant, memory enhancing, anti-inflammatory, anti-ulcerogenic, anti-parkinsonian and anti-carcinogenic properties. In the present study fat extract of Withania somnifera root has shown promising results in chronic studies but were less effective in the acute studies of experimental depression. These studies are valuable for identifying lead compounds for anti-depressant drugs, keeping in mind the side effects of presently used anti-depressants. The standardization of the extracts, identification and isolation of active principles along with pharmacological studies of these principles may be considered for further detail studies. Still further human studies are needed to prove the safety and efficacy of long term administration of fat extract of Withania somnifera root. In the light of observations made it may be envisaged that Withania somnifera can be used as a potential adjuvant in the treatment of depressive disorders.

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