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Study of Anxiolytic and Anti-Depressant Activities of Withaniasomnifera

Monika M.B
Department of Pharmacognosy,
Government College of Pharmacy,
Bengaluru - 560027, India.

Sujani Kamble
Department of Pharmacognosy,
Government College of Pharmacy,
Bengaluru - 560027, India.

Muhammed Majeed
Sami Labs Limited R&D Centre,
Peenya Industrial Area,
Bengaluru - 560058, India.

Lakshmi Mundukur
Sami Labs Limited R&D Centre,
Peenya Industrial Area,
Bengaluru - 560058, India.

Mahadev Nayak
Sami Labs Limited R&D Centre,
Peenya Industrial Area,
Bengaluru - 560058, India.

ABSTRACT

The present study was undertaken to investigate the anxiolytic & anti-depressant activity of Withania somnifera root extract (Shagandha™). The rats and mice were administered at a dose of 10 mg/kg, 20 mg/kg, and 40 mg/kg b.w. for a period of 14 days. Elevated plus Maze and Light Dark box Models were selected for evaluation of anti-anxiety using diazepam 5mg/kg b.w. as standard. Amitriptaline 10mg/kg was used as standard for comparison of the antidepressantactivity.Diazepam reduced anxiety due to altitude (acrophobia), while Shagandha™ showed protection at different levels. Individually Shagandha™ at 10 mg/kg showed better protection compared to the standard drug. In the light dark model of anxiety, treatment with diazepam, induced animals to spend more time in bright arena and the number of entries in to the light area were high. The time spent in light arena showed a dose dependent increase for Shagandha™ with the 40mg/kg dose showing highest protection.Shagandha™ at 20mg/kg was found to be highly effective in reducing anxiety with protection level better than the standard drug diazepam in despair swim test.

Keywords: ***Withania somnifera, Anti-depressant, Antianxiety.***

Introduction

The history of herbal medicine is as old as human civilization. India has an ancient heritage of traditional medicine. The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relay almost exclusively on traditional medicine for their primary health careneeds.

Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary plant metabolites in one or more parts of the plants. These plant metabolites are grouped as alkaloids, glycosides, essential oils, etc. India recognizes more than 2,500 plant species as having medicinal value.

Neuropsychiatric disorders and debilitating mental illnesses are mainly responsible for global burden of disease of about 28% aggregate in low, middle and high income countries. According to WHO (World Health Organization), anxiety and related disorders will become the second leading cause of disability in both developed and developing countries by the year 2020[1]. Anxiety disorders show a high co-morbidity with major depressive disorders ranging from 37% for separation anxiety disorder to 62% for generalized anxiety disorder, also called anxious depression[2].

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These disorders affect about ⅛th of population worldwide, and have become a very important area ofresearch[3].

Stress has also been reported to be the major cause in development of these psychiatric disorders[4]. Both acute and chronic stress can have numerous patho-physiological effects such as activation of neuro-endocrine and hormonal functions[5]. Sustained and persistent stressful conditions also lead to the excessive production of free radicals and oxidative burden, which can precipitate anxiety and lead to disorders such asdepression[6].

Peroxidation of membrane polyunsaturated fatty acids produces toxic Malondialdehyde (MDA), which results in decrease in membrane fluidity and damage in membrane proteins thereby inactivating receptors, enzymes and ion channels. As a result, oxidative stress can alter neurotransmission, neuronal function and overall brain activity[7].

Withania somnifera belongs to family Solanaceae, has hypotensive, immuno-modulatory potential. Benzodiazepines are used to treat several forms of anxiety. Although these compounds have well known benefits but their side effects are prominent, including sedation, muscle relaxation, ethanol potentiation, anterograde amnesia and seriousness of anxiety disorders. Depression is a heterogeneous disorder that affects a person's mood, physical health and behaviour. Treatments of major mood disorders have improved in recent years with the advent of newer antidepressant drugs like TCAs (Tricyclic anti-depressants) e.g. Amitriptyline, Imipramine, and SSRIs (Selective Serotonin Reuptake Inhibitors)e.g. Fluoxetine, MAO (Mono amine oxidase inhibitors) is a typical antidepressants, which are more selective with insignificant side effects. Despite their general safety and efficacy, they are not totally avoid of side effects and relatively expensive for long-term use. Therefore, the development of newer agents possessing anxiolytic effect and anti-depressant effect with minimal or no adverse effect would be a greater importance. The present study is anxiolytic and anti-depressant effect of *Withania somnifera* extractto get a highly potent and efficient result by using different ratios of Shagandha™extracts, screened by using selected models in laboratoryanimals.

Material and methods

Drugs, chemicals and animals:

- Amitriptyline was given as a gift sample from Micro Labs Ltd., Bengaluru.
- Shagandha™ was collected from Sami Labs Limited, Bengaluru.
- Diazepam was collected from **GCP,** Bengaluru.
- Wister Albino Female Rats and Swiss albino Male Mice were collected from **DTL**, Bengaluru.

Collection and extraction

The standardized extracts from *Withania somnifera* (Shagandha™) was kindly gifted from Sami Labs Limited, Bengaluru.Shagandha™: The dried Ashwagandha root powder was extracted with aq.ethanol (20%).The ethanolic extract was concentrated to 1/4th volume and was allowed for crystallization. The crystalized material was filtered, dried, and packed. The content of withanolides (2.5%) in Shagandha™ was analyzed by HPLC as per United State Pharmacopoeial (USP) method.

Animals

- Adult Wistar albino female rats weighing 150–200 g and Swiss albino male mice weighing 25-30g were used for evaluation. They were procured from the Drug Testing Laboratory, Bangalore. The animals were allowed with normal diet and water ad libitum. Animals were maintained in standard laboratory conditions (12h: 12h dark and light cycle).
- All procedures described were reviewed and approved by the Institutional Animal Ethical Committee.

ISSN No: 2348-4845
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Neuropharmacological activities

- Wistar albino rats and mice were subjected to anxiolytic activity evaluated using elevated plus maze and light and dark arena models with standard diazepam 5mg/kg and extracts with the dose of 10, 20 and 40 mg/kg b.w. of Shagandha™.

- Similarly, the antidepressant activity was evaluated using tail suspension test and despair swim models with standard amitriptyline 10mg/kg and extracts with the dose of 10, 20 and 40 mg/kgb.w. of Shagandha™.

EPM test

After administering the definite dose for each group of animals they were subjected for the elevated plus maze test. It consists of two open arms, 50×10×40cm, and two enclosed arms 50 × 10 ×40 cm with an open roof, arranged so that the two open arms are opposite to each other. The maze was elevated to a height of 50cm. After proper treatment each rat was placed at the center of the maze with its head facing the open arm. During the experiment, the behaviour of the rat was recorded at the interval of five minutes the number of entries into the open or closed arms and the time spent by the rat in each of the arm.

LDA test

This model consists of 5 groups each group consist of 6 mice. The first group receiving 2% gum acacia (Control group), the second group receiving standard (Diazepam 5mg/kg) b.w and the other three groups receivingShagandha™10mg20mg40mg/kg b.w. respectively for the evaluation of anti-anxiety activity

TST test

The tail suspension test is the second method for assessing the anti-depressant activity. Onehour after administration on the 5th day, the mouse was suspended by the tail from a lever (30 cm high) for 6 min with the movements of mouse being recorded by the observer.

The total duration of the test (6min) can be divided into agitation and immobility periods. The duration of immobility in the later 4 min represented the “behaviour despair”status.

DST test

- 5 groups of animals were taken as Standard, Control,Shagadha^{TM}10 mg, 20 mg, 40 mg. Mice were forced to swim individually for 15min in a glass beaker 12cm diameter & 15cm, height of 9cm at a temperature of 22±1°C (pre-test session). After 24h pre-test session, the animals were administered standard group and to control groups administered only test drug. Each animal were once again forced to swim in the same beaker for a period of 6 min in test session. The first 2min animal was allowed to adjust to the newconditions.
- The duration of immobility during the last 4 min was recorded.

Statistical analysis

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnets multiple comparisons. The results obtained were compared with the vehicle control group, in which $P<0.05$, and $P<0.001$ were considered to be statistically significant.

Results

Effect of Shagandha™ on anxiety behaviour of rats (EPM)

The control group subjected to elevated plus maze for 5 min duration was found to show anxiety-like behavior as characterized by their longer stay in closed arm of EPM and decreased percentage of time spent in open arms.

The standard drug Diazepam increased the time spent in open arm. Shagandha™ was found to reduceanxiety significantly as observed by increased time spent in open arm 43.16% and 42.1% at 10 mg/kg and 20 mg/kg compared to 26 % by control animals.

ISSN No: 2348-4845
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TABLE NO: 1

Sl.No	TREATMENT	PARAMETERS				
		Time spent in open arms (300sec)	NO. open arm entries	% Time in open arm	t-test	%Protection
1	Control Vehicle (2% gum acacia)	76 ± 46.64	3.16±1.07	25.33±15.53		
2	Standard Diazepam (5mg/kg)	119.5 ± 46.53	3.16±1.44	39.83±15.49	0.6603	28.618
3	Shagandha™ (10mg/kg)	129.5±39.62	3±0.44	43.16±13.19	0.8743	32.666
4	Shagandha™ (20mg/kg)	126.3±29.9	5±0.93	42.1±9.95	0.9068	62.206
5	Shagandha™ (40mg/kg)	59.3±23.64	1.83±0.40	19.76±7.87	0.3188	-32.031

Effect of Shagandha™ on the anxiety behaviour of mice in light-dark box model.

The control group, subjected to light dark arena for 5 min duration was found to show anxiety-like behaviour as characterized by their longer stay in the dark compartment decreases number of transfers to bright arena. The time spent in light arena showed a dose dependent increase in Shagandha™ with the 40mg/kg dose showing 195±15.59 and 127.3±20.31 respectively compared to control animals which spent only 81.5±28.13 sec in light. At 20 and 40 mg/kg it was found to afford higher protection compared to standard drug diazepam (39.6%). Overall Shagandha™ 20mg/kg b.w shown better percentage protection.

TABLE NO: 2

Sl. No	Treatment	PARAMETERS				
		Transfer latency(sec)	Time spent in bright arena (sec)	No. of transfers to dark arena	t-test	% protection
1	Control Vehicle (2% gum acacia)	82.67 ± 26.23	81.5±28.13	9.5±1.78		
2	Standard Diazepam (5mg/kg)	68.33±32.65	138.7±17.79	12.5±1.66	1.718	39.603
3	Shagandha™(10mg/kg)	27.33±3.7	94.5±19.66	9.5±1.60	1.666	27.643
4	Shagandha™(20mg/kg)	38.83±1.13	117.5±13.91	10.5±0.92	0.937	60.352
5	Shagandha™(40mg/kg)	29.5±2.37	127.3±20.31	11.67±1.25	0.419	47.459

TABLE NO: 3

Overall Percentage Protection of Shagandha™ for Anxiety

SL. NO	Treatment	Overall %Protection (ANXIETY)
1	Standard Diazepam (5mg/kg)	34.111
2	Shagandha™ (10mg/kg)	30.154
3	Shagandha™ (20mg/kg)	61.279
4	Shagandha™ (40mg/kg)	7.714

Effect of Shagandha™ on the depressant behaviour of rat in TST.

The control group when subjected to Tail Suspension Test for 6 min duration was found to show depressant behaviour as characterized by their increased immobility when suspended. Shagandha™ was found to increase mobility only at 40mg/kg to 157.8±18.26 sec.

TABLE NO: 4

Effect of Shagandha™ on the depressant behaviour of rat inDST

Sl.No	Treatment	PARAMETER			
		Immobility time in sec. values Mean±SEM	Mobility time in sec. values Mean±SEM	t-test	% Protection
1	Control Vehicle (2% gum acacia)	101.7±26.27	138.3±26.27		
2	Standard Amitriptaline (10mg/kg)	41.17±15.37	198.8±15.37	1.988	51.632
3	Shagandha™ (10mg/kg)	134.7±29.05	105.3±29.05	0.8426	-28.155
4	Shagandha™ (20mg/kg)	130.7±34.84	109.3±34.84	0.6646	-24.742
5	Shagandha™ (40mg/kg)	82.17±18.26	157.8±18.26	0.6096	16.652

The control groups, subjected to Despair swim test for 6 min duration were found to show depressant behaviour as characterized by their immobility when suspended decreases the mobility time. In this model of depression, Shagandha™ was found to have a dose dependent response. The mobility time was 109.5±26.09 sec at 10mg/kg and increased to 20mg/kg (144.83±20.07) and at 40 mg/kg (153±25.51).

ISSN No: 2348-4845
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TABLE NO: 5

SL.NO	Treatment	PARAMETER			
		Immobility time in sec. values Mean±SEM	Mobility time in sec. values Mean±SEM	t-test	% Protection
1	Control Vehicle (2% gum acacia)	135±5.47	105±5.47		
2	Standard Amitriptaline (10mg/kg)	98.83±26.71	141.16±26.71	1.327	30.615
3	Shagandha™ (10mg/kg)	130.5±26.09	109.5±26.09	0.1688	3.810
4	Shagandha™ (20mg/kg)	95.17±20.07	144.83±20.07	1.915	33.719
5	Shagandha™ (40mg/kg)	87±25.51	153±25.51	1.84	40.635

TABLE NO: 6

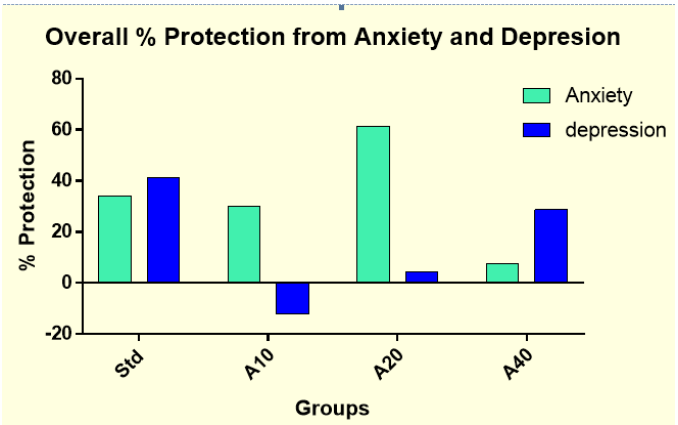
Overall Percentage Protection of Shagandha™ for Depression

SL. NO	Treatment	Overall
1	Standard Diazepam (5mg/kg)	41.124
2	Shagandha™ (10mg/kg)	-12.173
3	Shagandha™ (20mg/kg)	4.488
4	Shagandha™ (40mg/kg)	28.643

TABLE NO: 7

Over all protection of Shagandha™ extracts for anxiolytic and Anti-depressant activity

Treatment	Anxiety	Depression
Standard Diazepam (5mg/kg)	34.111	41.124
Shagandha™ (10mg/kg)	30.154	-12.173
Shagandha™ (20mg/kg)	**61.279**	4.488
Shagandha™ (40mg/kg)	7.714	28.643



Graph 1: legend for graph 1

Taking both the models together, It found that Shagandha™ 20mg/kg (61.28%) was better than standard drug diazepam (34.11%) in reducing anxiety.

DISCUSSION:

Anti-Depressant Activity

Ashwagandha is commonly available natural product used intreating several CNS disorders. In addition, traditionally these plants are used in treating rheumatisms, dementia, bradycardia, and immunomodulatory actions, gout, hypertension etc. Shagandha™ is a standardized extract containing2.5 % withanolides. Although several extract of *Withania somnifera* have been studied earlier, the standardized extract containing known concentration of the active molecules have not been subjected to any preclinical evaluation.

In the present study, we explored the anxiolytic and anti-depressant activity of Shagandha™ extract. Shagandha™ is subjected to preliminary phytochemical investigation so that various types of phytoconstituents can be identified quantitatively. As a part of standardization, the extracts were standardized by USP methods.

Anxiolytic activity of the study extracts was carried out with Elevated plus maze and Light dark arena Models. Both these models are classified under unconditional behaviour models, The elevated plus maze (EPM) is one of the most popular and frequently used behavioral tests for anxiety (Psychopharmacology (Berl). 1987; 92(2):180-5.) Itis based on the natural aversion of rodents to open spaces, and uses conflict between exploration and this aversion. The behavioral parameters in the mouse in plus maze provided measures of two independent factors, one reflecting anxiety and one reflecting motor activity. The percentage of open-arm entries and the time spent on the open arms are good measures of anxiety, while closed-arm entries provided a better measure of motor activity (*PharmacolBiochemBehav.* 1995; 52: 297–303). We assessed time spent on the open arms to understand the effect of herbal supplements in controlling anxiety. Shagandha™ was found to reduce anxiety in rats significantly.

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The light/dark (LD) test is based on the innate aversion of rodents to brightly illuminated areas, and on the spontaneous exploratory behaviour of rodents in response to mild stressors i.e. novel environment and light. The light/dark (LD) test is based on an approach-avoidance conflict between exploration of novel environments and avoidance of brightly lit, open spaces. Behaviour in unconditioned anxiety tests such as the LD test is thought to reflect impulsivity or risk taking in addition to anxiety, so the LD test may be a useful model to investigate neural systems relevant to adolescent risk taking (*Animal Behaviour*. 2002; 64:541–546). Five main parameters are assessed to test the anxiolytic profile of drug: the latency time for the first passage from the light compartment to the dark one, the number of transitions between the two compartments, the movement in each compartment, and the time spent in each compartment. The most consistent and useful for assessing anxiolytic-like activity action is the time spent in the light compartment, as this parameter provides the reliable dose-effect responses with different compounds (*PharmacolBiochemBehav*. 1998 Jul; 60(3):645-53).

Anti-Depressant Activity

Despite the prevalence of depression and its serious impacts, studies on the pathogenesis of depression are still preliminary as an ideal animal model for depression does not exist. Despair swimming test (DST), also known as forced swim testis a behavioral despair test.

Although it works in subacute condition, it remains highly reliable in predicting the therapeutic potential of the tested compounds (Psychopharmacology (Berl) 2005, 177: 245- 255). The test is based on the observation that animals develop an immobile posture in an inescapable cylinder filled with water. After AD administration, the animals will actively perform escape-directed behaviours with longer duration than animals with vehicle treatment. Shagandha™ 10, 20,40mg/kg was studied in the despair swim test in mice to measure the immobility and mobility time in seconds. Shagandha™ showed highest anti depressive activity at 40mg/kg. The tail suspension test shares a common theoretical basis and behavioral measure with the DST. The tails of rodents are suspended using adhesive tape to a horizontal bar for 6 min, and the time of immobility is recorded. Typically, the suspended rodents are immediately engaged in several agitation or escape-like behaviours, followed temporally by developing an immobile posture (NeurosciBiobehav Rev 2005, 29: 571-625). Although TST and DST share a common theoretical basis, there are many differences between them; therefore they could complement each other in some situations. For example, TST avoids problems of hypothermia or motor dysfunction that could interfere with the performance in swimming test, while FST could overcome the tail-climbing problem in TST [Psychopharmacology (Berl) 2003, 166: 373-382).

Overall our results show that Shagandha™ 20mg/kg (61.28%)was better than standard drug diazepam in reducing anxiety.

Conclusion:

The standardised extract of Shagandha™ was evaluated for the Anxiolytic an Anti-depressant activity. Among various doses Shagandha™ 20mg/kg b.w had shown better results.

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ISSN No: 2348-4845
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