A study on evalution of antidepressant effect of Imipramine adjunct with *Aswagandha* and *Bramhi*

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ABSTRACT

Depressive disorders increase the risks of self-harm or even suicide in patients. Indigenous drugs are being tried to treat such patient along with conventional antidepressant drugs. This study was planned to investigate the antidepressant action of *Ashwagandha* and *Bramhi* and also to confirm its efficacy in the behavioural despair animal model of depression. Normal saline as control (5ml/kg), Imipramine as standard (16,32,64 mg/kg) and *Ashwagandha* (50,100,150 mg/kg), *Bramhi* (20,40,80 mg/kg) as test drugs were introduced to the albino rats weighing between 200-250gm for 2 weeks, 1 hr before electric shock in Learned helplessness test (LHT) and swimming in Forced swimming test (FST). Effects of individual drugs as well as their combination were evaluated. Avoidance response, escape failure and immobility period in case of Imipramine and *Ashwagandha* showed highly significant ($p<0.01$) result on individual use. There was no significant result in case of *Bramhi* used alone except in escape failure and immobility period (FST), where at higher doses it showed significant ($p<0.01$) result. But combination of *Bramhi* and *Ashwagandha* in low doses with low dose of Imipramine gave a highly significant result ($p<0.01$) in all the parameters. *Ashwagandha* had significant antidepressant action, but *Bramhi* had not when used alone. Combination of these two indigenous drug with Imipramine showed high efficacy in animal model.

Keywords: *Ashwagandha*, *Bramhi*, Forced swimming test, Indigenous drug, learned helplessness test.

INTRODUCTION

Depression is a heterogeneous disorder that affects a person's mood, physical health and behaviour. Incidence of depressive mood disorders is rising in the modern stressful society leading to an increased risks of self-harm or suicide as well as increased mortality from related general medical conditions.[1] As many as 10-15% of individuals with this disorder exhibit suicidal tendency during their life time. These groups of patients respond well to anti depressant drugs and in severe cases to electro convulsive therapy (ECT).

Treatments of major mood disorders have improved in recent years with the advent of newer antidepressant drugs like TCAs (Imipramine), SSRIs (Fluoxetine), MAO-Is and atypical antidepressants, which are more selective with insignificant side effects. Generally the antidepressant agents are reserved for severe and otherwise incapacitating depressive disorders.[2] Despite their general safety and efficacy they are not totally avoid of side effects and relatively expensive for long-term use. Therefore evaluation of natural products for the management of major mood disorders was justified. Research trials are continuing to introduce numerous herbal formulation approved by food and drug administration for the treatment of several psychological conditions.[3] *Ashwagandha* (*Withania somnifera*), *Bramhi* (*Bacopa monniera*) have memory enhancing and anxiolytic effect along with improvement of cognitive function.[4] Nevertheless data relating to their antidepressant action is yet to be established. The aim of present study was to investigate the antidepressant actions of *Ashwagandha* and *Bramhi* in behavioural despair animal model of depression for confirmation of the already reported efficacy.

MATERIALS AND METHODS

Learned helplessness test (LHT)[5] and Forced swimming test (FST)[6] models were used for preparation of behavioral despair animal model. Albino rats weighing between 200-250gm were allowed free access to food and water *ad libitum* and were maintained under standard laboratory conditions with a natural light and dark cycle following CPCSEA guidlines. The animals were acclimatized for 5 days before behavioural experiments which were carried out between 09.00 A.M and 1.00 P.M.

Table-1: Study Groups drug administration and doses

Group	Drugs and Doses (mg/kg)	Group	Drugs and Doses (mg/kg)	Group	Drugs and Doses (mg/kg)
Group I	Normal saline (5ml/kg)	Group V	*Bramhi* (20)	Group IX	*Ashwagandha* (100)
Group II	*Imipramine* (16)	Group VI	*Bramhi* (40)	Group X	*Ashwagandha* (150)
Group III	*Imipramaine* (32)	Group VII	*Bramhi* (80)	Group XI	*Imipramine* (16)+*Bramhi* (20)
Group IV	*Imipramine* (64)	Group VIII	*Ashwagandha* (50)	Group XII	*Imipramine* (16) +*Ashwagandha* (50)
Following is the schedule for each group (n=6) receiving drug orally through pediatric nasogastric tube with mentioned dose in parenthesis for each test (LHT & FST).					

Apparatus – 1. Gemini Avoidance System; This apparatus is divided into two equal compartments by a retractable door. Floors of the chambers in the shuttle box consisted of stainless steel rods. This apparatus was for LHT. 2. Polypropylene vessel (45x40x30cm) for FST, 3. Stop watch, 4. Pediatric nasogastric tube.

Drugs: Pure powder form of the following drugs were obtained from different sources -

1. Imipramine (Torrent pharmaceuticals Ltd.)

2. Ashwagandha and *Bramhi* (Indian Herbs research and supply co Ltd., Saharanpur, Uttarpradesh).

Experimental protocol was approved by Institutional Animal Ethics Committee before starting the study. Design of this study was comparative and parallel groups. Animals were divided into 24 groups (12 groups for LHT & FSTeach) containing six rats in each group (Table-1). Normal saline as control, imipramine as standard, Ashwagandha and bramhi as test drugs were administered orally as per the study protocol. The doses of control, standard and the test drugs were selected after observing the response of pilot doses of each, calculated by considering their recommended dose for clinical use and on the basis of earlier studies available in literature respectively.[7] Freshly prepared suspension of the drugs with vehicle normal saline, was administered orally through a pediatric nasogastric tube followed by 5ml of distilled water. Each dose of individual and combinations drugs were given for 14 days, 1 hr before the experiment. Earlier studies[8-10] stated that repeated oral administration did not causes sign of intoxication.

Learned Helplessness test[5]: In this test, an animal is initially exposed to uncontrollable shock stress. When the animal is later placed in a situation in which shock is controllable (escapable), the animal not only fails to

Table-2: Effect of *Bramhi* and *Ashwagandha* alone and in combination with imipramine on Avoidance response and Escape failure, using learned helplessness test model

Group	Drugs (mg/kg,P.O)	Number of Avoidance response [mean ± SEM]	Increase of number of avoidance response (%)	Number of Escape failure [mean ± SEM]	Decrease of number of escape failure (%)
I	Normal saline (5 ml/kg)	13.33 ± 0.42	0	7.5 ± 0.43	0
II	Imipramine (16)	13.83 ± 0.60	3.75	6.33 ± 0.49	15.6
III	Imipramine (32)	17.67 ± 0.88[a]	32.56	3.83 ± 0.40[a]	48.93
IV	Imipramine (64)	20.33 ± 0.71[a]	52.51	3.17 ± 0.31[a]	57.73
V	*Bramhi* (20)	13.5 ± 0.43	1.27	6.33 ± 0.49	15.6
VI	*Bramhi* (40)	14 ± 0.58	5.03	6 ± 0.58	20
VII	*Bramhi* (80)	14.67 ± 0.49	10.05	5.83 ± 0.31[b]	22.27
VIII	*Ashwagandha* (50)	16.33 ± 0.42[a]	22.51	5.33 ± 0.33[a]	28.93
IX	*Ashwagandha* (100)	16.83 ± 0.40[a]	26.26	4.33 ± 0.42[a]	42.27
X	*Ashwagandha* 150)	19.17 ± 0.48[a]	43.81	3.66 ± 0.33[a]	51.2
XI	Imipramine (16) + *Bramhi* (20)	16.33 ± 0.88[a]	22.51	4.33 ± 0.33[a]	42.27
XII	Imipramine (16)+ *Ashwagandha* (50)	21 ± 0.47[a]	57.54	3 ± 0.36[a]	60
One Way ANOVA	Results are given as no of avoidance response mean ± SEM in learned helplessness test . a-p<0.001 ,b-p<0.01Vs simple control (NS) with shock. N=6 in each group.				

Table-3: Effect of *Bramhi* and *Ashwagandha* alone and in combination with Imipramine on Immobility, using forced 3swimming test.

Group	Treatment (mg/kg, P.O)	Duration of immobility mean (sec) ± SEM	% Reduction of duration of immobility
I	Normal saline (5 ml/kg)	185.33 ± 0.08	0
II	Imipramine (16)	180.17 ± 0.91	2.78
III	Imipramine (32)	132 ± 1.03[a]	28.78
IV	Imipramine (64)	90 ± 1.03[a]	51.33
V	*Bramhi*(20)	184.83 ± 0.60	0.27
VI	*Bramhi*(40)	180 ± 0.37	2.88
VII	*Bramhi*(80)	179 ± 0.58[b]	3.44
VIII	*Ashwagandha*(50)	173.5 ± 2.88[a]	6.38
IX	*Ashwagandha* (100)	127.83 ± 1.66[a]	31.02
X	*Ashwagandha* (150)	70.17 ± 2.59[a]	62.13
XI	Imipramine (16)+ *Bramhi* (20)	120.33 ± 0.49[a]	35.07
XII	Imipramine (16)+ *Ashwagandha* (50)	67.83 ± 2.64[a]	63.4
One way ANOVA	Results are given as mean duration of immobility ± SEM in last 4 min. of 6 min. test. a-p<0.001 Vs simple control, b- p<0.01 Vs simple control (N.S.).		

acquire the escape responses but also often makes no efforts to escape the shock at all. This escape deûcit is reversed by chronic antidepressant treatment.[11,12] Learned helplessness behavioral tests were performed with the Gemini Avoidance System. This apparatus is divided into two equal compartments by a retractable door. Floors of the chambers in the shuttle box consisted of stainless steel rods. Scrambled shocks were delivered through a shock generator.

The drugs were used at three dose levels. Low dose combination of imipramine, bramhi and ashwagandha was administered orally for 2wks, starting on day 1, 60min before the inescapable shocks, and on day 12, the rats were individually placed in the chamber and given 90 inescapable shocks (0.8 mA) of 15 s duration at 45sec intervals. Control rats were not given shocks. On day 14, the rats were subjected to the 30-trial escape test. The animals were individually placed in the shuttle box and given a 5-min adaptation period; a tone signal was given during the first 5sec of each trial (conditioned response). If there was no avoidance response within this period, the tone signal remained on and a 0.8mA shock (15sec duration) was delivered through the grid floor. If no escape response was made within this period, both the tone signal and the shock were automatically terminated. The intertrial interval was 5 s. The number of escape failures and avoidence responce which refers to a noncrossing-crossing response during the shock delivery, was recorded.

Forced swimming Test[6]. - The test was based on the method described by Porsolt. Rats were made to swim individually in a polypropylene vessel (45x40x30cm) with a water level of 20 cm height and maintain at a temperature 22± 1ÚC, this ensued that the rats' feet didn't touch the floor of the vessel and that it could not climb out of it. The entire study was divided in to two sessions.

A. Pretest session: Animals (rats) were forced to swim individually for 15 minutes in the polypropylene container for 14 days.

B. Test session: 24 hours latter i.e. on 15th day each animal was again forced to swim for a period of 6 minutes in a test session. Each animal made vigorous attempt to get out of the polypropylene container during the first couple of minutes and there after surrendered to experimental conditions and became immobile with occasional escape attempts (characterized by complete cessation of swimming with the head lifting just above the water level as noted). This immobility period, after frenzied attempts to escape is postulated to represent the "Behavioural despair" in an animal experimental model. The total duration of immobility during last 4 minutes of a 6 minutes test was recorded as period of despair.

Data were statistically analyzed by one way ANOVA followed by Dunnet's t-test. Results were expressed as mean number of each group + standard error of mean(SEM)[mean ± SEM]. 'P' value <0.05 was considered significant.

RESULTS

The results (Table-2 and 3) showed significant increase in the number of rats showing avoidance response also reduction in the number of rats showing escape failure and immobility period. Avoidance response and escape failure in case of imipramine and ashwagandha showed highly significant result on individual use. But there was no significant result in case of all doses of bramhi except in case of escape failure and immobility period, bramhi in higher doses showed just significant result. In combination doses there was highly significant increase

in the number of avoidance response also reduction in escape failure and in immobility period when compared to low doses of all drugs given alone.

DISCUSSION

Ashwagandha an indigenous herbal product of Indian origin at the given dose levels produces significant reduction in both the parameters. The potency of *Ashwagandha* against depression in animal models was demonstrated earlier by Griebel *et al*[13] who compared the above indigenous herbal products with that of the standard SSRI.

Bramhi is famous for its memory enhancing tranquilizing and antioxidant properties.[14] With *Bramhi* in low dose level, no significant reduction in any of the parameter were detected, which carroborate with the previous study.[15]

The positive effect of Imipramine in the learned helplessness test and Forced swimming test model seems to be due to increased availability of these neurotransmitters norepinephrine (NE) and serotonin (5HT) at the post synaptic site following their reuptake inhibition. But uptake blocked is not directly responsible for antidepressant action. Thus it is attributed to these coronary events that followed the primary drugs on presynaptic α_2, $5HT_{1a}$, $5HT_{1d}$ auto receptors and amines turn over in brain. However, the antidepressant action of a drug is due to the respective synaptic sites in the brain region, net effect is enhancement of noradrenergic and serotonergic transmission.[16]

Ashwagandha is an essential popular Indian medical plant with marked antiaging, immunomodulatory, anti anxiety and stress relieving property, acts by normalizing the augmented Lipoxygenese (LPO) activities and enhancing the activities Glutathione peroxidase.[17] From our study, we have seen that it has marked antidepressant effect and it also causes the enhancement of effect when combined with low dose of imipramine. This result corroborates with the results of Bhattacharya *et al*.[18]

The present study evaluated that *Ashwagandha* had significant antidepressant action but *Bramhi* had not when used alone. These two drug showed high efficacy in depression model when combined with low dose Imipramine. So these drugs may be tried in the treatment of depression and further scientific research should be under taken towords this dimension.

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