

EFFECT OF *WITHANIA SOMNIFERA* ON FORCED SWIMMING TEST INDUCED IMMOBILITY IN MICE AND ITS INTERACTION WITH VARIOUS DRUGS

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Abstract : The objective of the present study was to evaluate the antidepressant action of *Withania somnifera* (WS) as well as its interaction with the conventional antidepressant drugs and to delineate the possible mechanism of its antidepressant action using forced swimming model in mice. Effect of different doses of WS, fluoxetine and imipramine were studied on forced swimming test induced mean immobility time (MIT). Moreover effect of WS 100 mg/kg, i.p. was observed at different time intervals. Effect produced by combination of sub therapeutic doses of WS with imipramine (2.5 mg/kg, i.p.) as well as fluoxetine (2.5 mg/kg, i.p.) were also observed. Effect of WS (100 mg/kg, i.p.) as well as combination of WS (37.5 mg/kg, i.p.) with either imipramine (2.5 mg/kg, i.p.) or fluoxetine (2.5 mg/kg, i.p.) were observed in mice pretreated with reserpine (2 mg/kg, i.p.) and clonidine (0.15 mg/kg, i.p.). Effects of prazosin (3 mg/kg, i.p.) or haloperidol (0.1 mg/kg, i.p.) pre-treatment were also observed on WS induced decrease in MIT. WS produced dose dependent decrease in MIT. Maximum effect in MIT was observed after 30 min of treatment with WS 100 mg/kg, i.p. Combination of WS (37.5 mg/kg, i.p.) with imipramine (2.5 mg/kg, i.p.) or fluoxetine (2.5 mg/kg, i.p.) also produced significant decrease in the MIT. Clonidine and reserpine induced increase in MIT, was significantly reversed by treatment with WS (100 mg/kg, i.p.) as well as combination of WS (37.5 mg/kg, i.p.) with either imipramine (2.5 mg/kg, i.p.) or fluoxetine (2.5 mg/kg, i.p.). Pre-treatment with prazosin but not haloperidol, significantly antagonized the WS (100 mg/kg, i.p.) induced decrease in MIT. It is concluded that, WS produced significant decrease in MIT in mice which could be mediated partly through α adrenoceptor as well as alteration in the level of central biogenic amines.

Key words : *Withania somnifera* forced swimming test antidepressants

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INTRODUCTION

Withania somnifera (WS) is a herb that grows naturally in diverse areas ranging from Africa, the Mediterranean East and in India. It has earned the nickname "Indian Ginseng". The chemistry of WS, has been extensively studied and over 35 chemical constituents have been identified, extracted and isolated (1). The biologically active chemical constituents are alkaloids (Isopelletierine, Anaferine), steroidal lactones (Withanolides, Withaferins), saponins containing an additional acyl group (sitoindoside VII and VIII) and withanolides with a glucose at carbon 27 (sitoindoside IX and X). The withanolides are important constituent of WS. They are believed to be responsible for its various pharmacological actions e.g. the anticonvulsant (2), anti-inflammatory (3), antioxidant (4), antitumor (5), immunomodulatory (6) and anxiolytic (7) actions. Moreover, ancient ayurvedic literature as well as several studies has reported antidepressant action of WS (7, 8) which has also been attributed due to presence of the active constituent, withanolides.

This study was planned to evaluate the antidepressant action of *Withania somnifera* (WS) as well as its interaction with the conventional antidepressant drugs and to delineate the possible mechanism of its antidepressant action using forced swimming model in mice.

MATERIALS AND METHODS

(I) Selection of animals

Albino mice of either sex weighing 25 ± 3

gm raised in central animal house, Medical College, Vadodara were used for the study. They were maintained under standard laboratory conditions on 12-hour day/night cycle and with free access to food and water. The animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were carried out between 10:00 hours to 16:00 hour at ambient temperature. The animals were drawn at random for test and control groups.

(II) Forced swimming test

Forced swimming test (FST) model suggested by Porsolt (9) was used to evaluate

antidepressant activity of various drugs. We chose to work with the forced swimming test (FST) since its relevance has been demonstrated with antidepressants of a wide variety (10). Another reason for choosing this animal model is the correlation which is observed between results in this test and clinical potency, which is not found in any other model (10).

Briefly in this test, animals were forced to swim individually in a glass jar ($25 \times 12 \times 25$ cm) containing water of 15 cm height (11) at a temperature of $25 \pm 3^\circ\text{C}$ for a period of six min in a "Test session". Each animal made vigorous attempts to get out of water bath during first couple of minutes and thereafter surrendered to experimental conditions and assumed a typical immobile posture (which is defined, in the traditional Porsolt test, as when no additional activity is observed other than that required to keep the head above the water) with occasional escape attempts. The total duration of

immobility was recorded. Animals were removed from water, dried and kept in their respective cages.

Drugs used in the study

Drugs used in the study were

- *Withania somnifera* root extract (Dhanvantari, Anand)
- Imipramine (Sun Pharmaceutical Industries, Baroda)
- Fluoxetine (Sun Pharmaceutical Industries, Baroda)
- Clonidine (Sarabhai Piramal Pharmaceuticals Baroda)
- Reserpine (Loba- Chemie, Bombay)
- Prazosin hydrochloride (Sun Pharmaceutical Industries, Baroda)
- Haloperidol (Sun Pharmaceutical Industries, Baroda)

WS, imipramine and fluoxetine were dissolved in 0.9% normal saline. While clonidine, reserpine, prazosin and haloperidol were dissolved in 5% DMSO. All the drugs were administered intraperitoneally (i.p.), before the "Test session." For each group fresh animals were used in the study.

Study design

Animals were divided into four major groups according to the treatment they received and were further divided into different subgroups.

Control group

A single dose of WS (100 mg/kg, i.p.) was administered to four subgroups of mice 15 min, 30 min, 45 min and 60 min before FST respectively to detect time required for development of its peak effect on MIT and this time interval was used for the further study.

Different doses of WS 25, 37.5, 50, 100 and 200 mg/kg, imipramine 2.5, 5.0, and 10.0 mg/kg and fluoxetine 2.5, 5.0, and 10.0 mg/kg were administered to study their effect on MIT. Moreover combinations consisting of different doses of WS (25 mg/kg and 37.5 mg/kg) with either imipramine (2.5 mg/kg) or fluoxetine (2.5 mg/kg) were administered 30 min before FST.

All the drugs were administered intraperitoneally (i.p.) 30 min prior to FST.

Clonidine treated group

In order to study the interaction of WS (100 mg/kg, i.p.) and clonidine (0.15 mg/kg, i.p.) on MIT in mice, they were administered 30 min and 15 min prior to FST respectively. Similarly, effect of clonidine (0.15 mg/kg, i.p.) on combination consisting of WS (37.5 mg/kg, i.p.) with either imipramine (2.5 mg/kg, i.p.) or fluoxetine (2.5 mg/kg, i.p.) were also studied.

Reserpine treated group

Two subgroups of animals were reserpinised by administration of reserpine (2 mg/kg, i.p.) 4 and 24 hours before FST respectively. Such reserpinized mice were used to study effects of WS (100 mg/kg, i.p.)

alone or its combination with either imipramine (2.5 mg/kg, i.p.) or fluoxetine (2.5 mg/kg, i.p.) given 30 min before FST.

Treatment with prazosin and haloperidol

Either prazosin (3 mg/kg, i.p.) or haloperidol (0.1 mg/kg, i.p.) were administered 60 min before FST while WS 100 mg/kg, i.p. was administered 30 min after the administration of above drugs (i.e. 30 min before FST) in order to study its effect on MIT in the mice pretreated with above drugs.

Statistical analysis

All the data were analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple range tests. P values <0.05 were considered significant.

RESULTS

Treatment with the vehicles (normal saline and DMSO) given at different time interval, did not modify the duration of immobility in FST (data not shown).

WS (100 mg/kg, i.p.) produced maximum decrease in the immobility time at an interval of 30 min. This effect persisted up to 45 min of the drug administration (Fig. 1). WS, imipramine and fluoxetine reduced the duration of immobility in mice in a dose dependent manner (Table I, II) However, statistical significant reduction in mean immobility time was observed with doses of WS 50 mg/kg, and higher, imipramine 5 mg/kg and higher and fluoxetine 5 mg/kg and higher. (Table I, II)

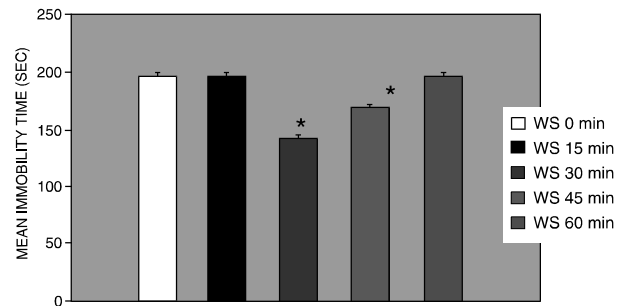


Fig. 1: Effects of *Withania somnifera* (100 mg/kg, i.p.) on FST induced immobility in control mice at different time intervals. The columns depict mean immobility time in sec and the vertical lines denote SEM (n=6).

Values are expressed as Mean±SEM.

*P<0.001 as compared to the corresponding value at '0' minutes using ANOVA and Tukey multiple comparisons tests. Degree of freedom: between columns: 4, within columns: 25; F=61.22.

WS = *Withania somnifera*

TABLE I: Effects of different doses of *Withania somnifera* on FST induced immobility in mice.

Treatment (mg/kg, i.p.)	Immobility time (sec)
Vehicle (saline)	198±2.5
<i>Withania somnifera</i> (25)	195.1±6.3
<i>Withania somnifera</i> (37.5)	194.2±2.2
<i>Withania somnifera</i> (50)	178.2±2.8*
<i>Withania somnifera</i> (100)	143.3±3.1**
<i>Withania somnifera</i> (200)	140.0±3.3**

Values are expressed as Mean±SEM, n=6.

*P<0.01, **P<0.001 as compared to the vehicle treated group using ANOVA and Tukey multiple comparisons tests. Degree of freedom: between columns: 5, within columns: 30; F = 53.71.

However, combination of subtherapeutic doses 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.) also produced significant (P<0.001) decrease in the mean immobility time (Table II) suggesting that, immobility reducing effect of imipramine as

TABLE II: Effects of different doses of imipramine, fluoxetine and combination of WS with either imipramine or fluoxetine on FST induced immobility in mice.

Treatment (mg/kg, i.p.)	Immobility time (sec)
Vehicle (saline)	198±2.5
Imipramine (2.5)	191.8±3.7
Imipramine (5.0)	177.3±2.4*
Imipramine (10.0)	154.2±4.2**
Fluoxetine (2.5)	196.2±3.4
Fluoxetine (5.0)	170.7±2.8*
Fluoxetine (10.0)	146.3±3.7**
WS (25) + Imi (2.5)	197.0±2.6
WS (37.5) + Imi (2.5)	178.8±2.5**
WS (25) + Flu (2.5)	195.2±4.6
WS (37.5) + Flu (2.5)	169.7±2.9**

Values are expressed as Mean±SEM, n=6.
 *P<0.01, **P<0.001 as compared to the vehicle treated group using ANOVA and Tukey multiple comparisons tests. Degree of freedom: between columns: 10, within columns: 55; F = 30.00.
 WS = *Withania somnifera* Imi = Imipramine
 Flu = Fluoxetine.

well as fluoxetine were enhanced by concomitant administration of WS.

Treatment with clonidine (0.15 mg/kg, i.p.) produced significant (P<0.001) increase in immobility time as compared to that of the vehicle treated animals. This effect was completely reversed by WS (100 mg/kg, i.p.) (Fig. 2). Combination consisting of sub therapeutic doses of WS (37.5 mg/kg, i.p.) with either imipramine (2.5 mg/kg, i.p.) or fluoxetine (2.5 mg/kg, i.p.), produced significant (P<0.001) reduction in mean immobility time in clonidine treated mice (Fig. 2).

Reserpine 2 mg/kg, i.p. produced significant (P<0.001) increase in immobility time after 4 hr and 24 hr of its treatment (Table III). WS (100 mg/kg) significantly (P<0.001) reduced reserpine induced

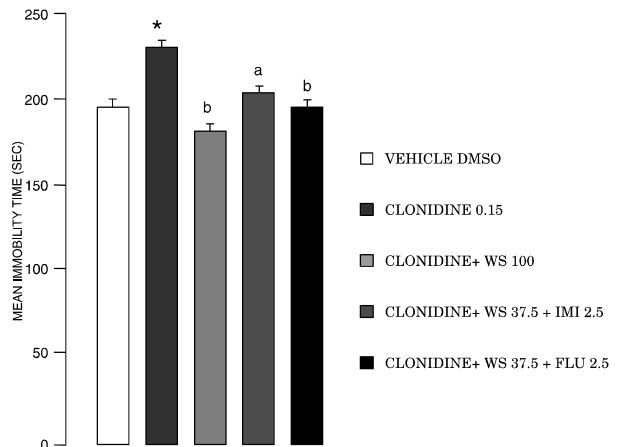


Fig. 2: Effects of various treatments on FST induced immobility in clonidine (0.15 mg/kg, i.p.) treated mice. The columns depict mean immobility time in sec and the vertical lines denote SEM. (n=6). Values are expressed as mean±SEM.
 *P<0.001 as compared to the vehicle treated group.
^aP<0.01 as compared to clonidine treated group.
^bP<0.001 as compared to clonidine treated group.
 Degree of freedom: between columns: 04, within columns 25; F=18.4.
 WS = *Withania somnifera*
 IMI = imipramine FLU = fluoxetine

TABLE III: Effects of various treatments on FST induced immobility in reserpine treated mice.

Treatment (mg/kg, i.p.)	Latency time (hr)	Immobility time (sec)
Vehicle (DMSO)	4	198.4±3.2
Vehicle (DMSO)	24	196.8±2.5
Reserpine (2)	4	297.8±2.4*
Reserpine (2)	24	296.8±2.8 [#]
Reserpine (2) + WS (100)	4	249.7±3.2 ^a
Reserpine (2) + WS (100)	24	249.2±3.5 ^b
Reserpine (2) + WS (37.5) + Imi (2.5)	4	274.7±3.3 ^a
Reserpine (2) + WS (37.5) + Imi (2.5)	24	274.1±2.4 ^b
Reserpine (2) + WS (37.5) + Flu (2.5)	4	265.2±2.8 ^a
Reserpine (2) + WS (37.5) + Flu (2.5)	24	264.1±2.7 ^b

Values are expressed as Mean±SEM.
 *P<0.001 as compared to the DMSO-4 treated group.
[#]P<0.001 as compared to the DMSO-24 treated group.
^aP<0.001 as compared to reserpine-4 treated group.
^bP<0.001 as compared to reserpine-24 treated group.
 Degree of freedom: between columns: 09, within columns: 50; F = 146.77.
 WS = *Withania somnifera*; Imi = Imipramine;
 Flu = Fluoxetine; DMSO = Dimethyl Sulphoxide.

immobility time. Moreover, combination consisting of sub therapeutic doses of WS (37.5 mg/kg) with either imipramine (2.5 mg/kg) or fluoxetine (2.5 mg/kg) also produced significant ($P < 0.01$) reduction in mean immobility time in both the groups of reserpine treated mice.

Pretreatment with haloperidol per se did not alter the mean immobility time; moreover, it also failed to alter effect of WS 100 mg/kg, i.p. on duration of mean immobility time (Table IV).

Prazosin per se also did not alter the mean immobility time in mice, but it significantly antagonized the immobility reducing action of WS 100 mg/kg, i.p. (Table IV).

TABLE IV: Effects of prazosin and haloperidol pretreatment on WS induced decrease in immobility time in mice.

Treatment (mg/kg, i.p.)	Immobility time (sec)
Vehicle (DMSO)	197.5±2.8
WS 100	142.0±3.28*
Prazosin	197.5±3.0
Prazosin+WS (100)	187.8±3.3 ^a
Haloperidol	196±3.4
Haloperidol+WS (100)	147±3.5

Values are expressed as Mean±SEM.

- * $P < 0.001$ as compared to group I.
- ^a $P < 0.001$ as compared to group II.

Degree of freedom: between columns: 06, within columns: 35; $F = 63.5$.

WS = *Withania somnifera*; DMSO = Dimethyl Sulphoxide.

DISCUSSION

Forced Swimming Test (FST) was developed by Porsolt and colleagues (9) in the rat and subsequently in the mouse. This test is the most widely used tool for assessing

antidepressant activity preclinically. Immobility in this model is reduced by a variety of treatments which are therapeutically effective in depression (12).

In the present study, different doses of WS, imipramine and fluoxetine produced dose dependant decrease in mean immobility time in mice. Decrease MIT produced by WS 100 mg/kg was comparable to that produced by either imipramine 10 mg/kg or fluoxetine 10 mg/kg.

Besides, combination consisting of sub therapeutic doses of WS (37.5 mg/kg) with either imipramine (2.5 mg/kg) or fluoxetine (2.5 mg/kg) produced significant reduction in MIT. These results suggest that WS enhances the effect of imipramine and fluoxetine on mean immobility time.

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI), while imipramine is a presynaptic uptake inhibitor of both noradrenaline as well as serotonin (13). Since catecholamines and 5-HT have been implicated in the aetiology of depression, the positive effect of these drugs in FST seems to be due to increased availability of these neurotransmitters at the postsynaptic receptor sites following their re-uptake inhibition. In the present study, WS 100 mg/kg, i.p. reversed increased immobility time induced by clonidine as well reserpine. Reserpine, a vesicular re-uptake blocker, which depletes catecholamines or lowers noradrenaline turnover in the brain produce depression like syndrome in animals (15). Since reserpine induced depressive state is found to significantly reverse by WS, it is tempting to suggest the involvement of biogenic amines in antidepressant action of WS.

Clonidine is α_2 adrenoreceptor agonist which reduces central sympathetic outflow. Clonidine has been previously shown to increase immobility time in mice, which was antagonized by α_2 blocker yohimbine (14). Reversal of clonidine induced increase in immobility time by WS indicates the facilitation of noradrenergic activity. Several studies (16, 17) have pointed out the role of α_1 adrenergic receptors in the immobility reducing effect of imipramine in FST. In the present work, α_1 adrenergic antagonist prazosin partially antagonized the immobility reducing action of WS. This observation suggests the possible involvement of α_1

adrenergic receptors in the immobility reducing action of WS.

In the present study pre-treatment with haloperidol failed to modify the immobility reducing action of WS. Haloperidol being a Dopamine₂ receptor agonist, this observation indicates that, dopaminergic receptors may not be involved in the immobility reducing effect of WS.

Thus on the basis of the above observations it is suggested that, WS has multiple sites of actions. Further detail studies are required to find out the exact mechanism of action of WS as an antidepressant agent.

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