



INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

ANTIDEPRESSANT EFFECTS: HYDROALCOHOLIC EXTRACT OF *WITHANIA SOMNIFERA*, *BERBERIS ARISTATA* AND *MUCUNA PRURIENS* ON TAIL SUSPENSION TEST IN MICE

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Accepted Date: 11/08/2014; Published Date: 27/08/2014

Abstract: Since ancient time, *Withania somnifera*, *Mucuna pruriens* and *Berberis aristata* are known for treatment of neurological disorders such as Parkinson's disease and depression in the Indian traditional medicine system, Ayurveda. Therefore, we aim to develop the polyherbal formulation from hydroalcoholic extract (HAE) of dried mature root, *Withania somnifera*, dried stem bark, *Berberis aristata* and dried seed, *Mucuna pruriens* for the treatment of depression. The HAEs of plants were prepared by continuous hot extraction process. The antidepressant activity of HAEs (5, 10, 20 and 40 mg/kg, p.o., for 1 hr) and polyherbal formulation (PHF, 10 mg/kg, p.o., for 1 hr) were evaluated in the mice using tail suspension test and photoactometer. The HAEs (40 & 10 mg/kg) of *Withania somnifera*, *Berberis aristata* and *Mucuna pruriens* showed significant decrease in locomotor activity and increase in active time (antidepressant behavior), respectively. PHF (10 mg/kg) significantly increased antidepressant behavior and did not affect the locomotor activity and this effects was significantly higher than HAEs of plants. Therefore, we conclude that HAEs of dried mature root, *Withania somnifera*, dried stem bark, *Berberis aristata* and dried seed, *Mucuna pruriens* possess antidepressant activity and CNS depressant activity at low (10mg/kg) and high dose (40mg/kg), respectively. The PHF, that made by mixing 1:1:1 proportion of each extract, show synergistic interaction on antidepressant activity.

Keywords: Depression, tail suspension test, hydroalcoholic extract, polyherbal formulation, *Withania somnifera*, *Mucuna pruriens* and *Berberis aristata*



PAPER-QR CODE

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How to Cite This Article:

Makwana S, Suhagiya B, Bhandari A, Chaudagar K; IJPRBS, 2014;
Volume 3(4): 815-822

INTRODUCTION

The psychiatric disorders are major burden for developing society. Among them, the depression is top ranked for disturbing society in 2030 according to WHO reports (1, 2). Depression is associated with imbalance in the activity of monoamine secreting neurons (3, 4). Based on this theme, many drugs, such as tricyclic antidepressants, serotonin reuptake inhibitor and monoamine oxidases are marketed for its treatment. Many clinical studies indicated the efficacy of such conventional antidepressant therapy compared to placebo groups (5, 6). However, they showed only partial attenuation of depression in other studies. Apart from these, conventional antidepressants cause serious side effects and decrease the patient adherence to the therapy (7). Therefore, there is need to established newer approach or drugs for the treatment of depression. Evidence based and preclinical studies provided the pivotal role of plants in the treatment of depression (8, 9).

Withania somnifera, known as ashwagandha, Indian ginseng and winter cherry, is a small woody shrub of the Solanaceae family that is widely used in the treatment of anxiety, cognitive and neurological disorders, inflammation, Parkinson's disease, bronchitis, asthma, ulcers, insomnia, and senile dementia. It is therapeutically well famous for adaptogenic and chemopreventive properties (10). It is a key ingredient of Ayurvedic antidepressant formulation, *Mamsyadi Kwatha* (11). There have been many studies indicated the chronic treatment concern antidepressant action of *Withania somnifera* that has been related to adaptogenic properties (12). However, there is lack of data related to dose dependent and acute effects of *Withania somnifera* and its extracts on depression.

Berberine is a alkaloid present in the various species of *Berberis*, such as *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), and *Berberis aristata* (tree turmeric). It is used for the treatment of cancer, inflammation, diabetes, depression, hypertension, and various infectious diseases such as bacterial diarrhea, intestinal parasite infections, and ocular trachoma infections (13-15). There is no detail report on antidepressant actions of *Berberis aristata* and its extracts.

Mucuna pruriens, also known as velvet bean, is a famous for the treatment of Parkinson's disease because it is rich in the L-dihydroxyphenylalanine (L-DOPA) (16). L-DOPA plays a crucial role in plant-plant interaction. *Mucuna pruriens* synthesizes and releases L-DOPA in the environment to inhibit the growth of surrounding plants (17, 18). In human, L-DOPA is a precursor for the synthesis of catecholamines. Decreased in the brain activity of catecholamines is associated with the depression (3). However, there is no report on antidepressant potential of *Mucuna pruriens*.

The combination of different plants or different extract of single plants showed unexpected synergistic interaction for treatment of disease in many preclinical and clinical studies (19). Therefore, we targeted the development of multiple plant-containing formulation using *Withania somnifera*, *Mucuna pruriens* and *Berberis aristata* for the treatment of depression.

MATERIALS AND METHODS

Materials

Plant materials, dried mature root of *Withania somnifera* (Ashwagandha), dried stem bark of *Berberis aristata* (Daruhaldi) and dried seed of *Mucuna pruriens* (Kauchapa) were purchased from Lalubhai Vrijlal Gandhi Healthcare Pvt. Ltd., India. These plant materials were authenticated with quality control standards laid down by The Ayurvedic Pharmacopoeia of India at Department of pharmacognosy, and phytochemistry, K.B. Institute of Pharmaceutical Education and Research, India (20). The fluoxetine was given as a gift sample by Unison Pharmaceuticals Ltd., India. Ethanol (95%) was obtained from Merck, India.

Preparation of hydroalcoholic extract and polyherbal formulation of plants

The powder of dried mature root of *Withania somnifera* (100gm), dried stem bark of *Berberis aristata* (100gm) and dried seed of *Mucuna pruriens* (100gm) were prepared by blender mixture. The coarse powder of each plant was extracted with hydroalcoholic mixture (7:3 = ethanol:distilled water) by Soxhlet apparatus (continuous hot extraction process) for 6hr. Each extract was dried by distillation. The %yield of HAE of dried mature root of *Withania somnifera* (WEE), dried stem bark of *Berberis aristata* (DEE) and dried seed of *Mucuna pruriens* (KEE) were 14.9, 10.5 and 9.2, respectively. The polyherbal formulation was prepared by mixing 1:1:1 proportion of HAE of *Withania somnifera*, *Mucuna pruriens* and *Berberis aristata* using mortar and pestle according to results of antidepressant activity of each extract on tail suspension test in mice.

Animals

Swiss albino mice (25–35g) of either sex were maintained at constant room temperature (18–24°C) with free access to water and food, under a 12:12h light:dark cycle for 1 week before experiment. Prior ethical approval was taken from IAEC, Sharda School of Pharmacy College, India and experiments were performed according to the guideline of CAPCSEA, India.

Experimental Design

Mice were divided into fifteen groups (n=20/group); Group 1 was treated with saline (5ml/kg, p.o., for 1 hr) and considered as a control group. Group 2 was treated with fluoxetine (20 mg/kg, p.o., for 1 hr) and considered as standard group. Group 3, 4, 5 and 6 were treated with *Withania somnifera* (5, 10, 20 and 40 mg/kg, p.o., for 1 hr), respectively. Group 7, 8, 9 and 10 were treated with *Mucuna pruriens* (5, 10, 20 and 40 mg/kg, p.o., for 1 hr), respectively. Group 11, 12, 13 and 14 were treated with *Berberis aristata* (5, 10, 20 and 40 mg/kg, p.o., for 1 hr), respectively. Group 15 were treated with polyherbal formulation (10 mg/kg, p.o., for 1 hr). Each group were further divided into two subgroups (n=10/subgroup) for evaluation of antidepressant activity using tail suspension test and locomotor activity using gross behavior test, respectively. For tail suspension test, mice were suspended in air on stand at height of 50 cm and immobility (passive time) and mobility behavior (active time) were observed and time were counted for duration of 6 mins. Mice were considered immobile only when they hung passively or stay completely motionless. For gross behavior test, mice were placed separately in a square (30 cm) closed arena equipped with infra-red light sensitive photocells using digital

photoactometer. Number of light cut off was recorded for a period of 5 min and the values were expressed as counts/5 min (21).

Statistical analysis

Data are expressed as MEAN±SEM. For statistical analysis of hydroalcoholic extract of plants and their polyherbal formulation, one way and two way ANOVA were performed followed by bonferoni multiple comparison, respectively. P<0.05 was considered as statistically significant.

RESULTS

The HAE of *Withania somnifera* (40 mg/kg), *Mucuna pruriens* (20 and 40 mg/kg) and *Berberis aristata* (40 mg/kg) significantly (p<0.05) decreased number of light cut off in gross behavior test as compared to vehicle treated group whereas other doses of HAE of *Withania somnifera* (5, 10 and 20 mg/kg), *Mucuna pruriens* (5 and 10 mg/kg), *Berberis aristata* (5, 10 and 20 mg/kg) and fluoxetine (20mg/kg) showed non-significant changes in locomotor activity in mice (Figure 1).

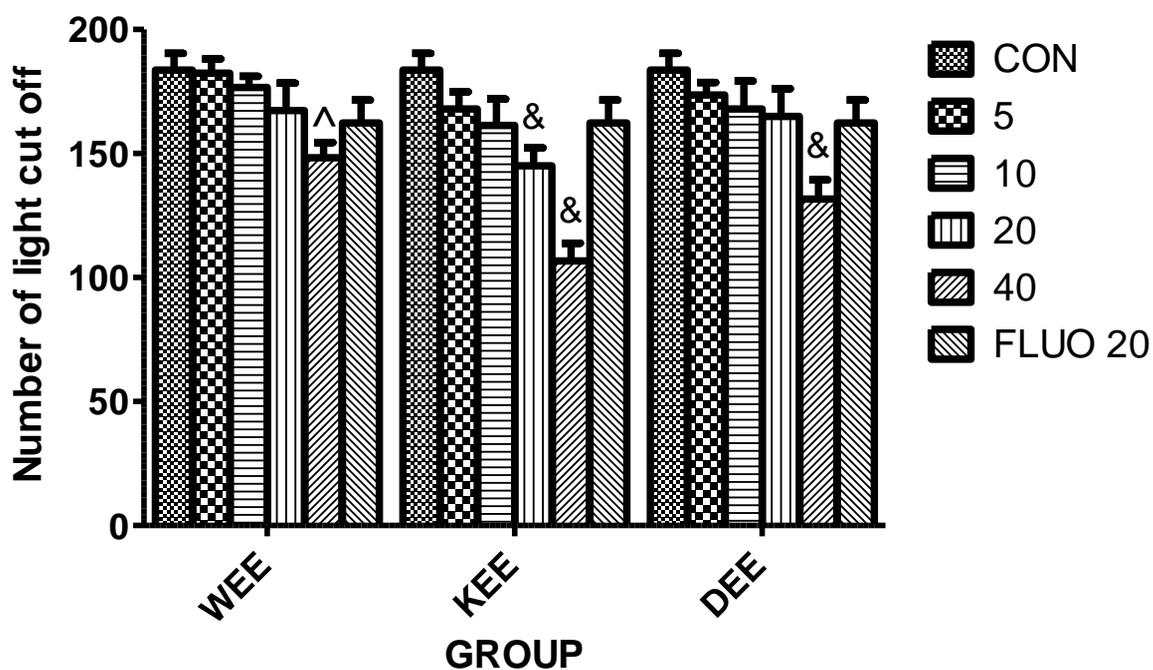


Figure 1: Effect of hydroalcoholic extracts of *Withania somnifera* (WEE-5mg/kg (5), 10mg/kg (10), 20mg/kg (20), 40mg/kg (40)), *Mucuna pruriens* (KEE-5mg/kg (5), 10mg/kg (10), 20mg/kg (20), 40mg/kg (40)), *Berberis aristata* (DEE-5mg/kg (5), 10mg/kg (10), 20mg/kg (20), 40mg/kg (40)), vehicle (0.05%v/v ethanol (CON)) and fluoxetine (20 mg/kg (FLUO 20)) on locomotor activity of mice in photoactometer. Values are expressed as MEAN±SEM. ^p<0.001 and &p<0.01 vs. CON.

The HAE of *Withania somnifera* (10 and 20 mg/kg), *Mucuna pruriens* (10 mg/kg), *Berberis aristata* (10 and 20 mg/kg) and fluoxetine (20mg/kg) significantly (p<0.001) increased duration of active time in tail suspension test as compared to vehicle treated group whereas other doses of HAE of *Withania somnifera* (5 and 40 mg/kg), *Mucuna pruriens* (5, 20 and 40 mg/kg), *Berberis aristata* (5 and 40 mg/kg) did not affect depression status of mice in tail Suspension test (Figure 2).

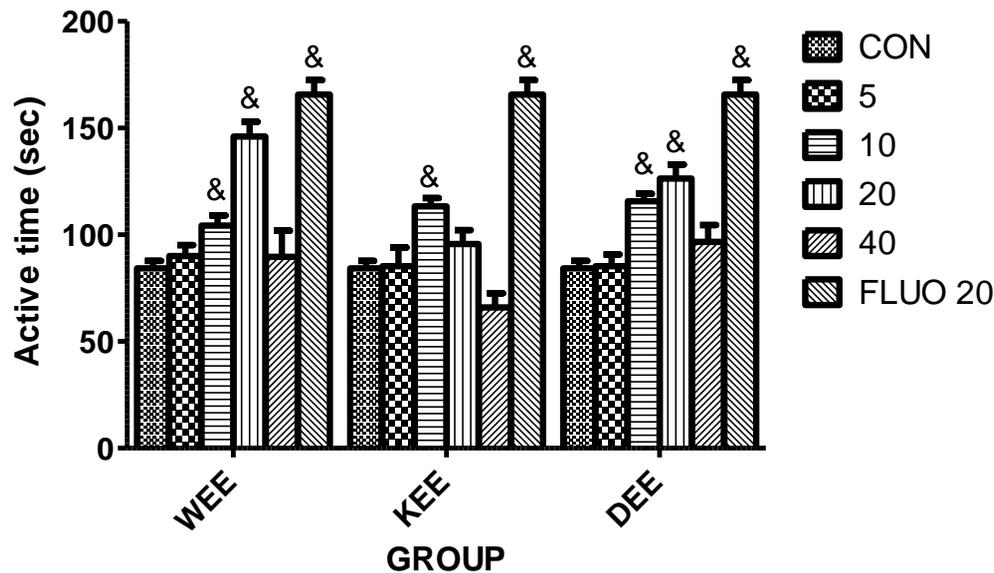


Figure 2: Effect of hydroalcoholic extracts of *Withania somnifera* (WEE-5mg/kg (5), 10mg/kg (10), 20mg/kg (20), 40mg/kg (40)), *Mucuna pruriens* (KEE-5mg/kg (5), 10mg/kg (10), 20mg/kg (20), 40mg/kg (40)), *Berberis aristata* (DEE-5mg/kg (5), 10mg/kg (10), 20mg/kg (20), 40mg/kg (40)), vehicle (0.05%v/v ethanol (CON)) and fluoxetine (20 mg/kg (FLUO 20)) on locomotor activity of mice in photoactometer. Values are expressed as MEAN±SEM. & p<0.001 vs. CON.

Polyherbal formulation (10 mg/kg) showed significant (p<0.001) increase in duration of active time in tail suspension test as compared to vehicle, HAE of *Withania somnifera* (10 mg/kg), *Mucuna pruriens* (10 mg/kg) and *Berberis aristata* (10 mg/kg) treated group whereas it was non-significant as compared to fluoxetine (20 mg/kg) treated group (**Figure 3b**). Polyherbal formulation (10 mg/kg) did not affect locomotoer activity in mice (**Figure 3a**).

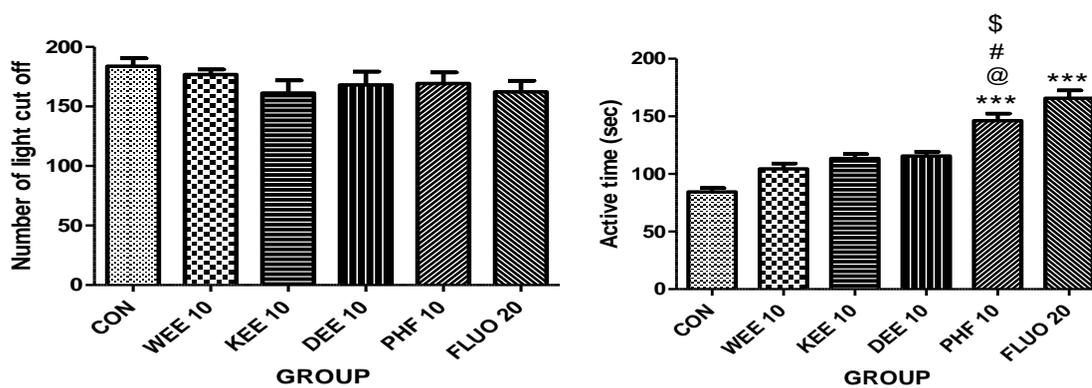


Figure 3: Effect of hydroalcoholic extracts of *Withania somnifera* (10mg/kg (WEE 10)), *Mucuna pruriens* (10mg/kg (KEE 10)), *Berberis aristata* (10mg/kg (DEE 10)), vehicle (0.05%v/v ethanol (CON)), polyherbal formulation (10mg/kg (PHF 30)) and fluoxetine (20 mg/kg (FLUO 20)) on (a) locomotor activity in photoactometer and (b) active behaviour in tail suspension test. Values are expressed as MEAN±SEM. *** p<0.001 vs. CON. @ p<0.01 vs. WEE 10. # p<0.01 vs. KEE 10. \$ p<0.01 vs. DEE 10.

DISCUSSION

In present study, we found that hydroalcoholic extracts of dried mature root of *Withania somnifera* (Ashwagandha, 10 & 20mg/kg), dried stem bark of *Berberis aristata* (Daruhaldi, 10 & 20mg/kg) and dried seed of *Mucuna pruriens* (Kauchapa, 10mg/kg) showed antidepressant activity whereas CNS depressant activity at dose of 40, 40 and 20 mg/kg. The polyherbal formulation (10 mg/kg) showed the synergistic effects on antidepressant activity for the combination (1:1:1) of hydroalcoholic extracts of *Withania somnifera*, *Berberis aristata*, *Mucuna pruriens* without affecting the locomotor activity in the photoactometer.

The tail suspension test is one of the most widely for assessing antidepressant-like activity in mice in which animals are subjected to the short-term, inescapable stress of air suspension by their tail. This leads to immobile posture and indicates the depressive behavior (21). The marketed antidepressant such as fluoxetine showed decrease in this behavior. In consistent to this, the HAE of *Withania somnifera*, *Berberis aristata*, *Mucuna pruriens* decreased immobile posture and showed antidepressant activity at low dose (10 & 20mg/kg) in our study. The antidepressant activity of HAE, *Withania somnifera* might be related to its adaptogenic properties, monoamine oxidase inhibition (MAOI), anti reserpine activity and modulation in the sensitivity of serotonin receptor (5-HT₂ and 5-HT_{1A}) (11, 12, 22-24). The antidepressant activity of HAE, *Berberis aristata* might be due to the presence of berberine and its actions on the indirect monoaminergic and corticotrophin releasing factor system stimulation via involvement of nitric oxide and sigma receptor (25, 26). The antidepressant activity of HAE, *Mucuna pruriens* might be related to increase in synthesis of catecholamine (3, 17).

The tail suspension test showed false negative and false positive results with CNS depressant agents and psychostimulants, respectively (9). We found the CNS depressant activity of HAE, *Withania somnifera*, *Berberis aristata*, *Mucuna pruriens* at dose of 40mg/kg that could affect their antidepressant activity in the tail suspension test. Therefore, we had selected the low antidepressant dose (10mg/kg) of HAE, *Withania somnifera*, *Berberis aristata*, *Mucuna pruriens* for the study of PHF and development of PHF in consistent to other reported studies on synergism of herbs (19). The synergistic antidepressant activity of PHF at 10mg/kg that might be related to indirect monoaminergic system stimulating activity of *Withania somnifera* and *Berberis aristata*; and direct synthesis enhancing activity of *Mucuna pruriens* in present study.

CONCLUSION

The hydroalcoholic extracts of dried mature root of *Withania somnifera* (Ashwagandha), dried stem bark of *Berberis aristata* (Daruhaldi) and dried seed of *Mucuna pruriens* (Kauchapa) have antidepressant activity and CNS depressant activity at low (10mg/kg) and high dose (40mg/kg), respectively. The polyherbal formulation, that made by mixing 1:1:1 proportion of each extract, show synergistic interaction on antidepressant activity.

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