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ANTIDEPRESSANT ACTIVITY OF AQUEOUS EXTRACT OF *WITHANIA SOMNIFERA* ROOT IN MICE - AN *IN VIVO* DESIGN

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ABSTRACT

Depression is one of the major mental disorders characterized with symptoms such as regular negative moods, decreased physical activity, feelings of helplessness, sluggish thought, and cognitive function. Depression is a common illness worldwide, which affects around 350 million people. It has become a major psychiatric disorder and imposes a substantial health burden on the society. The therapeutic agents derived from plants are justified by the emergence of diseases and the growth of scientific knowledge about herbal medicines as important alternatives or complementary treatment of diseases. Many studies have shown that medicinal plants contain coumarins, flavonoids, phenolics, alkaloids, terpenoids, tannins, essential oils, lectin, polypeptides, and polyacetylenes. The main objective of the present research is to screen the antidepressant activity of aqueous extract of *Withania somnifera* root in mice by using tail suspension test and forced swim test. Swiss albino mice of either sex weighing 20-30g were used. Sixty mice were divided into two arms. Each arm was further divided into five groups (n=6). Drugs were given orally once daily. Group 1 was the control group and received saline. Group 2 received standard drug-imipramine (15 mg/kg). Group 3 received WSRE (100 mg/kg). Group 4 received WSRE (200 mg/kg). Group 5 received WSRE (400 mg/kg). The study showed significant reduction in immobility time in both forced swim test and tail suspension test in the WSRE group when compared with the control group. The present study suggested that WSRE possessed potential antidepressant effects which could be of therapeutic interest for using in the treatment of patients with depression.

INTRODUCTION:

Plants have been used for thousands of years to flavour and conserve food, to treat health disorders and to prevent diseases including epidemics. The knowledge of their healing properties has been transmitted over the centuries within and among human communities. Active compounds produced during secondary vegetal metabolism are usually responsible for the biological Properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases [1]. Depression is the most common of the affective disorders defined as disorders of mood); it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions. Worldwide,

depression is a major cause of disability and premature in addition to the significant suicide risk, depressed individuals are more likely to die from other causes, such as heart disease or cancer. Depression is a heterogeneous disorder, with patients presenting with one or more core symptoms, and depression is often associated with other psychiatric conditions, including anxiety, eating disorders, schizophrenia, Parkinson's disease and drug addiction. The symptoms of depression include emotional and biological components. Several theories have been proposed to explain the causes of depression. None fully explain all of the observations and evidence of pathological changes that occur with depression. Here we summarise the main theories as they relate to the mechanisms of action of current drug therapies. Progress in unravelling the neurochemical mechanisms is, as in so many areas of psychopharmacology, limited by the lack of good animal models of the clinical condition. There is no known animal condition corresponding to the inherited form of depression in humans. Procedures involving mild stress (e.g. the forced swim test, inescapable foot shock) produce behavioural states in animals (withdrawal from social interaction, loss of appetite, reduced motor activity, etc.) that mimic aspects of human depression. In such tests, current antidepressant drugs reverse the symptoms of depression. However, we need neve drugs to treat forms of depression that late resistant to current drugs and thus new animal models are Genetically modified mice (e.g. knock-down quired of 5-HT, noradrenaline and glutamate transporters, mutations or down of 5-11 receptors, etc.) have been extensively knock-d studied to mimic various aspects of the disorder. However, a good animal model of drugresistant depression has still to be developed [2-5]. *Withania somnifera*, known commonly as ashwagandha or winter cherry is an evergreen shrub in the Solanaceae or nightshade family that grows in India, the Middle East, and parts of Africa. Several other species in the genus *Withania* are morphologically similar. The plant, particularly its root powder, has been used for centuries in traditional Indian medicine. Although used in herbal medicine and sold as a dietary supplement, there is insufficient scientific evidence that *W. somnifera* is safe or effective for treating any health condition or disease. *Withania somnifera* is a small shrub or herb grown as an annual in zones colder than 8, but in its native habitat it grows as a ground covering perennial. The native habitats include open and disturbed areas. It plays a similar role as ginseng in China, leading to one of its common names, Indian Ginseng.

Figure 1: *Withania somnifera* plant

In the Ayurvedic system of medicines, roots and leaves of the plant were considered phytotherapeutic agents to cure various ailments. Various clinical and preclinical trials exhibited the plant’s potential in curing hepatotoxicity, neurological disorders, anxiety, Parkinson’s disease, and hyperlipidemia. The fruits contained considerable amounts of saponins and leaves possessed insect repellent properties. Phytochemical analysis of *W. somnifera* revealed the presence of pharmacologically active steroidal lactones named withanolides. Withanine, a group of alkaloids isolated from the roots of the plant, forms 38% of the total weight of alkaloids. The principal withanolides extracted from *W. somnifera* in India were withanolide D and

withaferin A which exhibited antitumor and cytotoxic properties. In addition to alkaloids, the plant also consisted of steroids, saponins, phenolics, This Photo by Unknown Author is flavonoids, phytophenols, and glycosides. Also, it is widely used in traditional medicine formulations as an antipyretic, analgesic, adaptogenic, and anti-inflammatory agent [6-9]. The main aim of the present research is to assess the antidepressant activity of aqueous extract of *Withania somnifera* root powder in mice by using tail suspension test and forced swim test.

Figure 2: *Withania somnifera* root powder

MATERIALS AND METHODS

The study was carried out in the Department of Pharmacology, Siddhartha Institute of Pharmacy, Narapally, Ghatkesar, Hyderabad, Telangana, India.

Ethical approval: The protocol was approved by the Institutional Animal Ethics Committee of Siddhartha Institute of Pharmacy, Narapally, Ghatkesar, Hyderabad, bearing approval no. 2280/PO/Re/S/2024/CCSEA.

Collection of plant material [10,11]: The dried root powder of *Withania somnifera* was purchased from local market at Hyderabad, Telangana. The powder was boiled with distilled water and obtained residue was stored in the refrigerator for current research.

Chemicals and reagents: The standard drug imipramine hydrochloride was purchased from Sura Labs, Hyderabad, Telangana, India. All chemicals used were LR grade.

Animals [12,13]: Swiss Albino mice (weighing around 20-25 g) of either sex, from the animal house of the Department of Pharmacology, Siddhartha Institute of Pharmacy, Hyderabad were used in the present research. The animals were kept in the laboratory at 22 ± 1^0C with free access to food and water. One animal was used only once in this study. All procedures in this study were performed in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. The mice (n=60) were divided into two arms which was further divided into five groups, each group having six mice. Drugs were given orally after 12 h of fasting every day, for ten days. The drugs were prepared and administered per oral (0.1 ml/10g). Group 1 was administered normal saline (10 ml/kg). Group 2 was given standard drug imipramine (15 mg/kg). Group 3, 4 and 5 received 100 mg/kg, 200 mg/kg, 400 mg/kg doses of the test compound of *Withania somnifera* root extract (WSRE) respectively.

Pharmacological assessment [13-18]

Forced swim test (FST): The mice were individually forced to swim in a vertical plexiglass cylinder (capacity: 5L, height: 50cm diameter: 18cm) containing l5 cm of water maintained at temperature: 25 ^{0}C. Mice were subjected to pre-screening, which lasted for 15 min. 24 h after pre-screening, the trial was performed for 6 min of which the first 2 min were not recorded, and the periods of immobility for the latter 4 min was measured (in seconds) with a stopwatch. Mice were considered to be immobile when they made only the bare necessary movements to stay afloat, or when they were motionless. The mice were taken out of the plexiglass cylinder after 6 min. They were dried with a dry towel, and kept under a dim lamp for drying. The water was discarded after every test, and fresh water was used for the next mouse.

Tail suspension test: Antidepressants that are used in practice are able to reduce the period of immobility of mice when they try to escape when suspended by their tail. This

test was a reliable screening method for antidepressants, including those involving serotonergic system. Mice ware hung on a wooden rod, 50 cm above the table, by attaching them from their tail end with the use of an adhesive tape. The first 2 min were not recorded, and the periods of immobility for the latter 6 min was recorded (in seconds) with a stopwatch. Mice were considered to be immobile only when they were motionless and not attempting to escape.

Statistical analysis: The recorded data was entered in Microsoft Excel. The variables recorded followed normal distribution; hence, results have been expressed as mean (in seconds) ± standard error of mean (SEM). The data was analysed using one way ANOVA followed by post-hoc Dunnet's test. Probability $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Imipramine (15 mg/kg) and test drug WSRE (100 mg/kg, 200 mg/kg, 400 mg/kg) showed significant reduction in immobility times when compared to control in both FST and TST (Table 1). In this study, both imipramine and WSRE showed a reduction in immobility times in both FST and TST. Lowest immobility times were recorded with WSRE at 100 mg/kg dose in most recordings, and at times, it showed comparable or even better reduction in immobility times than imipramine in both tests.

Table 1: Immobility time in tail suspension test and forced swim test

Dose	Tail suspension test (TST)	Forced swim test (FST)
Normal saline	232.4(±19.54)	138.5(±6.42)
Imipramine 15 mg/kg	176.4(±5.35)*	105.53(±5.84)*
WSRE 100 mg/Kg	168.9(±13.7)*	95.63(±7.67)*
WSRE 200 mg/Kg	182.4(±9.82)*	112.73(±3.00)*
WSRE 400 mg/Kg	117.5(±6.62)*	109.16(±5.92)*

*Immobility time shown in seconds as mean (± SEM), *denotes statistically significant value.*

Antidepressants act by increasing the availability of the monoamine transmitters; norepinephrine (NE), dopamine (DA), and 5- hydroxy tryptamine (5-HT). This is achieved by either preventing the metabolism of these neurotransmitters (inhibitors of the enzyme monoamine oxidase) or by blocking the transporter-mediated reuptake of the neurotransmitters (tricyclic antidepressants, selective serotonin reuptake inhibitors, selective norepinephrine reuptake inhibitors). Several other mechanisms have also been described to be responsible for the antidepressant effect. But it is difficult to comment on the exact mechanism of antidepressant-like action of WSRE seen in this study. Further studies are needed to be conducted to gather in-depth information.

CONCLUSION

Natural medicines have been used to boost health since the time of immemorial and the success of modern medical science largely depends on drugs originally obtained from natural resources. In the past, a large number of antimicrobial compounds were discovered from synthetic and natural products for the treatment and control of infectious agents. However, only a few of them were reachable to the needy world's market. However, more extensive pharmacological studies of this plant are required for complete understanding of the antidepressant activity of aqueous extract of root extract of *Withania somnifera*. *Withania somnifera* root extract possesses antidepressant effect in animal models of depression which was comparable to that of imipramine as demonstrated in this study. Further studies would be necessary to evaluate the contribution of active chemical constituents for the observed antidepressant activity as it still remains to be determined which components were responsible for these effects.

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