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ANALGESIC AND NEUROPHARMACOLOGICAL ACTIVITY OF *WITHANIA SOMNIFERA ROOT*

Mohammad Shahriar[1]*, Fariha Alam[1] and Mir Muhammad Nasir Uddin[2]

[1]Phytochemistry Research Laboratory, University of Asia Pacific, Dhaka, Bangladesh
[2]Department of Pharmacy, University of Chittagong, Chittagong, Bangladesh

***Corresponding author e-mail:** shahriar@uap-bd.edu

ABSTRACT

This study was designed to evaluate the analgesic and neurophramacological investigations of root extracts of *Withania somnifera* in Swiss albino mice following oral administration. *In-vivo* analgesic activity test was evaluated by acetic acid induced writhing method and tail immersion test. *In-vivo* neurophramacological investigations were determined by open field and swimming test. There is no scientific report on analgesic activity and neuropharmacological activity of *Withania somnifera,* therefore the present study was undertaken to examine the possible *in-vivo* analgesic activity and neuropharmacological activity of this plant extracts. *In-vivo* analgesic activity test showed that methanol, ethanol and chloroform extract inhibited writhes in a dose dependent manner. But ethanol extract at 150 mg/kg showed highest inhibition (70.56%) which is even higher than the standard drug (25.55%). And in case of tail immersion test the basal reaction time was more for standard drug when compared to plant extracts. The ethanol extracts showed more reaction time and that is recovered after 120 min the order of potency in 60 min after dose administration is diclofenac Na > ethanol > chloroform > methanol > control. This plants root extracts also exhibit significant neuropharmacological activity.

Keywords: *Withania somnifera*, analgesic activity, neurophramacological activity

INTRODUCTION

The plants which are useful for healing diseases are called medicinal plant. According to the WHO, "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." There are more than 500 medicinal plants growing in our country [1]. Recently World Health Organization (WHO) estimated that 80% of people worldwide rely on herbal medicines partially for their primary health care. It has been recorded that about 450 to 500 plants growing or available in Bangladesh have therapeutic values [1, 2]. In Bangladesh, people living in the remote hilly areas, such as, ethnic communities rely mostly on herbal medicines. Bangladesh, a country fertile deltaic land has a rich diversity of flora of medicinal plants scattered throughout the forests, crop fields, roadsides gardens and wastelands. *Withania somnifera* is a small and erect evergreen woody under shrub that grows up to a height of 1-m tall and belongs to the family of solanaceae locally known as Ashwagandha. This plant is capable of growing wildly not only in all the drier parts of the subtropical Bangladesh i.e. in Nator, Savar, and North-western parts of Bangladesh but also in India, Congo, South Africa, Egypt, Morocco, Jordan, Pakistan and Afghanistan. The roots are the main portions of the whole plant as they possess wide number of the therapeutic agents. The crude aqueous extract of the plant contains the phenolics and flavonoids which are said to be the potent antioxidants [3]. Ashwagandha is found to be a major ingredient of various adaptogenic and anti-stress tonics [4]. A methanolic extract of the various parts of *Withania somnifera* had showed a potent anti-inflammatory activity. *Withania somnifera* is found to be a unique plant where a wider range of biological activities has been demonstrated including

antagonism with several inflammatory factors and the immune modulation.

MATERIALS AND METHOD

Plant material: The plant roots were collected during July, 2013, from Savar, Dhaka, Bangladesh. Then the plant sample was submitted to the National Herbarium of Bangladesh, Mirpur, Dhaka. One week later its voucher specimen was collected after its identification (Accession No. 35903) which was identified and authenticated by taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka. The roots were sun dried for seven days. The dried plant part was then ground in coarse powder using high capacity grinding machine.

Preparation of extract: Hot solvent extraction process was used for extraction of the plant material. Soxhlet extractor was used for the extraction procedure. Plant material was extracted by the solvent- methanol, ethanol and chloroform. After extraction, was kept at petri dishes and dried at room temperature. After drying, extracts were stored in petri dishes and kept in refrigerator for further use.

Preparation of animals: Adult Swiss albino mice (BALB/c) weighing between (12-30) gm of either sex were used for the studies. The animals were maintained under normal laboratory condition & kept in standard cages at room temperature of 30°C±2°C and 60% to 65% relative humidity and provided with standard diet & water *ad libitum.* The experimental protocols were approved by institutional Animal Ethical Committee to carry out and complete this study.

Analgesic activity test: Analgesic activity was evaluated by acetic acid writhing test and tail immersion test.

Acetic acid induced writhing: The acetic acid writhing test in mice as described by Koster *et al.,* 1959 [5] was employed with slight modification. Mice were divided into 5 groups of 6 mice each. The first group was given 10ml\kg of 1% Tween 80 i.p. and served as control. Group 2 received Diclofenac Sodium 100 mg\kg of body weight and served as standard, groups 3, 4 and 5 received methanol, ethanol & chloroform extracts of root of *Withania somnifera* 100, 150 mg\kg of body weight respectively. Thirty minutes later after oral administration, each mouse was injected intraperitoneally with 0.7% acetic acid at a dose of 10 ml/kg body weight. Full writhing was not always completed by the animal, because sometimes the animals start to give writhing, but they do not finish it. This incomplete writhing was taken as a half writhing. Accordingly, two half writhing were considered as one full writhing. The number of writhing responses was recorded for each animal during a subsequent 5 min period after 15 min IP administration of Acetic acid and the mean abdominal writhing for each group was obtained.

The percentage inhibition was calculated using the formula

% Inhibition = [1- (No. of writhing of standard or sample/ No. of writhing of control)] X 100

Tail immersion test: The tail immersion method was used to evaluate the central mechanism of analgesic activity. Here the painful reactions in animals were produced by thermal stimulus that is by dipping the tip of the tail in hot water [6]. On the test day, albino swiss mice were divided into 5 groups of 6 mice each. Here diclofenac Na (50 mg/kg) is used as standard drug as well. Animals were fasted for 16 hours with free access to water. After administration of standard and test drugs, the basal reaction time was measured by immersing the tail tips of mice (last 1-2 cm) in hot water of water bath, where temperature was previously adjusted at 51ºC. The actual flick response of mice that is time taken in second to withdraw it from hot water source was calculated and results were compared with control group. The latent period of the tail-flick response was determined at 0, 30, 60, 90 and 120 minute after the administration of drugs.

Neuropharmacological study: ***Open field test:*** According to Gupta *et al.,* 1971[7] open field was performed and test to monitor behavioral responses in mice that were placed in a novel and bright arena. Rodents tend to stay away from brightly illuminated areas. The experiment also assesses a range of anxiety-induced, locomotor activity and exploratory behaviors. The animals were divided into 5 groups of 6 mice each. The first group was given 10ml\kg of 1% Tween 80 orally and served as control. Groups 3, 4 and 5 received methanol, ethanol & chloroform extracts of root of *Withania somnifera* 100, 150 mg\kg of body weight respectively, while the group 2 was given 2mg Diazepam per kg body weight orally and served as standard. The test was carried out according to the technique described by Gupta *et al.,* 1971[21] with slight modification. The open field apparatus is made of hardboard (60cmx60cm; 40cm walls).Blue lines drawn on the floor divide the floor into 36 squares (10cm x 10cm squares alternatively colored black and white and Central Square (10cm x 10cm) in the middle clearly marked. The number of squares visited by the

animals was calculated for 2 min, at 0, 30, 60, 90, 120 and 150 min subsequent to oral administration of the experimental crude extracts.

Forced swimming test, FST: According to Porsolt *et al.*, 1978 [8] swimming test was performed. Animals were randomly divided into 5 groups (n=6). The first group was given 10ml\kg of 1% Tween 80 orally and served as control. Groups 3, 4 and 5 received methanol, ethanol & chloroform extracts of root of *Withania somnifera* 100, 150 mg\kg of body weight respectively, while the group 2 was given 10 mg imipramine per kg body weight orally and served as standard. The forced swim test was carried out on mice individually forced to swim in an open acquire water tank apparatus (29cm x 19cm x 20cm), containing 9 cm of water at 25±1 °C; the total duration of immobility during the 6-min test was scored as described. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. The duration of immobility was recorded. Decrease in the duration of immobility during the FST was taken as a measure of antidepressant activity.

Statistical analysis: Data was expressed as Mean ± SEM (Standard error of Mean).

RESULTS AND DISCUSSION

Analgesic activity test:

Acetic acid induced writhing method: The result of Acetic acid induced writhing method with root extracts of *Withania somnifera* is shown in **Table 1.** Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids [9]. The constriction response of abdomen produced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics. It has been associated with prostanoids in general, for example, increased levels of PGE_2 and PGF_{2a} in peritoneal fluids [10, 11] as well as lipoxygenase or cyclo-oxygenases products [12, 13] and acid sensing ion channels [14].

Table 1 represent the effect of different extracts of *Withania somnifera* in Acetic acid induced writhing test. Methanol, ethanol and chloroform extract inhibited writhes in a dose dependent manner. But ethanol extract at 150 mg/kg showed highest inhibition (70.56%) which is even higher than the standard drug (25.55%).

Tail immersion test: Tail immersion method, the heat itself acts as a source of pain. The different concentrations of methanol, ethanol and chloroform extract of plant (100 and 150 mg/kg) and diclofenac Na (50 mg/kg) were administered to mice and observed the basal reaction time in different time intervals. The basal reaction time increased with increasing the concentrations along with increasing the time. The basal reaction time was more for standard drug when compared to plant extracts. In case of plant extracts the ethanol extracts shows more reaction time and that is recovered after 120 min the order of potency in 60 min after dose administration is diclofenac Na > ethanol > chloroform > methanol > normal saline **(Table 2).**

Neuropharmacological study: *Open field test:* This experiment was performed to assay general locomotor activity levels. After investigation with root extracts of *Withania somnifera* following data was observed **(Table 3, 4, 5 & 6).** According to Hall, 1936 [15] originally proposed that measuring aspects of rat behavior in a contained arena would indicate the emotional reactivity of the subjects. Many reports have validated open field tests as useful measures of emotional reactivity [16, 17] e.g., reviewed by Sandnabba, 1996 [18] for Turku aggressive mice; others have not found differences in open-field activity despite differences in other anxiety measures e.g., MHC-congenic [19]. Nevertheless, the open-field test remains a standard behavioral assay reported in the literature [20]. The standard Open field test is commonly used to assess locomotor, exploratory and anxiety like behavior in laboratory animals (rats/mice) [21]. The open field test is designed to examine responses of mice or rats to a new and unfamiliar environment (novel environment).Rodents demonstrate anxiety, fear and curiosity when placed in a new environment [22]. In response to the novel environment the rodents tend to explore the surrounding. The exploration capacity might be considered to be an index of anxiety although it is difficult to separate it from motor activity [22]. However, rodents are also fear to go to the open and illuminated space which is also a sign of anxiety. So the novel environment induces anxiety and fear in rodents which is clearly demonstrated by their rearing, grooming, defecation, locomotor and so on. These parameters are well utilized to assess anxiety and fear in rodents. Inhibition of such behaviors is indicative of centrally acting depressant or sedatives [23]. **Table 3, 4, 5 & 6** represents the effect of different extracts of *Withania somnifera* on various parameter of Open field test. Chloroform extract decreased movement of rodents in a dose dependent manner but could not reach significance; whereas

diazepam decreased movement significantly. Diazepam also decreased standing significantly. But extracts failed to exert any effect on standing and entrance in the center of the open field. The effect of higher dose of methanol 150 mg and dose of chloroform 150 mg on defecation was like diazepam. The results show that the methanol and chloroform extract has not the ability to relieve stress and had an anxiolytic effect on the rodents like diazepam did. On the other hand, lower dose of methanol and both dose of chloroform decreased defecation which cannot support the previous results.

Forced swimming test: Forced Swimming test was performed to evaluate the effect of antidepressant effect of root extracts of *Withania somnifera* on mice. After investigation root extracts of *Withania somnifera* following data was observed **(Table 7).**
Literature revealed that the FST was designed by Porsolt *et al*., 1978 [8] as a primary screening test or antidepressants. It is still one of the best models for this procedure. This is a low-cost, fast and reliable model to test potential antidepressant treatments with a strong predictive validity. However, the low face and construct validities should not forbid the use of this model for neurophysiological studies. It has a great sensitivity with all the antidepressant classes and all the mechanisms of action of treatments could be determined, but clinical correlations should be considered very carefully. When rodents are forced to swim in a confined place, they tend to become immobile after vigorous activity (struggling). This stressful inescapable situation can be evaluated by assessing different behavioral strategies and immobility during the test could be an efficient adaptive response to the stress [24]. The development of immobility when the rodents are placed in an inescapable container of water reflects the cessation of persistent escape directed behavior. The CNS depressant effect of the extracts may be attributed to chemical constitute other than flavonoids and alkaloids because flavonoids are responsible for the decrease in immobile phase in the swim test [24] and so does alkaloid as well [25].

CONCLUSION

After pharmacological studies with root extracts of *Withania somnifera* for analgesic activity and neuropharmacological investigation, plant root has significant analgesic activity and neuropharmacological action.

Table 1: Effect of different extracts of *Withania somnifera* in acetic acid induced writhing test

Samples	Doses (mg/kg)	No. of Writhing	% of Inhibition
Control	10 ml/kg of 1% Tween 80	2.17 ± 0.21	-
Std (Diclofenac)	100	2.31 ± 0.19	25.55
Methanol Extract	100	2.63 ± 1.88	14.33
	150	2.56 ± 0.27	16.61
Ethanol Extract	100	9.56±0.67	53.87
	150	6.10±0.28	70.56
Chloroform Extract	100	2.17 ± 0.90	29.32
	150	12.82 ± 0.49	38.13

Number of writhing values are mean ± S.E.M., (n=6)

Table 2: Effects of various extracts of *Withania somnifera* on latency time in tail immersion test

Groups	Doses (mg/kg)	Latency time (s)				
		-30 min	+30 min	+60 min	+90 min	+120 min
Control	-	3.094±1.40	2.618±1.07	4.644±1.14	4.14±1.24	4.398±1.75
Standard	50	2.952±0.93	2.516±1.69	2.964±1.35	3.438±1.37	3.00±1.39
Methanol Extract	100	2.56±0.93	2.460±1.47	3.224±0.57	4.456±1.44	3.342±1.48
	150	3.806±2.30	4.258±1.08	3.708±0.69	4.214±1.09	3.43±1.08
Ethanol Extract	100	3.806±1.23	3.494±1.11	2.892±0.54	4.09±1.01	2.738±1.11
	150	1.442±0.32	3.622±1.11	6.962±0.94	4.86±1.54	4.324±1.22
Chloroform Extract	100	4.596±1.47	4.31±1.64	4.022±0.92	4.694±0.61	4.326±1.31
	150	4.048±1.1	3.674±0.54	4.304±0.93	4.806±1.42	4.396±1.05

Number of writhing values are mean ± S.E.M., (n=6)

Table 3: Effect of different extracts of *Withania somnifera* in Open Field test (Movement)

Samples	Doses (mg/kg)	-30 mins	+30 mins	+60mins	+90 mins	+120 mins	+150 mins
Control		16.33±11.57	25.17±5.05	31.17±10.	34.17±19.7	31.5±15.46	18.3±11.63
Diazepam	2	4.33±3.09	24.5±5.06	27±5.97	22.83±6.04	24.17±3.29	16.33±7.50
Methanol Extract	100	5.33±2.43	18.33±7.43	23.36±7.3	21.16±8.66	25±3.36	28.33±6.82
	150	6.16±1.21	24.83±9.54	30.33±8.7	34±6.73	32.33±11.45	33.16±10.4
Ethanol Extract	100	0.66±0.74	4±1.15	3.16±1.86	3.16±1.86	4.16±1.34	3.83±1.46
	150	0.16±0.37	4.33±1.69	3.16±1.34	3.5±2.06	3.16±1.07	3±1.30
Chloroform Extract	100	2.66±0.74	20.83±9.28	16.83±8.7	16±4.47	10.5±6.42	12.83±4.25
	150	3.16±1.21	20.66±11.16	13.16±6.9	12.5±13.42	7.5±8.38	11.66±15.1

Values are mean ±S.E.M. (n=6)

Table 4: Effect of different extracts of *Withania somnifera* in Open Field test (Standing)

Samples	Doses (mg/kg)	-30 mins	+30 mins	+60 mins	+90 mins	+120 mins	+150 mins
Control		0.17±0.37	2.83±2.19	3.5±1.98	3.83±2.19	2.83±2.27	2.17±1.57
Std(Diazepam)	2	0.17±0.37	6.83±1.46	5±1	5.17±1.34	4.16±0.69	4.67±2.05
Methanol Extract	100	0.83±0.68	6.33±2.05	5.83±1.21	4±0.57	5.16±1.95	4.66±2.21
	150	0.16±0.37	3.5±0.95	4.33±1.37	4.5±1.25	4±0.81	4.5±1.89
Ethanol Extract	100	0.66±0.74	4±1.15	3.16±1.86	3.16±1.86	4.16±1.34	3.83±1.46
	150	0.16±0.37	4.33±1.69	3.16±1.34	3.5±2.14	3.16±1.06	3±1.29
Chloroform Extract	100	0.5±0.5	3±0.81	2.5±1.25	2.33±1.10	2.16±1.34	2.16±1.34
	150	0.16±0.37	3±1.63	2±1.39	2.5±2.14	1.66±1.06	1.83±1.21

Values are mean + S.E.M., (n=6)

Table 5: Effect of different extracts of *Withania somnifera* in Open Field test (Centre)

Samples	Doses (mg/kg)	-30 mins	+30 mins	+60 mins	+90 mins	+120 mins	+150 mins
Control		1±0	0.17±0.37	0.5±1.6	0.33±0.99	0.5±1.6	0.5±1.05
Std(Diazepam)	2	1±0	0.17±0.37	0.33±0.47	0.83±0.37	0.17±0.37	0.5±0.5
Methanol Extract	100	0.66±0.74	0.16±0.37	0.33±0.47	0.16±0.37	0±0	0±0
	150	0.16±0.37	0.16±0.37	0.16±0.37	0.5±0.76	0.83±0.89	0±0
Ethanol Extract	100	0.66±0.74	0.33±0.74	0.33±0.47	0.16±0.37	0.33±0.74	0.33±0.74
	150	1.16±0.68	1.33±0.47	0.16±0.37	0±0	0±0	0.33±0.47
Chloroform Extract	100	1.33±1.37	0.5±0.76	0.16±0.37	0±0	0.16±0.37	0.33±0.74
	150	0.66±0.47	0.16±0.37	0±0	0.16±0.37	0±0	0.33±0.74

Values are mean ± S.E.M., (n=6)

Table 6: Effect of different extracts of *Withania somnifera* in Open Field test (Stool)

Samples	Doses	-30 mins	+30 mins	+60	+90 mins	+120	+150
Control		0.83±0.68	0±0	0±0	0±0	0±0	1.67±1.21
Std(Diazepam)	2	0.83±0.68	0±0	0±0	0±0	0.17±0.37	0.17±0.37
Methanol Extract	100	1.33±1.11	0.17±0.37	0.33±0.47	0±0	0.5±0.76	0.17±0.37
	150	0.5±0.76	0.33±0.47	0±0	0±0	0.33±0.47	0.33±0.47
Ethanol Extract	100	0.66±0.47	0.33±0.47	0±0	0±0	0.33±0.47	0.33±0.47
	150	1±1	0±0	0±0	0±0	0.17±0.37	0.17±0.37
Chloroform Extract	100	1.12±0.98	0.17±0.37	0.33±0.47	0±0	0.17±0.37	0.17±0.37
	150	1±1.14	0.17±0.37	0.17±0.37	0.33±0.47	0.67±1.49	0.17±0.37

Values are mean ± S.E.M., (n=6)

Table 7: Effect of different extracts of *Withania somnifera* in swimming test

Samples	Doses (mg/kg)	Duration of Immobility (s)
Control	—	34.14 ± 3.15
Std (Imipramine)	10	38.25 ± 0.74
Methanol Extract	100	33.33 ± 6.86
	150	39.69 ± 6.12
Ethanol Extract	100	39.47 ± 6.02
	150	39.39 ± 1.97
Chloroform Extract	100	36.72 ± 6.65
	150	37.36 ± 5.46

Values are mean ±S.E.M. (n=6)

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