Indian J.Sci.Res.3(2) : 1-7, 2012

INVESTIGATION OF ANALGESIC AND NEUROPHARMACOLOGICAL ACTIVITIES OF *Asparagus racemosus* ROOT EXTRACTS

ROBIN KUMAR AICH[a], MOHIUDDIN AHMED BHUIYAN[b] AND MOHAMMAD SHAHRIAR[c1]

[a]Department of Pharmacy, University of Asia Pacific, Dhanmondi, Dhaka, Bangladesh
E-mail: robikha@gmail.com
[b]Department of Pharmacy, University of Asia Pacific, Dhanmondi, Dhaka, Bangladesh
E-mail: headpharm@uap-bd.edu
[c]Department of Pharmacy, University of Asia Pacific, Dhanmondi, Dhaka, Bangladesh
E-mail: shahriar_12@yahoo.com

ABSTRACT

The present study was undertaken to evaluate the analgesic activity and neropharmacological activity of different root extracts of *Asparagus racemosus.* In acetic acid-induced writhing method the methanolic extract showed significant analgesic activity at the doses of 150 mg/kg ($p<0.05$) as compared to control group, when analyzed statistically by independent sample t-test. The neropharmacological activity was performed by open field test and forced swimming test. In open field test four parameters were utilized to assess anxiety and fear in rodents. But methanol and chloroform extracts could not show any significant effect on movement, standing and center. Rather methanol extracts increased locomotor activity in a dose dependent manner. Interestingly higher dose of methanol and both dose of chloroform showed significant effect on defecation ($p<0.05$) as compared to control group, when analyzed statistically by independent sample t-test. In forced swimming test the methanol and chloroform extract showed significant anti-depressant activity consecutively at the dose of 150 mg/kg and 100 mg/kg ($P<0.05$) as compared to control group, when analyzed statistically by independent sample t-test.

KEYWORDS: Analgesic activity, neropharmacological activity, *Asparagus racemosus*

Asparagus racemosus is commonly known as Shatamuli, which is widely used in traditional medicine in Bangladesh due to possessing high medicinal value. It is an all-round tonic and rejuvenative which can be given to a person with any type, constitution, males or females, youngsters or elders. Indeed, Shatavari is the Universal Rasayana. The name Shatavari is symbolic which means one who possesses one hundred husbands .The plant is widely used in diarrhoea and dysentery. It also possesses anthelmintic and antiseptic properties (Sinha and Biswas,2011). The root extract of *A.racemosus* has been used in ulcer, diabetes and immunomodulation. Several nervous disorders, dyspepsia, tumors, inflammation, neuropathy, hepatopathy, cough, bronchitis, hyperacidity and certain infectious diseases can also be treated by this medicinal plant. The modulatory activity of *A. racemosus* has been observed on plasma glucose, insulin, insulin resistance index and metabolic liver enzymes in young and aged rats (Velavan and Begum, 2007).In addition to this, methanol root extract of *Asparagus racemosus* shows cerebroprotective activity due to reduction of oxidative stress (Nandagopal et al., 2011). The root extract of the plant has significant effect in increasing milk secretion during lactation. It also shows the antihepatotoxic and antineoplastic activities (Chawla et al., 2011).

As The plant have phytochemical constituents such as alkaloids, triterpenes and saponins, Ethanol and aqueous extract of *A. racemosus* root has to be tested for its analgesic activity and neuropharmacological activity. Therefore, the present study was undertaken with the objective to investigate the analgesic and neuropharmacological activities of the root extracts of *A. racemosus* in a scientific manner using Swiss albino mice.

MATERIALS AND METHODS

Plant Collection and Identification

The root of *Asparagus racemosus* was collected during November, 2011, from southern part of Bangladesh .The plant was identified and authenticated by taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka. (DABC; Accession number-35902).

[1]**Corresponding author**

Preparation of Plant Material

The roots were sun dried for two days. Then dried by hot air oven at 45°C temperature for five days. It is helpful for better grinding. The dried roots then grinded in coarse powder using high capacity grinding machine which was then stored in air tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

Extraction

The powdered plant material (30 gm) was successively extracted in a soxhlet extractor at elevated temperature using 300 ml of methanol (40-60)°C which was followed by petroleum ether and chloroform. All extracts were filtered individually through filter paper and poured on petridishes to evaporate the liquid solvents from the extract to get dry extracts. After drying, crude extracts were stored in stock vials and kept in refrigerator for further use. Yield of extraction of roots of *A. racemosus* in different solvents were 40.0% with methanol, 0.56% with chloroform and 0.25% with petroleum ether.

Experimental Animals

Swiss albino mice weighing 15-25 g of either sex were used for the research. The mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B) Mohakhali, Dhaka. The animals were maintained under standard hygienic conditions (temperature 27 ± 1.0 C, relative humidity 55-65% and 12 h light/ 12 h dark cycle) and had free access to feed and water adlibitum. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

Analgesic Study

The acetic acid writhing test in mice as described by Koster *et al.*, 1959 was employed with slight modification. Mice were divided into 8 groups of 4 mice each. The first group was given 10ml\kg of 1% Tween 80 intraperitoneally and served as control. Groups 3, 4 received methanol extract of roots of *A. racemosus* (ARM) 100, ARM 150 mg\kg of body weight, groups 5, 6 received chloroform extract of roots of *A. racemosus* (ARC) 100, ARC 150mg\kg of body weight, groups 7, 8 received petroleum ether extract of roots of *A. racemosus* (ARP) 100, ARP 150mg/kg of body weight, while the group 2 was given 100 mg Diclofenac per kg body weight intraperitoneally and served as standard. Thirty minutes later 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 intraperitoneally administration of Acetic acid and the mean abdominal writhing for each group was obtained.

The percentage inhibition of writhing was calculated using the following formula:

$$\% \text{ Inhibition} = \left(1 - \frac{\text{No. of writhing (Drug/Standard)}}{\text{No. of writhing (Control)}}\right) \times 100$$

Neuropharmacological Study

Open Field Test

According to Gupta et al., 1971 we used open field 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 6 groups of 4 mice each. The first group was given 10ml\kg of 1% Tween 80 orally and served as control. Groups 3, 4 received ARM 100, ARM 150 mg\kg of body weight, and groups 5, 6 received ARC 100, ARC 150 mg\kg of body weight, 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 with slight modification. The open field apparatus is made of hardboard (70cmx70cm; 30cm walls). Blue lines drawn on the floor divide the floor into twenty five 14cm x 14cm squares alternatively colored black and white and Central Square (14cm x 14cm) 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.

Swimming Test

According to Porsolt et al., 1977 swimming test were performed. Animals were randomly divided into six groups (n=4). Group I (control) received 1% Tween 80,10ml/kg orally. Group II received reference drug 10 mg/kg Imipramine. Groups 3, 4 received ARM 100, ARM 150 mg\kg of body weight, and groups 5, 6 received ARC 100, ARC 150 mg\kg of body weight. The forced swim test was carried out on mice individually forced to swim in an open acquire water tank apparatus (29cm x 18cm x 18cm), 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.

RESULTS AND DISCUSSION

Acetic Acid Induced Writhing Method

Methanol, chloroform and petroleum ether extracts inhibited writhes in a dose dependent manner. But methanol extract at 150 mg/kg showed highest inhibition (70.83%) ($p< 0.05$) which is even higher than the standard drug (54.16%) ($p< 0.05$) in table,1. Literature revealed that the 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 (Ahmed et al., 2006). 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 PGE2 and PGF2a in peritoneal fluids (Ronaldo et al., 2000) as well as lipoxygenase or cyclooxygenases products (Dhara et al., 2000).

Table1: Effect of different extracts of *A. racemosus* in Acetic Acid Induced Writhing test

Group	Doses (mg/kg)	No of Writhing	Inhibition (%)
Control	-	6±1.79	-
Diclofenac	100	2.75±1.47*	54.16
ARM	100	4.75±2.70	20.83
	150	1.75±0.94*	70.83
ARC	100	5.75±1.23	4.16
	150	3.75±2.81	37.5
ARP	100	5.75±1.23	4.16
	150	4.5±2.33	25

(N.B, ARM=Methanol extract of roots of A. racemosus, ARC= Chloroform extract of roots of *A.racemosus,* ARP= Petroleum ether extract of roots of *A. racemosus)* Number of writhing values are mean ± S.E.M., (n=4). *$p< 0.05$, significantly different from control; done by independent t-test.

Open Field Test

Methanol extracts increased locomotor activity in a dose dependent manner. Chloroform extract decreased movement of rodents in a dose dependent manner but could not reach significance, whereas diazepam decreased movement significantly ($P<0.01$) in table,2.

Table 2: Effect of different extracts of *A. racemosus* in Open Field test (Movement)

		Movement					
Group	**Doses (mg/kg)**	**-30min**	**+30min**	**+60min**	**+90min**	**+120min**	**+150min**
Control	-	52.75±5.62	31.5±11.58	25.75±4.41	27.5±5.80	23.75±9.41	18.5±3.05
Diazepam	2	54.75±22.78	19.75±11.13	11.5±9.07	7.75±5.33**	12.25±11.55	10.5±5.46
ARM	100	58.5±4.56	21.25±5.57	35.75±8.85	25.25±9.03	18±7.96	12.75±7.48
	150	55.75±19.87	37.25±21.70	42.75±39.31	44.75±31.19	25.25±19.46	18.25±17.57
ARC	100	49.25±19.24	29.5±11.04	22±10.94	17.75±13.54	17±7.55	14.5±10.04
	150	55.5±22.44	36.25±22.59	27.25±9.65	20.5±8.33	11.5±3.62	8.25±3.03

Values are mean ±S.E.M. (n=4). **P<0.01, significantly different from control; done by independent sample t-test.

Diazepam and extracts failed to exert any effect on center entrance in the open field in table,3.

Table 3: Effect of different extracts of *A. racemosus* in Open Field test (Centre)

		Centre					
Group	**Doses (mg/kg)**	**-30min**	**+30min**	**+60min**	**+90min**	**+120min**	**+150min**
Control		0.5±0.57	0.25±0.49	0.25±0.49	0±0	0.25±0.49	0.5±0.98
Diazepam	2	0.5±0.57	0±0	0±0	0±0	0±0	0.25±0.49
ARM	100	0.75±0.49	0.5±0.57	0.25±0.49	0.25±0.49	0.25±0.49	0±0
	150	1±1.39	0.5±0.57	1.25±1.47	1.25±0.94	0.5±0.98	0.25±0.49
ARC	100	1.75±2.81	0.5±0.57	0.75±1.47	0.5±0.57	0.25±0.49	0±0
	150	0.5±0.57	0±0	0.25±0.49	0±0	0±0	0±0

Values are mean + S.E.M. (n=4)

Table 4: Effect of different extracts of *A. racemosus* in Open Field test (Standing)

Standing							
Group	**Doses (mg/kg)**	**-30min**	**+30min**	**+60min**	**+90min**	**+120min**	**+150min**
Control		9.25±3.03	5.75±4.76	5±3.75	5.25±2.17	4.75±2.17	2.75±1.67
Diazepam	2	11.25±2.93	2.5±0.57	2.25±2.58*	2.75±2.45	0.75±0.49	1.5±1.70
ARM	100	9.5±8.06	11±2.77	11±2.53	9.5±5.80	3.25±2.17	1.25±0.94
	150	12.75±3.52	3.5±1.27	5.25±2.70	4.5±3.35	3±3.49	2±2.65
ARC	100	6.5±4.83	2.75±1.67	3.25±0.94	2.25±0.94	2.25±1.47	1.25±0.94
	150	6.25±5.62	3.5±3.96	3.75±2.81	2.5±1.27	1.5±2.33	1.75±2.81

Values are mean ± S.E.M., (n=4). *p<0.05, significantly different from control; done by independent sample t-test

Diazepam decreased standing significantly (p<0.05).But extracts failed to exert any effect on standing in the open field in table 4.

Table 5: Effect of different extracts of *A. racemosus* in Open Field test (Stool)

Stool							
Group	**Doses (mg/kg)**	**-30min**	**+30min**	**+60min**	**+90min**	**+120min**	**+150min**
Control		2.5±0.98	1±0.80	0.75±0.49	0.75±0.94	1±0.40	0.75±0.28
Diazepam	2	1.75±0.49	0.75±0.94	0.5±0.57	00±00	00±00*	00±00*
ARM	100	2.75±1.23	1.25±0.94	1±0.80	1.12±1.29	0.25±0.49	0.5±0.57
ARM	150	2.35±1.76	1.5±0.57	1±0.80	0.25±0.49	00±00*	00±00*
ARC	100	2±0.80	0.25±0.49	0.5±0.57	0.65±1.22	00±00*	00±00*
ARC	150	2.75±0.49	1.25±1.23	1.75±0.94	1.2±1.23	0.15±0.24*	0.5±0.98

Values are mean ± S.E.M.,(n=4). *p<0.05, significantly different from control; done by independent sample t-test

The effect of higher dose of methanol (ARM 150) and dose of chloroform (ARC 100, ARC 150) on defecation was like diazepam. Among all parameter only defecation showed diazepam like effect in table,5. So it is understood that the methanol and chloroform extract has not the ability to relieve stress and had an anxiolytic effect on the rodents like diazepam did. But interesting effect of methanol and chloroform i.e. decreased defecation which was due to the

physiological and environmental factor, or it might be chemical constitute present other than flavonoids and alkaloids in root extract of *A. racemosus* (Butterweck et al., 2000). Literature revealed that the Hall, 1934, 1936 originally proposed that measuring aspects of rat behavior in a contained arena would indicate the emotional reactivity of the subjects. Others have not found differences in open-field activity despite differences in other anxiety measures (Brown et al., 1999). 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 (Datusalia and Kalra, 2008). 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 (Datusalia and Kalra, 2008). 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 (Brown et al., 1999).

Swimming Test

Table 6 : Effect of different extracts of *A. racemosus* in Swimming test

Group	Doses (mg/kg)	Duration of Immobility(s)
Control		110±27.96
Impiramine	10	49.5±24.85*
ARM	100	80±40.05
	150	63.25±22.41*
ARC	100	47.25±23.09*
	150	68±12.73

Values are mean ±S.E.M. (n=4). *P<0.05, significantly different from control; done by independent sample t-test.

The higher dose of methanol extract (ARM 150) and lower dose of chloroform (ARC 100) (P<0.05) decreased Immobile phase like Imipramine (10 mg/kg) (P<0.05). It indicates the Anti-depressant like effect of these extracts in table,6.

Literature revealed that the FST was designed by Porsolt 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 (Porsolt et al., 1978). 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 (Butterweck et al., 2000) and so does alkaloid as well (Silva et al., 2005).

CONCLUSION

Different crude extracts of *Asparagus racemosus* root were subjected to analgesic and neuropharmacological investigations to validate the traditional use. In the present study methanol extract possess remarkable analgesic activity. The significant analgesic activity may be due to the presence of flavonoids. Flavonoids are known to target prostaglandins, which are involved in the late phase of acute inflammation and pain perception. One the other hand methanol and chloroform extracts showed antidepressant activity. But it has no sedative and hypnotic effect on various rodent behavioral models. Methanol extracts increased locomotor activity and chloroform extract decreased movement of rodents but could not reach significance. Hence, *A. racemosus* shall be considered as safe and effective medicinal plant. *A. racemosus* plants have been raw material for the synthesis of many drugs and they remain an important source of new therapeutic agents. Therefore, further chemical and pharmacological studies with extracts of *A. racemosus* for anti-inflammatory activity, antipyretic activity, diuretic effect in rats, hypoglycemic effect in rabbits, isolating new bioactive compounds, chronic toxicity profile might be the next steps to be followed to eventually find new led compounds.

REFERENCES

Ahmed F., Hossain M.H. and Rahman A.A., 2006. Antinociceptive and sedative effects of the bark of *Cerberaodollam Gaertn*. J. Oriental Pharmacy Exp. Med.,**6**: 344-348.

Brown R.E., Corey S.C. and Moore A.K., 1999. Differences in measures of exploration and fear in MHC-congenic C57BL/6J and B6-H-2K mice. Behav. Genet., **29**:263-271.

Butterweck V., Jurgenliemk G. and Nahrstedt A., 2000. Flavonoids from *Hyperium perforatum* show antidepressant activity in forced swim test. Plnta. Med.,**66**: 3-6.

Chawla A., Chawla P., Mangalesh P. and Roy R.C., 2011. *Asparagus racemosus* (Willd): Biological activities and its active principles. Indo-Global J. Pharm. Sci., **1**: 113-120.

Datusalia A.K. and Kalra P., 2008. Anxiolytic and antiseizure of Sidatiagiibhandri. J. Helth. Sci.,**54**(5):544-550.

Dhara A.K., Suba V., Sen T., Pal S. and Nag Chaudhuri A.K., 2000. Preliminary studies on the anti-inflammatory and analgesic activity of the methanolic fraction of the root extract of Tragia involucrate. J. Ethanopharmacology.,**72**: 265-268.

Gupta B.D., Dandiya P.C. and Gupta M.L., 1971. Psycho-pharmacological analysis of behaviour in rats . JpnJ Pharmacol., **21**: 293.

Koster R., Anderson M. and DeBeer E., 1959. Acetic acid for analgesic screening. Federation Proceedings,**18**: 412418.

Nandagopal M., Muralidharan P. and Thirumurugan G., 2011. Cerebroprotective effect of root extract of *Asparagus racemosus* Willd in global cerebral ischemia in rats. J. Pharmacol. Toxicol,**6**: 49-61.

Porsolt R.D., Bertin A. and Jalfre M., 1978. Behavioural despair in rats and mice, strain differences and the effects of imipramine. Eur. J. Pharmacol.,**51**:291-294.

Porsolt R.D., Bertin A. and Jalfre M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther., **229**:327-336.

Ronald A.R., Mariana L.V., Sara M.T., Adriana B.P.P. and Steve P., 2000. Involvement of resident macrophages and mast cell in the writhing noceptive response induced by zymosan ad acetic acid in mice .Eur. J. Pharmacol., **387**:111-118.

Silva G.N., Martins F.R. and Matheus M.E., 2005. Investigation of anti-inflammatory and antinociceptive activities of *Lantana trifolia*. J. Ethnopharmacol.,**100**: 254-259.

Sinha S. and Biswas M., 2011. Effect of extracts from *Asparagus racemosus* Willd root against pathogenic bacteria. Int. J. Applied Biol. Pharm. Technol.,**2**: 312-314.

Velavan S. and Begum V.M.H., 2007. Modulatory role of *Asparagus racemosus* on glucose homeostasis in aged rats. Int. J. Pharmacol., **3**: 149-154.