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EVALUATION OF ANTI-ANXIETY ACTIVITY OF *ASPARAGUS RACEMOSUS* (SATAVARI) IN RATS BY USING LIGHT AND DARK MODEL

Rajat Yadav[*1], Kamal Kishore Maheshwari[2], Amit Gupta[3] and Ishan Bansal[1]

[1]Shri Ram Murti Smarak College of Engineering & Technology (Pharmacy), Bareilly- 243202 (Uttar Pradesh), India
[2]Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly- 243006 (Uttar Pradesh), India
[3]BIU College of Pharmacy, Bareilly International University, Bareilly- 243006 (Uttar Pradesh), India

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ABSTRACT

Present research work was performed to evaluate the anxiolytic activity of methanol extract of *Asparagus racemosus* (Satawari) in rat by using light and dark model. Anti-anxiety potentials of extracts of *Asparagus racemosus* were compared with diazepam. Rats of either sex were divided into seven groups of 6 animals in each group. Group were distilled water, vehicle medium as 10% carboxy methyl cellulose (C.M.C), diazepam 2mg/kg, extract of *Asparagus racemosus* (50mg/kg), extract of *Asparagus racemosus* (100mg/kg), extract of *Asparagus racemosus* (200mg/kg) and diazepam + effective dose of *Asparagus racemosus (*200 mg/kg). All the test solutions were freshly prepared daily and administered to animals for 7 days by oral route. After 7th day, each animal was checked to anxiety by using light and dark model. The extracts of *Asparagus racemosus* 100 mg/kg, 200 mg/kg and diazepam 2 mg/kg + *Asparagus racemosus* 200 mg/kg treated group shows significant ($p<0.05$) different reduced spend time in dark chamber of light and dark model as comparison with control group. Extracts of *Asparagus racemosus* 50mg/kg did not show significant ($p<0.05$) different reduced spend time in dark chamber of light and dark model as comparison with control group. Extract of *Asparagus racemosus* 200mg/kg with diazepam 2mg/kg gives synergetic anxiolytic activity in rats.

KEYWORDS: *Asparagus racemosus*, Satavari, Anxiety, Diazepam, Light and Dark Model and Anxiolytic activity

Corresponding Author

Mr. Rajat Yadav,
Assistant Professor, Shri Ram Murti Smarak College of Engineering & Technology (Pharmacy), Bareilly- 243202 (Uttar Pradesh), India

E-mail: yadavm.pharm@gmail.com

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INTRODUCTION

Anxiety can be defined as a psychological state characterized by emotional, cognitive, somatic and behavioral components. These components combine to create an unpleasant feeling condition that is typically associated with uneasiness, apprehension, fear or worry. It is generalized mood condition that can often occur without an identifiable triggering stimulus [1]. Anxiety states are controlled by both inhibitory and facilitatory mechanism that either counter or favor anxiety states. These neurochemical and neuropeptide systems have been shown to have effects on distinct cortical and sub cortical brain areas that are relevant to the mediation of the symptoms associated with anxiety disorders.

The ultimate objective is to develop substances that are as effective as benzodiazepines, which have been the traditional treatment for anxiety for over 40 years. The various search has lead to development of unconventional agents, which are partial benzodiazepine GABA receptor antagonist or target specific subunits of the $GABA_A$ receptor or manipulate GABA levels, agents that effect the serotonin and nor epinephrine systems, corticotrophin-releasing factor and substance P [2].

Mice and humans share more than 90% of their genes, and animal models seems to be a useful tool in biomedical sciences, as evidenced by a notable increase in the number of active laboratories working in the field [3]. The development of animal models of anxiety and stress has helped to identify the pharmacological mechanisms and potential clinical effects of several drugs. Animal models of anxiety are based on conflict situations that can generate opposite motivational states induced by approach avoidance situations.

There are various animal models which are available for evaluating anti-anxiety agents for rodents like Elevated plus maze, Elevated zero maze, Elevated T maze, Light dark model, Open field test, Stair case test, Vogel test, Hole board test, Active/passive avoidance, Defensive burying test, Four plate test, Geller conflict test, Ultrasonic vocalization test (adult), Human threats, etc. are used frequently for testing of anti-anxiety activity. These tests are categorized into two categories conditioned responses and unconditioned responses. Light and dark model comes under the category of unconditioned responses. It is simple less time-consuming test for evaluation of anti-anxiety agents [4].

Some part of anxiety is normal in our day to day life but treatment is needed when it becomes severe, usually the drugs used for treatment of anxiety are benzodiazepines class of drugs i.e. diazepam but these class of drugs are also having large number of side effects so a new class of drug should be developed which have minimum side effects or no side effects. So, this class of drugs can only be developed from herbal categories, there are vast range of plants are available which proves to have beneficial effect for the treatment of anxiety. *Asparagus racemosus* is one of the common species which is easily found in India.

In Indian system of medicine *Asparagus racemosus* is an important medicinal plant and its root paste or root juice has been used in various ailments and as health tonic. *Asparagus racemosus* is also reported to have many beneficial effects for the treatment of various diseases like learning and memory, antioxidant, anti-epileptic, adaptogenic activity, anti-tussive activity, glactogogue activity, hepatoprotective activity, etc. [5] So, *Asparagus racemosus* was evaluated for its anti-anxiety activity with the help of light and dark model, following parameters were evaluated like time spend in light chamber, time spend in dark chamber and number of entries between light and dark chamber. On behalf of this study it can observed that *Asparagus racemosus* having anti-anxiety activity or not.

MATERIALS AND METHODS

Plant Collection and Authentication

The roots of *Asparagus racemosus* was collected in the month of October from Karamcharinagar, Distt-Bareilly, Uttar Pradesh. The plant was taxonomically identified and authenticated by Dr. A. K. Jaitly, Head, Department of Plant Science, M. J. P. Rohilkhand University, Bareilly, 243001, Uttar Pradesh (Reference No. RU/PS/2016/415)

Preparation of Methanolic Extract of *Asparagus racemosus*

The shade dried roots of *Asparagus racemosus* were powdered with the help of grinder. Extraction was performed by packing the coarsely powdered (80mg) drug in Soxhlet assembly (with round bottom flask containing 95 % methanol as solvent and reflux condenser on top). Round bottom flask was kept on heating mantle at the temperature up to 60 °C. After that solvent was removed through evaporation and then a semi-solid extract was obtained.

Animals

All experiments were carried out using healthy albino rats of either sex (150-250gm) obtained from the animal house of the Department of Pharmacy, M. J. P. Rohilkhand University, Bareilly, Uttar Pradesh. The experimental protocol was approved by the Institutional Animal Ethical Committee and conducted according to the CPCSEA guideline on the use and Care of experimental animals (Approval No: CPCSEA//IAEC/MJPRU/16/011).

Animals were kept at an ambient temperature of 25 ± 2 °C and 55-65% relative humidity with a 12hour light and dark cycle. The animals had free access to standard pellet chow and tap water. Animals were housed in groups for at least 1 week before using them for experiment.

Drugs and Chemicals

Diazepam (Pushkar Pharma. Pvt., Ltd.) as standard drug, distilled water, methanol, Carboxy methyl cellulose (suspending agent) is

taken from the store Department of Pharmacy, M. J. P. Rohilkhand University.

Glassware's and Apparatus Used

Beaker, funnel, measuring cylinder, glass rod, Soxhlet apparatus, round bottom flask, condenser, heating mantle and weighing balance.

Light and dark Chamber Model

The light and dark test is based on innate aversion of rodent to brightly illuminated areas and on the spontaneous exploratory behavior of rodent in response to mild stressor, that is, novel environment and light [6]. A natural conflict situation occurs when an animal is exposed to an unfamiliar environment or novel objects. The conflict is between the tendency to explore and the initial tendency to avoid the unfamiliar (neophobia). The exploratory activity reflects the combined result of these tendencies in the novel situation.

Figure 1: Light and Dark Apparatus

Thus, in the light/dark test, two-compartment box, in which a large white compartment is illuminated, and a small black compartment is darkened, is suggested as index of anxiolytic activity. An increase in transitions without an increase in spontaneous locomotion is considered to reflect anxiolytic activity [7]. The light and dark box consists of two compartments: one light area (27L × 27W × 27H cm) in dimension was illuminate by 100 W desk lamps and the other dark area (18L × 27W × 27H cm) was painted black. The two compartments were separated by partition with tunnel (7.5 × 7.5 cm) to allow passage from one compartment to other [8].

Behavior Recoding Procedure

Rats will be placed individually in the illuminated part of the cage facing in opposite direction of the opening of dark chamber and following parameters will be recorded during the test session of 5 minutes, total no. of crossings, total time spent in the illuminated part of the cage and time spent in the dark part of the cage.

In light and dark apparatus, to evaluate anti-anxiety activity three parameters were observed during the test session of 5 min:

- ✓ Time spent in light chamber.
- ✓ Time spent in dark chamber.
- ✓ Total number of entries from dark to light chamber.

Experimental Protocol

Seven groups and each group comprising of six rats of either sex were employed in this present study.

- **Group I, n=6:** Control group, normal control treated with distilled water orally for seven days.
- **Group II, n=6:** Vehicle control, animals treated with carboxy methyl cellulose (C.M.C) orally for seven days.
- **Group III, n=6:** Standard drug (Diazepam), animals treated with standard drug diazepam 2mg/kg, intra peritoneal, before 30 min starting the experiment.
- **Group IV, n=6:** Extract I, animals treated with methanolic extract of *Asparagus racemosus* (50mg/kg), orally for seven days.
- **Group V, n=6:** Extract II, animals treated with methanolic extract of *Asparagus racemosus* (100mg/kg), orally for seven days.
- **Group VI, n=6:** Extract III, animals treated with methanolic extract of *Asparagus racemosus* (200mg/kg), orally for seven days.
- **Group VII, n=6:** Diazepam + effective dose of *Asparagus racemosus (*200 mg/kg), animals treated with diazepam (2mg/kg, intra peritoneal) + effective dose of *Asparagus racemosus extract (*200 mg/kg), orally for seven days.

Statistical Analysis

All results were expressed as mean ± SEM. Data was analyzed using one-way ANOVA followed by Dennett's test and Student t-test. $P<0.05$ was considered to be statistically significant.

RESULTS

Control group (Saline water) and vehicle control (10% CMC) give no significant difference in animal (rat) behavior during dark and light chamber anxiety test. Diazepam (2mg/kg) treated group was significantly increasing time spend in light chamber with increasing number of entry into light chamber and decreasing time spend in dark chamber as compared to control (distilled water) treated group, during dark and light chamber anxiety test. Extract I (*Asparagus racemosus* 50mg/kg) treated group was no significant difference in animal (rat) behavior during dark and light chamber anxiety test as compared Vehicle control (10% CMC) treated group. Extract II (*Asparagus racemosus* 100mg/kg) treated group was significantly increasing time spend in light chamber with increasing number of entry into light chamber and decreasing time spend in dark chamber as compared to vehicle control (10% CMC) treated group, during dark and light chamber anxiety test. Extract III (*Asparagus racemosus* 200mg/kg) treated group was significantly increasing time spend in light chamber with increasing number of entry into light chamber and decreasing time spend in dark chamber as compared to vehicle control (10% CMC) treated group, during dark and light chamber anxiety test. Extract III (*Asparagus racemosus* 200 mg/kg) +Diazepam (2mg/kg) treated group was significantly increasing time spend in light chamber with increasing number of entry into light chamber and decreasing time spend in dark chamber as compared to Extract III (*Asparagus racemosus* 200mg/kg)treated group, during dark and light chamber anxiety test **(Figure 2 Chart A, B and C)**.

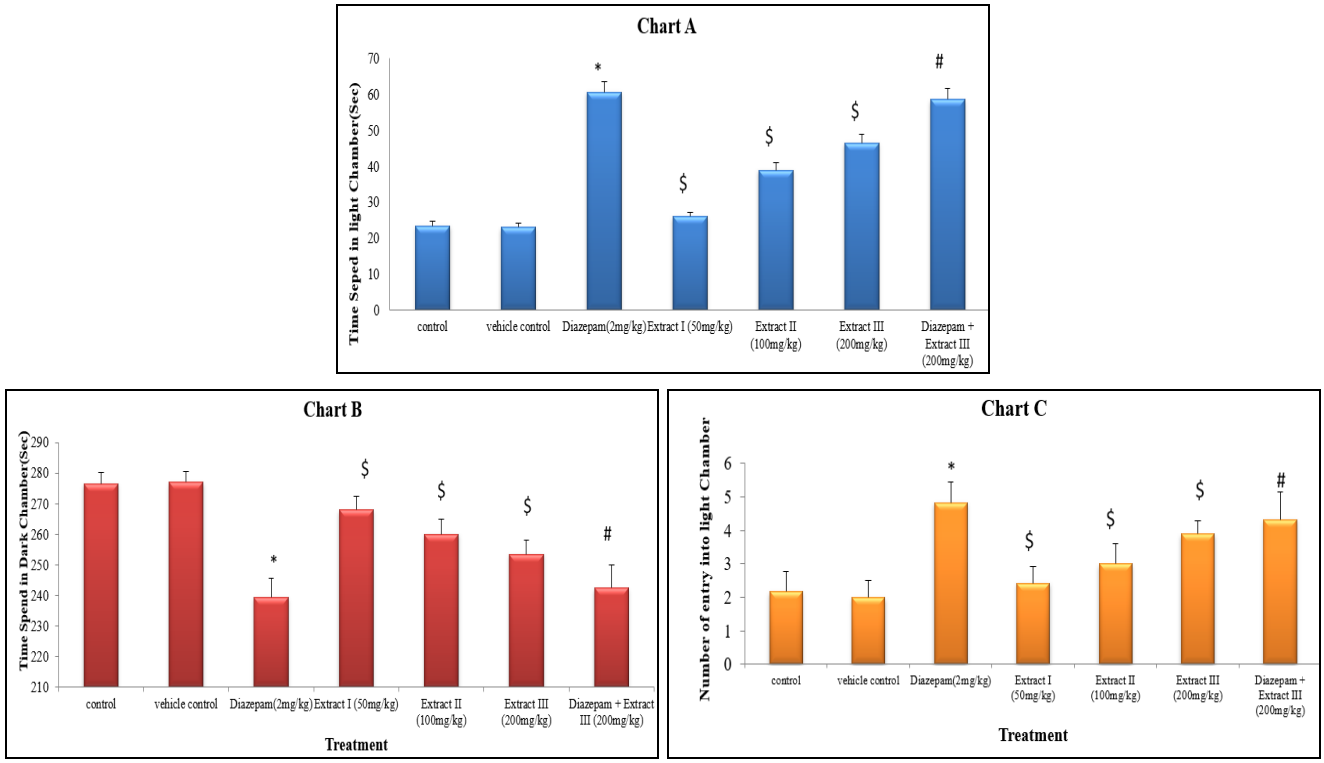

Figure 2: Effect of Different Treatment on- (Chart A) Time Spend in Light Chamber; (Chart B) Time Spend in Dark Chamber; (Chart C) Number of entries into light chamber. [Values are expressed as mean ± SEM. *$P \leq 0.05$ as compared to control group, $$P \leq 0.05$ as compared to vehicle control group, #$P \leq 0.05$ as compared to Extract III (200 mg/kg) group]

DISCUSSION

In the present study *Asparagus racemosus* is investigated for its anti-anxiety activity. Anxiety is feeling of uneasiness, discomfort, apprehension, or fearful concern accompanied by a host of autonomic and somatic manifestations. Anxiety is a normal, emotional, reasonable and expected response to real and potential danger. However, if the symptoms of anxiety are prolonged, irrational, disproportionate or severe, occur in absence of stressful events or stimuli or interfere with everyday activities, then, these are called anxiety disorders. Anxiety disorder is among the most common mental, emotional, behavioural problems of the world [9].

After some specific point in anxiety disease treatment is needed, so the frequently use class of drugs which are chose for treatment are Benzodiazepines like diazepam, lorazepam, alprazolam. As these drugs have beneficial effect, they also have large number of side effects. So, a new class of drug should be used for treatment of anxiety which have minimum or no side effects. This type of drugs can only be obtained from plants sources. In the present study *Asparagus racemosus* is investigated for its anti-anxiety activity. This plant is already having many activities like learning and memory [10], anti-parkinsonism activity [11], adaptogenic activity [12] etc. To evaluate anxiety various animal models are available which can be categorized into two categories conditioned responses and unconditioned responses mostly the models which are used comes under the conditioned responses categories as they are simple, less time consuming and quick.

So, we have selected light and dark apparatus to evaluate anxiety as this apparatus is simple, less time consuming and gives good results. The light and dark model is based on innate aversion of rodent to brightly illuminated areas and on the spontaneous exploratory behavior of rodent in response to mild stressor, that is, novel environment and light [13]. A natural conflict situation occurs when an animal is exposed to an unfamiliar environment or novel objects. The conflict is between the tendency to explore and the initial tendency to avoid the unfamiliar (neophobia). The exploratory activity reflects the combined result of these tendencies in the novel situation.

Thus, in the light/dark test, two-compartment box, in which a large white compartment is illuminated, and a small black compartment is darkened, is used to test anti-anxiety drugs. The evaluation method which is used to test anxiety is to note three parameters time spent in light area, time spent in dark area and number of entries. An increase in time spent in light box, number of entries and decrease in time spent in dark box is considered to reflect anxiolytic activity [14]. This apparatus is quick and simple no prior trials are required for this apparatus and this apparatus comes under the category of unconditioned responses. On the basis of evaluation methods following results are obtained from various groups of rats by using light and dark apparatus.

Control group and vehicle control group (Distilled water group and carboxy methyl cellulose group) animals give no significant difference between both the means when compared in graph pad prism software. Diazepam treated group consist of six animals which are treated with Diazepam (2mg/kg, i.p.) 30 min before starting the experiment. Diazepam is one of the most frequently prescribed drugs for anxiety treatment; it is a benzodiazepine class of drug. It is observed that time spent in light chamber significantly increases, number of entries also increases, and time spent in dark chamber decreases. Diazepam shows better result in light and dark apparatus in comparison to control, vehicle control (carboxy methyl cellulose), methanolic extract of *Asparagus racemosus* (100 mg/kg, 150 mg/kg and 200 mg/kg).

Thus, result shows that diazepam is having a potent anti-anxiety activity in rats. This contention is supported by earlier observation with diazepam for anti-anxiety test in light dark apparatus [15]. Extract I (methanolic extract of *Asparagus racemosus* 100 mg/kg) treated group consist of six animals which are treated with extract I orally for seven days. The result showed that there is no significant difference in time spent in light chamber, dark chamber but there is very little significant difference in number of entries, when they are compared with control group (distilled water).

So, extract I (methanolic extract of *Asparagus racemosus* 100 mg/kg) does not show antianxiety activity in rats. Extract II (methanolic extract of *Asparagus racemosus* 150 mg/kg) treated group consist of six animals which are treated with extract II orally for seven days. Here the time spent in light chamber increases, number of entries across the tunnel also increases and the time spent in dark chamber decreases in comparison to control group (distilled water). There is significant difference in time spent in light chamber, time spent in dark chamber and number of entries.

All the parameters highlight that methanolic extract of *Asparagus racemosus* dose 150 mg/kg is having a anti-anxiety activity. So, extract II shows anti-anxiety activity in rats [16]. Extract III (methanolic extract of *Asparagus racemosus* 200 mg/kg) treated group consist of six animals which are treated with extract III orally for seven days.

The time spent in light chamber and number of crossings across the tunnel is much higher in comparison to control (distilled water), methanolic extract of *Asparagus racemosus* dose 100 mg/kg and 150 mg/kg. The time spent in dark chamber decreases in comparison to the above-mentioned groups. There is significant different between control (distilled water) group and methanolic extract of *Asparagus racemosus* 200 mg/kg in time spent in light chamber, time spent in dark chamber and number of entries. So, the result showed that dose 200 mg/kg is having more anti-anxiety activity in comparison to dose 100 mg/kg and 150 mg/kg, but it is not as potent as diazepam (2 mg/kg).

On behalf of this study it can determine methanolic dose of *Asparagus racemosus* 200 mg/kg to have anti-anxiety activity in rats. This activity might be due to the enhanced level of serotonin and norepinephrine neurotransmitters. This study is supported by earlier studies of *Asparagus racemosus* on anxiety [17]. Diazepam + effective dose of *Asparagus racemosus* treated group consist of six animals, which are treated with methanolic extract of *Asparagus racemosus* 200 mg/kg and diazepam (2mg/kg).

The time spent in light chamber, number of entries increases and the time spent in dark chamber decreases in comparison to *Asparagus racemosus* 200 mg/kg group and there is significant difference between both the groups in time spent in light chamber, time spent in dark chamber and number of entries. The result showed that extract of *Asparagus racemosus* 200mg/kg with diazepam 2mg/kg gives synergetic anxiolytic activity in rats.

CONCLUSION

It may be concluded that, methanolic extract of *Asparagus racemosus* 150 mg/kg exhibit anti-anxiety activity but in comparison to other doses it is lesser and dose 200 mg/kg proves to be the effective dose then all other doses of methanolic extract of *Asparagus racemosus* but it is not as potent as standard drug diazepam (2 mg/kg) in light and dark apparatus. The methanolic extract of *Asparagus racemosus* 200mg/kg with diazepam give synergetic anxiolytic activity.

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CONFLICT OF INTEREST

None

REFERENCES

1. Pathak NL, Kasture SB, Bhatt NM and Patel RG. “Experimental modeling of anxiety”. Journal of Applied Pharmaceutical Science (JAPS), 2011; 1(3), pp. 6-10.
2. Kaur S and Singh R. “Role of different neurotransmitters in anxiety: a systemic review”. Int J Pharm Sci Res (IJPSR), 2017; 8(2): pp. 411-21.
3. Gilhotra N and Dhingra D. “Neurochemical modulation of anxiety disorders”. International Journal of Pharmacy & Pharmaceutical Sciences (IJPPS), 2010; 2(1), pp. 1-6.
4. Maheshwari KK. “Drug Screening techniques, pharmacological methods”. Vallabh Prakashan, Delhi, Edition 1st, 2015; pp. 57.
5. Chawla A, Chawla P and Roy RC. “*Asparagus racemosus* (wild) biological activities and its active principals”. Indo-Global Journal of Pharmaceutical Sciences (IJPS), 2011; 1(2), pp. 113-120.
6. Crawley J and Goodwin KK. “Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines”. Pharmacol. Biochem. Behav (PBB), 1980; 13, pp. 167–170.
7. Serchov T, Calker DV and Biber K. “Light/dark transition test to assess anxiety-like behavior in mice”. Bio-protocol (BP), 2016; 19, pp. 20-24.
8. Hascoet M and Bourin M. “A new approach to the light or dark test procedure in mice”. Pharmacol. Biochem. Behav (PBC), 1998; 60, pp. 645-653.
9. Kesser RC, Berglund P, Demler O and Walters EE. “Lifetime prevalence and age of onset distributions of DSM-IV disorders in the National Comborbidity Survey replication”. Archves of Ggeneral Psychiatry (AGP), 2005; 62, pp. 617-709.
10. Saxena G, Singh M, Meena P, Sharma D, Ayushi J and Bhatnagar M. “Neuroprotective effects of *Asparagus racemosus* Linn. root extract: an experimental and clinical study”. Journal of Herbal Medicine and Toxicology (JHMT), 2010; 4, pp. 69-76.
11. Parihar MS and Hemnani T. “Experimental excitotoxicity provokes oxidative damage in mice brain and attenuation by extract of *Asparagus racemosus”*. Jo of Neural Transmission (JNT), 2004; 111, pp. 1-12.
12. Garg R, Gupta VB, Farnsworth NR and Soejarto DD. “In the adaptogenic activity of milk and aqueous decoction of *Asparagus racemosus*. In acute and chronic stress paradigms in mice”. Journal of Cell and Tissue Research (JCTR), 2010; 10, pp. 2281-2286.
13. Belovicova K, Bogi E, Csatlosova K and Dubovicky M. “Animal tests for anxiety-like and depression-like behavior in rats”. Interdiscip Toxicol (IT), 2017; 10(1), pp. 40–43.
14. Hascoet M and Bourin M. “A new approach to the light or dark test procedure in mice”. Pharmacol. Biochem. Behav (PBB), 1998; 60, pp. 645-653.
15. Wei X, Yang J and Wu C. “Anxiolytic effect of Biacilin in mice”. Asian Journal of Traditional Medicine (AJTM), 2006; (1), pp. 3-4.
16. Joshi T, Saha S and Singh A. “Anti-stress activity of ethanolic extract of *Asparagus racemosus* Willd roots in

mice". Indian Journal of Experimental Biology (IJEB), 2012; (50), pp. 419-424.
17. Devapriya G. and Sairam K. "*Asparagus racemosus* attenuates anxiety like behavior in experimental animal models". Cellular and Molecular Neuribiology (CMN), 2014; 43(4), pp. 511-521.

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