Original article

EVALUATION OF SPONTANEOUS LOCOMOTOR ACTIVITY OF AQUEOUS EXTRACT OF *AZADIRACHTAINDICA* IN MICE.

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ABSTRACT

Aim and Objectives: The objective of this study would be to evaluate spontaneous locomotor activity of aqueous extract of *Azadirachtaindica* in mice.

Materials and Methods: This is an animal interventional study where 48 mice were divided to 8 groups. Each group was given the respective drug either aqueous extract of *Azadirachtaindica* or standard drug or control drug. Spontaneous locomotor activity was recorded before and after 10 days of administration of drugs using actophotometer.

Results: There was no statistically significant difference in spontaneous locomotor activity of aqueous extract of *Azadirachtaindica*, standard drug and control drug before and after administration respectively.

Conclusions: The results of this study clearly demonstrate that aqueous extract of *Azadirachtaindica* leaves doesn't possess CNS psychostimulant activity or sedative action.

Keywords: Azadirachtaindica, psychostimulant.

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INTRODUCTION:

Since animal behavior appears as the total of cognitive function, psychological status and physical condition, its assessment for experimental animals is often applied in wide-ranged scientific fields including pharmacology, toxicology and physiology.[1]

There are several established experiments to investigate emotion, recognition, and memory of experimental rodents under special environment. For example, elevated plus maze test is used to assess their anxiety.[2] Forced swim test is used to assess their depression.[3] Water maze test is used to examine their memory.[4] In addition to these behavioral tests, spontaneous locomotor activity (SLA) is often measured to assess basic status of animal.SLA is the extent of free movement in the familiar environment, which directly reflects the animal's physical and mental conditions.[1]

Approximately two-thirds of the depressed patients respond to the currently available treatments (Tricyclic antidepressants, Selective serotonin reuptake inhibitors, etc.), but the magnitude of improvement is still

disappointing. Regular use of antidepressants makes changes in CNS monoamines leading to deterioration of cognitive functioning, psychomotor impairment, confusion, physical dependence and tolerance[5]. Currently available treatment of depression is often associated with several undesirable side effects like sedation, anticholinergic effects, postural hypotension, and weight gain and cheese reaction with chronic use of antidepressantsand it is effective only in a certain portion of the patients[6]. A search for novel pharmacotherapy from herbal plants for psychiatric diseases has progressed considerably within the past decade. A large number of different herbal preparations for antidepressant drug activity have been evaluated in a variety of animal models[7].

Though effective drugs are available for depression, the adverse effects produced by their respective drugs are significant. Hence, plant products like *Azadirachta indica*, claimed to be free from all those adverse effects and less toxic than synthetic drugs may be used to effectively treat the condition, if found to be effective in experimental animal models. Hence, the objective of this study is to evaluate spontaneous locomotor activity (SLA) in all mice for ruling out stimulant activity using actophotometer apparatus.

MATERIALS AND METHODS:

This is an animal interventional study in which there was 8 groups, where each group containing 6 male albino mice weighing between 20-30g were obtained from the registered (1081/a/07/CPCSEA) Central Animal House facility of Pondicherry Institute of Medical Sciences (Pondicherry, India). Hence, a total of 48 mice were used in this study. The animals were housed in clean polypropylene cages, in groups, maintained under standard laboratory conditions with natural 12 hours light and dark cycles and ambient room temperature, with free access to food and water. The animals were acclimatized to laboratory conditions every time before testing. Experiments were conducted between 10.00 and 17.00 hours. All procedures in the study were reviewed and approved by the Institutional Committee for Ethical use of animals. The care of animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

The test drug *Azadirachta indica* was procured from CL Baidmetha College of pharmacy, Chennai, India. Phytochemical analysis of *Azadirachta indica* showed the presence of Alkaloids, Steroids, Flavonoids, Glycosides, Terpenoids, Carbohydrates and Antiquinones. The extract was reconstituted in distilled water and then fed to the animals according to the appropriate doses mentioned below in each group.

- **Study Medications:**

Standard- Purified form of fluoxetine was obtained from Sigma Chemical Company, St. Louis, Missouri, United states of America. The powdered form was reconstituted in distilled water and then fed to the animals according to the appropriate doses mentioned below in each group.

Control- Distilled water

- **Instruments:** Digitalactophotometer for measuring locomotor activity.
- **Principle:** Locomotor activity estimates whether a test substance possesses

psychostimulant or sedative activity. Locomotor activity is considered as an index of alertness and a decrease in that indicates a sedative effect [8]. This helps to rule out any stimulant activity of the drugs involved in this study.

- **Procedure:** The locomotor activity was measured by using an actophotometer. The actophotometer contains a square arena (30×30 cm) with walls that are fitted with photocells just above the floor level [9]. The photocells were checked before the beginning of the experiment. The number of times each animal crossed the light beam was recorded automatically for a period of 5 minutes. All groups of mice as shown below were checked for locomotor activity before and after giving the respective drug to rule out stimulant activity.
  - **Group A:** Control (Distilled water) per oral (p.o)
  - **Group B:** Standard Drug (Fluoxetine 20mg/kg) per oral (p.o)
  - **Group C:** Test Drug [Neem leaf extract (NLE) 25mg/kg] per oral (p.o)
  - **Group D:** Test Drug (NLE 50mg/kg) per oral (p.o)
  - **Group E:** Control (Distilled water) per oral (p.o)
  - **Group F:** Standard Drug (Fluoxetine 20mg/kg) per oral (p.o)
  - **Group G:** Test Drug (NLE 25mg/kg) per oral (p.o)
  - **Group H:** Test Drug (NLE 50mg/kg) per oral (p.o)

- **Followed Order Of Procedures:**

Day 1-7: All groups of mice were acclimatized in the departmental laboratory and locomotor activity was recorded.

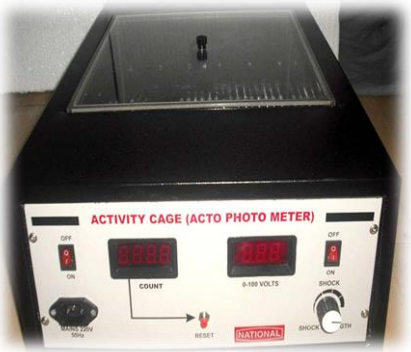
Day 8-17: 8 AM to 10 AM, respective drugs were administered orally for all mice once daily for 10 days.

Day 18 onwards: 10 AM to 5 PM, blinding was done for all groups of mice and locomotor activity was recorded.

STATISTICAL ANALYSIS:

All the parameters recorded from the above models were tabulated and expressed as mean ± SEM (standard error of mean). One-way ANOVA (analysis of variance) followed by Dunnett's test was used for analysis of data between different groups. For analysis of data between same group, Students paired "t" test was used for seven groups which followed normal distribution and Wilcoxon signed ranks test was used for one group since it did not follow normal distribution. For all inferential statistical tests, a two tailed P value of <0.05 was considered to be statistically significant and P value of <0.01 was considered to be extremely statistically significant. Graph Pad in Stat software of version 3.06 was used for analysis of data.

Figure 1: Locomotor activity using actophotometer



RESULTS:

Table 1: Evaluation of Locomotor Activity of mice in Groups A, B, C, H (Mean ± SEM) n (Number of mice) = 6 in each group;[NS] – Not significant before vs. after, * data was analyzed by using Wilcoxon signed ranks test, ** data was analyzed by using Paired t test.

Groups	Drugs	Locomotor Activity (counts) (Mean ± SEM)	
		Before drug	After drug
A**	Control (Distilled water)	206 ± 8.25	204.64 ± 7.89 [NS]
B*	Standard Drug (Fluoxetine 20mg/kg)	204.84 ± 7.76	204.47 ± 8.15 [NS]
C**	Test Drug (NLE 25mg/kg)	268.17 ± 7.59	267.7 ± 8.14 [NS]
H**	Test Drug (NLE 50mg/kg)	189.17 ± 19.27	187.04 ±19.45 [NS]

From table 1, it is evident that, the test drug NLE at both doses after giving it for 10 days (chronic study) did not show any statistically significant difference ($p>0.05$) in the total counts of locomotor activity when compared with that of before giving the NLE. The standard drug fluoxetine also after giving it for 10 days (chronic study) did not show any statistically significant difference ($p>0.05$) in the total counts of locomotor activity when compared with that of before giving the standard drug.

Table 2: Evaluation of Locomotor Activity of mice (Mean ± SEM) n = 6 in each group;[NS] – Not significant before vs. after, ** data was analyzed by using Paired t test.

Groups	Drugs	Locomotor Activity (counts) (Mean ± SEM)	
		Before drug	After drug
E**	Control (Distilled water)	223.5 ± 15.61	221.87 ± 15.22 [NS]
F**	Standard Drug (Fluoxetine 20mg/kg)	170.84 ± 11.64	170.92 ± 12.08 [NS]
G**	Test Drug (NLE 25mg/kg)	215.5 ± 26.96	214.64 ± 26.71 [NS]
D**	Test Drug (NLE 50mg/kg)	195.5 ± 28.20	194.52 ± 27.91 [NS]

From table 2, it is evident that, the test drug NLE at both doses after giving it for 10 days (chronic study) did not show any statistically significant difference ($p>0.05$) in the total counts of locomotor activity when compared with that of before giving the NLE. The standard drug fluoxetine also after giving it for 10 days (chronic study) did not show any statistically significant difference ($p>0.05$) in the total counts of locomotor activity when compared with that of before giving the standard drug.

DISCUSSION:

In spite of all the advancements in modern medicine, traditional medicines have always been practiced, because of their natural origin, easy availability, cost effectiveness, lesser side effects and better tolerability [10]. *Azadirachta indica*, commonly known as neem worldwide, has been most commonly used in traditional medicine for several ailments. In both acute and sub-acute toxicity studies of aqueous extract of neem leaf, there was no impact on mortality up to a dose of 2.5 g/kg in mice. There were no significant changes in tissues or body weight, water and food intake. For 28 days with treatment of 1 g/kg dose of aqueous extract of neem leaf, blood parameters and different kidney and liver functional tests in rats were found to be within normal limits[11].

Locomotor activity is assessed usually by actophotometer and is used to estimate whether a substance has any CNS psychostimulant or sedative action. Thus, locomotor activity is considered as an index of alertness and a decrease in that indicates a sedative effect. For example, drugs like barbiturates and alcohol which are known CNS depressants reduces the locomotor activity whereas drugs like caffeine and amphetamines which are known CNS stimulants increases the locomotor activity [8]. If the test substance has a psychostimulant activity, this can give rise to a false positive result. Thus, it is ideal to rule out any CNS psychostimulant activity before checking for antidepressant like property. From the results it is clear that both standard fluoxetine and NLE does not have any CNS psychostimulant activity nor sedative property. By this finding it is ascertained that there won't be any false positive outcomes in checking for antidepressant like property using experimental animal models.

CONCLUSION:

In conclusion, this study shows that aqueous extract of *Azadirachta indica* leaves doesn't possess CNS psychostimulant activity or sedative action. Further studies are needed to be done to evaluate antidepressant property of aqueous extract of *Azadirachta indica* leaves using experimental animal models. The problems with the conventional antidepressants like delay in the onset of their efficacy and other unacceptable side effects are needed to be investigated in further studies to see if it lacks with *Azadirachta indica*.

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