Original Article

Journal of Pharmaceutical and Medicinal Chemistry
Volume 10 Number 2, July-December 2024
DOI: http://dx.doi.org/10.21088/jpmc.2395.6615.10224.1

Anti-anxiety Activity of *Achyranthes aspera* Linn. Stem Extracts on the Elevated Plus-maze Model on Wistar Rats

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How to cite this article:
Shubhangi B. Dorle, Fulchand V. Kajale, *et al.,* Anti-anxiety Activity of *Achyranthes aspera* Linn. Stem Extracts on the Elevated Plus-maze Model on Wistar Rats. J Pharmaceut Med Chem. 2024;10(2): 43-47.

Abstract

Achyranthes aspera Linn. has been broadly used as traditionally medicine in several countries. All parts of this plant have been used in treatment and prevention of several complications. Taxonomically *Achyranthes aspera* Linn. belong to family Amaranthaceae. In the present study, stem of *Achyranthes aspera* Linn. were subjected to Pharmacognostic evaluation. Various methods including macroscopic, microscopic, physiochemical and phytochemical methods were applied to determine the features for the identification and standardization of intact and powdered drug of stem of *Achyranthes aspera* Linn. This analysis is proved useful to differentiate it from other drug material. The powdered plant material was removed by Soxhlet extractor in Ethyl acetate and ethanol after defatting with petroleum ether.Phytochemical testing of extracts discovered presence of glycosides, flavonoids, carbohydrates, etc. The total phenolic and flavonoid content of plant extracts was expressed as Gallic acid equivalents and as quercetin equivalents separately. Anti-oxidant activity was carried out by 2,2-diphenyl-1-picrylhyrazyl (DPPH) assay. DPPH scavenging assay were performed to evaluate the antioxidant activity which was found maximum at 100 microgram concentration for both extracts. The results of acute oral toxicity studies of plant extracts as per standard references revealed median lethal dose (LD_{50}) could be greater than 2000mg/kg body weight in rats. Accordinglysafe experimental dose was calculated as 100 and 200mg/kg. The evaluation of *in-vivo* anxiolytic activity in rats was done using elevated plus maze model. The results showed increases locomotor activity in both extracts at dose of 200mg/kg compared to vehicle group.

Keywords: Anti-anxiety, *Achyranthes aspera* Linn, Elevated plus maze, Anti-oxidant activity

INTRODUCTION

Anxiety is an emotional state, unkind in nature, associated with nervousness, uneasiness and fear or fear about some defined or undefined future threat. Some degree of anxiety is a part of normal life. Treatment is needed when it is unequal to the situation and excessive[1]. Anxiety disorders are among the most common psychiatric disorders that affect all age groups of the general population[2].

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Received on: 01.11.2024 **Accepted on:** 06.12.2024

Anxiety disorders are the most common mental disease in the U.S. affecting 40 million adults in the United stages age 18 and older, or 18.1% of the population every year[3]. Anxiety is a normal behaviour. When it is simple and/ or long-lasting, however, it becomes pathological and can rapid or aggressive cardiovascular and psychiatric disorders. Although many drugs are available in allopathic medicine to treat anxiety disorders, they provide various systemic side effects or exhibit tolerance upon chronic use. In ayurvedic medicine, many plant products have been requested to be free from side effects and less toxic than synthetic drug. Benzodiazepines (BZD) are the major class of compounds used in anxiety and they remain the most commonly prescribed drugs in anxiety. However, the BZD's have many unwanted side effects that has encouraged many researchers to evaluate new compounds in the hope of identifying other anxiolytics with fewer side effects[4].

Worldwide anxiety statics

It is expectable that 268 million adults around the globe have anxiety of these adults[5], 185 million were female (63%) and 105 million were male (37%)[6].The frequency of all mental disorders improved by 50% worldwide from 416 million to 615 million between 1990 and 2013[7].

MATERIAL AND METHODS

Plant material

The fresh stem of ***Achyranthes aspera* L**. were collected from local region from Bhokarand were authenticated by **Dr. Shrirang S. Bodke**, Associate professor and Head of Botany and Horticulture, Yashwant Mahavidyalaya, Nanded.The fresh stems of plant *Achyranthes aspera* were subjected to shade drying and further crushed to coarse powder passed through mesh no. 14 and stored in air tight container for further use.

Table 1: Taxonomical Classification of *Achyranthes Aspera* Linn[8]

Kingdom	Plantae
Sub-division	Viridiplantae
Super division	Embryophyta
Division	Tracheophyta
Class	Magnoliopsida
Order	Caryophyllus
Family	Amaranthaceae
Genus	Achyranthes
Species	*Achyranthes aspera*

Traditional uses of plant

The root paste helps to decreases the pain, skin rashes, itching, insect bites and helpful in earache. Daily consumption of *Achyranthes aspera* plant supplement powder with jaggery improves digestion[9]. For snake bites the seeds are beneficial. *Achyranthes aspera* plant ash added with potash has been used for washing clothes.***Achyranthes aspera*** L. used in treatment of asthma, bleeding in helping delivery, boils, bronchitis, cold, cough, dog bite and skin diseases[10].

Preliminary Phytochemical evaluation

The extracts obtained by successive extraction were subjected to quantitative tests for the identification of various secondary metabolites such as carbohydrates, proteins, tannins, steroids, flavonoids, alkaloids and glycosides.[11,12]

ELEVATED PLUS MAZE TEST[13]

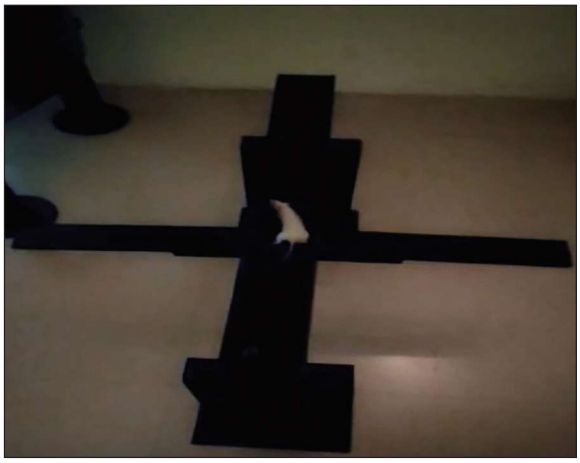


Fig. 1: Elevated plus maze model

I. The elevated plus maze consist of two intersecting arms which are two open arms 35× 15 × 15 cm and two enclosed arms both 35 × 15 × 15 cm with an open rooftop, arranged so that the two open arms are opposite to each other.
II. The maze is elevated to a height of 50 cm and arm is 10 cm wide.
III. Eighty-four male & female rat (200–250 g body weight) of approximately two months of age were used as experimental animal.
IV. During this time the rats was handled by the investigator on alternate days to reduce stress.
V. Animals were divided into 6 groups of 6 rats. Control, Standard drug treated, and four extract treated group. (Higher & Lower)

VI. Thirty min after oral and i.p. administration of the test & standard drug respectively, the rat was placed in the centre of the maze, facing one of the enclosed arms.

VII. The procedure and observation are typically performed by video recording and the video camera mounted directly above the maze.

IAEC Approval

Selection and procurement of animals

The experiment was performed with the approval of Institutional Animal Ethics Committee (IAEC) following guidelines of CPCSEA. The male and female wistar rats with 200-250 g body weight were selected for study using elevated plus maze model[14]

Animals used

Male and female wistar rats

Route of administration

Standard: Intravenous rote

Test: Orally

Animal Grouping

Group A- Control: DMSO (0.5%)

Group B- Standard Diazepam (2mg/kg)

Group C- Test Dose 1: AAEAE (100MG/KG)

Group D- Test Dose 2: AAEAE (200MG/KG)

Group E- Test Dose 1: AAETH (100MG/KG)

Group F- Test Dose 2: AAETH (200MG/KG)

Anti-anxiety activity

Table 1: ***Achyranthes aspera*** stem extracts mean reading from Elevated plus maze model

Treatment	Time spent in open arm (sec)	Time spent in enclosed arm (sec)	Number of entries in open arm	Number of entries in enclosed arm
Control	110 ± 6.54	173 ± 2.36	2.5 ± 0.22	5.66 ± 0.21
Diazepam (2mg/kg)	240.16 ± 1.66**	68.5 ± 1.10**	32.33 ± 0.78**	7.8 ± 0.46**
AAEAE (100mg/kg)	138 ± 2.88**	146.68 ± 2.38**	18.16 ± 0.80**	4.16 ± 0.45**
AAEAE (200mg/kg)	198.12 ± 2.87**	82.31 ± 2.86**	20.5 ± 0.80**	4.66 ± 0.32**
AAETH (100mg/kg)	148.16 ± 1.40**	142.2 ± 1.54**	14.5 ± 0.40**	6.40 ± 0.32**
AAETH (200mg/kg)	210.66 ± 1.32**#	72.81 ± 1.72 **#	22.33 ± 0.82 **#	8 ± 0.38**#

RESULTS

Each value represents the mean ± S. E. M (n=6)

*Significant difference when standard and test compound with control (P < 0.05);

**Highly significant difference when test compared with control (p <0.001); # No significant difference when test compared with standard.

The vehicle treated rat spent less time in open arm (110 ± 6.54) and more time in enclosed arm (173 ± 2.36) with 2.5 ± 0.22 entries in open arm and 5.66 ± 0.21 entries in enclosed arm. The AAEAE (200mg/kg) and AAETH (200MG/KG) show highly significant decrease in time spent in enclosed arm. Administration of AAEAE (200mg/kg), AAETH (100mg/kg), AAETH (200mg/kg) and diazepam (2mg/kg) show significant (p- 0.001) increase in the occupancy in open arm indicating antianxiety activity of AAEAE (200mg/kg) and AAETH (2000mg/kg) extract as compared to AAEAE (100mg/kg) and AAETH (100mg/kg) extract.

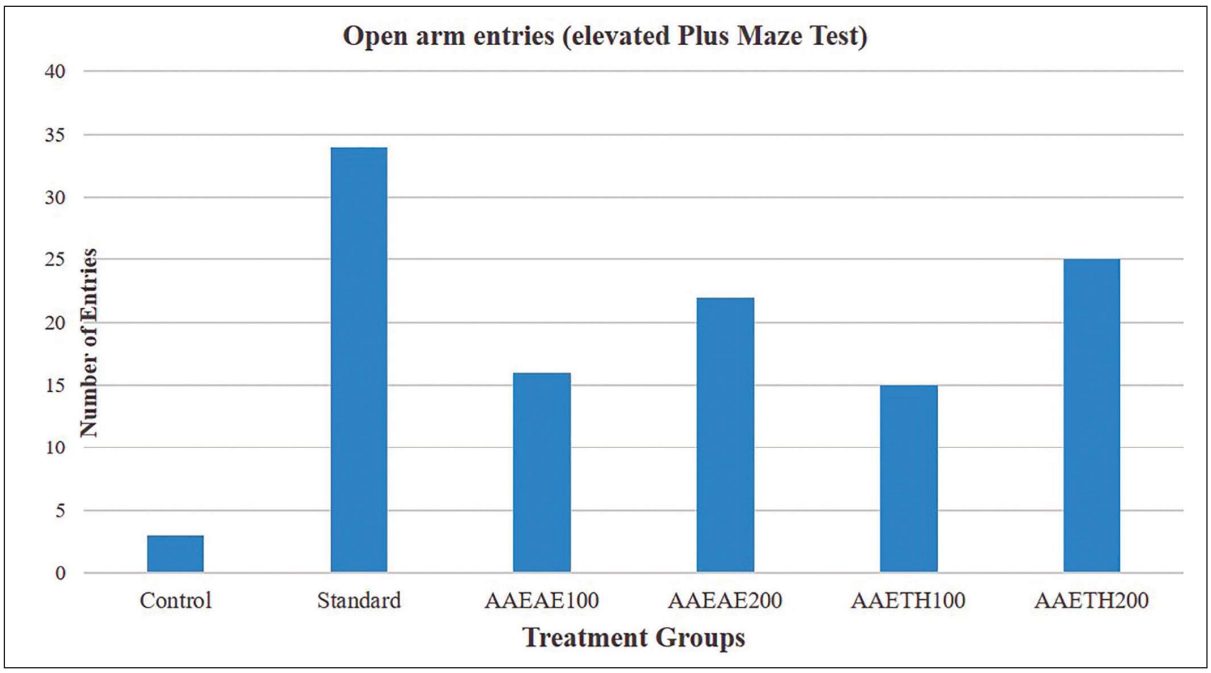
Safe Dose Calculation[15]

For acute oral toxicity studies literature survey has been carried out for referencing (LD50) range as per OECD (423) guidelines of selected plant extracts and accordingly 1/10th of LD50 will be used as maximum experimental safe dose.

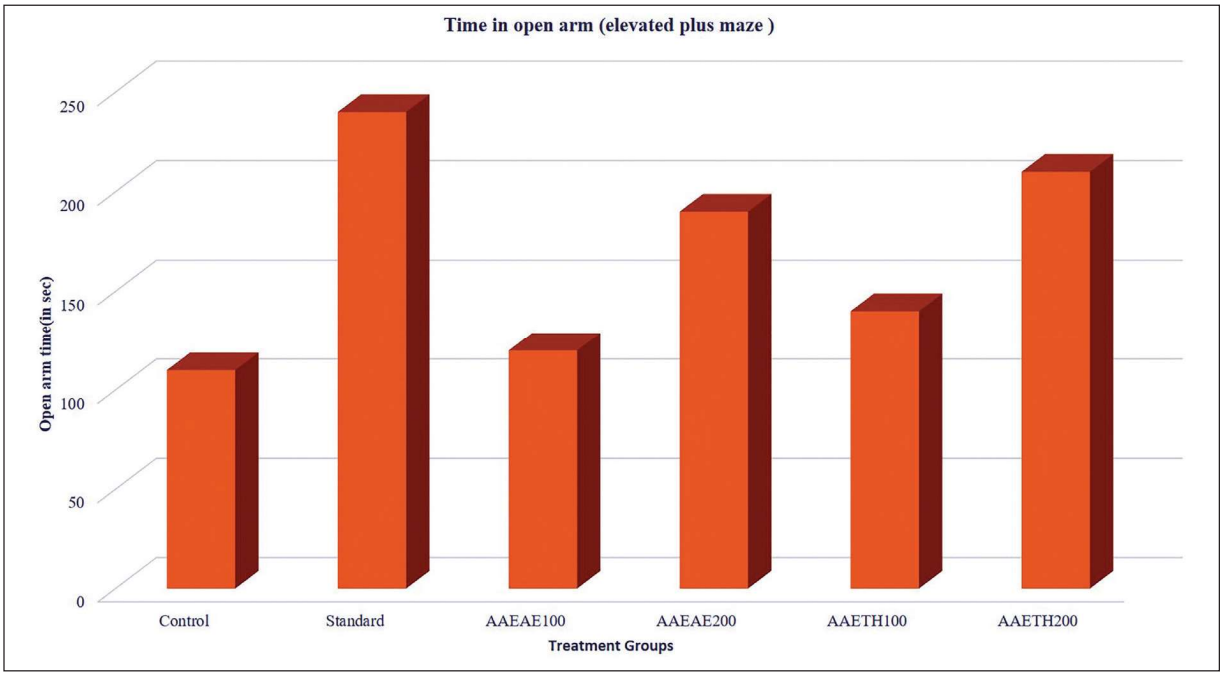
LD50 value of plant extracts: 2000mg/kg

Selected doses:

1. Lower dose: 100mg/kg
2. Higher dose: 200mg/kg



Graph 1: Total number of entries in open arm in elevated plus maze test



Graph 2: Time Spent in open arm (in sec) in elevated plus maze test

DISCUSSION

The stem of *Achyranthes aspera* Linn were collected from Local region of Bhokar. Stem was identified and authenticated from Yeshwant College, Nanded and herbarium was submitted to Nanded Pharmacy College, Nanded. The collected stems were dried and used for extraction. Extraction was done by continuous hot method using Soxhlet apparatus. Two extracts namely Ethyl acetate and ethanolic were obtained. The percentage yield of each extract was calculated and stored for further use. Pharmacognostic evaluation of the powdered stem was done with parameters like loss on drying, total ash value, acid insoluble ash value.

The extracts were subjected to phytochemical screening, both extracts showed the presence of carbohydrates, proteins, flavonoids, alkaloids, steroids. The TLC fingerprinting of extracts using various solvent proportions showed different coloured spots. The Rf values of the spots were leisurely. Acute toxicity studies (OECD 423: Acute Oral Toxicity Class Method) of *Achyranthesaspera* Linn. conducted by researchers revealed that the graded doses administration of extracts (up to a dose of 2000mg/kg) did not produce significant changes in behaviour such as diarrhoea, convulsions, coma and in appearance of the animals. No death was verified up to the dose of 2000mg/kg body weight. The result of such studies showed that in single dose; the plant extract had no adverse effect, indicating that the medium lethal dose (LD_{50}) could be greater than 2000mg/kg body weight in mice/ rats. Accordingly for further screening of extracts.

For studying *In-vivo* anti-anxiety activity, elevated plus maze used as animal model. Eighty-four animals were required. Animals were divided into eleven groups; control, Standard, and twelve test groups (100, 200mg/kg) each group containing six animals. The anti-anxiety activity of ethyl acetate and ethanol extracts of *Achyranthes aspera* was studied by using elevated plus maze model. The obtained results were compared with standard drug diazepam (2mg/kg).

In this model, rats were allowed to move in maze which consists of two open arms and enclosed arms for a period of 5min. Fear due to height induces anxiety in animals when placed in the maze. The anxiety and fear in animals is exhibited by decreases in locomotor activity and preference to be at safter places. Parameters like number of entries in open arm and closed arm, time spent in open arm, closed arm. These parameters were measured by video tracking system. The results showed that the higher dose (200mg/kg) of both extracts, have highly significant anti-anxiety activity. It was observed that Ethyl acetate and ethanol extracts of plant show significant difference as compared to vehicle treated group, and shows same behavioural effects produced by standard drug diazepam.

From the results it was observed that ethyl acetate (200mg/kg) and ethanol (200mg/kg) extracts showed effective anti-anxiety activity[8].

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