



PHARMACOLOGICAL EVALUATION OF CITHAREXYLUM SERRATUM FOR ITS ANXIOLYTIC AND MUSCLE RELAXANT ACTIVITIES ON MICE

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ABSTRACT

Key Words

Citharexylum Serratum, anxiolytic, muscle relaxation, Photoactometer, Open field test, Elevated Plus Maze, Chimney test, IR Actimeter, Hole board apparatus, Circular tangle.



Objective: To carry out the preclinical evaluation of methanolic extract of the leaves of *Citharexylum Serratum* for anxiolytic and muscle relaxation activity. **Methodology:** The preclinical evaluation of standardized diazepam and methanolic extract of the leaves of *Citharexylum serratum* for anxiolytic and muscle relaxation activity was carried out by using the following experimental models: a) Elevated Plus Maze b) Chimney test c) IR Actimeter d) Hole board apparatus e) Photo actometer f) Open field test g) Circular tangle. **Results:** The models for studying drugs or conditions that affect muscle relaxation and anxiolytic activity was standardized and evaluated by using leaf extracts of *Citharexylum serratum*. The methanolic leaf extract of *Citharexylum serratum* has shown significant muscle relaxation and anxiolytic activity by all the employed experimental models. **Conclusion:** Screening models for studying drugs or conditions that affect anti anxiety and muscle relaxation was standardized and evaluated by using methanolic extract of *Citharexylum Serratum*. The leaf extract has shown significant activity of anxiolytic and muscle relaxant when compared with standard treatment group.

INTRODUCTION:

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of

modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine. Much of the

medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledgebase. They methodically collected information on herbs and developed well defined herbal pharmacopoeias. *Citharexylum serratum* has a wide distribution in the Caribbean. It is most common in the Florida Keys and has a scattered presence in pinelands and hammocks along Florida's east coast, north to Brevard County and to Manatee County on the west coast. It is a nuisance plant in Bermuda, Australia and on several Pacific Islands including Inflorescences, early January.

MATERIALS AND METHODS

The commonly employed technique for separation of active substance from crude drug is called as 'Extraction' which involves the use of different solvents. The plant material used for extraction should be properly authenticated or identified. The choice of the plant Material for extraction depends upon its nature and the components required being isolated. The dried powdered plant material is commonly used for extraction. The solvent used for extraction is called menstruum and the residue is known as marc. Methanol was used as a solvent for the extraction process as it confers a good polarity.

EXPERIMENTAL ANIMALS

Mice (25-30g) were maintained for 7 days in the animal house of Chalapathi Institute of Pharmaceutical Sciences, Guntur under standard conditions temperature ($24 \pm 10^\circ\text{C}$), relative humidity (45-55%) and 12:12 light: dark cycle. The animals were fed with standard pellet and water ad libitum. The animals were allowed to acclimatize to laboratory conditions 48 h before the start of the experiment. 5 mice/group were used in all sets of experiments. All the experiments were conducted after obtaining permission

from the Institutional Animal Ethics Committee (IAEC) Chalapathi Institute of Pharmaceutical Sciences, Guntur.

SELECTION OF DOSE AND TREATMENT PERIOD

Mice (25-30g) of either sex were divided into four groups containing five animals in each. A control group received normal saline solution, while second group received standard drug (Diazepam) and other group the standardized methanolic leaf extracts of "*Citharexylum serratum*" (obtained from Pharmacognosy Department of Chalapathi Institute of Pharmaceutical Sciences) at doses 100 mg /kg-1 p.o, respectively.

- Group-1- Control group (0.9% Normal saline 2ml/kg orally)
- Group-2 – Standard (Diazepam at a dose of 2 mg/ kg i.p)
- Group-3 – Methanolic extract (100mg/kg orally)

EQUIPMENT

The following equipment was used to evaluate anxiolytic and muscle relaxant activity:

- a) Elevated Plus Maze
- b) Photoactometer
- c) IR Actimeter
- d) Hole Board Apparatus
- e) Circular Tangle
- f) Chimney Test
- g) Open Field Test

Elevated Plus Maze Test

Mice were carried into the test room in their home cages and were handled by the base of their tails at all times. Mice were placed in the central square of the Plus-Maze facing an open arm and were then allowed to explore the apparatus for 5 minutes. An observer sitting quietly about 1 m from the apparatus recorded the behaviour of the animals on the maze.

Hole Board Apparatus

The HB apparatus is an area with walls around it to prevent escape.

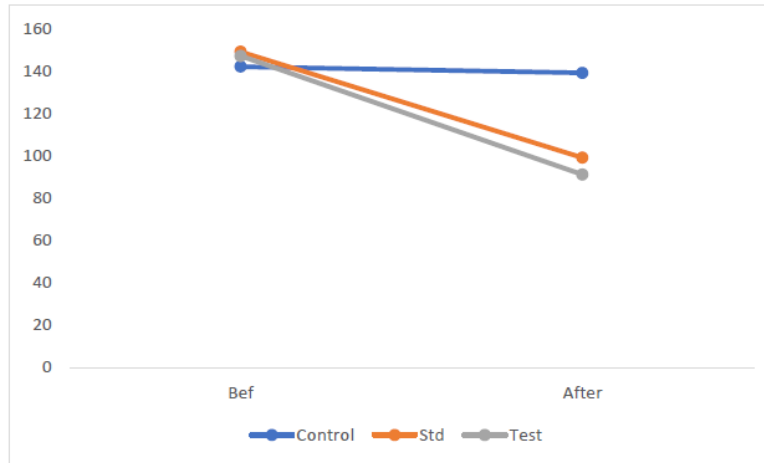


Fig 1: Observations of treatment groups for Photoactometer

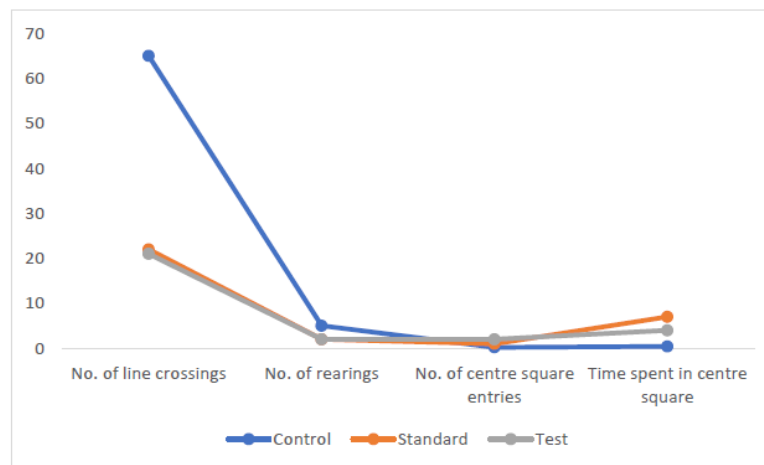


Fig 2: Observations of treatment groups for Open field test

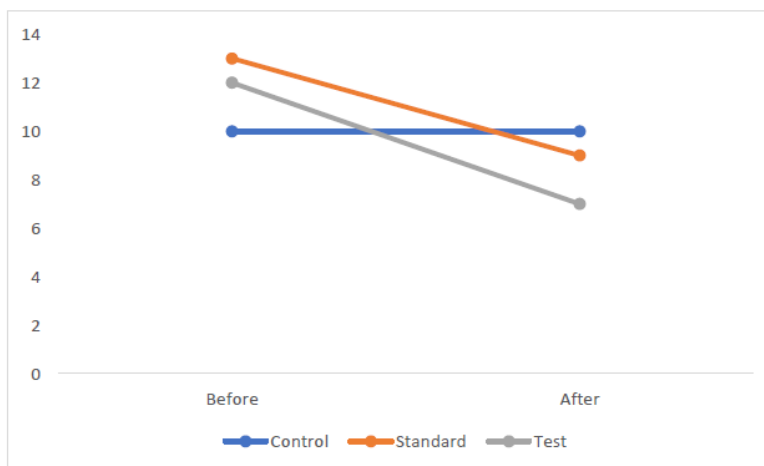


Fig 3: Observations of treatment groups for Hole Board test



Fig 4: Observations of treatment groups for IR actimeter

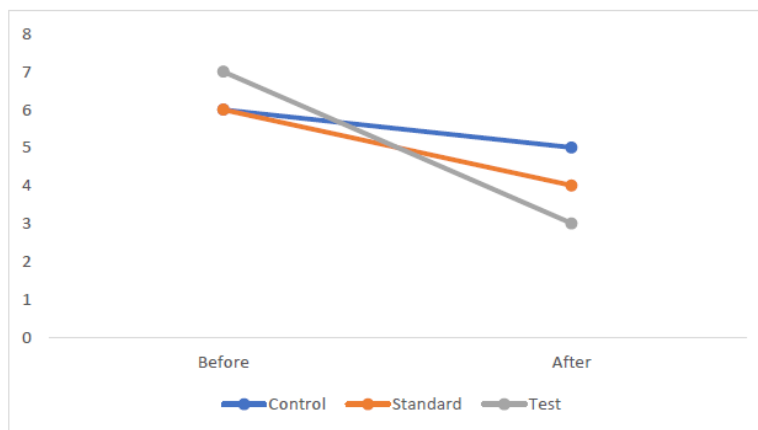


Fig 5: Observations of treatment groups for Chimney test

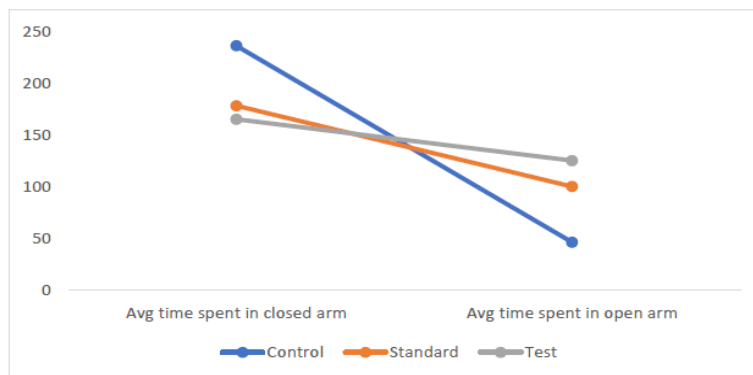


Fig 6: Observations of treatment groups for Circular tangle

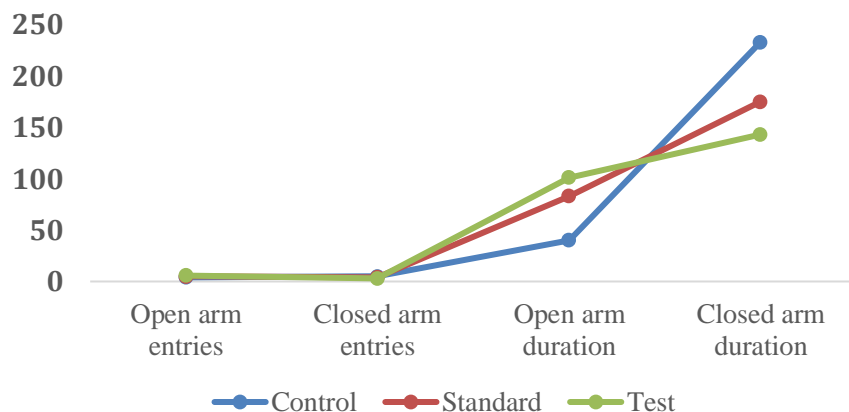


Fig 7: Observations of treatment groups for Elevated plus maze

The floor of the area is covered with holes. Over the years the HB apparatus has become more complex. Modern devices are now monitored by computer and have infrared beams. The common evaluation parameters are head dipping and rearing. Head dipping is commonly defined as when the animal puts his head into the hole until its ears are level with the floor or in modern devices when it breaks the infrared beam.

IR Actimeter

Infrared (IR) Actimeter allows the study of spontaneous locomotor activity, exploration in rodents. Each frame counts with 16 x 16 infrared beams for optimal subject detection. Each frame may be used for evaluation of general activity (one or more animals), locomotor, stereotypic movements.

Open field

Mice were carried to the test room in their home cages and were handled by the base of their tails at all times. Mice were placed into the center or one of the four corners of the open field and allowed to explore the apparatus for 5 minutes.

Behaviours scored: Line crossings, time spent in centre squares, number of centre square entries and rearing.

Photoactometer

In a digital photoactometer, continuous beam of light falls on photoelectric cells.

When the reading is considered as zero, any cut off in the continuity of light by the animal, is recorded on a digital counter in the form of counts.

Circular Tangle

Mice were placed in the circle facing an open area and were then allowed to explore the apparatus for 2 minutes. Rearing, head-dips, grooming, stretch attends and line crosses were scored by the experimenter, whereas measures of open and dark entries and the time in each area was recorded.

Chimney Test

This was mainly used to evaluate muscular activity of mice where a measuring cylinder was used to identify the withstand capacity. Mice was introduced into the measuring cylinder facing towards closed side, then turn the measuring cylinder inversely in order to set it for an inclined plane. The time when the mice released from the measuring cylinder was noted as evaluation parameter.

CONCLUSION

Screening models for studying drugs or conditions that affect anti anxiety and muscle relaxation was standardized and evaluated by using methanolic extract of *Citharexylum Serratum*. The leaf extract has shown significant activity of anxiolytic and muscle relaxant when compared with standard treatment group.

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