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Research Article

SCREENING ANXIOLYTIC ACTIVITIES OF METHANOLIC FRUIT EXTRACTS OF *SPONDIA PINNATA* PLANT

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ABSTRACT

This Present study was surpassed to investigate the methanolic extracts of *Spondias pinnata* fruit for anxiolytical activities. *S. pinnata* is a plant of Anacardiacea family. The methanolic crude extract were screened for anxiolytic activities using hole board test method (***p < 0.001) and elevated-plus maze test method (***p < 0.001) in mice. Test results found 40.25±2.98 times for 400mg/ml extract in hole-board and 152.5±44.78, 147.5±, 9.25±1.79 times in elevated plus maze respectively as comparison with standard. The use of crude extracts of fruits of *Spondias pinnata* as anxiolytics have been confirmed that the fruits extracts displayed potential anxiolytic activity against mice for head dipping and stay in open and enclosed arms in the study. Various concentrations of extracts were tested and results were expressed in terms of time. In conclusion, the methanol extract of the fruits and plant of *Spondias pinnata* displayed anxiolytic activity.

Keywords: *Spondias pinnata*, Anacardiacea, Anxiolytic.

INTRODUCTION

Anxiety is one of the serious health issues all around the world. It is defined as the expectation of future threat, a feeling of uneasiness and worry, usually generalized and unfocused as an overreaction to a situation that is only subjectively seen as menacing. This “alarm reaction” takes place when the body’s natural alarm system (the “fight-flight-freeze” response or “adrenaline response”) has been triggered. This response prepares human body to defend itself. Anxiety does not only affect your body, it also affects patient’s thoughts and behavior. Anxiety may become persistent, seemingly uncontrollable, and overwhelming. If it’s an excessive, irrational dread of everyday situations, it can be disabling. When anxiety interferes with daily activities, people may have an anxiety disorder.

Anxiety disorders are the most common mental illness in the U.S., affecting 40 million adults in the United States age 18 and older, or 18% of the population.[1] People with an anxiety disorder are three to five times more likely to go to the doctor and six times more likely to be hospitalized for psychiatric disorders than those who do not suffer from anxiety disorders. Anxiety disorders cost the U.S. more than $42 billion a year, almost one-third of the country's $148 billion total mental health bill[2]. More than $22.84 billion of those costs are associated with the repeated use of health care services; people with anxiety disorders seek relief for symptoms that mimic physical illnesses[2]. The prevalence of anxiety disorder is worldwide. A comprehensive study of anxiety published by researchers at the University of Queensland across 91 countries, involving more than 480,000 people show that clinical anxiety is a serious health issues all around the world. Clinical anxiety affected around 10 percent of people in North America, Western Europe, and Australia/New Zealand compared to about 8 percent in the Middle East and 6 percent in Asia. According to the Global Burden of Disease (GBD) Study anxiety is the most common of all mental disorders—currently affects about one in 13 people (7.3 percent)[3,4].

Nature has been a source of medicinal treatments for thousands of years, and plants-based systems continue to play an essential role in the primary health care of 80% of the world's underdeveloped and developing countries[5,6,7,8]. Medicinal herbs are indispensable part of the traditional medicine practiced all over the world due to easy access, low cost and ancestral experience. *Spondias pinnata* is also known as Hogplum or Aamra in Bengali is a deciduous tree which is distributed throughout Bangladesh, India, Srilanka and South-East Asian countries. Its accession number is 40254 as per the Bangladesh National Herbarium. Fruits, leaves, bark of *S. pinnata* are strong anti-scorbutic agents. Roots of the plants are traditionally used for regulating menstruation. All parts of this plant have been used in folkloric medicine as an anti-tubercular agent, while the unripe fruits were used as an aphrodisiac[9]. Pharmacologically *S.pinnata* was tested for many important activities such as: hypoglycemic activity[9], anthelmintic activity[9], anti-cancer activity[9], anti-microbial activity[9], anti-oxidant activity[9], cytotoxic activity[9], hepatoprotective activity[9]. However, no activity of the plant parts has been examined for its CNS effects. The present study was undertaken to evaluate the anxiolytic activity of extracts of the plant fruits in animal models.

MATERIALS & METHODS

Collection of plant materials

The fruits of *Spondias pinnata* (Family: Anacardiaceae) was collected from Chaumuhani, Noakhali, Bangladesh on April, 2015. The Local Name of *S.pinnata* is Amra, Amna, Deshi-amra; Piala, Pial (Chittagong). Its accession number is 40254 as per the Bangladesh National Herbarium.

Extraction
After collection fruits were thoroughly washed with water. The collected plant parts (fruits) were separated from undesirable materials or plants or plant parts. Then the collected plant materials were cut, dried, and pulverized. About 500g of the powdered materials was soaked in 1.5 liter of 99% methanol at room temperature for 16 days with occasional stirring. Then the solution was filtered using filter cloth and Whitman's filter paper and evaporated by using traditional spontaneous natural vaporization method at room temperature covered with aluminum foil with small pores to facilitate evaporation of methanol. It was kept there for 3 months which rendered a gummy concentrate of dark greenish color. The gummy concentrate was designated as crude methanolic extracts.

Experimental Animal
Swiss-albino mice of either sex, aged 4-5 weeks, obtained from the Animal Resource Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR,B) were used for the experiment. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2ºC; relative humidity 60-70%) in a 12 h light-dark cycle and fed ICDDR; B formulated rodent food and water (ad-libitum). As these animals are very sensitive to environmental changes, they are kept before the test for at least 3-4 days in the environment where the experiment will take place.

Anxiolytic Activity test

Hole board test
Hole board test is a generally used method for screening the potential anxiolytic character of drugs. It is a rodent model test. The rodent when placed in the apparatus, is free to deep its head through the holes in the floor; the frequency and duration of this behavior, known as 'head-dipping' is thought to measure levels of neophilla. The test is based on the assumption, that head-dipping activity of the animals is inversely proportional to their anxiety activity state as changes in head-dipping activity have been considered to be related anxiety by some researchers. So, we tested this assumption by measuring the head-dipping activity of animals' in environments with different levels of adverse character.

Materials & Apparatus
Test sample, Hole board machine, Weighing machine, Syringe, Gloves, Glass rod, Beaker, Distilled water.

Preparation of the sample
Sample is prepared by mixing 200 and 400 mg extract in 10 ml distilled water in separate beaker. Concentration is maintained as (200-400mg /10 ml) carefully.

Test Method
The hole-board consists of a wooden box (40 x 40 x 25 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the floor in a greed pattern. The apparatus was elevated to the height of 35 cm. One hour after treatment with distilled water (10 ml/kg p.o), plant extract (200 and 400 mg/kg) and diazepam (1 mg/kg), each mouse was placed in turn at one corner of the board with the animal subsequently moving about and dipping its head into the holes. The number of head dips during a 5 min period was recorded for individual mouse [10-16].

Elevated plus maze test
The elevated plus maze is a model of anxiety that usually uses rodents as a screening test for putative anxiety compound and as a general research tool in neurobiological anxiety research. The model is based on the test animal's aversion top open spaces and tendency to be thigmotaxic (a preference to remain near to, or touching, vertical surfaces).In the EPM, this expressed as the animal spending more time in the enclosed arms.

Materials & Apparatus

Test sample, Swiss Albino mice, Weighting machine, Test machine, Sonicator machine, Distilled water, Beaker, Glass rod, Marker.

Preparation of test sample
Extract is taken separately as 200mg and 400mg in 10 ml beaker and 10 ml distilled water ia than added to make a concentrated extract solution(200/400mg10/ml).

Elevated plus-maze Test Method
The elevated plus-maze was based on that described by Lister (1987).The EPM consisted of two open arms (35 x 5 cm) crossed with two closed arms (35 x 5 x 15 cm). The arms were connected together with a central square of 5 x 5 cm. The apparatus was elevated to the height of 40 cm in a dimly illuminated room. Swiss albino mice (20–25 g) fasted overnight were divided into four groups of five animals each. Distilled water (10 ml/kg, p. o), plant extracts (200 and 400 mg/kg, i.p) and diazepam (1 mg/kg, i.p) were administered to groups of four mice each. All the four groups were given respective treatment and after 1hour, mice were individually placed in centre square facing either one of the open arms. The time spent in both the open and closed arms was recorded for 5 min. The numbers of entries into the open and closed arms were also counted during the test. An entry was defined as having all four paws within the arm [11-16].

Table 1: Effects of fruit extract on mice stay in Hole board

Treatment	Dose (mg/kg)	Number of head dipping
Control	10ml/kg	10.50±13
Diazepam	1mg/kg	14.83±1.6*
Extract	200mg	31.75±4.49***
	400mg	40.25±2.98***

All values are mean ± SEM (n = 4); *p < 0.05, ***p < 0.001 when compared tocontrol. One- way ANOVA, Tukey Kramer post hoc test.

Table 2: Effects of fruit extract on mice stay in the open and enclosed arms of the elevated plus-maze

Sample/Drug	Dose	Time spent in the open arms(s)	Time spent in the enclosed arm (s)	Entries into enclosed arm
Control	10ml/kg	30.8 ±6.4	243.5±8.8	14.7±1.0
Diazepam	1mg/kg	107±12	169±13.1	9.7±0.9
Extract	200mg	103 ±22.11***	197±22.11***	8.25±0.85***
	400mg	152.5±44.78***	147.5±44.78***	9.25 ±1.79***

All values are mean ± SEM (n = 4); *p < 0.05, ***p < 0.001 when compared to control. One- way ANOVA, Tukey Kramer post hoc test.

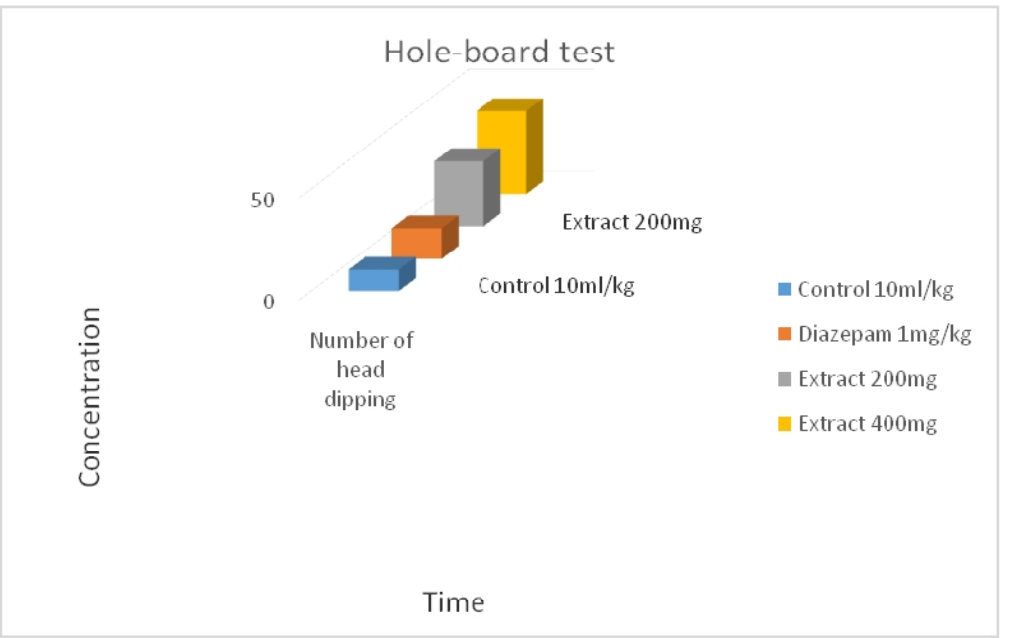

Figure 1: Effect of fruit extract on mice stay in Hole-board. Head dipping at 400mg/kg is slightly in an increase than the standard & control value which indicates a significant effect of anxiolytic activity.

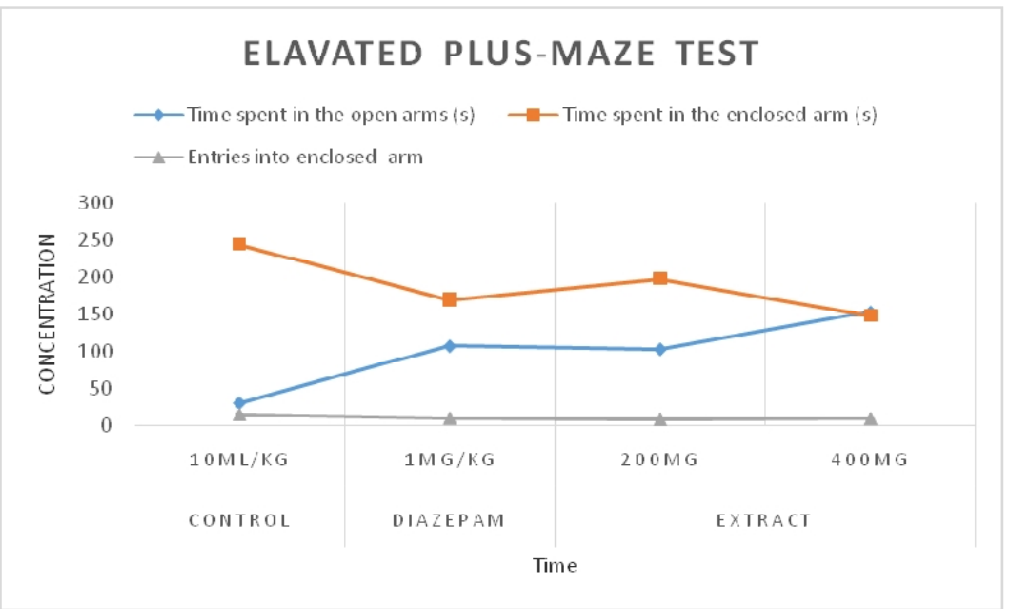

Figure 2: Effects of fruit extract on mice stay in the open and enclosed arms of the elevated plus-maze. Time spent in enclosed arm at 400mg/kg is slightly in decrease than the standard & control value which indicates a marked effect of anxiolytic activity.

RESULT & DISCUSSION

In this study, the hole-board, elevated plus maze tests were used to investigate the anxiolytic effect of extract in mice.

Result & Discussions of Hole board test

The test result of the methanolic extract of fruit as plant part of *Spondias pinnata* shows an increase of head dipping in mice which exhibit a significant effect of anxiolytic activity. Results shown in Table 1 and Figure 1. Head dipping at 400mg/kg is slightly in an increase than the standard & control value which indicates a significant effect of anxiolytic activity.

Result & Discussions of Elevated plus-maze test

The test result of the methanolic extract of fruit as plant part of *Spondias pinnata* shows an increase of time spent in open arms in mice which exhibit a significant effect of anxiolytic activity. Results are shown in Table 2 & Figure 2. Time spent in enclosed arm at 400mg/kg is slightly in decrease than the standard & control value which indicates a marked effect of anxiolytic activity.

Statistical analysis

Results obtained were expressed as mean ± SEM (n = 4). The data were analyzed using one-way ANOVA followed by Dennett's post-hoc test using Graph Pad Prism 4 Software. Results were considered significant when $P < 0.05$ (Figure 1 & 2)

CONCLUSION

Crude extract of fruit *Spondias pinnata* used for determination of anxiolytic activity. Two complementary test systems, namely Hole board test and Elevated-plus maze test. Such experiments are used in the field of behavior pharmacology. These pharmacological models have been employed in the evaluation of medicinal plant *S. pinnata* for neuro-pharmacological activities towards the identification of botanicals and drugs with beneficial effects in the treatment of anxiety as diverse CNS disorders. The choice of test methods not only determines effectiveness but in some instances also gives an indication of the mechanism(s) of the test substance. On the basis of above result and available reports, methanolic crude extracts of *Spondias pinnata* had potent anxiolytic activity. Due to the anxiolytic activity; it will be useful for the treatment of anxiety disorder. In the end, it can be concluded that the experimental evidence obtained in the laboratory test model could provide a rationale for the traditional use of this plant. The plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of chemically interesting and biologically important drug candidates.

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