Research Article

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ACUTE ANXIOLYTIC EFFECT OF ETHANOLIC EXTRACT OF *SARACA ASOKA* BARK IN WISTAR ALBINO RATS

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Abstract: Anxiety is defined as a psychological state characterized by abnormal cognitive, somatic, emotional, and behavioral components. Anxiety is normal reaction to stress. However, when it becomes excessive, falls under the classification of an anxiety disorder. Even though Benzodiazepines (BZDs) are the major class of compounds used in anxiety; long-term use of the same may cause many adverse psychological and physical effects. In the present study, we have attempted to evaluate the acute anxiolytic effect of ethanolic extract of *Saraca asoka* Bark (EESAB) in Wistar Albino Rats. The rats weighing 150–200gm were divided into 5 groups containing 6 animals for each dose and were housed for 10 days prior to testing. In this study, control (1% Gum acacia), test drug EESAB (100, 200 & 400mg/ kg) and standard drug Diazepam (1.0mg/kg) were administered orally. One hour after oral administration of the drugs / vehicle, the experiment were conducted by Elevated Plus Maze (EPM). Our results suggest that, behavioral dis-inhibitory effects of EESAB exhibited anxiolytic activity at the dose of 200 and 400 mg/kg compared to control.

Keywords: Ashoka bark, Anti-anxiety, Diazepam.

INTRODUCTION

Anxiety, is a state of excessive fear which is characterized by motor tension, sympathetic hyperactivity, apprehension and vigilance syndromes.[1] It also includes debilitating physical manifestations as headaches, uncontrolled trembling, sweating, muscle tension and aches.[2] Among world population, about one-eighth is suffering from anxiety and it has become a very important area of research interest in psychopharmacology. Benzodiazepines have remained the most commonly prescribed treatment for anxiety and anxiety related disorders, inspite of the unwanted side effects such as sedation, muscle relaxation, ataxia, amnesia and tolerance. [3] Herbal medicines have been used to treat central nervous system (CNS) disorders from many centuries. Importance of plant-derived medications that affect the "mind" is growing globally. Different herbal medicines have been used as anxiolytic drugs in different parts of the world. [4] Medicinal herbs are moving from fringe to mainstream use with a great number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals.[5] Natural sources as medicinal products contain organic substances and could be obtained in both primary and secondary metabolic process since the ancient times. Especially, plant kingdom has proved to be the most useful in the treatment of many diseases and they provide an important source of all the drugs in the world. Bioactive constituents of these plants like steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides etc. have served a valuable starting material for drug development.[6]

Ashoka tree, universally known by its binomial Latin name *Saraca asoca* (Roxb.), De.wild or *Saraca indica* belonging family *Caesalpinaceae*. It is one of the endangered trees called in english as Asok tree. It is also known as Kankeli (Sanskrit), Ashoka (Assamese), Ashoka (Bengali), Ashoka (Gujrati), Ashoka (Hindi), Ashokadamara (Kannada), Ashok (Kashmiri), Asokam (Malayalam), Ashok (Marathi), Ashoka (Oriya), Ashok (Punjabi), Asogam (Tamil), Ashokapatta (Telugu). [7-9] Saraca *asoca* is a small evergreen tree which grows to a height of 7-10 m. It occurs above the altitude of 750 m. Leaves are parpinnate 15-20 cm long and the leaflets 6-12 in number, oblong and rigidly sub-coriaceous. Leaves are narrowly lanceolate, cork like at the base and with a short pestistipules are intra-petiolar and completely united. The bark is dark brown or grey or almost black with warty surface. Stem bark are rough and uneven due to the presence of rounded or projecting lenticles. Bark channeled, smooth with circular lenticles and traversely ridged, sometimes cracked. Flowers are saffron coloured, fragrant, polygamous apetalous, laterally placed corymbose, axillary panicles, bract small, deciduous and calyx petaloid. Seeds are usually 4-8 in number, ellipsoid-oblong and compressed. [10-14]

MATERIALS AND METHODS

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Yenepoya Medical College, Yenepoya University, Mangalore, India.

Animals

Adult male and female Wistar Albino rats weighing

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150-200 gm from our breeding stock were used in this study. The animals were housed at 24±20C with 12:12 hr light and dark cycle. They had free access to food and water *ad libitum*. The animals were acclimatized for a period of 10 days before the study. The study was conducted according to CPCSEA guidelines.

Sample Size, Grouping and Dose of the Drugs:

Animals will be divided in to 5 groups (Control, Standard & Test drug) containing 6 animals making a total number of 30 animals (Table 1).

Table.1: Showing Drugs/Dose of the drugs, groups and number of rats in each groups

Drugs / Dose	Groups	Number of Rats (n=6)
Control (1% Gum acacia)	I	6
Diazepam (1.0mg/kg)	II	6
EESAB	III	6
EESAB	IV	6
EESAB	V	6

Authentification:

The bark of *Saraca asoca* was authentified by Prof. (Dr) Krishna Kumar. G, Chairman, Dept of Applied Botany, Mangalore University, Mangalore, Karnakata, India.

Bark:

The bark of *Saraca asoca* was collected from campus of Yenepoya University, Derlakatte, Mangalore, Karnakata, India.

Extraction:

About 1000gm of shade dried bark of *Saraca asoca* was powdered and was extracted with ethanol in a Soxhlet extractor for 36 hours. It was concentrated to dryness under reduced pressure and controlled temperature (40-50^{o}C) using rotary evaporator. The Ethanolic Extract yielded a brownish mass weighing 165g. Extracts were concentrated by vacuum distillation to dryness; the yield obtained was 16.5% w/w with respect to dried powder.

Elevated Plus Maze (EPM):

The wooden maze consisted of two open arms (length 50 cm X breadth 10 cm) and two closed arms of the same size (height 40 cm). The arms of the same type are opposite to each other, with a central square of 10cm. The maze was elevated to a height of 50 cm above the floor. The apparatus consisted of an open top wooden box. [15]

Behavioral assessment:

For acute study the animals received drugs or vehicle 60 mins before the experiment and each animal was placed in the centre square of the Elevated Plus Maze, facing one of the closed arms. Time spent in open and closed arms and the numbers of rears in open arm in a five-minute period were noted.

Statistical Analysis:

The data were analysed by one-way ANOVA and Post-hoc comparisons were performed by applying Dunnet's multiple comparison test. P <0.05 was considered statistically significant.

DISCUSSION

Even though, anxiety is a normal phenomenon in day to day stress full conditions of life, when exceeds, it becomes anxiety disorder. Anxiety has become the most frequent mental disorder. In this condition the most commonly used anxiolytics are benzodiazepenes, but it has adverse effects on long run. Hence there is a search for newer anxiolytic agent at present status. EPM is one of the experimental models for preclinical screening of anxiolytic drugs in rodents. The open arms are more fear provoking than the closed arms in the EPM. The number of entries, time spent and rearing behaviour in open arms to closed arms reflects the safety of closed arms when compared to fearfulness of open arms.

The reduction in entry, time spent, rearing in open arms, ratio of open arm to total arm entries and increased defecation are the indications of high level of fear or anxiety. Anxiolytic drugs tend to increase the proportion of entries, time spent and rearing in open arms. They also increase the ratio of open arm to total arm entries. [16]

In the present study, group IV and V that received EESAB at the dose of 200mg/kg and 400mg/kg showed a significant increase in the time spent and the rears in open arms with the p value of p <0.05 and p< 0.01 respectively (Table 2, Fig 1 & Fig 3). Both the doses have also shown a decrease in time spent in closed arms (Table 2 & Fig 2). All these suggest that decreased fear, an increased exploratory behavior and the behavioral dis-inhibitory effect of EESAB at the dose of 200mg/kg and 400mg/kg comparable to diazepam, the standard anxiolytic.

Table.2: Showing Time Spent in Open Arms, Closed Arms and Number of Rears in Open Arms

Groups / Dose	Time spent in open arms (Sec)	Time spent in closed arms (Sec)	Number of rears in open arms
Control (1% Gum acacia), p.o	15.27±0.93	267.78±6.61	4.66±0.98
Diazepam (1.0mg/kg), p.o	105.93±4.57***	188.72±2.47***	19.83±1.44***
EESAB 100 mg / kg, p.o	57.94±16.55*	242.05±16.55**	5.16±0.79*
EESAB 200 mg/kg, p.o	65.18±12.00**	234.81±12.00**	9.66±1.20**
EESAB 400 mg/kg, p.o	128.35±18.04***	171.65±18.04***	20.83±1.40***

N=6, Observations are Mean±SEM. *p>0.05, **p<0.05, ***p<0.01
ANOVA followed by Dunnet's multiple comparison test
EESAB-Ethanolic Extract of *Saraca asoka* Bark, p.o-per oral

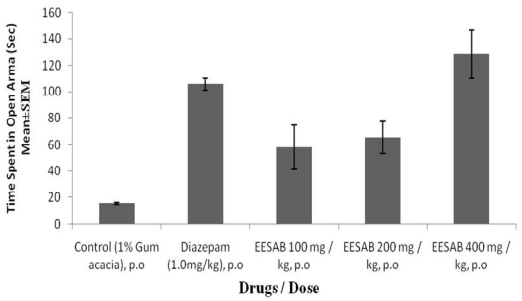

Fig.1: Time spent in open Arms

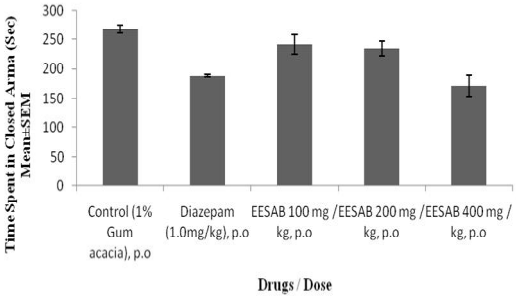

Fig.2: Time spent in closed Arms

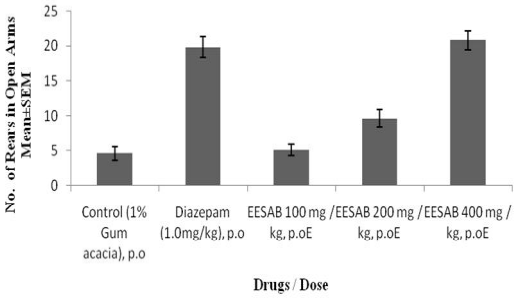

Fig.3: number of rears in open armst

The Ethanolic Extract of *Saraca asoka* bark contains total phenol and tannin content.[17] Tanins have neuro-protective functions capable of reversing 6-hydroxydopamine induced toxicity. Tannic acid has shown as a good therapeutic agent in patients with neurologic disease. [18]

Our preliminary animal study suggests that, the Ethanolic Extract of *Saraca asoka* bark has anxiolytic activity at the dose of 200mg/kg and 400mg/kg comparable to diazepam, the standard anxiolytic. However further research are required to evaluate the exact mechanism of action of extract of *Saraca asoka* bark as a potent and efficacious anxiolytic agent.

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