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Chronic anxiolytic activity of ethanolic extract of *Saraca asoca* bark in wistar albino rats

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Abstract

Objective: Anxiety is characterized by psychologically affected abnormal cognitive, somatic, emotional, and behavioral components. Anxiety is usually a normal reaction to stress in day to day life. But, when it becomes excessive, it comes under anxiety disorder. Commonly benzodiazepines (BZDs) are the major class of drugs used in anxiety and related disorders but, long-term use of the same may cause many adverse psychological and physical effects which vary from mild to severe. The objective of our study was to evaluate the chronic anxiolytic effect of Ethanolic Extract of Saraca asoca Bark (EESAB) in Wistar Albino Rats. **Material and Methods:** The rats weighing 150–200 gm 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 & 400 mg/ kg) and standard drug Diazepam (1.0 mg/kg) were administered orally once a day for ten days and one hour before the experiment on tenth day. Experiments were conducted by Elevated Plus Maze (EPM). **Results and Conclusions**: Our results suggest that, behavioral disinhibitory effects of EESAB exhibited chronic anxiolytic activity at the dose of 200 and 400 mg/kg compared to control.

Keywords: Anti-anxiety, Diazepam, Ethanolic Bark Extract, *Saraca asoca*.

Introduction

Anxiety is a cardinal symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions.[1] Anxiety is a normal emotional behavior, when it is severe and/or chronic and disturbs the day-to-day activities, it becomes pathological and can precipitate or aggravate cardiovascular and psychiatric disorders. Although many drugs are available in modern medicine to treat anxiety disorders, they produce various systemic side effects or exhibit tolerance upon chronic use.[2]

Despite the advent of new molecules in the pharmacotherapy of anxiety, it is unfortunate that this disorder goes undiagnosed and untreated. Although the currently prescribed molecules provide some improvement in the clinical condition of the patient, it is at the cost of having to bear the burden of their adverse effects. Hence, finding newer and safer therapeutic agents would benefit the existing treatment modalities.[3]

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).[4]

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.[5]

This plant is proven to have antimicrobial, anti-oxytocic and anticancer properties in various preclinical studies. Phytochemical analysis of the study has shown that *Saraca asoca* bark extract possess Phytosterols, Triterpenoids, Carbohydrate, Glycosides, Flavonoids, Phenolic compound and Tannins.[6]

Our previous study has revealed acute anxiolytic activity of the *Saraka asoca* bark in preclinical models.[7] Hence the preset study was carried out to evaluate the chronic anxiolytic activity of EESAB in Wistar albino rats using elevated plus maze.

Materials and Methods

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Yenepoya Medical College, Yenepoya University, Mangalore 575018, India.

Animals

Adult male and female Wistar Albino rats weighing 150-200 gm from our breeding stock were used in this study. The animals were housed at 24±2ºC 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 Test Drugs

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

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 campus, Derlakatte, Mangalore 575018, Karnakata, India.

Extraction

About 1000 gm 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 (40-50ºC) 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.

Apparatus

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 10 cm. The maze was elevated to a height of 50 cm above the floor. The apparatus consisted of an open top wooden box7.

Behavioural assessment

All the drugs were given once daily orally and on tenth day of experiment drug and vehicle were administered 60min 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.[7]

Statistical Analysis

The data were analysed by one-way ANOVA followed by Dunnet's multiple comparison test. $P < 0.05$ was considered statistically significant.

Results and Discussion

Generally anxiety is a normal phenomenon in day to day life. It becomes anxiety disorder only when it exceeds normal behavior. In present world anxiety has become the most frequent psychological disorder. In this condition the most commonly used anxiolytics are benzodiazepines. But adverse effects of the same on long run are well documented. Hence there is a search for newer anxiolytic agent at present scenario.

EPM is a proven experimental model for preclinical screening of anxiolytic drugs in rodents. The open arms are more fear provoking than the closed arms in the EPM as rodents are nocturnal animals. The number of entries, time spent and rearing behavior 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.

In the present study, group IV and V that received EESAB at the dose of 200 mg/kg and 400 mg/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. Both the doses have also shown a decrease in time spent in closed arms. (Table 2 & Figure 1). All these suggest that decreased fear, an increased exploratory behavior and the behavioral dis-inhibitory effect of EESAB at the dose of 200 mg/kg and 400 mg/kg comparable to diazepam, the standard anxiolytic.

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), p.o	I	6
Diazepam (1.0 mg/kg) p.o	II	6
EESAB (100 mg/kg) p.o	III	6
EESAB (200 mg/kg) p.o	IV	6
EESAB (400 mg/kg) p.o	V	6

Table 2: Effect of Chronic Administration of EESAB on Behaviour of Wistar albino rats in Elevated Plus Maze test

Drug groups (n=6)	Time spent in open arms(Sec)	Time spent in closed arms(Sec)	No. open arms entries	No. Closed arm entries	No. rears in open arms
1% Gum acacia (10.0 ml/kg)	20.40±2056	267.85±3.68	1.83±0.75	3.83±0.75	2.00±0.89
Diazepam (1.0 mg/kg)	116.34±9.25***	167.28±10.01****	11.66±1.86***	4.16±0.75***	8.66±1.21***
EESAB (100mg/kg)	45.54±17.80*	245.79±17.1*	2.66±0.81	4.50±1.04	3.00±1.41
EESAB (200mg/kg)	89.90±21.52***	195.26±20.72***	6.16±0.75***	4.00±1.09***	6.16±0.75***
EESAB (400mg/kg)	118.91±24.10***	168.06±25.11***	10.66±1.21***	3.66±1.21***	8.16±0.75***

All values are expressed as mean±S.E.M. ANOVA followed by Dunnet's multiple comparison test $P<0.05$ was taken as significance. *p>0.05, **p<0.05, *** p<0.001 compared to control. EESAB-Ethanolic Extract of *Saraca asoca* Bark

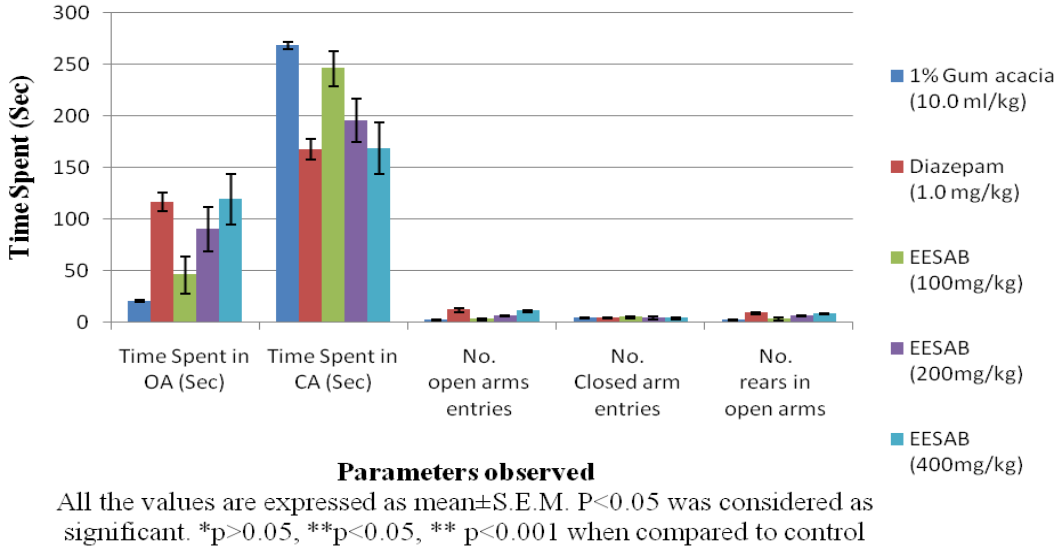

Figure 1: Effect of Chronic Administration of EESAB on Behaviour of Wistar albino rats in Elevated Plus Maze test

Conclusion

Our results suggest that, behavioral dis-inhibitory effects of EESAB exhibited chronic anxiolytic activity at the dose of 200 and 400 mg/kg compared to control (Table 1).

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