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Evaluation of antistress activity of *Cassia auriculata* seed extract

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ABSTRACT

Objective: The present study was designed to investigate the anti-stress activity of *Cassia auriculata* ethanolic seed extract in mice. **Methodology:** The anti-stress effect was evaluated by using Elevated plus maze (EPM), Force swimming test (FST) and Tail suspension test (TST). The ECS at a doses (250,500 and 1000 mg/kg p.o.) and standard (diazepam 2 mg/kg i.p and fluoxetine 20 mg/kg i.p) was administered. **Results:** The extract showed the increased in the number of entries and time spent in open arm in Elevated plus maze and decreased in the immobility time in both Force swimming test and Tail suspension test. **Conclusion:** The effect of ECS on animal behavior was concordant with a significant regulation of GABA and stress hormones. Therefore, this study was attempted to demonstrate the preventive potential of ECS against stress disorders at *in vivo* levels.

Keywords: *Cassia auriculata* seeds, Elevated plus maze, force swimming test, tail suspension test.

INTRODUCTION

Stress is a complex, dynamic process that effects the homeostasis. It is the condition in which one reacts physically, mentally and emotionally to the various conditions. Stress may caused by constant pressure both at work and home are a modern phenomenon. Susceptibility to illness rises progressively with increase in the severity of life-crisis from mild to severe [1]. *Cassia auriculata* (family:Caesalpiniaceae) has medicinal properties and that grows in various topical regions. The root, stem, leaves, flowers and unripe fruit are used for treatment, especially in Ayurvedic medicine. It is used for eye infections, joint and muscle pain (rheumatism), constipation, jaundice, diabetes, liver disease and urinary tract problems. *Cassia auriculata* has been reported to possess antimicrobial activity [2] anti diabetic function [3], Antibacterial, anti oxidant [4], Anti obesity [5], Antioxidant activity [6], Anti-ulcer activity [7], Anxiolytic Activity [8], antihelminthic activity [9], Anti-inflammatory and analgesic [10], Immunomodulatory activity [11]. However majority of to overcome ADR the world today is carrying out many research works on herbal many phytochemicals of significant use. In addition, *Cassia auriculata* has been widely used in Ayurvedic medicine. The present study involves the seeds of *Cassia auriculata* were extracted with ethanol and evaluated for antistress activities by using diazepam and fluoxetine as standard drugs.

MATERIALS AND METHODS

Animals

Albino Mice (20g) of either sex were used for the study. Animals were obtained from Bhaskar medical college (1758/PO/ERe/S/14CPCSEA). The study was conducted in accordance with the guidelines issued by CPCSEAA authority regulating animal experiments and was approved by the Institutional Animal Ethics Committee.

Plant material

The Seed of *Cassia auriculata* were collected from local areas of Ananthagiri, Vikarabad, Telangana, India and authenticated by Department of Pharmacognosy, Jawaharlal Nehru Technological University, Hyderabad.

Preparation of extract

*Cassia auriculata*ethanolic seed extract (ECS) was prepared by using ethanol, by maceration technique for 72hrs at room temperature. The extract was concentrated by simple evaporation at room temperature.

A suspension of ECS in water was prepared for oral administration.

Acute toxicity study

The different extracts of ECS were screened for acute toxicity studies with the standard procedure (OECD: 423). Animals were maintained at laboratory conditions. The dose of all extract was prepared with water and was administered orally. The acute toxicity was tested upto dose of 5000mg/kg.

Elevated Plus Maze Model

The elevated plus maze model was used to evaluate the antistress activity in mice. The maze consists of two enclosed arms (50×10 cm) having 40 cm high walls crossed with opposite open arms of the same dimension with a central square (10×10 cm) giving the apparatus the shape of plus sign. The whole setup was kept at a height of 50 cm above the floor^[15]. Mice were divided into groups of five and received the compounds cassia auriculata at different doses viz. 250, 500 and 1000mg/kg control and diazepam (2mg/kg) was used as standard drug. One hour post administration each mice were placed individually in the centre of maze, facing an open arm where the number of entries and time spent on the open and enclosed arms were noted for 5 min. An arm entry is considered only when all the four paws of mice were in the arm^[12].

Forced swimming test

The FST is the most widely used pharmacological in vivo model for assessing antistress activity. Mice were individually placed in a cylinder (45×20 cm) containing 15 cm water (25±2°C), so that it could not touch the bottom of the cylinder with its hind limb or tail, or climb over the edge of the chamber.¹ Mice were divided into groups of five and received the compounds *cassia auriculata* at different doses viz. 250, 500 and 1000mg/kg control and Floxutine (20mg/kg) was used as standard drug. One hour post administration each mice were placed individually in a tank. Period of immobility (i.e. the total time the animal remained floating in water without struggling and making only those movements necessary to keep its head above water) during the 6 min test period was measured^[13].

Tail suspension method

Each mouse in the group were suspended individually by the end of tail (50 cm above the floor) with adhesive tape placed approximately 1cm from the tip of the tail. Mice were divided into groups of five and received the compounds *cassia auriculata* at different doses viz. 250, 500 and 1000mg/kg control and Floxutine (20mg/kg) was used as standard drug on the test day after 60mins of the administration of last dose. The Duration of immobility was observed for a period of 8 minutes. After the early escape oriented actions, the rat rapidly turns out to be immobile and immobility (when it did not show any movement of body and hanged passively) was recorded during last 5 mins of observation period^[14].

Statistical Analysis

The data were expressed as mean ± standard error mean (SEM). The significance of differences among the groups was assessed using one way analysis of variance (ANOVA). The test was followed by Dunnett's test, p values less than 0.05 were considered as significance.

RESULTS

Preliminary Phytochemical Analysis

Qualitative phytochemical studies were performed on *Cassia auriculata* ethanolic seed extract using suitable chemicals and reagents to confirm the presence of flavonoids, tannins, lipids, polyphenols, triterpenoids and steroids.

Acute toxicity studies

The albino mice were fasted over night, the *Cassia auriculata* ethanolic seed extract was administered to the animals orally up to the dose 5000 mg/kg. No considerable sign of toxicity and mortality observed in all test animals when they were subjected to acute toxicity studies upon oral administration of 5000mg/kg for 13 days. It was conformed that the test drug ECS is practically non-toxic in normal mice. 1/10th of dose was considered as therapeutic dose and to identify the dose dependent action the 50% and 200% of therapeutic dose was considered as minimum and maximum dose for further pharmacological evaluation in animal model.

Elevated Plus Maze Model (EPM)

The mean number of entries and time spent by mice in open and closed arms after the drug administration are given in Table 1. The results showed that the number of open arm entries and time spent in the open arms were increased and number of closed arm entries and time spent in the closed arms were decreased significantly in the extract treated groups which was comparable with control group.

The mean number of entries into open arm by control, test doses (250, 500, 1000mg/kg) and standard drug was 3.1±1.06, 5.5±1.14, 7.25±1.34, 9.8±1.49 and 12.4±1.51 respectively. The mean number of entries into closed arm by control, test doses (250, 500, 1000mg/kg) and standard drug was 15.4±0.39, 10.1±0.14, 9.2±0.14, 8.1±0.24 and 7.02±1.52 respectively. The mean time spent in open arm by control, test doses (250, 500, 1000mg/kg) and standard drug was 13.8±0.42, 22.1±0.44, 29.1±0.49, 42.1±0.81 and 72.2±1.82 respectively. The mean time spent in closed arm by control, test doses (250, 500, 1000mg/kg) and standard drug was 212±1.21, 181±0.69, 172.1±0.69, 131.1±0.85 and 98.1±1.49 respectively. It was found that the mean number of entries into open arm was moderately significant for test doses (250 and 1000mg/kg), and significant for test dose (500mg/kg) and standard compared to control group.

Forced swimming test

In forced swim test, the immobility time of control, test (250, 500 and 1000 mg/kg) and standard was 151±1.25, 90.5±2.6*, 68 ±6.2*, 61.2±2.54* and 55.01 ±.54*** respectively. The immobility time of test and standard was significant (**p < 0.01) and more significant (**p < 0.001) respectively. The immobility time decreases with dose dependent manner. The immobility time of test was gradually decreases when compared to control.

Tail suspension test

In tail suspension test, the immobility time of control, test (250, 500 and 1000mg/kg) and standard was 248.2±2.5, 210.5±4.6, 201.3±4.2, 189.5±2.54 and 178.6±1.54 respectively. The immobility time of test

and standard was significant (** $p < 0.01$) and more significant (***) ($p < 0.001$) respectively. The immobility time decreases with dose dependent manner. The immobility time of test was gradually decreases when compared to control.

DISCUSSION

Present day lifestyle has made human beings to be exposed to stressful conditions which results in physical and physiological abnormalities. So, one has to improve one's own adaptability to various stressful conditions. Chemical substances like neurotransmitters are functionally involved in the regulation of stress responses and are meant to provide resistance against stressful conditions. This phenomenon is called adaptability. If the stress conditions are prolonged it results in ineffective adaptation leading to reduced stamina or mood. Though a few synthetic drugs are available, they are expensive and are associated with many side effects. Therefore, many alternative methods like yoga, herbal medicines have become the present day's interest to treat stress [15]. Medicinal plant research, worldwide has progressed gradually denoting the pharmacological effectiveness of various plant species in different animal models [16]. Since the introduction of adaptogens, several plants that had once been used as tonics have been investigated in Ayurvedic medicine for their adaptogenic and rejuvenating properties [17]. The present study aims at evaluating antistress activity of *Cassia auriculata* seed extract. Stressful situations may produce gastric ulcers which have been observed to have multifactorial pathophysiology [18].

Elevated plus maze test is one of the widely used experimental methods to evaluate antistress activity. Animals when placed facing open arm tend to spend most of the time in closed arm due to stress when exposed to the new environment. After administration of the drug significantly decreased time spent in closed arm and increase in time spent in open arm is noted due to antistress activity of drug. Mice received the standard diazepam showed significant time spent in open arm at a dose of 2 mg/kg i.p. The mean time spent by the ECS treated mice in open arm, indicate that the antistress activity has increased in a dose dependent manner and was found significantly highest for those treated with 1000 mg/kg dose of test drug.

The forced swimming test is the most widely used method for the evaluation of antistress property of a novel compound. Mice when forced to swimming in a restricted space, become immobile after an initial period of vigorous activity, indicating the stress. Mice pretreated with ECS show significant improvement in the swimming time [19].

This method is based on the observation that animals forced to swim in water eventually assumed a characteristic immobile posture, devoid of any activity. The appearance of immobility, therefore indicate a state of tiredness, fatigue, reduced stamina or a lowered mood (hopelessness). These signs represent the core symptoms observed in individuals under intense stress. It is well known that drugs with anti-stress properties reduce the duration of immobility in animals [20].

The pretreatment with ECS increases swimming endurance in mice. Mice with ECS shown significant improvement in the swimming time. The antistress effect of the ECS was prominent at 1000mg/kg. In the forced swimming test all the doses administered were able to reduce immobility time and simultaneously enhance swimming. In the tail suspension test, the mice shown immediate sign of struggles or escape like behaviors when they were suspended in the air followed by temporary increasing periods of immobility. The tail suspension method revealed in the present study that anti stress activity increases with decrease in immobility time compared to control mice of ECS [21].

Cortisol is also released in response to fear or stress as a part of fight or flight mechanism. The elevated levels of cortisol may also interfere with learning and memory, lower bone density and immune function, increased blood pressure, weight gain, heart diseases and cholesterol. Gamma amino butyric acid (GABA) plays a major role in the central integration of the hypothalamic-pituitary-adrenocortical (HPA) stress responses. GABAergic neurons in the bed nucleus of the striaterminalis, preoptic area, and hypothalamus can directly inhibit paraventricular nuclei outflow, and thereby, reduce adrenocorticotrophic hormone secretion. Thus, GABA produces a marked inhibitory effect on HPA axis activity. However the decreased utilization of amines in various regions of brain also helps to reduce stress, as they are responsible for causing stress.

ECS may decrease the release of Cortisol or CRF or ACTH from the HPA axis and increase the level of GABA that has inhibitory effect on HPA axis. Hence increased GABA activity leads to hindrance to the stimulation of hypothalamus for CRF release. In turn it decreases the release of ACTH and cortisol from pituitary and adrenal gland respectively. The results revealed that ECS having higher antistress activity. Due to presence of various phytoconstituents like alkaloids, tannins, flavonoids, triterpenoids, lipids and steroids in ECS shown antistress activity. Various phyto chemical present in ECS also increases the enzyme succinate dehydrogenase in the brain which is responsible for consumption and maintenance of energy in the cellular system of the organism, which helps in adaptive processes during stress.

Table 1: Effect of *Cassia auriculata* seed extract on animals in Elevated plus Maze Model

Group	Treatment	Mean no. of entries in		Mean time spent in (sec)	
		Open arm	Closed arm	Open arm	Closed arm
I	Control- water	3.1±1.06	15.4±0.39	13.8±0.42	212±1.21
II	ECS 250 mg/kg. p.o	5.5±1.14*	10.1±0.14*	22.1±0.44*	181±0.69*
III	ECS 500 mg/kg. p.o.	7.25±1.34**	9.2±0.14*	29.1±0.49*	172.1±0.69*
IV	ECS 1000 mg/kg. p.o.	9.8±1.49*	8.1±0.24*	42.1±0.81*	131.1±0.85*
V	Diazepam-2mg/kg. i.o.	12.4±1.51**	7.02±1.52**	72.2±1.82**	98.1±1.49*

$n = 6$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (one way ANOVA followed by Dunnett's 't' test)

Table 2: Effect of *Cassia ariculata* seed extract on animal in Forced swim test

Group	Treatment	Immobility time (sec)
I	Control- water	151±1.25
II	ECS 250 mg/kg. p.o.	90.5±2.6*
III	ECS 500 mg/kg. p.o.	68.2 ±6.2**
IV	ECS 1000 mg/kg. p.o.	61.2±2.54*
V	Fluoxetine – 20 mg/kg. i.o.	55.1 ±0.54**

n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dunnett's 't' test)

Table 3: Effect of *Cassia ariculata* seed extract on animal in Tail suspension test

Group	Treatment	Immobility time (sec)
I	Control- water	248.2±2.5
II	ECS 250 mg/kg. p.o.	210.5±4.6**
III	ECS 500 mg/kg. p.o.	201.3±4.2*
IV	ECS 1000 mg/kg. p.o.	189.5±2.54**
V	Fluoxetine – 20 mg/kg. i.o.	178.6±1.54**

n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dunnett's 't' test)

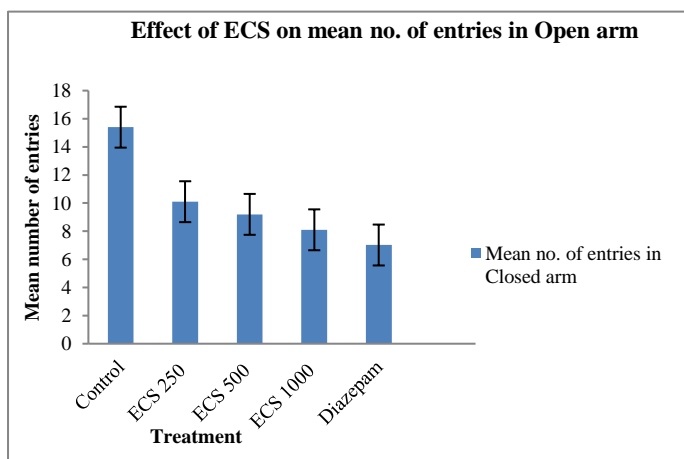


Figure 1: Effect of ECS on Mean No. of Entries into Open Arm

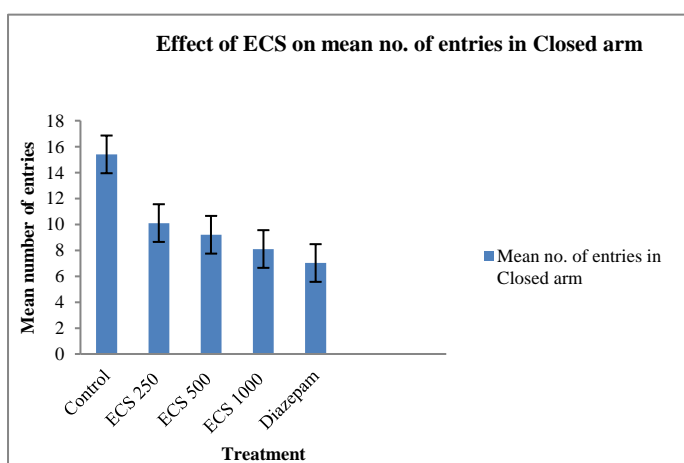


Figure 2: Effect of ECS on Mean No. of Entries into closed Arm

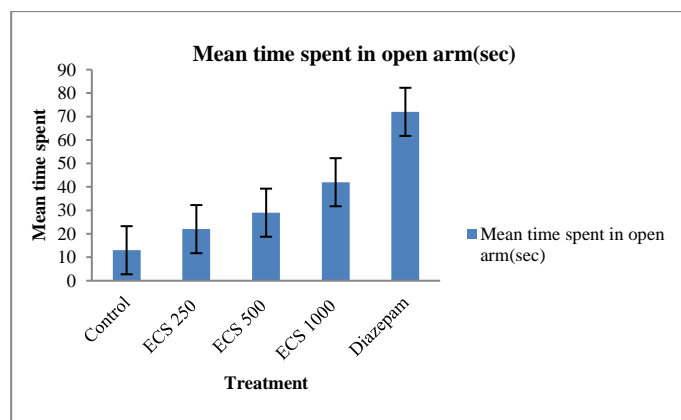


Figure 3: Effect of ECS on Mean time Spent in open arm

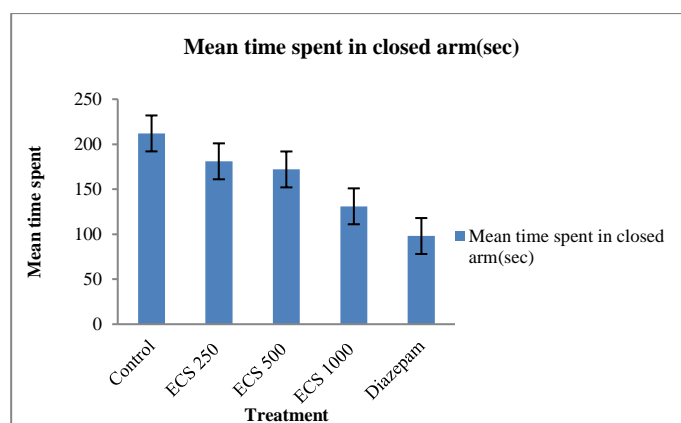


Figure 4: Effect of ECS on Mean time Spent in closed arm

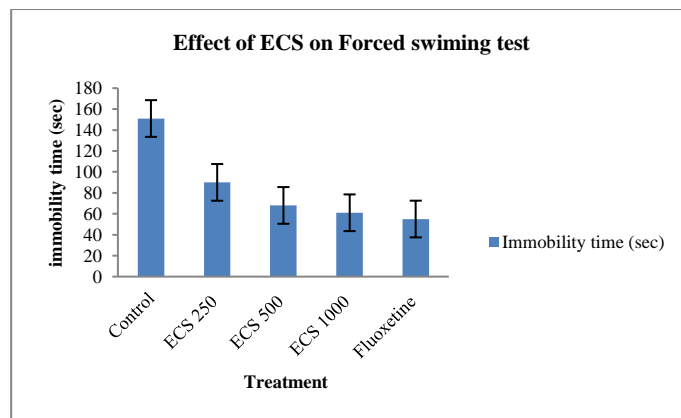


Figure 5: Effect of ECS on Forced swimming test

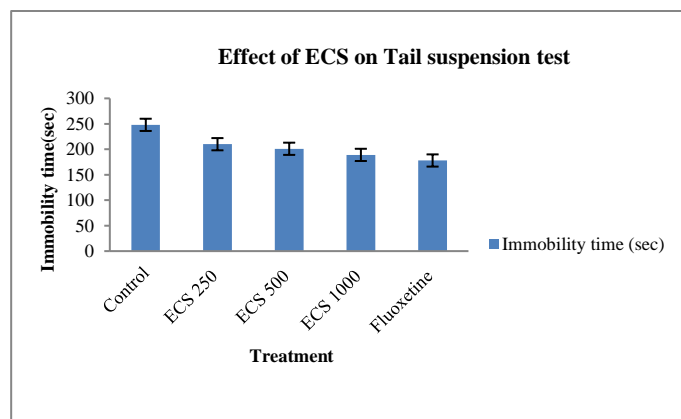


Figure 6: Effect of ECS on Tail suspension test

CONCLUSION

From the present investigation, the effectiveness of the extract depends upon the effect of active constituents. *Cassia auriculata* seed extracts showed anti stress activity against Elevated plus maze, Forced swimming test and Tail suspension test model *in vivo*. Further, studies with purified isolated phytochemical constituents are needed to understand the complete mechanism of anti stress activity.

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