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Research Article

EVALUATION OF ANTIANXIETY AND ANTIDEPRESSANT ACTIVITY OF *CASSIA OCCIDENTALIS* LEAVES

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ABSTRACT

Anxiety and Depression are widespread psychiatric disorders affecting around 5% of the population. Furthermore, it is difficult to predict which patient will respond to any given treatment. In the traditional systems of medicine, many plants and formulations have been used to treat anxiety and depression for thousands of years. The present study was designed to evaluate the antianxiety and antidepressant activity of the ethanolic and aqueous extracts of *Cassia occidentalis* leaves in rodents. Antianxiety activity was tested by exposing rats to unfamiliar aversion in different methods like elevated plus maze model and actophotometer. The results infer that reduced aversion fear elicits antianxiety activity. The antidepressant activity was tested by using despair swim test and tail suspension test. The results infer that reduced immobility time elicits antidepressant activity. It was concluded that ethanolic and aqueous extracts of *Cassia occidentalis* leaves having antianxiety and antidepressant activity. Ethanolic extract of *Cassia occidentalis* leaves showing more significant activity over the aqueous extract.

Keywords: *Cassia occidentalis*, Antianxiety activity, Antidepressant activity, Elevated plus maze, Actophotometer, Despair swim test.

INTRODUCTION

According to the World Health report [1], approximately 450 million people suffer from mental or behavioral disorders, yet only a small minority of them receives even the most basic treatment. This amounts to 12.3% of the global burden of disease and will rise to 15% by 2020 [2]. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide has progressed constantly demonstrating the pharmacological effectiveness of different plant species in a variety of animal models [3]. Anxiety and depression are extremely dramatic and debilitating multifacetic disorders and it is now becoming clear that without knowledge of clinical and biological aspects of anxiety and depression, it is impossible to offer effective treatment strategies for the patients. Over the past decades, there has been intensive study of a variety of neurobiological aspects of depression and anxiety. Currently the most widely prescribed medications for anxiety disorders are benzodiazepines. But the clinical applications of benzodiazepines as antianxiety agents are limited by their unwanted side effects. Therefore the development of new pharmacological agents from plant sources are well justified. *Cassia occidentalis* a native plant of southern India, called as Kasmard in Sanskrit, Kasondi in Hindi and Coffee senna in English belongs to family Caesalpiniaceae. Its common name is ponnavari. The parts used are leaves, seeds and roots. It is used for fever, menstrual problems, tuberculosis and as a tonic for general weakness and illness [4]. *Cassia occidentalis* leaf extracts have anti bacterial [5,6,7], antiplasmodial [8], antimutagenic [9], hepatoprotective[10] and anti-diabetic activity[11]. The purpose of the present study was to evaluate the antianxiety and antidepressant activity of ethanolic and aqueous extracts of *Cassia occidentalis* leaves.

MATERIALS AND METHODS

Plant material

Collection and authentication of plant materials

The leaves of *Cassia occidentalis* belonging to the family Caesalpiniaceae were collected in the month of april 2011 from the local areas of Anantapur district, Andhra Pradesh, India. The plant material was identified and authenticated by Dr. J. Raveendra Reddy, M.Pharm., PhD, Department of Pharmacognosy, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur and voucher specimen (13/11) was preserved in Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, India.

Processing of sample Leaves were dried in shade for 25 days and then powdered to get a coarse powder. This powder was stored in an air tight container and used for successive extraction.

Preparation of extracts

Preparation of ethanolic extract of *Cassia occidentalis* leaves

The leaves of *Cassia occidentalis* were shade dried and reduced to coarse powder. The standardized coarse powder was evenly packed in the soxhlet apparatus and subjected to ethanol extraction. The extract was filtered and filtrate was concentrated by vaccum distillation. Percentage yield of ethanolic extract was found to be 13.8%.

Preparation of aqueous extract of *Cassia occidentalis* leaves

The leaves of *Cassia occidentalis* were powdered and extracted by maceration process by using 300ml of distilled water. In maceration procedure, total amount of 50g of powdered leaves were macerated for 3 days it was occasionally stirred at regular intervals of time. It was filtered and concentrated. Then it was dried by rotary evaporator. The percentage yield of aqueous extract was found to be 11.3%.

Phytochemical analysis

Both the ethanolic and aqueous extracts of *cassia occidentalis* were subjected to preliminary phytochemical screening. [12]

Experimental animals

Wistar rats (150-200 g) and swiss albino mice (18-22g) of either sex were procured from Bioneeds, Bangalore and acclimatized at the animal house of Raghavendra Institute of Pharmaceutical Education and Research, Anantapur. All the animals were maintained under standard conditions, that is room temperature 26 ± 1°C, relative humidity 45 - 55% and12:12 h light – dark cycle.

Acute toxicity studies

Swiss albino mice of either sex (18-22 g weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD and animals were observed for mortality and behavioural changes[13].

Ethical approval

The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Raghavendra Institute of Pharmaceutical Education and Research, Anantapur. (878/ac/05/CPCSEA/014/2011) and all the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Drugs and chemicals

Fluoxetine (Crescent Therapeutics limited, Himachal Pradesh), Diazepam (Ranbaxy Laboratory limited.), Ethanol (Loba Chemicals Mumbai.).

Antianxiety and antidepressant activity

The ethanolic and aqueous extracts of *Cassia occidentalis* leaves were tested for antianxiety activity using elevated plus maze and actophotometer and antidepressant activity using despair swim test and tail suspension test.

Treatment

Animals were divided into four (I-IV) groups.

Group I - Control group received distilled water (1ml, p.o).
Group II - Standard group received Diazepam (5mg/kg i.p).
Standard group received Fluoxetine (10mg/kg i.p).
Group III - Test group received ethanolic extract of *Cassia occidentalis* (500mg/kg p.o).
Group IV - Test group received aqueous extract of *Cassia occidentalis* (500mg/kg p.o).

ANTIANXIETY ACTIVITY

Elevated plus maze (EPM) model

The apparatus comprises of two open arms (35x5cm) and two closed arms (30x5x15cm) that extend from a common central platform (5x5cm). The floor and walls of the closed arms are made of wood and painted black. The entire maze is elevated to a height of 50 cm above the ground level. Rats weighing (150 – 200gms) were housed in a pair of 10 days prior to the test in the apparatus. During this time the rats were handled by the investigator on alternate days to reduce stress. 30 min and 60min after oral administration of the drug treatment, each rat was placed in the center of the maze facing one of the enclosed arms. During five minutes session, number of entries into open arm and time spent in the open arm were noted[14,15]. The procedure was conducted preferably in a sound attenuated environment.

Locomotor activity

The locomotor activity can be easily studied with the help of actophotometer, the rats were grouped and treated with drugs. Each animal was placed individually in actophotometer and the basal activity score of all the animals were recorded for 10 mins after 30 and 60 min of drug treatment[16].

ANTIDEPRESSANT ACTIVITY

Despair Swim Test Apparatus

For the determination of antidepressant activity, forced swim test (FST) protocol was employed. During the test, animals were individually placed in a glass cylinder (20 cm in height, 14 cm in diameter) filled with water up to a height of 10cm, at 25 ± 2°C. All animals were forced to swim for 5 min and the duration of immobility was observed and measured during the 5 min interval of the test. Immobility period was regarded as the time spent by the rats to float in water with no struggle and making only those movements necessary to keep its head above the water. In order to check the fitness level of each test animal, a pre-test was carried out 24 h before the FST by subjecting each test animal to a session of 15 min swimming.

Tail suspension test

Tail suspension test was performed based on the method prescribed[17]. The mice were suspended 58cm above the floor by means of an adhesive tape, placed approximately 1cm from the tip of the tail. The total duration of immobility was quantified during a test period of 5min. Mice were considered immobile when they were completely remain motionless.

RESULTS

Physical properties of the extracts

The colour, texture and the percentage yield of the ethanolic and aqueous extracts of leaves of *cassia occidentalis* were tabulated in table 1.

Acute toxicity

The acute toxicity study revealed the non toxic nature of all the extracts even at a higher dose of 4 g/kg body weight of mice for oral route of administration. For the present study the dose is being selected as 500mg/kg p.o.

Phytochemical analysis

After subjecting to screening, both the ethanolic and aqueous extracts of leaves of *cassia occidentalis* revealed the presence of flavonoids, glycosides, tannins and saponins. The details of phytochemical constituents are given in table 2.

Table 1: Physical properties of *Cassia occidentalis* leave extracts.

Plant part	Type of extract	% Yield	Texture	Colour
Cassia occidentalis leaves	Ethanolic extract	13.8	Gummy	Reddish brown
	Aqueous extract	11.3	Gummy	Greenish

Table 2: Phytochemical analysis of *Cassia occidentalis* leaves extracts

Phytochemicals	Ethanolic extract	Aqueous extract
Flavonoids	+	+
Glycosides	+	+
Saponins	+	+
Tannins	+	+
Alkaloids	-	-

+ indicates presence; – indicates absence of the phytochemical constituents which were screened using various identification tests.

Table 3: Effect of Ethanolic and Aqueous Extracts of *Cassia Occidentalis* Leaves on Locomotor Activity (Actophotometer) In Rats at Different Time Intervals.

Group	Treatment	Photo cell count	(Count/600Sec)	% change in activity	
		30 min	60 min	30min	60min
I	CONTROL (VEHICLE)	306±5.032	307.7±4.842	NA	NA
II	Diazepam (5mg/kg i.p)	119*±1.528	84.67*±2.690	61.1(↓)	72.45(↓)
III	Ethanolic extract (500 mg/kg p.o)	145.7*±4.910	97.33*±1.856	52.4(↓)	68.35(↓)
IV	Aqeous extract (500 mg/kg p.o)	167.3*±2.404	118.3*±1.453	45.32(↓)	61.53(↓)

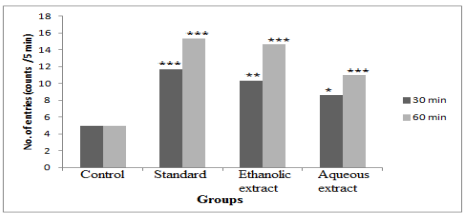
NA- Not Applicable, *P<0.001 when compared to Control

Assessment of antianxiety activity

Elevated plus-maze model

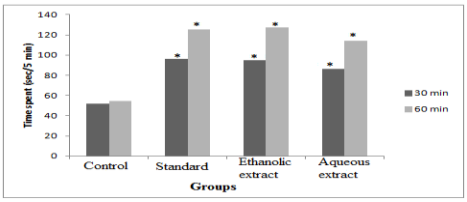
In elevated plus-maze test (EPM), the ethanolic and aqueous extracts of *cassia occidentalis* leaves at a dose of 500 mg/kg p.o. significantly increased the number of entries and time spent into the open arm.

The magnitude of the antianxiety effects 500mg/kg p.o. of ethanolic and aqueous extracts of *cassia occidentalis* was comparable to that of diazepam 5 mg/kg i.p. (Figure 1 and 2).



*****P<0.001, **P<0.01 *P<0.1 when compared to Control**

Fig.1: Effect of ethanolic and aqueous extracts of *Cassia occidentalis* leaves on number of entries (open arm) in elevated plus maze.



***P<0.001 when compared to Control**

Fig . 2 :Effect of ethanolic and aqueous extracts of *Cassia occidentalis* leaves on time spent (open arm) in elevated plus maze

Actophotometer

The average of basal activity scores in the control group after 30 and 60min of administration of ethanolic and aqueous extracts of *cassia occidentalis* leaves 500 mg/kg p.o. significantly reduced the locomotor activity. It may be due to the CNS depressant property of the drug (Figure 3).

Assessment of antidepressant activity

Despair swim test apparatus

In despair swim test apparatus, the ethanolic and aqueous extracts of leaves of *cassia occidentalis* at a dose of 500 mg/kg p.o. significantly decreased the immobility time. The magnitude of the antidepressant effects of 500 mg/kg p.o. of ethanolic and aqueous extracts of leaves of *cassia occidentalis* was comparable to that of fluoxetine 10 mg/kg i.p. (Table 4).

Tail suspension test

In tail suspension test, the ethanolic and aqueous extracts of leaves of *cassia occidentalis* at a dose of 500 mg/kg p.o. significantly decreased the immobility time. The magnitude of the antidepressant effects of 500 mg/kg p.o. of ethanolic and aqueous leaves of *cassia occidentalis* was comparable to that of fluoxetine 10 mg/kg i.p. (Table 5).

Table 4: Effect of Ethanolic and Aqueous Extracts Of *Cassia Occidentalis* Leaves on Despair Swim Test in Rats at Different Time Intervals

Group	Treatment	Duration of immobility (sec/5min)		% Change in activity	
		30 min	60 min	30min	60min
I	CONTROL (VEHICLE)	186.7±4.410	188±4.583	NA	NA
II	Fluoxetine (10mg/kg i.p)	88.33*±1.453	56.33*±1.453	52.6(↓)	70.0(↓)
III	Ethanolic extract (500 mg/kg p.o)	98.67*±2.028	68*±2.082	47.15(↓)	63.8(↓)
IV	Aqeous extract (500 mg/kg p.o)	111.7*±3.383	83.3*±2.404	40.19(↓) 55.69(↓)	55.69(↓)

NA-Not Applicable, *P<0.001 when compared to Control

Table 5: Effect of Ethanolic and Aqueous Extracts of *Cassia Occidentalis* Leaves on Tail Suspension Test in Mice At Different Time Intervals

Group	Treatment	Duration of immobility (sec/5min)		% Change in activity	
		30 min	60 min	30min	60min
I	CONTROL (VEHICLE)	105.7±3.480	107.3±3.528	NA	NA
II	Fluoxetine (10mg/kg i.p)	38.3*±1.764	36.6*±1.202	63.73(↓)	68.89(↓)
III	Ethanolic extract (500 mg/kg p.o)	35.33*±1.764	27.33*±2.906	66.57(↓)	74.55(↓)
IV	Aqeous extract (500 mg/kg p.o)	58.67*±1.764	44.33*±2.33	44.50(↓)	58.99(↓)

NA-Not Applicable, *P<0.001 when compared to Control

DISCUSSION

The fear due to height induces anxiety in the animals when placed on the EPM. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. Antianxiety agents are expected to increase the motor activity, which is measured by the time spent by the animal in the open arms. The spontaneous decrease in basal activity score implicates the reduced anxiety recorded using actophotometer. The widespread use of FST is mainly due to its ability to detect a broad spectrum of antidepressant agents. The test is based on the observation that rodents following initial escape-oriented movements develop an immobile posture when placed inside an inescapable cylinder filled with water. The immobility is thought to reflect either a failure of persistence in escape-directed behavior (i.e., despair behavior) or the development of a passive behavior, meaning the loss of the animal's ability to cope with stressful stimuli. Markedly showed a significant decrease in the time spent immobile by rodents. By performing tail suspension test, the reduced immobility time directed the antidepressant effect. The antianxiety and antidepressant effects may be due to the flavonoid[18] content present in both the extracts of *Cassia occidentalis*. However, further studies are required to identify the phytoconstituents responsible for the observed antianxiety and anti-depressant effect.

CONCLUSION

From the results it was concluded that both ethanolic and aqueous extracts of leaves of *Cassia occidentalis* showed antianxiety and anti-depressant activity. These findings suggest that the ethanolic extract of *Cassia occidentalis* leaves posses more significant antianxiety and anti-depressant activity compared to aqueous extract.

Statistical analysis

The results were expressed as mean ± S.E.M. The differences were compared using one way analysis of variance (ANOVA) and subsequently followed by Bonferroni's test.

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