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Research Article

Antidepressant like effect of *couroupita guianensis* aubl. Flowers in animal model of depression

Gupta V.H *, Wankhede S.S, Gunjal M.A, Juvekar A.R

Department of Pharmacology, Institute of Chemical technology, Nathlal Parikh marg, Matunga, Mumbai-400019, India.

ABSTRACT

Depression is a most serious stress related mental disorder affecting 450 million people worldwide. Due to clinical limitations and adverse effects researchers have focussed on novel pharmacotherapy for safe and efficient treatment of depression. The aim of present study was to evaluate the aqueous and methanolic extracts of *Couroupita guianensis* Aubl. flower for its antidepressant like activity. It has been observed from our study that both the extract at higher concentration showed significant reduction in immobility in tail suspension and forced swim model of depression comparable to imipramine. However further study is needed to understand mechanism of action and to identify active component responsible for antidepressant like activity.

Key words: Anti depressant activity, *Couroupita guianensis*, Forced swim test, Tail suspension test

INTRODUCTION

Depression is a most serious stress related mental disorder that affects a person's mood, physical health, behaviour, lack of motivation and physical energy. Patients with major depression had symptoms that reflect change in neurotransmitter, specifically nor-epinephrine (NE), serotonin and dopamine [1]. According to World Health Organization approximately 450 million people suffer from mental and behavioural disorder [2], which amounts to 12.3% of global burden of disease and will rise to 15% by 2020 [3]. An estimated 5.8% men and 9.5% women experience the depressive episodes in their lifetime [4]. Approximately, two third of depressed patients experience suicidal thoughts and 10-15% of them attempt suicide [5].

Although, several classes of antidepressants like tricyclic antidepressants (TCAs), selective reversible inhibitors of monoamine oxidize A (RIMAs), selective serotonin reuptake inhibitors (SSRIs) and specific serotonin nor-adrenaline reuptake inhibitors (SNRIs) are currently being used, but mainly associated with adverse effects such as problematic interaction and relatively low response [1]. Due to clinical limitations and adverse effect there is critical interest in development of efficient and safe drugs for the treatment of depression [6]. This consideration implicate search for new antidepressant agents having lesser side effect with quick onset of action. Recently, researchers have focussed significantly on novel pharmacotherapy from medicinal plants for their psychopharmacological disorders.

Couroupita guianensis Aubl. (Family: Lecythideaceae) commonly known as Shivalingam (Hindi) and Nagalingam (Tamil) is a large tree of 20-30m in height with wide spreading branches bearing a peculiar flower. It is an evergreen tree allied to the Brazil nut and is native to tropical northern South America and to the southern Caribbean. The infusion of its flower had been used to treat cold, intestinal gas formation, stomachache [7], barks are used to treat hypertension, tumours, pain and inflammatory process [8]. Different extracts of flower have been screened for immunomodulatory activity [9]. Methanolic extract of the plant exhibited antimicrobial activity [10], whereas petroleum ether and chloroform extract showed larvicidal activity against vector [11]. In flowers, mainly eugenol, linalool and stigma sterol were identified [12]. Leaves of *C. guianensis* are widely used as an analgesics medicine by the Brazilian rural population [13].

To the best of our knowledge, this is the first study to evaluate the antidepressant activity of *Couroupita guianensis* Aubl. flower, aqueous (CGA) and methanolic (CGM) extract in forced swim and tail suspension animal model of depression in mice.

MATERIALS AND METHODS

Plant Material: *Couroupita guianensis* [CG] (Aubl. Lecythidaceae) flowers were collected during rainy season in the month of July – August which is it flowering season from our ICT garden, Mumbai, India. The plant material was taxonomically identified and authenticated by Dr. Ganesh Iyer, Professor in Botany at Ruia College of Science, Mumbai University.

Preparation of extract: The flowers after collection were shade dried and powdered using a mechanical grinder and passed through 40 mesh sieve. Powder (100 g) was defatted using 1.5L of petroleum ether (bp: 60- 80 ºC)

Author for correspondence: Email: vinodhgupta@gmail.com

Table 1 Phytochemical evaluation of CGA and CGM extract

Test/Reagent Used	CGM	CGA
Alkaloids		
Dragendroff's test	+	+
Hager's test	+	+
Wagner's test	+	+
Carbohydrates and Glycosides		
Molisch's test	+	+
Fehling's test	+	+
Barfoed's test	+	+
Benedicts test	+	+
Saponins		
Foam test	-	-
Phenolic Compounds and Tannins		
Ferric chloride test	+	+
Lead acetate test	+	+
Proteins and Amino Acids		
Biuret test	-	-
Ninhydrin test	-	-
Flavonoids		
Shinoda's test	+	+

Present: +, Absent: -

CGA: *Couroupita guianensis* aqueous extract

CGM**:** *Couroupita guianensis* methanolic extract

and subjected to extraction in a Soxhlet apparatus using methanol for 12h. To obtain aqueous extract distilled water were used.. Both the CGA and CGM fraction were concentrated under reduced pressure and controlled temperature (40-50 ºC). The yielding ratio of aqueous and methanol extract of CG were 19.65% w/w and 15.3% w/w respectively. Both the extracts were stored in tightly closed container in refrigerator and were screened for phytochemicals.

Animals: Swiss male albino mice (weighing around 18-25 g) obtained from Haffkine Biopharmaceutical Corporation Ltd. Parel, Mumbai were used in the study. They were maintained at 22 ± 5 ºC, relative humidity 55 ± 5 ºC with free access to food (M/s D. S. Trading Ltd., Mumbai) and water *ad libitum*, under a 12:12 light /dark cycle (light on at 8:00 h). All manipulations were carried out between 9:00 and 15:00 h. with each animal used only once.

The experimental protocol was approved by the Institutional Animal Ethics committee of institute as per the direction of the Committee for the Purpose of Control and Supervision of Experimental on Animals (CPCSEA No 87/1999). The animals were acclimatized for a period of 7 days before the study. All efforts were made to reduce the number of animals used and treated humanely to minimize their pain and discomfort.

Acute toxicity study: The acute toxicity study in mice was performed as per the OECD guidelines (No. 423) to evaluate the undesirable effects or toxicity of both, CGA and CGM extracts. Swiss male albino mice were divided into the groups of 3 animals per group and were administered once orally with dose of 2000 mg/kg of CGA and CGM extract. The mice were then critically observed for clinical signs, gross behavioural changes and mortality after 30min, 1hr, 2hr, 3hr and then after 24hr. These observations were continued for a period of 7 days.

Drugs and Chemicals: The following drugs were used: Imipramine hydrochloride (Sarabhai Piramal Pharma Ltd, Vadodara), Sodium carboxy methyl cellulose (Na-CMC**)** (Loba Chemie, Mumbai). All the drugs were suspended in 0.1% Na-CMC [Vehicle control] just before use. All the other chemicals and reagents used for the study were of analytical grade procured from approved organization.

Experimental Design: Mice were randomly divided into 8 groups. Each group contained 6 mice. Group I was assigned as vehicle control received 0.1% Na-CMC (10ml/kg, *p.o.*), group II received imipramine (25mg/kg, *p.o.*) as a positive control, group III, IV and V received CGA extract in doses of 100, 250 and 500 mg/kg and group VI, VII and VII received CGM extract in doses of 100, 250 and 500 mg/kg *per orally*. Mice were starved for 24 hours and divided into control and experimental groups.

The animals were pre-treated orally with 0.1% Na CMC suspension of CGA and CGM extracts for 7 days daily at the doses of 100, 250 and 500 mg/kg/day. Control mice also received orally the vehicle (0.1% Na CMC suspension) and Imipramine in the dose of 25 mg/kg. Experiments were conducted one hour after last day drug administration. The antidepressant activity of the CGA and CGM extract were evaluated using forced swim test and tail suspension test animal models of depression.

Forced Swimming test (FST): Behaviour despair was proposed as a model to test antidepressant activity by Porsolt *et al.,* (1977) [14]. Mice were forced to swim individually in a glass jar (25x15x25 cm^3) containing fresh water of 15 cm height and maintained at 25°C±1°C after an initial 2 minute period of vigorous activity, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limb necessary to keep its head above water [14]. Animals were given pre-test session of 15 min 24 hour prior to the experiments. After 24 h, vehicle control (0.1%Na CMC suspension, *p.o*) or Imipramine (25 mg/kg, *p.o*) or CGA and CGM extract (100, 250 and 500 mg/kg, *p.o*) were administered to animals. After one hour of the treatment mice were again forced to swim in glass jar under same condition as mentioned above. The total duration of immobility was recorded during the next 4 minutes of a total 6 minute test. The percent reduction in the immobility time of test animals was calculated as compared to the control animals.

Table 2 Effects of CGA and CGM (100, 250 and 500 mg/kg) and imipramine (25 mg/kg) on duration of immobility (sec) in forced swim test

Groups	Treatment	Dose (kg $^{-1}$)	Immobility time (sec)	Percent reduction
I	Vehicle Control	10ml	142.5±21.85	------
II	Imipramine	25 mg	62.83±5.94**	55.9
Aqueous extract of *C.guaenensis*				
III	CGA	100 mg	50.66±7.36**	64.45
IV	CGA	250 mg	48.00±2.44**	66.31
V	CGA	500 mg	29.83±2.63**	79.06
Methanolic extract of *C.guaenensis*				
VI	CGM	100 mg	39.66±3.61**	72.17
VII	CGM	250 mg	20.33±1.96**	85.73
VIII	CGM	500 mg	16.00±2.89**	88.77

Values are expressed as mean ± SD, n=6, **P < 0.01, when compared to vehicle control group by one-way ANNOVA followed by Dunnett's test.
CGA: *Couroupita guianensis* aqueous extract,
CGM: *Couroupita guianensis* methanolic extract

Tail suspension test (TST): Tail suspension test is behaviour despair model of depression employed in rodents as a facile means of evaluating potential antidepressant by decreasing immobility period produced by several different classes of antidepressant drugs. The total duration of immobility induced by tail suspension was measured according to the method described by Steru *et al.,* (1985) [15]. Mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from tip of the tail. Immobility time was recorded during a 6 minute period. Animal was considered to be immobile when it did not show any movement of the body, hanged passively and completely motionless [15].

STATISTICAL ANALYSIS

Values are given as mean ± SD. and significances calculated using one way analysis of variances followed by Dunnett's test. The *p-values* <0.05 were considered statistically significant.

RESULTS

Phytochemical evaluation: CGA and CGM extract were subjected to various test and showed the presence of alkaloids, glycosides, tannins and flavonoids as represented in table 1.

Effect of CGA and CGM flower extract against FST induced depression like behaviour in mice: In the forced swim test oral pre-treatment of mice with both CGA and CGM extracts at doses of 100, 250 and 500 mg/kg showed significant(p<0.01) reduction in the , duration of immobility time in dose dependant manner compared to the control group (Table 2). The percent reduction in immobility time for CGA (100, 250 and 500 mg/kg) extract were 64.45 %, 66.31 % and 79.06 % respectively and 72.17 %, 85.73 % and 88.77 % respectively for CGM (100, 250 and 500 mg/kg) extract. It was observed that the values obtained for the CGA and CGM extracts were much lower than standard reference drug imipramine (55.90 %, at 25 mg/kg, *p.o*).

Effect of CGA and CGM flower extract against TST induced depression like behaviour in mice: As shown in Table 3, animals pre-treated with CGA and CGM extract at doses of 100, 250 and 500 mg/kg showed significant (p<0.01) reduction in the duration of immobility time in dose dependant manner compared to control group. The percent reduction in immobility time for CGA (100, 250 and 500 mg/kg) extract were 49.15%, 55.61 % and 58.79 % respectively and 53.49 %,61.01 % and 61.65 % respectively for CGM (100, 250 and 500 mg/kg) extract. Similarly, animals treated with imipramine (25mg/kg) as expected showed a significant (p<0.01) decrease in the immobility time (26.80 % at 25 mg/kg, *p.o*)

DISCUSSION

In the present study, antidepressant activity of *Couroupita guianensis* Aubl. flower extracts have been demonstrated. Extracts were found to be safe as no mortality was observed following treatment with doses as high as 2000mg/kg. Pre-treatment of mice with both CGA and CGM extracts for 7 days in the dose of 100, 250 and 500 mg/kg showed antidepressant activity in the forced swim

Table 3 Effects of CGA and CGM (100, 250 and 500 mg/kg) and imipramine (25 mg/kg) on duration of immobility (sec) in tail suspension test.

Groups	Treatment	Dose (kg $^{-1}$)	Immobility time (sec)	Percent reduction
I	Vehicle Control	10ml	157.33±13.90	------
II	Imipramine	25 mg	115.16±7.98 **	26.8
Aqueous extract of *C.guaenensis*				
III	CGA	100 mg	80.0±7.01**	49.15
IV	CGA	250 mg	69.83±5.19**	55.61
V	CGA	500 mg	64.83±4.40 **	58.79
Methanolic extract of *C.guaenensis*				
VI	CGM	100 mg	73.16±8.51**	53.49
VII	CGM	250 mg	61.33±7.99**	61.01
VIII	CGM	500 mg	60.33±6.08 **	61.65

Values are expressed as mean ± SD, n=6, **P < 0.01, when compared to vehicle control group by one-way ANNOVA followed by Dunnett's test.
CGA: *Couroupita guianensis* aqueous extract, **CGM**: *Couroupita guianensis* methanolic extract

and tail suspension test animal model. CGA and CGM extracts significantly reduced immobility period in the forced swimming test indicating antidepressant activity and its efficacy was found to be similar to Imipramine [14].

In FST assay, mice were forced to swim in a restricted space from which there is no escape, and after certain periods of agitation, cease attempts to escape and become immobile. The immobility exhibited by test animal in these models is an indicative of a behavioural despair which reflects depressive state [16]. These models are based on the fact that animals will develop an immobile posture when subjected to inescapable stress of being suspended by their tail or being dropped into water [14].

To validate further, tail suspension test were employed for anti depressant activity in animal model. It is based on the principle that suspending mice upside down leads to a characteristic behaviour of immobility after initial momentary struggle. This behaviour reflects a state of despair which can be reduced by several agents that are therapeutically effective in human depression [15]. In the present study, CGA and CGM extracts at the dose of 100, 250 and 500 mg/kg administered to mice 1 h before experiments. Both the extracts showed dose dependant significant reduction in immobility when subjected to tail suspension test. In both the antidepressant model, imipramine a standard reference drug also showed a significant anti-immobility. It is interesting to note that the most active extract was the CGM suggesting that the active principle are mainly concentrated in this fraction.

Depression has been linked to perturbations in the neurotransmission involving brain 5-HT, norepinephrine (NE) and dopamine activity [17]. The precise mechanism by which *C. guianensis* produced antidepressant like effect is not well characterized. Phytochemical screening of *C. guianensis* extracts showed the presence of alkaloids, glycosides, tannins and flavonoids. Anti depressant like activity may be due to flavonoids and glycoside as they are hydrolysed into aglycons and converted to conjugated metabolites by enzymes in the intestine during absorption process [18, 19].

CONCLUSION

The results suggest that both aqueous and methanolic extracts of *C. guianensis* showed antidepressant like effect in forced swimming and tail suspension animal model of depression. The methanolic extracts at dose of 500mg/kg being the most effective. Further studies are required to identify the active components present in the extract responsible for its antidepressant like activity.

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