COUROUPITA GUIANENSIS AUBL: EVALUATION OF ITS ANTIDEPRESSANT ACTIVITY IN MICE

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Summary

Couroupita guianensis Aubl. (Family: Lecythidaceae) popularly known as "cannon ball tree" contains triterpenoids which have been implicated in antidepressant activity. Therefore the present research was aimed to evaluate the potential antidepressant activity of methanolic extract of Couroupita guianensis (CGM) root in mice. This extracts was administered orally in a dose range of 125, 250 and 500 mg/kg of the body weight. The antidepressant activity was evaluated using tail suspension test (TST), forced swim test (FST) and reserpine antagonism in mice. Results of the activity showed significantly decrease in the immobility time in TST and FST, similar to that of the imipramine (10 mg/kg) which served as a positive control. In reserpine antagonism showed significantly decrease in duration of catalepsy & degree of ptosis in tested mice. The significance of difference among the various treated groups and control group were analyzed by means of one-way ANNOVA followed by Dunnett's tests. In conclusion, methanolic extract of *Couroupita guianensis* root possesses potential antidepressant activity (through noradrenergic mechanisms) and has therapeutic potential in the treatment of CNS disorders and provides evidence at least at a preclinical level.

Key Words: *Couroupita guianensis* Aubl. , Antidepressant Activity, tail suspension test, forced swim test, reserpine antagonism.

Introduction

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Among ancient civilisations, India has been known to be rich repository of medicinal plants, which are largely collected as raw materials for manufacture of drugs and perfumery products (1).

Couroupita guianensis (Aubl.) family Lecythidaceae, commonly known as cannon ball tree, locally known as "Kailashpati" and found throughout India in plains. It is widely distributed in tropical America and West Indies (2). The major phytoconstituents of the plants are triterpenes, tannins (3) and alkaloids (4). Isolation of α,β-amyrin, stigmasterol, β-sitosterol, campesterol, linoleic acid, eugenol, linalool, farnesol, nerol, tryptanthrin, indigo, indirubin, isatin, caretenoids etc., from flowers, seeds,fruits,leaves and leaves have been reported earlier (5). However, there is no systematic scientific report published showing its anti-depressant activity and no work reported on the root. Therefore, objective of present study was to evaluate anti-depressant activity of *Couroupita guianensis* by different pharmacological screening methods.

Depression is a common disorder associated with high rates of chronicity, relapse, and recurrence; psychosocial and physical impairment; and a high suicide rate. Currently available therapy for depression treatment is often associated with several undesirable side effects, and it is effective only in a certain portion of the population (6, 7). Therefore, the identification of alternative therapeutic tools for the treatment of depression is still needed. Herbal therapies may be effective alternatives in the treatment of depression (8, 9, 10) and the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses, including depression, has progressed significantly in the past decade (11).

Materials and Methods

Chemicals

Imipramine (Sarabhai Piramal Pharma Ltd, Vadodara); reserpine (Boehringer Ingelheim BI) was used to induce depressive states in mice and all other chemicals used were of analytical grade.

Plant material

The root of *Couroupita guianensis* was collected from the from K.E.M. Hospital and Research Centre, Parel, Mumbai, INDIA in December 2008. The collected sample was authentified by conducting macro and microscopic studies by Dr. A. M. Mujumdar, Plant Science Division, Agharkar Research Institute, Pune, INDIA. A voucher specimen (3/386/2008) has been preserved in laboratory for future reference. The root was dried under shade and then powdered with a mechanical grinder and stored in an airtight container.

Preparation of Extract

The dried powder material was defatted with petroleum ether (60^0-80^0C) and subsequently extracted with methanol by using Soxhlet extractor method. The solvent was completely removed by drying and methanolic extract of *Couroupita guianensis* (CGM) was obtained (yield 13.7%). The extract was stored at room temperature in a sealed container till required. Solution of CGM was prepared freshly in distilled water and used for the present study.

Phytochemical screening

The CGM extract was screened for the presence of various phytochemical constituents i.e. steroids, alkaloids, tannins, flavonoids, glycosides, etc by employing standard screening tests(12).

Animals

Male albino mice (Swiss, 22–25 g) were housed in groups of six under standard laboratory conditions of temperature, humidity and lighting. Animals had free access to food and water, except during experiment. They were deprived of food but not water 12 h before the drug administration. Each group consisted of six animals. All experiments were carried out during the light period. The studies were carried out in accordance with the guidelines given the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (India) and the Institutional Animal Ethical Committee approved the study.

Acute Toxicity Study

Acute toxicity study was performed according to OECD-423 guidelines (13). *Swiss albino* mice of either sex were used for study. The animals were fasted for 4 h, but were allowed free access to water *ad libitum* throughout. The animals were divided into six groups containing six animals each. CGM was dissolved in distilled water and administered orally as a single dose to mice at different dose levels viz. 500, 750, 1000, 1250, 1500 and 2000 mg/kg of body weight (b.w.). Mice were observed periodically for the symptoms of toxicity and death within 24 hours and then daily for next 14 days.

Evaluation of antidepressant activity

Tail Suspension Test

The tail suspension test (14) has been described as a facile means of evaluating potential antidepressants. Six groups of six mice each were used. Mice were treated with CGM orally in a dose range of 125, 250 and 500 mg/kg of the body weight 30 minutes prior to testing. Reserpine (5 mg/kg) and imipramine (10 mg/kg) was used for negative and positive control respectively. For the test the mice were suspended on the edge of a shelf 58 cm above a tabletop by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for a period of 5 minutes. Mice were considered to be immobile when they hung passively and completely motionless.

Duration of immobility was measured in controls and animals treated with various doses of a test drug or standards. Compared with the immobility score of the control group.

Forced Swim Test

The studies were carried out on mice according to the method of Porsolt (15). It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviour of immobility. It consists of a plexiglass cylinder/ plastic tub (height 40 cm, diameter 18 cm) containing 15 cm of water maintained at 25°C. Sixty minutes after administration of the CGM, the male mice were placed in plexiglass cylinder/ plastic tub containing 9 cm of water maintained at 25°C. After allowing one minute for acclimatization, immobility of each mouse was rated every 30s from second minute onwards for another five minutes. An animal was judged to be immobile whenever it remained floating passively in water in a slightly hunched but upright position, its nose just above the surface. Standard drug imipramine (10 mg/kg) and reserpine (5 mg/kg) were chosen as positive control and negative control agents respectively for the experiment. Duration of immobility was measured in controls and animals treated with various doses of test drug or standards. Compared with the immobility score of the control group.

Reserpine Antagonism in Mice (16**)**

Five groups of six mice each were used. Mice were treated with CGM orally in a dose range of 125, 250 and 500 mg/kg. Sixty minutes after oral administration of the test compound and control, 5 mg/kg reserpine was injected s. c. The test started 15 minutes after reserpine administration was continued for 2hrs. Catalepsy and ptosis were used as criteria for evaluation. Thirty seconds after replacement, the degree of ptosis was scored: eyes closed =4, eyes ¾ closed =3, eyes ½ closed =2, eyes ¼ closed= 1, eyes open =0.Similarly cataleptic effect was scored according to the duration of catalepsy. Catalepsy more than 60 s =5, between 30s and 60s =4, between 10 and 30 s =3, between 5 and 10 s =1, less than 5 s =0.

The scores of reserpine controls were taken as 100% and accordingly the percentage was calculated for the treated animals. Imipramine 25 mg/kg oral was used as a positive control agent.

Statistical analysis

Results are presented as mean ± standard error of mean (SEM). Data were subjected to analysis of variance followed by Dennett's test using Graph pad prism 5 software. $P \leq 0.05$ were considered significant.

Results

Phytochemical Screening

Preliminary phytochemical screening of the *Couroupita guianensis* (CGM) revealed the presence of triterpenoids, flavonoids, alkaloids and glycosides.

Acute Toxicity Test

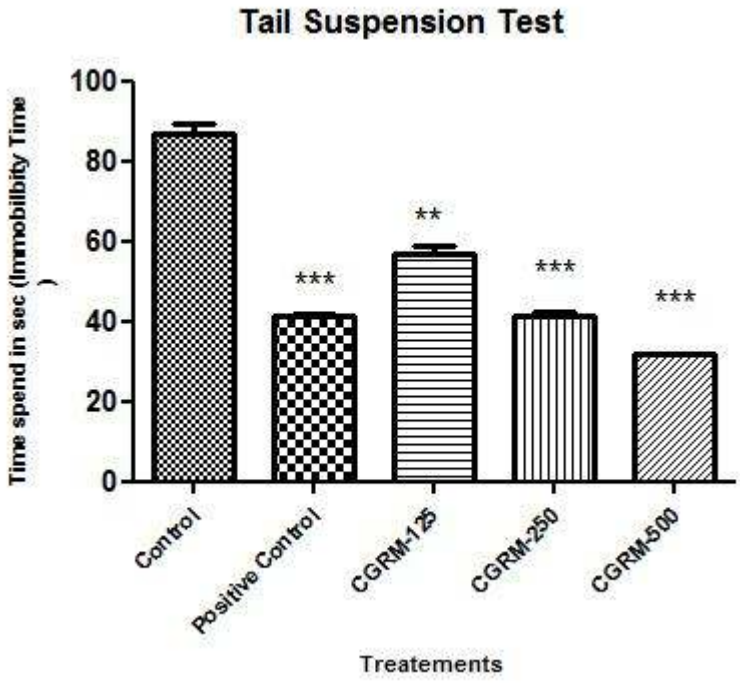
In the acute toxicity study no deaths were observed during the 72 h period at the doses tested. At these doses, the animals showed no stereotypical symptoms associated with toxicity, such as convulsion, ataxy, diarrhoea or increased diuresis. The median lethal dose (LD_{50}) was determined to be higher than the dose tested i.e. 2.0 g/ kg.

Evaluation of antidepressant activity

Tail Suspension Test

The effects of oral administration of the extract of CGM and imipramine on the immobility time in the TST is shown in Fig. 1, at doses of 125,250 and500 mg/kg significantly($P \leq 0.05$) decreased the immobility time as compared to the control group. As a positive control, the antidepressant imipramine also produced a significant reduction in the immobility time in the TST.

Figure 1

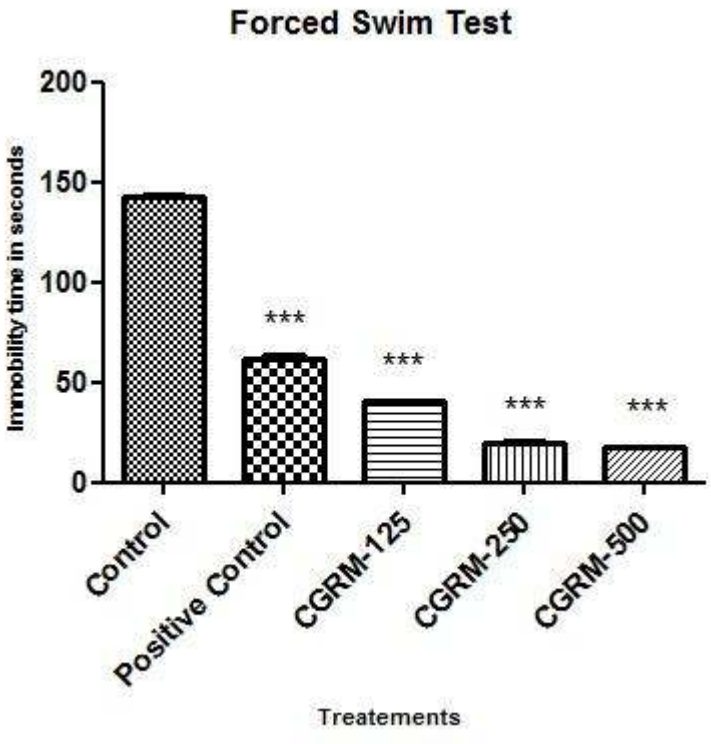


Effects of CGM on immobility time (seconds) is representing on y axis. Each point represents the mean ± S.E.M. for 6 animals. The asterisks denote the significance levels compared with control groups. Significantly different from controls, $P \leq 0.05$.

Forced Swim Test

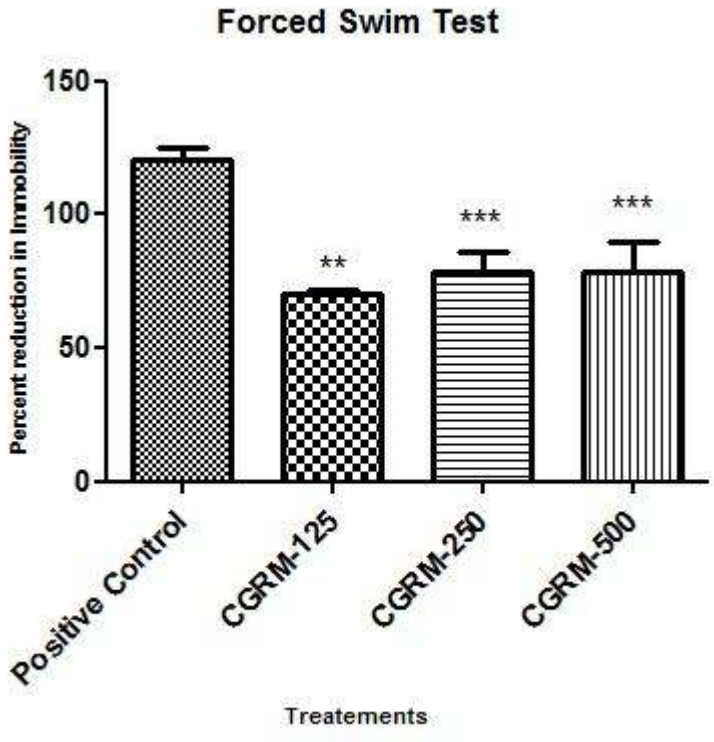
Effects of oral administration of the aqueous extract of CGM and imipramine on the duration of immobility in the mouse forced swimming test were shown in Figure 2. The extracts at doses of 125,250 and 500 mg/kg significantly ($P \leq 0.05$) decreased the duration of immobility in a dose-dependent manner after 7-day treatment. The percent reduction in it was 71.83%, 85.91% and 90.47 % respectively for the states doses as shown in Fig.2A. Imipramine at the dose of 10 mg/ kg significantly showed reduction in immobility and percent reduction (113.6%) when compared with control group.

Figure 2



Effects of CGM on immobility time (seconds) is representing on y axis by TST. Each point represents the mean ± S.E.M. for 6 animals. The asterisks denote the significance levels compared with control groups. Significantly different from controls, $P \leq 0.05$.

Figure 2A

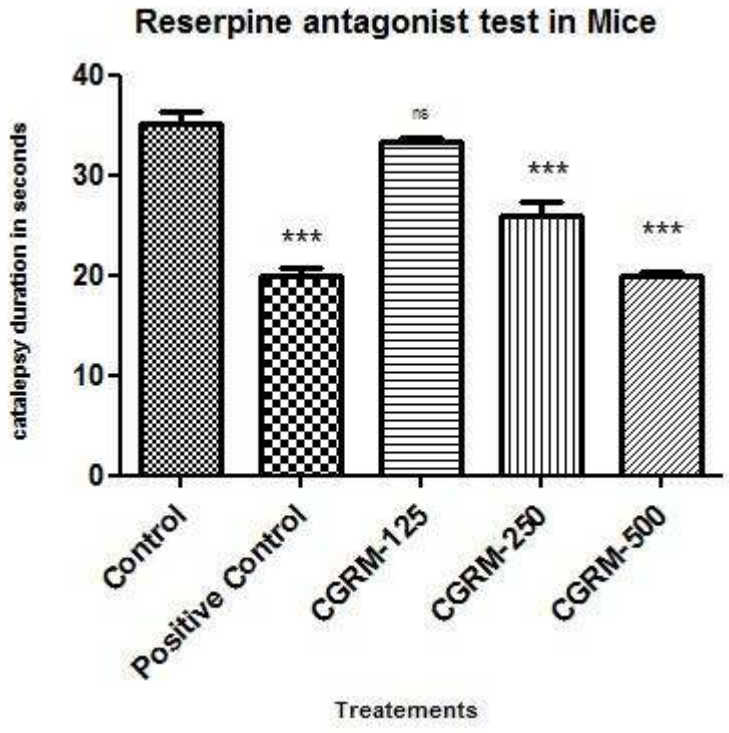


Effects of CGM on percent reduction immobility time (seconds) is representing on y axis by FST. Each point represents the mean ± S.E.M. for 6 animals. The asterisks denote the significance levels compared with control groups. Significantly different from controls, $P \leq 0.05$.

Reserpine Antagonism in Mice

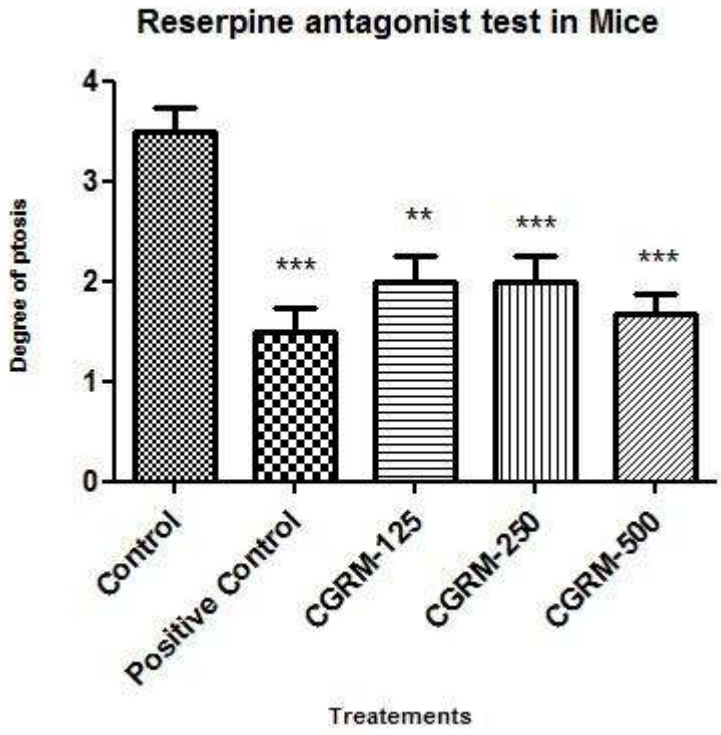
Results of the study showed in Fig.3 that CGM extract at the doses of 125, 250 and 500 mg/kg reduced significantly ($P \leq 0.05$) the catalepsy duration 2±0.63, 2±0.63 and 1.66±0.51 respectively. Imipramine showed marked decrease in catalepsy duration 1.5±0.54. The degree of ptosis as stated in Fig. 3A, in reserpine antagonism in mice significantly ($P<0.05$) when results were compared with vehicle control

Figure 3



Effects of CGM on catalepsy (seconds) is representing on y axis by FST. Each point represents the mean ± S.E.M. for 6 animals. The asterisks denote the significance levels compared with control groups. Significantly different from controls, $P \leq 0.05$.

Figure 3A



Effects of CGM on degree of ptosis is representing on y axis. Each point represents the mean ± S.E.M. for 6 animals. The asterisks denote the significance levels compared with control groups. Significantly different from controls, $P \leq 0.05$.

Discussion

Depression is a common, debilitating, life-threatening illness with a high incidence. Numerous antidepressant compounds are now available, which presumably act via different mechanisms involving the serotonergic, noradrenergic and/or dopaminergic systems. Heterogeneity of clinical response to antidepressant and mood-stabilizing drugs and susceptibility to adverse effects are major clinical problems (17). Therefore, new drugs are still needed for the control of depression-related disorders.

The present study has shown that oral administration of the methanol extract of *Couroupita guianensis* was effective in producing significant antidepressant effects in the tail suspension test, forced swimming test and in reserpine antagonism in mice, as is evident from the reduction in the immobility time in mice (15) and reduction in duration of catalepsy & degree of ptosis respectively.

The tail suspension test has been described by Steru et al(14). as a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair, which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by tail.

In FST, mice were forced to swim in a restricted space from which there was no escape, and will, after periods of agitation, cease attempts to escape and become immobile. It is accepted that immobility seen in rodents during swimming reflects behaviour despair as seen in human depression and that the antidepressant drugs are able to reduce the immobility time in mice (15). The chronic treatment of the extract significantly reduced the immobility time and increased the swimming behaviour at high dosage (500 mg/kg). This shows that the methanolic extract of the *Couroupita guianensis* possesses antidepressant activity and its specificity towards particular behavior may depend on the concentration of the extract. There are reports to indicate that immobility, swimming and climbing behaviors are enhanced by different groups of antidepressant drugs (18). The NE-selective uptake inhibitors like desipramine (DMI) and maprotiline (MAP) enhances the climbing behavior where as the serotonin specific reuptake inhibitors (SSRIs) like fluoxetine (FLX), sertraline (SRT) and paroxetine (PRX) enhance swimming but not climbing behavior. However, both the types of antidepressants reduce immobility behavior. Also recent studies show that the dopaminergic activation is also involved in struggling (climbing) behavior (19, 20, 21).

This implies that it contain active chemical constituents to elicit specific types of behavior in TST and FST through noradergnic, serotonergic and dopaminergic systems and thereby acting as an antidepressant agent. It also suggests that the activation of these systems may depend on the concentration of the extract. Further, there was no remarkable change in the ambulatory behavior on chronic treatment. The ambulatory behavior decreased in comparison to the control group but no significant difference was found. This ensures that any increases in mobility observed in the FST, after chronic treatments.

Reserpine induces profound depletion of catecholamine in brain and produce depression in animals and humans. Depression produced by reserpine in humans is similar to naturally occurring depression; hence reserpine is used as an agent to induce depression in animal models for testing antidepressant drugs. Since antidepressant drugs are known to prevent or antagonize the effects of reserpine, prevention of reserpine induced ptosis and catalepsy can be used for evaluation of antidepressants.

Preliminary phytochemical analysis carried out with the methanol extract revealed the presence of triterpenoids, flavonoids, alkaloids and glycosides (3, 4). Since antidepressant effects have been observed in several triterpenoids from different medicinal plants, it is possible that these polyphenolic substances might be responsible, at least in part, for the observed antidepressant activity in our study. Although the precise mechanism involved in the observed antidepressant activity is not yet clear, the experimental observations suggest a possible direct or indirect facilitation of the central serotonergic transmission for the species studied. Further studies should be carried out to correlate the pharmacological activities with the chemical constituents.

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