

**ANXIOLYTIC EFFECT OF METHANOLIC EXTRACT
OF ROOT OF *COUROUPITA GUIANENSIS* AUBL.**

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Summary

Couroupita guianensis Aubl. a member of the Lecythidaceae family, vernacularly known as cannon ball, locally Kailashpati, grows in tropical areas of the India, South America and the Caribbean. The plant has rich source of triterpenoids which have been concerned with anxiolytic activity. Therefore the present research was aimed to evaluate the potential anxiolytic activity of methanolic extract of *Couroupita guianensis* (CGRM) root in mice. This extracts was administered orally in a dose range of 125, 250 and 500 mg/kg of the body weight. The anxiolytic activity was evaluated using light and dark model (LDM), elevated plus maze (EPM) and hole board test (HBT) in mice. Results of the activity showed significantly increase in number of entries in light room in LDM. In EPM there was significant increase in number of entries and time spent in open arm in dose dependent manner, similar to that of the diazepam (3 mg/kg) which served as a positive control. Also, it was observed that in HBT there is increase in number of head dipping as compared to normal vehicle control. 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 anxiolytic activity (through its action on GABA/ benzodiazepine receptors) and has therapeutic potential in the treatment of CNS disorders and provides evidence at least at a preclinical level.

Key Words: *Couroupita guianensis* Aubl. , anxiolytic Activity, light and dark model, elevated plus maze and hole board test.

Introduction

Anxiety is very important area of research interest in behavioural pharmacology this decade. This increased interest is due to a swift growth of scientific studies and the discovery of new anxiolytic drugs that alter anxiety in animal models (1). The World Health report (2) states that approximately 450 million people suffer from a mental or behavioural disorder, however only a small minority of them receive even the most basic treatment for these ailments. According to Reynolds survey, this amounts to 12.3% of the global burden of disease, and will rise to 15% by year 2020 (3).

Currently, the most widely prescribed medications for anxiety disorders are the benzodiazepines. However, the clinical uses of benzodiazepines are limited by their side effects such as psychomotor impairment; potentiate other central depressant drugs and dependence liability. Therefore, the development of new medications possessing anxiolytic effect without the complications of benzodiazepines would be of great importance in the treatment of anxiety-related disorders.

In the search for new therapeutic products for the treatment of neurological and behavioural disorders, medicinal plant research has progressed constantly in all areas of world, demonstrating the usefulness in pharmacological activity of different plant species in a variety of animal models (4).

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 (5). The major phytoconstituents of the plants are triterpenes, tannins (6) and alkaloids (7). 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 (8). 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 anxiolytic activity of *Couroupita guianensis* by different pharmacological screening methods.

Materials and Methods

Chemicals

Diazepam (Zepose ® 5 mg, Cipla Limited, Mumbai) at dose of 3mg/kg was used as a positive control agent in anxiolytic agent; 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 authenticated 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 (60⁰-80⁰C) and subsequently extracted with methanol by using Soxhlet extractor method. The solvent was completely removed by drying and methanolic extract of *Couroupita guianensis* (CGRM) 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(9).

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 (10). *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 anxiolytic activity**Light and Dark Model**

The activity cage apparatus was modified in two compartments of 20 cm×10 cm×14 cm, by making partition with the help of thermacol. One compartment of cage was darkened with a cover and separated with a wall from otherwise brightly illuminated area. A round hole (Diameter 6 cm) allows the animal to pass from illuminated to darkened compartment. A 100W bulb placed 30 cm above the floor of the transparent compartment was the only light source in the room. One hour after oral treatment in a dose range of 125, 250 and 500 mg/kg of the body weight, the mouse was placed at the centre of the plus-maze facing one closed arm and was observed for 5 min. Diazepam(3mg/kg) was used as a positive control.

A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into the dark box (11,12,13). The apparatus was cleaned thoroughly between experiment.

Elevated Plus Maze

The plus-maze for mice consisted of two perpendicular open arms (30cm×5 cm) and two perpendicular closed arms (30cm×5cm×25 cm). The whole apparatus was made of wood, and the maze was 50 cm above the floor (14). One hour after oral treatment in a dose range of 125, 250 and 500 mg/kg of the body weight, the mouse was placed at the centre of the plus-maze facing one closed arm and was observed for 5 min. Diazepam(3mg/kg) was used as a positive control. The parameters observed were number of entries in the open and closed arms and time of permanence in the open arms. The first and the last parameters were expressed in percentage. A mouse was considered to have entered an arm when all four legs were on the arm. The number of entries in the closed arms was considered as the locomotor activity index and the percentage of the time spent and percentage of entries on the open arms as the anxiety index (14, 15, 16).

Hole Board Test

The apparatus was composed of a thermacol box (50 cm×50 cm×50 cm) with four equidistant holes 3 cm in diameter in the floor (17, 18) fabricated in the laboratory. The centre of each hole was 10 cm from the nearest wall of the box. The floor of the box was positioned 15 cm above the ground and divided into squares of 10 cm×10 cm with a water-resistant marker. One hour after oral treatment in a dose range of 125, 250 and 500 mg/kg of the body weight, the mouse was placed at the centre of the plus-maze facing one closed arm and was observed for 5 min. Diazepam (3mg/kg) was used as a positive control. An animal was placed in the centre of the hole-board and allowed to freely explore the apparatus for 5 min.

The total locomotor activity (numbers of squares crossed), and the number and duration of head-dippings were recorded. A head dip was scored if both eyes disappeared into the hole.

Statistical analysis

The data obtained were analyzed using the GraphPad software program Version 5.0 and expressed as mean \pm S.E.M. Statistically significant differences between groups were calculated by the application of an analysis of variance (ANOVA) followed by the Dennett's test. *P*-values less than 0.05 ($P < 0.05$) 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 (LD₅₀) was determined to be higher than the dose tested i.e. 2.0 g/ kg.

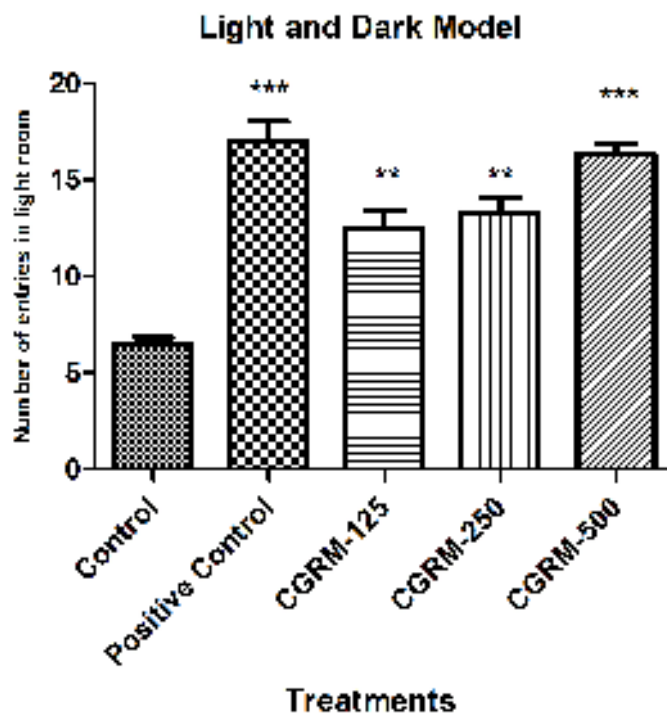
Evaluation of anxiolytic activity

Light and Dark Model

Effects of oral administration of the CGRM and diazepam on number of entries in light compartment in the mouse forced swimming test were shown in Figure 1. The extracts at doses of 125,250 and 500 mg/kg significantly ($P < 0.05$) increased the number of entries in a dose-dependent manner after 7-day treatment. The number of entries in it was 12.5 ± 0.9574 , 13.33 ± 0.7149 and $16.33 \pm 0.4944\%$ respectively for the states doses as shown in Fig.1. Diazepam at the dose of 3 mg/ kg significantly

showed increased number of entries in light compartment 17.00 ± 1.033 when compared with control group.

Figure 1 Effect of extracts on number of entries in light room in Light and Dark Model

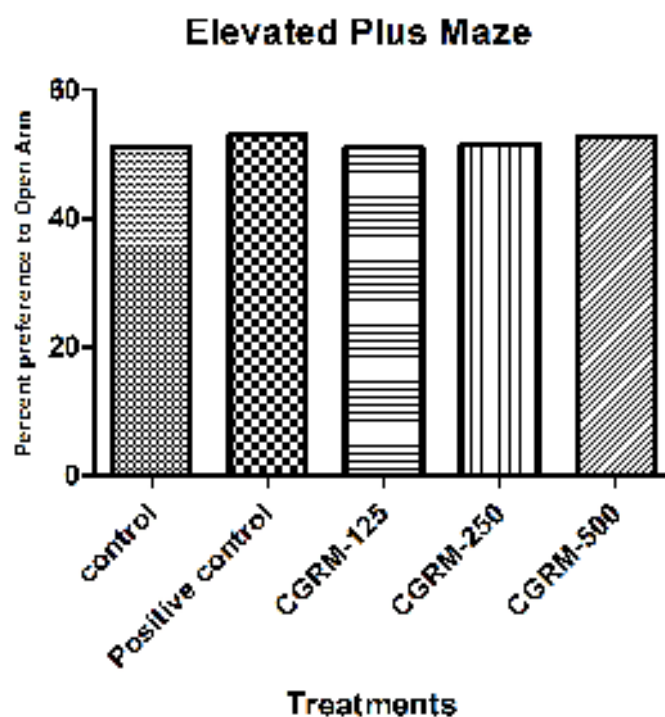


Effects of CGM on number of entries in light room representing on y axis. Each point represents the mean \pm S.E.M. for 6 animals. The asterisks denote the significance levels compared with control groups. Significantly different from controls, $P \leq 0.05$.

Elevated Plus Maze

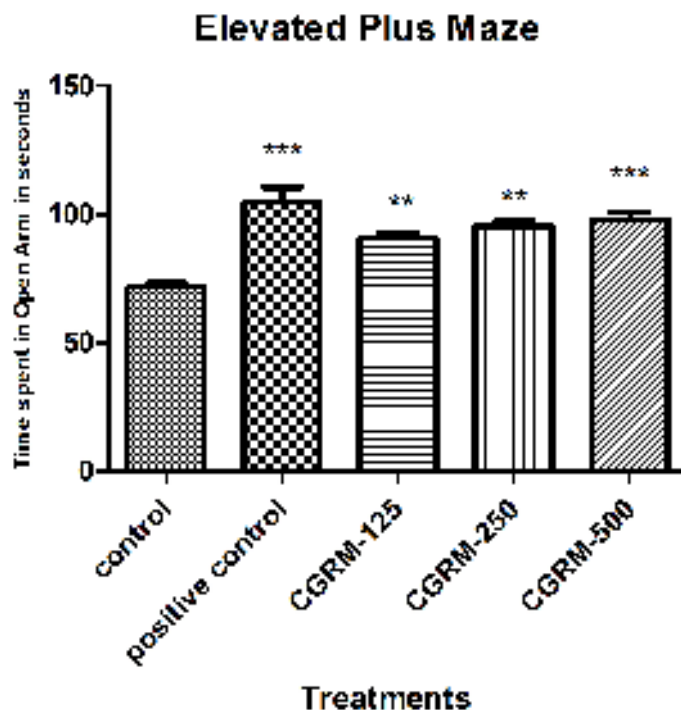
The effects of oral administration of the extract of CGRM and diazepam on percent preference to open arm, time spent in open arm in the EPM is shown in Fig. 2 and Fig. 2A respectively, at doses of 125,250 and500 mg/kg significantly ($P < 0.05$) increase the percentage and retention time in open arm as compared to the control group. As a positive control, the antidepressant imipramine also produced a significant reduction in the immobility time in the TST.

Figure2. Effect of extracts on percent preference to open arm in Elevated plus Maze



Effects of CGM on percent preference in open arm representing on y axis. Each point represents the percentage for 6 animals.

Figure2A. Effect of extracts on time spent in open arm (sec.) in Elevated Plus Maze

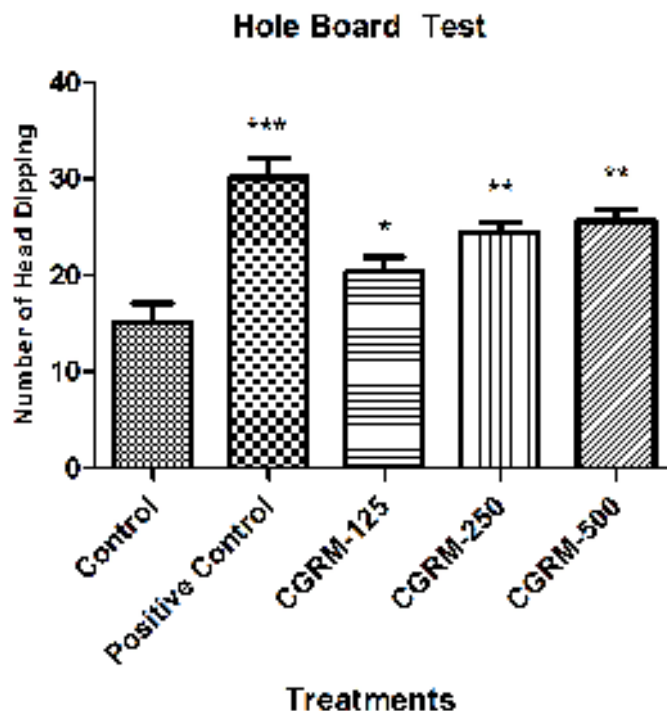


Effects of CGM on time spent in open arm (sec.) representing on y axis. Each point represents the mean \pm S.E.M. for 6 animals. The asterisks denote the significance levels compared with control groups. Significantly different from controls, $P \leq 0.05$.

Hole Board Test

Results of the study showed in Fig.3 that CGRM extract at the doses of 125, 250 and 500 mg/kg reduced significantly ($P < 0.05$) increase the head dipping in HBT 20.33 ± 1.585 , 24.5 ± 0.922 and 25.67 ± 1.174 respectively. Diazepam showed marked increase in head dipping 30.17 ± 1.99 when results were compared with vehicle control.

Figure 3 Effect of extracts on number of head dipping in Hole Board Test



Effects of CGM on number of head dipping representing on y axis. Each point represents the mean \pm 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

Preliminary phytochemical analysis carried out with the methanol extract revealed the presence of triterpenoids, flavonoids, alkaloids and glycosides (3, 4). Since anxiolytic 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 anxiolytic activity is not yet clear, the experimental observations suggest a possible direct or indirect facilitation of the central serotonergic transmission for the species studied. The anxiolytic effect was also evidenced through the light–dark test, based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors, that is, a novel environment and light. It has been assumed that the time mice spend in the illuminated side of the box is the most useful and consistent parameter of anxiety. It has been reported that simply the measurement of the time spent in the light area, but not the number of transfers, is the most consistent and useful parameter for assessing an anxiolytic action (19). The present study showed that CGRM (125,250 and 500 mg/kg) could increase the time in the light area, suggesting CGRM possesses anxiolytic properties.

In EPM test, the percentage of time that mice spend in the open arms as well as the percentage of entries into these arms was increased. As with the EPM test, this model is useful for modelling anxiety, and it has been developed for predicting the potency of clinically used compounds for treating this disease. The lack of dose-dependent effect could be attributed to the biological variability, as well as to the chemical complexity of the crude extract. The decreased aversion to the open arms is due to an anxiolytic effect expressed by an increased number of open arm entries in, EPM (20). Therefore, the behavioural alterations induced by the CGRM in the EPM are consistent with an anxiolytic effect. The anxiolytic effect brought about by the oral administration CGRM suggests that they contain liposoluble principle that might be acting on the specific central recognition sites coupled to GABA_A receptors, facilitating GABAergic transmission involved in the physiological expression of anxiety (21). The hole-board test provides a easy method for measuring the reaction of an animal to an unusual environment and is generally used to evaluate emotionality, anxiety and responses to stress in animals.

The head-dipping behaviour was sensitive to changes in the emotional state of the animal, and suggested that the expression of an anxiolytic state in animals may be reflected by an increase in head-dipping behaviour (22). In the present study, CGRM (125,250 and 500 mg/kg) increased head-dip counts without changing locomotion. These results indicate that CGRM has a significant anxiolytic effect in this paradigm.

Regarding the medical treatment of psychiatric disorders, the results obtained in this work became important because not only anxiolytic effects were observed; antidepressant activity (23) was already proved by the extract. The pharmacological mechanism that might account for the anxiolytic effect of CGRM has not been clearly identified. It has been hypothesised that CGRM can interact with GABA_A receptors, suggesting that the GABAergic system is at least partly involved in the pharmacological activity of methanolic root extract.

Further studies should be carried out to correlate the pharmacological activities with the chemical constituents.

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