

ANXIOLYTIC AND CNS DEPRESSANT ACTIVITIES OF METHANOL EXTRACT OF CURCUMA CAESIA RHIZOME

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Summary

Curcuma caesia Roxb. (Zingiberaceae), called black turmeric in English, is a perennial herb found throughout the Himalayan region, North-East and Central India. The plant has been traditionally used in India for several medicinal purposes. Present studies were carried out to evaluate the methanol extract of *Curcuma caesia* (MECC) rhizome for CNS depressant activities. MECC was studied for Hypnotic activity, Forced swim test and Tail suspension test. MECC (50 and 100 mg/kg; i.p.) produced significant ($p < 0.05$) and dose-dependent reduction in the onset and prolongation of sleep duration induced by pentobarbitone. MECC on immobility period in both FST and TST at the doses of 50 and 100 mg/kg, i.p for 7 successive days to mice decreased the immobility periods significantly in a dose dependent manner, indicating significant antidepressant like activity.

Key words: *Curcuma caesia*, Hypnosis, Immobility, Forced swim test and Tail suspension test.

Introduction

Depression is considered as an affective disorder characterized by change in mood, lack of interest in the surroundings, psychomotor retardation and melancholia. At present, anxiety and depression are the most frequent psychiatric conditions commonly found. A number of the population suffers from these conditions at some time during their life. According to the World Health report (1), approximately 450 million people suffer from a mental or behavioral disorder, 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).

Stress has been shown to induce a marked rise in the brain levels of biogenic amines such as adrenaline and nor-adrenaline. These chemical substances are release in response to stress signals and are meant to assist the organisms to cope with stress (3). However, increased utilization of the amines resulting in their depletion in prolonged severe stress is responsible for fatigue, reduced stamina, lowered mood (hopelessness) or despair seen in individuals under intense stress. It is has been reported that drugs with anti-stress properties induce a state of non-specific resistance against stressful conditions (4). Amphetamine, caffeine and anabolic steroids are the most widely used drugs by people to combat stress. However, the incidence of toxicity and dependence has limited the therapeutic usefulness of these drugs in the control of stressful events. The potential utilities of safer and cheaper herbal medicines as anti-stress agents have been reported in literature (3).

These considerations implicate the search for new anxiolytic and antidepressant agents that have a fast onset of action present with less side effects and a wider safety margin. It has lead scientists to investigate plants, which are commonly employed in traditional and alternate system of medicine for sleep disorders and related diseases (5). Therefore, herbal therapies should be considered as alternative/complementary medicines. Recently, the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly (6).

Black Turmeric (*Curcuma caesia*) is a perennial herb with bluish-black rhizome, native to North-East and Central India. It is also sparsely found in Papi Hills of East Godavari, the root hills of the Himalayas and North Hill forest of Sikkim. The rhizomes of Black Turmeric have a high economical importance owing to its putative medicinal properties. The rhizomes are used in the treatment of hemorrhoids, leprosy, asthma, cancer, epilepsy, fever, wound, vomiting,

menstrual disorder, smooth muscle relaxant activity (7), anthelmintic, aphrodisiac, inflammation, gonorrhoeal discharges, etc. (7, 8).

The aim of the present study was to evaluate anxiolytic and antidepressant effect of the methanol extract of *C. caesia* in different Swiss albino mice models.

Materials and methods

Plant Materials

The rhizome of *C. caesia* plant was collected from the upper hill region of Sikkim, India. Air dried whole rhizomes (225 g) were powdered in a mechanical grinder and the plant materials was extracted by methanol using Soxhlet extraction apparatus. The solvent was completely removed under reduced pressure in a rotary evaporator. The concentrated extract was obtained by lyophilization and stored in vacuum desiccators (20°C) for further use. The yield of the methanol fraction was about 21.51%.

Qualitative analysis

Preliminary qualitative analysis has been performed to know the type of compounds present in the extracts. Chemical group test were performed for Alkaloids, Flavonoids, Saponins, Tannins, Steroids (9, 10).

Animals

Male Swiss albino mice (20-25 g) were taken from Rita Ghosh & Co. Kolkata, India. The mice were grouped and housed in poly acrylic cages (38 cm × 23 cm × 10 cm) with not more than six animals per cage. The animals were maintained under standard laboratory conditions (temperature 25-30°C and 55-60% relative humidity with dark/light cycle 14/10h) and were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The mice were acclimatized to laboratory conditions for 7 days before commencement of the experiment. All the procedures described were reviewed and approved by the University Animal ethical Committee.

Toxicity study

Determination of LD₅₀

As per reported method (Organization for Economic Co-operation and Development 420, 2000), fasted animals of single sex were dosed in a stepwise

procedure using the fixed dose of 5, 50, 300 and 2000 mg/kg orally (11, 12). All the animals were observed for any signs of toxicity or mortality least 24 hr. All the animals were subjected for sharp observation for a period of 14 days. MECC shows no mortality or toxic effect up to 2000 mg/kg body weight in mice.

Pentobarbitone induced sleeping time

Albino mice (20–25 gm) were grouped of six each. Group I received normal saline, groups II and III received the MECC (50 and 100 mg/kg, *i.p.*) and group IV received reference drug (Chlorpromazine hydrochloride 3 mg/kg, *i.p.*). Animals were administered with sodium pentobarbitone (40 mg/kg, *i.p.*) 30 min later and index of hypnotic effect recorded. The effects were recorded as follows: Time elapsed between the administrations of pentobarbitone until loss of righting reflex was recorded of as the onset of sleep, while the time from the loss to its recovery was considered as the duration of sleep (13).

Forced swim test (FST)

Behavior despair was proposed as a model to test for antidepressant activity by (14). The procedure was essentially the same as described by (15). Mice were forced to swim individually in a glass jar ($25 \times 12 \times 25 \text{ cm}^3$) containing fresh water of 15 cm height and maintained at $25^\circ\text{C} (\pm 3^\circ\text{C})$. After an initial 2 min 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 limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 min of a total 6 min test. The changes in immobility duration were studied after administering drugs in separate groups of animals. Each animal was used only once. The mouse was considered immobile when it floated motionlessly or made only those moments necessary to keep its head above the water surface.

Tail Suspension Test (TST)

The total duration of immobility induced by tail suspension was measured according to the standard method (16). Mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility was recorded during the next 4 min of a total 6 min test. Mice were considered to be immobile only when they hung passively and were completely motionless.

Data analysis

All the data are given as the mean \pm SEM of three individual measurements. Data on all the experiment were analyzed using analysis of variance (ANOVA) and the group means were compared by Dunnett's by Graph Pad Prism software, version 4.03. A probability of $p < 0.05$ was considered as significant.

Results

Preliminary phytochemical screening of crude methanol extract of *Curcuma caesia* demonstrated strong positive test for flavonoids and tannin, additionally, alkaloids and saponins were also present (Table-1).

MECC produced significant ($p < 0.01$) and dose-dependent reduction in the onset and prolongation of sleep duration induced by pentobarbitone, MECC at the dose of 50 and 100 mg/kg, *i.p.* showed a significant prolongation of sleep duration, where as MECC at high dose 100 mg/kg, *i.p.* is more significant when compare of 50 mg/kg and the result is comparable to that produced by chlorpromazine (Table-2).

MECC at the doses 50 and 100 mg/kg intraperitoneal administered for 7 successive days to mice decreased the immobility periods significantly in a dose dependent manner in both FST and TST, indicating significant antidepressant like activity as shown in Fig-1 and Fig-2. MECC at a dose 100 mg/kg showed most potent antidepressant like activity as indicated by highest decreased immobility period as compared to the standard drug imipramine (15 mg/kg, *i.p.*).

Table 1: Preliminary phytochemical screening MECC.

| Extract | Steroid | Tannin | Saponin | Flavonoid | Alkaloid |
|----------|---------|--------|---------|-----------|----------|
| Methanol | + | + | + | + | + |

(+) indicate the presence of phytoconstituents.

Table 2: Effect of MECC on righting reflex (hypnosis).

| Gr. | Treatment | Dose | Onset of sleep (Min) | Duration of sleep (Min) |
|-----|----------------|------------------------|----------------------|-------------------------|
| I | Normal saline | 5 ml/kg, <i>i.p.</i> | 8.5 ± 0.43 | 52.17 ± 1.49 |
| II | MECC | 50 mg/kg, <i>i.p.</i> | 5 ± 0.89** | 63.33 ± 2.11** |
| III | MECC | 100 mg/kg, <i>i.p.</i> | 3.83 ± 0.31*** | 70.33 ± 2.86*** |
| IV | Chlorpromazine | 1 mg/kg, <i>i.p.</i> | 3.17 ± 0.31*** | 73.33 ± 2.01*** |

Results expressed as mean±SEM (n=6); **indicates $p<0.01$, ***indicates $p<0.001$.

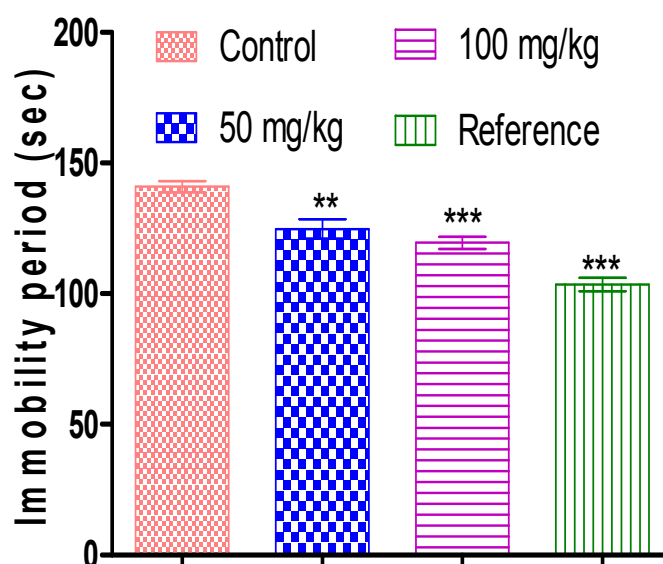


Fig-1: Forced swim test.

Results expressed as mean ± SEM (n=6); **indicates $p<0.01$, ***indicates $p<0.001$

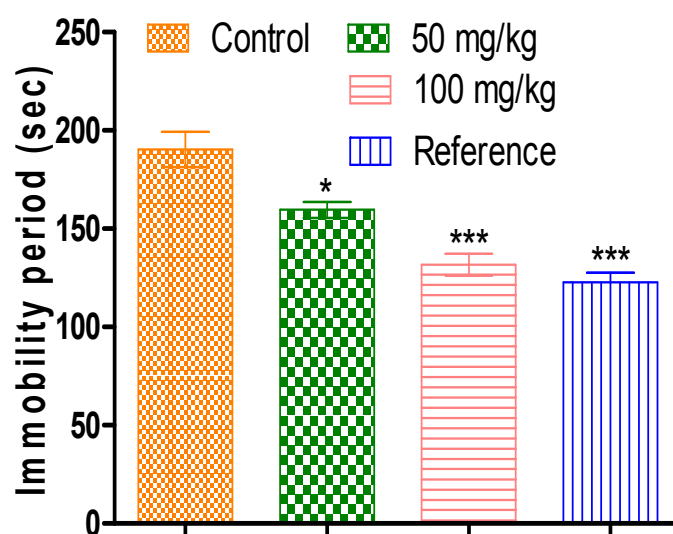


Fig-2: Tail suspension test.

Results expressed as mean \pm SEM (n=6); *indicates $p < 0.05$, **indicates $p < 0.01$, ***indicates $p < 0.001$

MECC also potentiated pentobarbitone induced sleeping time. The exact mechanism by which MECC potentiated PB induced sleeping time is unknown. It has been reported that the saponins show a potent sedative activity when tested in similar models and also inhibit spontaneous motor activity in mice (17). Therefore, the saponin content of this extract might be contributing in part to the experimental pharmacological effects.

In the present study we have evaluated the antidepressant activity of MECC in TST and FST. These tests are quite sensitive and relatively specific to all major class of antidepressant drugs including tricyclics, serotonin-specific reuptake inhibitors, MAO inhibitors, and atypical (18). The development of immobility when rodents are suspended by their tail during TST and when they are placed in an inescapable cylinder of water during FST reflects the cessation of their persistent escape-directed behavior. Conventional drugs reliably decrease the duration of immobility in animals during these tests. This decrease in duration of immobility is considered to have a good predictive value in the evaluation of potential antidepressant agents (19).

In the present study, MECC in the highest dose tested (100 mg/kg) was superior to imipramine in both the experimental models and this effect seems most likely to be mediated through an interaction with adrenergic and dopaminergic systems. Exact mechanisms underlying the antidepressant action cannot be concluded at the moment due to the presence of large number of phytochemicals in the MECC. Preliminary photochemical analysis of the plant extract revealed the presence of tannin, steroids, saponin, and flavonoids (20). However, the antidepressant activity may be attributed to the presence of flavonoids and tannic acid. Tannic acid has been shown to be a non selective inhibitor of monoamine oxidase, thereby increasing the levels of monoaminergic neurotransmitters in the brain (21). Another possible mechanism of action is the attenuation of oxidative stress produced during depression, by the tannic acid present in MECC.

Therefore, one of the antidepressant mechanism of *C. caesia* is thought to involve flavonoids, saponin and tannic acid which reach the brain tissues through the metabolizing process, protecting brain function from CNS disturbance and consequently, exerting an antidepressant effect. Thus, extracts of *C. caesia* may have potential therapeutic value for the management of depressive disorders. Further study is required to identify the particular components present in this extract responsible for its antidepressant like activity.

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