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Anti-depressant like effect of curcumin and its combination with piperine in unpredictable chronic stress-induced behavioral, biochemical and neurochemical changes

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ABSTRACT

Curcumin, a yellow pigment extracted from rhizomes of the plant *Curcuma longa* (turmeric), has been widely used as food additive and also as a herbal medicine throughout Asia. The present study was designed to study the pharmacological, biochemical and neurochemical effects of daily administration of curcumin to rats subjected to chronic unpredictable stress. Curcumin treatment (20 and 40 mg/kg, i.p., 21 days) significantly reversed the chronic unpredictable stress-induced behavioral (increase immobility period), biochemical (increase monoamine oxidase activity) and neurochemical (depletion of brain monoamine levels) alterations. The combination of piperine (2.5 mg/kg, i.p., 21 days), a bioavailability enhancer, with curcumin (20 and 40 mg/kg, i.p., 21 days) showed significant potentiation of its anti-immobility, neurotransmitter enhancing (serotonin and dopamine) and monoamine oxidase inhibitory (MAO-A) effects as compared to curcumin effect *per se*. This study provided a scientific rationale for the use of curcumin and its co-administration with piperine in the treatment of depressive disorders

1. Introduction

Depression is an incapacitating psychiatric ailment that has been estimated to affect 21% of the world population (Schechter et al., 2005). It is characterized by a pervasive low mood, loss of interest in usual activities and diminished ability to experience pleasure. A large number of clinical observations have suggested that stress can act as a precipitating factor in the onset of affective illness, especially major depression (Bidzinska, 1984). Chronic stress can induce depressive disorders, and animal stress models are widely used in pre-clinical antidepressant evaluation (Garcia, 2002).

Curcumin, a yellow pigment extracted from rhizomes of the plant *Curcuma longa* (turmeric), has been widely used as food additive and also as a herbal medicine throughout Asia. In India, it is one of the extensively consumed spices. Curcumin is reported to possess antioxidant (Ruby et al., 1995; Sharma, 1976; Sugiyama et al., 1996), anti-inflammatory (Srimal et al., 1973), hepato- and nephro-protective (Kiso et al., 1983; Venkatesan et al., 2000), antimicrobial (Jordan and Drew, 1996; Mahady et al., 2002; Reddy et al., 2005), anticarcinogenic (Kuttan et al., 1985), and thrombosis suppressing (Srivastava et al., 1985) properties. Xu et al. (2005a,b) have recently reported the antidepressant activity of curcumin. In the present study, we have examined the antidepressant-like effect of chronic administration of curcumin in unpredictable stress paradigm in rats. Since curcumin has low bioavailability, attempts were also made to administer piperine, a bioavailability enhancer, and curcumin together to enhance the anti-stress activity of curcumin.

2. Materials and methods

2.1. Animals

Female Wistar rats (200–250 g) bred at Central Animal House (CAH) Panjab University, Chandigarh, were used. They were housed under standard (25±2 °C, 60–70% humidity) laboratory conditions, maintained on a 12 hour natural day–night cycle, with free access to standard food and water. Animals were acclimatized to laboratory conditions before the test. All behavioral experiments were carried out between 1000 and 1400 h. The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) and conducted according to the CPCSEA guidelines on the use and care of experimental animals.

2.2. Chronic stress procedure

The rats were subjected to stress as described by Molina et al. (1990) and Murua et al. (1991) with some modifications. Animals

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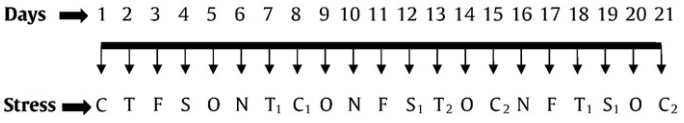
were subjected to stress paradigm once a day over a period of 21 days between 0900 and 1300 h. The order of stressors used was as follows:



C — Cold swim (8 °C, 5 min); T — Tail pinch (1 min); F — Food and water deprivation (24 h); S — Swimming at room temperature (24±2 °C, 20 min); O — Overnight illumination; N — No stress; T_1 — Tail pinch (1.5 min); C_1 — Cold swim (10 °C, 5 min); S_1 — Swimming at room temperature (24±2 °C, 15 min); T_2 — Tail pinch (2 min); C_2 — Cold swim (6 °C, 5 min).

On day 21 (60 min after drug administration), animals were subjected to different behavioral (FST), biochemical and neurochemical tests. For biochemical and neurochemical investigations, rats were sacrificed by decapitation and brains were isolated. Brains samples were then stored in phosphate buffer (0.1 M, pH 7.4) at −80 °C till further investigations.

2.3. Forced-swim test (FST)

The animals were individually forced to swim in a 60×30×45 cm (L×B×H) filled with water (23–25 °C) up to a height of 25 cm. After the initial 2–3 min of vigorous activity the animals showed period of immobility by floating with minimum movements. An animal is considered to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its nose above the water surface. The total immobility period during the 6 min test was recorded with the help of stop-watch (Kulkarni and Mehta, 1985).

2.4. Drugs and treatment

Both curcumin (Sanat Products Ltd, Delhi, India) and piperine were dissolved in rice bran oil. The drugs were administered daily for 21 days by intraperitoneal route (i.p.). Rice bran oil was administered to the vehicle treated group. The doses of the drugs used were selected according to the previous studies conducted in our laboratory (Bishnoi et al., 2008; Kuhad et al., 2007; Kumar and Singh, 2008; Sharma et al., 2006) and as reported in the literature (Wattanathorn et al., in press). Behavioral observations were made 60 min after the last dose of drug treatment.

2.5. Measurement of monoamine oxidase enzyme activity

The MAO activity was assessed spectrophotometrically (Schurr and Livne, 1975). The buffer washed brain samples were homogenized in 10 volume of sodium phosphate buffer (0.1 M, pH 7.4) and centrifuged (Biofuge primo-R, Germany) at 15,000 g for 20 min. Pellets were discarded. Supernatant was pipetted out and used for the estimation of MAO-A and MAO-B activity. For estimating MAO-A activity, 2.75 ml Tris buffer (0.1 M, pH 7.4) and 100 µl of 4 mM 5-hydroxytryptamine were mixed in quartz cuvette which was then placed in double beam spectrophotometer (UV-Pharmaspec-1700, Shimadzu, Japan). This was followed by the addition of 150 µl solution of brain homogenate to initiate the enzymatic reaction and the change in absorbance was recorded at wavelength of 280 nm for 5 min against the blank. For estimating MAO-B activity, 2.75 ml Tris buffer (0.1 M, pH 7.4) and 100 µl of 0.1 M benzylamine were mixed in quartz cuvette which was then placed in double beam spectrophotometer. This was followed by the addition of 150 µl solution of brain homogenate to initiate the enzymatic reaction and the change in absorbance was recorded at wavelength of 249.5 nm for 5 min against the blank containing Tris buffer and 5-hydroxytryptamine.

2.6. Measurement of biogenic amines

Biogenic amines (norepinephrine, serotonin and dopamine) were estimated by HPLC with electrochemical detector by the method of Beyer et al. (2002). Waters® standard system consisting of a high pressure isocratic pump, a 20 µl sample injector valve, C18 reverse phase column and electrochemical detector was used. Data was recorded and analyzed with the help of Empower® software. Mobile phase consists of 0.15 M sodium dihydrogen phosphate, 0.25 mM ethylenediaminetetraacetic acid, 1.75 mM 1-octane sulfonic acid, 2% isopropanol and 10% methanol (pH 4.8). Electrochemical conditions for the experiment were +0.800 V, sensitivity ranges from 1–100 nA. Separation was carried out at a flow rate of 1 ml/min. Samples (20 µl) were injected manually. Brain samples were homogenized in homogenizing solution containing 0.1 M perchloric acid. After that, samples were centrifuged at 24,000 g for 15 min. The supernatant was further filtered through 0.25 µm nylon filters before injecting in the HPLC injection pump.

2.7. Statistical analysis

One specific group of rats was assigned to one specific drug treatment condition and each group comprised six rats (n=6). All the values are expressed as means±S.E.M. The data were analyzed by One Way ANOVA followed by Tukey's test. $p \leq 0.05$ was considered as statistically significant.

3. Results

3.1. Effect of curcumin and its combination with piperine on immobility period in FST

As shown in Fig. 1, chronically stressed rats exhibited significant increase in immobility period as compared to control animals. Chronic curcumin administration (20 and 40 mg/kg, i.p.) dose dependently reversed the increase in immobility period in stressed rats. When curcumin (20 and 40 mg/kg, i.p.) and piperine (2.5 mg/kg, i.p.) were co-administered, significant potentiation was observed in the anti-immobility effect of curcumin. The combination of curcumin

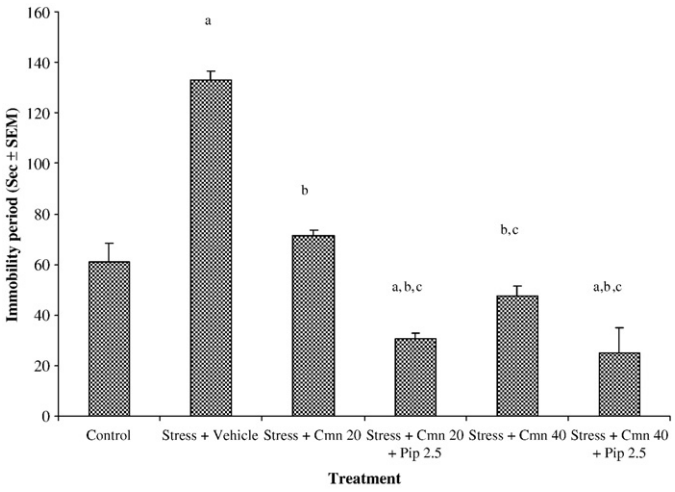


Fig. 1. Effect of curcumin and its combination with piperine on forced swim-induced immobility period in rats. [a]$p \leq 0.05$ as compared with control group; [b]$p \leq 0.05$ as compared with stress (S)+vehicle group, [c]$p \leq 0.05$ as compared with stress (S)+curcumin (20).

(20 mg/kg, i.p.) and piperine (2.5 mg/kg, i.p.) was even more potent than curcumin (40 mg/kg, i.p.) *per se*.

3.2. Effect of curcumin and its combination with piperine on monoamine oxidase (MAO) activity

Chronic stress procedure resulted in significant increase in monoamine oxidase (MAO-A and MAO-B) enzymatic activity. Chronic curcumin (20 and 40 mg/kg, i.p.) dose dependently reversed the MAO-A enzymatic activity. However, reversal in MAO-B activity was not dose dependent. The combination of curcumin (20 and 40 mg/kg, i.p.) and piperine (2.5 mg/kg, i.p.) showed potentiation in MAO-A enzyme inhibitory activity. The enzyme inhibitory effect of these combinations were comparable to curcumin (20 and 40 mg/kg, i.p.) alone. The combination of curcumin (20 and 40 mg/kg, i.p.) and piperine (2.5 mg/kg, i.p.) did not show any potentiation in MAO-B enzyme inhibitory activity (Fig. 2).

3.3. Effect of curcumin and its combination with piperine on brain monoamine levels

21 day stress procedure resulted in significant depletion of brain monoamine levels (norepinephrine, dopamine and serotonin). Curcumin (20 and 40 mg/kg, i.p., 21 days) reversed the chronic stress-induced neurotransmitter changes. However, increases in neurotransmitter levels (serotonin and dopamine) were significant only at curcumin (40 mg/kg, i.p.). There was no change in the levels of norepinephrine. When sub-threshold dose of piperine (2.5 mg/kg, i.p., 21 days) was combined with curcumin (20 mg/kg, i.p., 21 days), there was significant increase in monoamine levels as compared to curcumin (20 mg/kg, i.p., 21 days) alone. The effect was comparable with curcumin (40 mg/kg, i.p., 21 days) *per se* (Fig. 3).

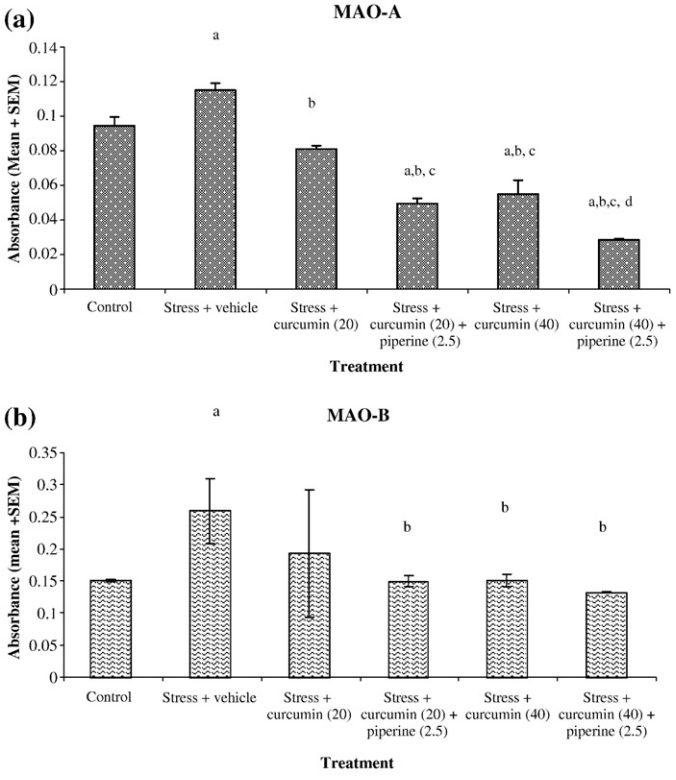


Fig. 2. Effect of curcumin and its combination with piperine on brain monoamine oxidase (MAO) activity. (a): $^{a}p \leq 0.05$ as compared with control group, $^{b}p \leq 0.05$ as compared with stress+vehicle group; $^{c}p \leq 0.05$ as compared with stress+curcumin (20), $^{d}p \leq 0.05$ as compared with stress+curcumin (20)+piperine (2.5) and stress+curcumin (40). (b): $^{a}p \leq 0.05$ as compared with control group; $^{b}p \leq 0.05$ as compared with stress +vehicle group.

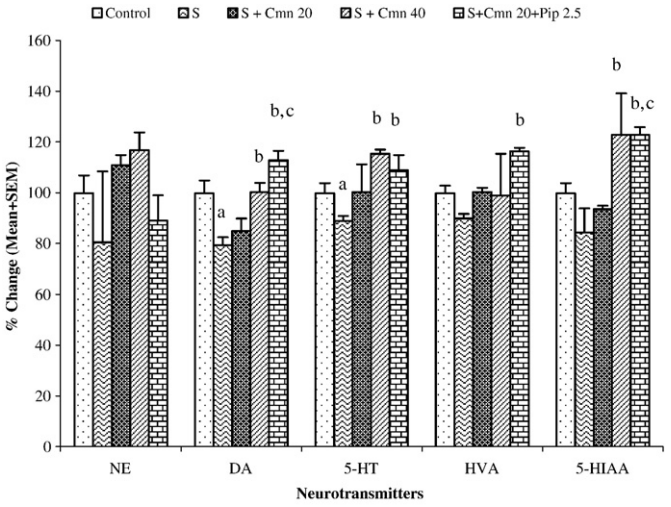


Fig. 3. Effect of curcumin and its combination with piperine on brain monoamine levels. $^{a}p \leq 0.05$ as compared with control group, $^{b}p \leq 0.05$ as compared with stress (S)+vehicle group, $^{c}p \leq 0.05$ as compared with stress (S)+curcumin (20).

4. Discussion

There is a complex relationship among stressful situations, mind and body's reaction to stress, and the onset of clinical depression. Some stress-provoked disturbances seem to be associated with the pathophysiology of depression (Kioukia-Fougia et al., 2002). There is growing body of evidence showing that the chronic administration of various uncontrollable stresses, a procedure known as "chronic uncontrollable stress", is an appropriate model for the pre-clinical evaluation of antidepressants (Katz and Schmaltz, 1980; Willner, 1991; Willner et al., 1992). Both variability and unpredictability during stress regime are critical triggers in the introduction of depressive-like behavior, such as escape deficit in shuttle box task (Murua et al., 1991; Soblosky, 1986). The theoretical premise behind this method is that depression is the outcome of an eventual inability to cope with a stream of dissimilar unpleasant stimuli imposed by the environment. To stimulate this effect in animals, stressors are used to induce behavioral deficits which can subsequently be reversed by antidepressant treatments (Katz and Hersh, 1981; Kennett et al., 1986; Maier, 1984). So, we used chronic unpredictable stress model to determine whether chronic curcumin administration can alleviate or reverse the stress-induced depressive-like behavior in rats. We used mainly female rats in this study as majority of the patients of depression are female. Nevertheless our earlier study was in male mice which reveals no gender specific effect of curcumin (Kulkarni et al., 2008).

Chronic unpredictable stress prolongs learned helplessness behavior and increase plasma corticosterone levels (Chen et al., 2006). It also inhibits the brain monoamine oxidase (MAO-A and MAO-B) enzyme activity (Lin et al., 2005) which may further result in the depletion of brain monoamine levels. Various antidepressant drugs, either by inhibiting MAO enzyme or by inhibiting reuptake mechanism, increase the central monoamine levels and reverse the stress induced depressive-like behavior. Chronic tramadol and desipramine treatments increase the level of norepinephrine (NE) and its metabolite 3-methoxy-4-hydroxy-phenylglycol (MHPG) in the locus coeruleus but not in the cerebellum, whereas only MHPG level is increased in the hypothalamus (Yalcin et al., 2007). Chronic citalopram treatment exerts antidepressant-like effect by increasing 5-HT levels in chronic unpredictable stressed animals (Tõnissaar et al., 2008).

Various studies have reported the antidepressant action of curcumin in forced swim, olfactory bulbectomy (Xu et al., 2005a,b).

It has been suggested that curcumin reverses the effects of chronic stress on behavior, the HPA axis, BDNF expression, and phosphorylation of CREB (Xu et al., 2006). It also increases serotonin receptor 1A mRNA in chronically stressed rats (Xu et al., 2007). Recent reports have suggested the involvement of serotonin receptors (5-HT_1 and 5-HT_2) in mediating its antidepressant activity (Wang et al., 2008). Studies conducted in our laboratory also revealed the antidepressant-like effect of curcumin in forced swim and reserpine-induced depression models in mice. In the present study, rats that were exposed to a regime of chronic stress exhibited greater immobility period as compared to control animals. The increase in immobility time was significantly reversed by curcumin (20 and 40 mg/kg, i.p., 21 days) administration. It significantly inhibited monoamine oxidase (MAO-A and MAO-B) enzyme activity. Chronic curcumin administration also reversed the stress-induced neurotransmitter alterations and resulted in increased levels of serotonin and dopamine. Thus, curcumin exerted antidepressant-like effect in chronic unpredictable stress induced depression model, which is attributed to the inhibition of monoamine oxidase (MAO) enzyme activity and the resulted increase in brain monoamine levels (serotonin and dopamine).

Bioavailability of curcumin is a major concern which limits its therapeutic utility. Various studies have reported that curcumin undergoes extensive reduction, most likely through alcohol dehydrogenase, followed by conjugations like sulfation and glucuronidation at various tissue sites mainly in liver and intestine (Garcea et al., 2004; Hoehle et al., 2006; Wahlstrom and Blennow, 1978). Although, advanced drug delivery systems (nanoparticles, liposomes, micelles and phospholipid complexes) may increase the bioavailability, but the simultaneous administrations of adjuvants, which can block metabolic pathways of curcumin, are still the major means to improve bioavailability of curcumin. Piperine, a known inhibitor of hepatic and intestinal glucuronidation, increases the bioavailability of many drugs including curcumin (Atal et al., 1985; Shoba et al., 1997). During the course of studies we performed HPLC for the detection of curcumin in brain tissue. After the administration of curcumin 20, 40, 100 mg/kg, we could not detect curcumin. At 200 and 400 mg/kg dose dependent detection of curcumin was there. On co-administration of piperine (2.5 mg/kg) we were able to detect the curcumin levels in brain after 100 mg/kg administration (data not presented). When we combined sub-threshold dose of piperine (2.5 mg/kg, i.p., 21 days) with curcumin (20 mg/kg, i.p., 21 days), potentiation was observed in the pharmacological (FST), biochemical (MAO) and neurochemical activities of curcumin as curcumin (20 mg/kg, i.p., 21 days) showed effects comparable with curcumin (40 mg/kg, i.p., 21 days) *per se*. It suggests that piperine increased the bioavailability of curcumin. In the case of neurotransmitter release curcumin does not affect all the neurotransmitters. It usually increases the concentration of serotonin and dopamine (Kulkarni et al., 2008). In the present study also the trend is the same and curcumin as well as curcumin + piperine significantly increases the levels of serotonin and its metabolite and dopamine.

In conclusion, curcumin exerted antidepressant-like effect in chronic unpredictable stress induced depression model in rats and this effect may be mediated by the central monoaminergic neurotransmitter system (5-HT and dopamine). Further this finding provides a scientific rationale for the co-administration of piperine and curcumin, which may act as a useful and potent combination in the treatment of depressive disorders.

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