



Protective Effect of *Curcuma longa* Rhizomes against Physical Stress-induced Perturbations in Rats

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Abstract

Curcuma longa (Turmeric) is a bright yellow ancient spice native to Asian countries. It has been used as traditional remedy dating back to 600 BC. Turmeric is well known for its applications as a cosmetic, condiment and flavoring agent. The present study was an attempt to explore the protective effect of *Curcuma longa* rhizomes against physical stress-induced perturbations in rats. Animals were pre-treated with extracts of *C. longa* rhizomes (crystallized ethylacetate extract; and byproduct-oleoresin) at doses of 200 and 400 mg/kg for 21 days. The effect on swimming endurance followed by post-swimming muscle co-ordination and spontaneous motor activity was evaluated. Estimation of brain monoamine levels in rats and HPLC analysis were carried out. Pre-treated rats with *C. longa* extracts showed dose dependant significant enhancement in swimming endurance time, increased the duration (sec) of stay on rota-rod apparatus and increased the count (actophotometer score) in spontaneous motor activity. In addition, the pre-treated rats were found to possess normalizing activity against physical stress induced changes in norepinephrine, dopamine and serotonin. Curcuminoids was identified by HPLC analysis and it was one of the active principles responsible for the adaptogenic activity. Extracts of *C. longa* rhizomes exhibited adaptogenic activity against physical stress model followed by post-swimming muscle co-ordination and spontaneous motor activity, which could be due to the presence of curcuminoids content. In conclusion, the results of the present investigation emphasized the protective effect of *C. longa* rhizomes against physical stress-induced perturbations in rats.

Keywords: Adaptogenic, actophotometer, brain monoamines, *Curcuma longa*, swimming endurance, rota-rod

1. Introduction

In modern era, stress and stress-related disorders are a significant cause of disease and contributing to perhaps 75% of all illnesses [1]. Stress is a pattern of metabolic and behavioural reactions occurs in response to physical, chemical, biological and emotional changes [2]. It has played a vital role in the etiopathogenesis of a diverse variety of diseases ranging from psychiatric disorder such as anxiety and depression, immunosuppression, male sexual dysfunction, cognitive dysfunctions, peptic ulcer, hypertension, ulcerative colitis and endocrine disorders including diabetes mellitus [1].

Adaptogens are biologically active substances derived from plants that appear to induce a state of non-specific increase of resistance of the organism to diverse assaults that threaten internal homeostasis and improve physical endurance [3]. They mainly focus on its ability to reduce stress reactions during the alarm phase of stress response, prevent or at least delay the state of exhaustion. Hence adaptogens provide a certain level of protection against long term stress [4]. Ayurveda, the Indian traditional system of medicine uses the plant, plant products and active ingredients present in plants for treating various disorders. Several plants are being investigated for management of number of disorders including antistress

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activity and have been reported for adaptogenic and rejuvenating properties [5].

Curcuma longa L., commonly known as turmeric is widely consumed in the countries of its origin for a variety of uses, including as a dietary spice and dietary pigment [6]. In Ayurveda, Unani, and Siddha medicine *C. longa* is used as a home remedy for various diseases including biliary diseases, cough, hepatic diseases, wound healing. It is used in the textile and pharmaceutical industries and in Hindu religious ceremonies in one form or another [7]. In old Hindu texts, turmeric has been described as an aromatic stimulant and carminative. As a household remedy, turmeric powder mixed with slaked lime can be used for the treatment of sprains and swelling caused by injury, applied locally over the affected area. In some parts of India, the powder is taken orally for the treatment of sore throat. This non-nutritive Phytochemical is pharmacologically safe, considering that it has been consumed as a dietary spice [6]. Turmeric has a long traditional use in the Chinese and Ayurvedic systems of medicine, particularly as an anti-inflammatory and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage, and colic. Research has also focused on turmeric's antioxidant, hepatoprotective, anti-inflammatory, anticarcinogenic and antimicrobial properties, in addition to its use in cardiovascular disease and gastrointestinal disorders [8]. With this view, present study was an attempt to explore the protective effect of *Curcuma longa* rhizomes against physical stress-induced perturbations in rats.

2. Materials and Methods

2.1 Study Drug Extract

Extracts of *C. longa* rhizomes (crystallized ethylacetate extract and byproduct-oleoresin) were obtained as a gift sample from Olive Lifesciences Pvt Ltd, Tumkur, India.

2.2 Experimental Animals

Sprague-Dawley rats (180–220 g) of both sexes were used in the present study. Animals were kept in standard cages, provided with a standard diet supplied by Pranav Agro Industries Ltd., Sangli, India and drinking water *ad libitum*. Animal housing conditions were maintained at $25 \pm 2^\circ\text{C}$ with relative humidity of $65 \pm 10\%$ under 12-hour light/dark cycles. The procedures were carried only

after obtaining the approval of the Institutional Ethics Committee.

2.3 Acute Toxicity Study

The acute toxicity study was performed according to the Organisation for Economic Co-operation and Development (OECD) guideline 425. A dose limit of 2000 mg/kg of *Curcuma longa* rhizomes each extract was administered in five healthy female adult Sprague-Dawley rats. Rats were fasted overnight from food, but not water, prior to dosing and weighed before the extract was administered orally. A dose of 2000 mg/kg was given to one animal, and this rat was observed for mortality and clinical signs (behaviours: unusual aggressiveness, unusual vocalisation, restlessness, sedation and somnolence; movements: twitch, tremor, ataxia, catatonia, paralysis, convulsion, fasciculation, prostration and unusual locomotion; convulsion) for the first hour, then hourly for 3 h and, finally periodically until 48 h. If the animal survived, then four additional animals were given the dose 2000 mg/kg sequentially at 48 h intervals. All of the experimental animals were maintained under close observation for 14 days, and the number of rats that died within the study period was noted. The LD_{50} was predicted to be greater than 2000 mg/kg if three or more rats survived [9].

2.4 Swimming Endurance and Post-swimming Motor Function Tests

The rats were randomly assigned to seven groups of six rats each. The treatment schedule was as follows:

Group I: Normal control

Group II: Stress control

Group III: *Withania somnifera* 50 mg/kg p.o.

Group IV & V: Crystallized ethylacetate extract (CEE) 200 and 400 mg/kg, p.o. respectively

Group VI & VII: Byproduct-oleoresin (BPO) 200 & 400 mg/kg, p.o. respectively.

Treatment was given to rats for 21 days. On 21st day 1-h after drug administration, all the rats except normal control group were subjected to swimming endurance test (physical stress). Rats were allowed to swim in a water tank (140 × 60 × 45 cm) maintained at room temperature ($30 \pm 2^\circ\text{C}$). The end point was taken till the animals got

exhausted and the moment they drowned. The mean swimming time for each group was calculated [10].

The animals were removed, allowed to recover and dry for about 5 min. All animals including normal control group were subsequently tested for muscle co-ordination on a rota rod rotating at 15 rpm and the duration of stay on the rod was recorded. Immediately after muscle co-ordination test, rats were tested for spontaneous motor activity in actophotometer for 05 min. Thereafter, the animals were sacrificed by cervical dislocation; whole brain was dissected to estimate the brain monoamine levels [Norepinephrine (NE), Dopamine (DA) and 5-hydroxytryptamine (5-HT)] [10].

2.5 Estimation of Brain Monoamine Levels in Rats

Brain monoamine levels were estimated by spectrophotometrically. Immediately after dissection of brain tissues, washed in ice-cold saline and homogenized in 10 ml acidified butanol. Tissue homogenate (4 ml) was mixed with 10 ml of 10% heptane and 5 ml of 0.003 N HCl. Then the mixture was shaken for 5 minutes and centrifuged at 2000 rpm for 10 minutes. Acid layer (4.5 ml) was eluted and mixed with 100 mg alumina and 1 ml of 2 M sodium acetate. Then the mixture was shaken for 5 minutes and centrifuged at 2000 rpm for 10 minutes and the resultant supernatant was taken for the estimation of 5-HT and the precipitate was used for the estimation of NE and DA.

- Estimation of Serotonin (5-HT)

To the supernatant obtained previously, 3 ml of 10% isobutanol and 2 ml of boric acid buffer (pH 10) was added. After shaking the mixture, 2 ml of 10% heptane was added to the butanol phase and 5 ml of 0.1 N HCL was added followed by shaking again and 1 ml of 0.3 N HCL was mixed. Then it was taken for spectrofluorometric reading of 5-HT.

- Estimation of Dopamine and Norepinephrine

Cold distilled water (5 ml) was added to the precipitate, shaken well and centrifuged at 2000 rpm for 30 seconds. 3 ml acetic acid (0.33 N) was added and centrifuged at 2000 rpm for 3 minutes. Supernatant was transferred to glass stoppered centrifuge tube. 1.2 ml of freshly prepared ethylenediamine and ethylene diammonium dihydrochloride mixture (7:5) was added to it and

incubated at 50°C for 40 mins. Mixture was cooled at room temperature and saturated with sodium chloride and 4 ml of 10% isobutanol was added. It was then centrifuged at 2000 rpm for 3 mins. Supernatant was taken for the estimation of DA and the precipitate was mixed with 4 ml cold distilled water for the estimation of NE.

The fluorescence of 5-HT, DA and NE was measured in the Perkin Elmer MPF 44B Fluorescence spectrophotometer, USA with activation and emission wavelength set at 295 nm and 550 nm (for 5-HT), 320 nm and 370 nm (for DA) and 385 nm and 485 nm (for NE) [11].

2.6 HPLC Analysis

Extracts of *C. longa* rhizomes were dissolved separately in 10 ml acetonitrile and filtered through a membrane filter with a pore size of 0.45 μm prior to analysis. The HPLC profile of *C. longa* was analyzed by means of an HPLC system (Shimadzu Lab Solutions) with a photodiode array detector. A Phenomenex Luna (5 μm) C18 column was used (4.6 mm i.d. \times 250 mm) and for elution of the constituents, mobile phase (phosphate buffer and acetonitrile) was employed. The flow rate used was 0.1 ml/min and the injection volume was 20 μl . The column oven was set at 27°C and the elutant was monitored at wavelength of 420 nm. The retention times and UV spectra of major peaks were analysed. The HPLC analyses were carried out in the R & D Centre, Olive Lifesciences Pvt. Ltd., Tumkur, Karnataka, India.

2.7 Statistical Analysis

Data is expressed as mean \pm SEM; analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison tests. Results were considered significant when $p \leq 0.05$.

3. Results

No symptoms or signs of toxicity or death were observed in the experimental rats when they were subjected to toxicity study. Thus the LD₅₀ was considered to be greater than 2000 mg/kg. Extracts of *C. longa* rhizomes at doses 200 and 400 mg/kg exhibited significant ($p < 0.01$) increase in swimming performance time in rats in dose related manner. In post-swimming muscle

coordination (anti-fatigue) test, extracts of *C. longa* rhizomes increased the duration (sec) of stay on rota-rod in rats which was also dose dependant. Actophotometer reading for spontaneous motor activity (anti-fatigue test) was increased in rats treated with extracts of *C. longa* rhizomes which were also dose dependant (Table 1).

Pre-treated rats with extracts of *C. longa* rhizomes were found to be significantly ($p < 0.01$) prevent the stress induced depletion of norepinephrine and dopamine levels when compared to stress control group, thus helping the animals to cope up better during stress (Table 2). Increase in brain norepinephrine and dopamine levels after treatment with extracts indicate stimulation of sympathetic outflow as a result of stress. In addition, pre-treatment with extracts was also found to be significantly ($p < 0.01$) reduce the stress induced rise in brain serotonin level when compared to stress control group (Table 2).

HPLC profile of *C. longa* rhizomes

Three peaks were appeared in the standard curcuminoids chromatogram at 420 nm wavelength with retention times of 3.66, 3.94 and 4.24 min (Figure 1a). The chromatograms of extracts of *C. longa* rhizomes (CEE & BPO) were matched with standard curcuminoids chromatogram (Figures 1b–c). Thus, confirming the presence of curcuminoids in extracts of *C. longa* rhizomes.

4. Discussion

Stress alters the normal functioning of the body in special contrivance, when an animal forced to swim in water eventually assume a characteristic immobile posture which reflects a state of tiredness, fatigue, reduced stamina or depressed mood. These signs represent the core symptoms observed in depressed patients and in individual under

Table 1: Effect of *C. longa* rhizomes on swimming endurance and post-swimming motor function tests

Groups	Swimming time (min)	Rota-rod test (falling of time in sec)	Spontaneous motor activity (Actophotometer score)
Normal control	–	41.16 ± 0.47	185.16 ± .47
Stress control	166.3 ± 0.88	15.17 ± 0.30	78.67 ± 0.88
<i>Withania somnifera</i> 50 mg/kg	226.3 ± 1.14**	35.67 ± 0.55**	171.3 ± 1.14**
CEE-I (200 mg/kg)	195.5 ± 0.76**	26.17 ± 0.30**	132.5 ± 1.33**
CEE-II (400 mg/kg)	210.7 ± 0.71**	30.17 ± 0.30**	140.0 ± 1.06**
BPO-I (200 mg/kg)	185.2 ± 0.83**	23.17 ± 0.30**	104.5 ± 0.76**
BPO-II (400 mg/kg)	190.2 ± 0.70**	24.50 ± 0.42**	110.9 ± 0.73**

Data is expressed in mean±SEM (n=6); **p<0.01 as compared to stress control.

CEE: Crystallized Ethylacetate Extract; BPO: ByProduct-Oleoresin.

Table 2: Effect of *C. longa* rhizomes on brain monoamine levels in physical stress induced rats

Treatment	Concentrations ng/g in brain tissue		
	Norepinephrine	Dopamine	5-Hydroxy tryptamine
Normal	358.5 ± 1.43	175.5 ± 0.76	189.5 ± 0.76
Stress control	299.5 ± 1.25	130.2 ± 0.94	270.5 ± 0.76
<i>Withania somnifera</i> 50 mg/kg + stress	464.2 ± 1.42**	241.5 ± 1.25**	165.5 ± 0.56**
CEE-I (200 mg/kg)+stress	360.8 ± 1.07**	171.2 ± 0.60**	190.2 ± 0.70**
CEE-II (400 mg/kg)+stress	370.0 ± 1.15**	182.3 ± 1.05**	181.0 ± 0.73**
BPO-I (200 mg/kg)+stress	334.5 ± 0.95**	155.5 ± 0.42**	225.5 ± 0.76**
BPO-II (400 mg/kg)+stress	340.3 ± 0.88**	161.2 ± 0.47**	220.3 ± 0.98**

Data is expressed in mean±SEM (n=6); **p<0.01 as compared to stress control.

CEE: Crystallized Ethylacetate Extract; BPO: ByProduct-Oleoresin

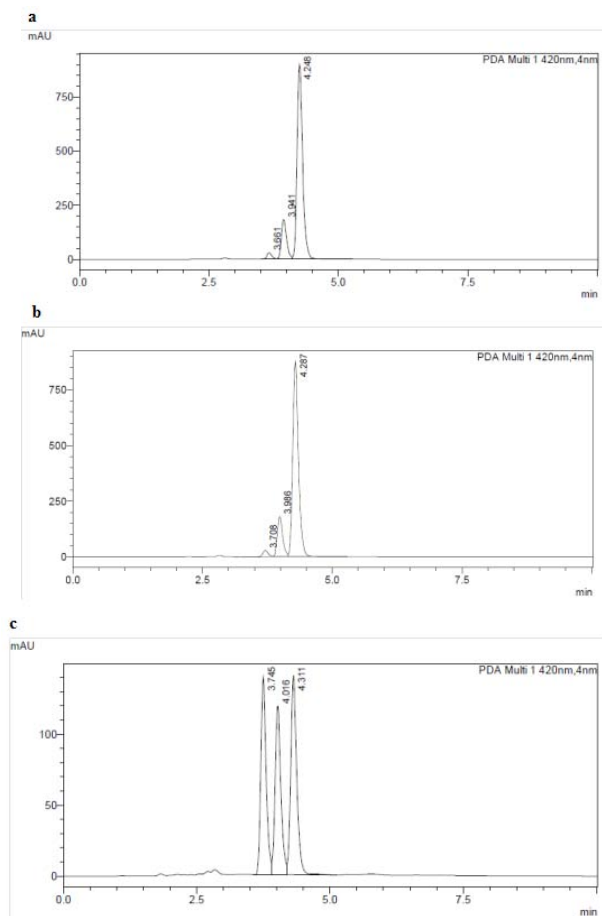


Fig.1. HPLC profile of *C. longa* rhizomes, (a) chromatogram of standard curcuminoids. (b) Crystallized ethylacetate extract of *C. longa* rhizomes. (c) ByProduct-Oleoresin of *C. longa* rhizomes.

intense stress. Drugs with antistress property increase the duration of swimming performance in animals [2]. In the present study, extracts of *C. longa* rhizomes (CEE and BPO) increased labor efficiency as evident by the increase of swimming performance.

Evaluation of muscle co-ordination (muscle strength and grip) was carried out using horizontal rotating rod (rota rod) test, which determines an animal's ability to support its own body weight by holding on to the rotating rod. The loss of muscle grip is an indication of muscle relaxation which is recorded by fall-off time [12]. The stress control animals showed an early fall from rota rod which interprets the reduced muscle co-ordination. In the present study, animals treated with extracts of *C. longa* rhizomes (CEE and BPO) increased muscle co-ordination as evident by the increase of fall

off time, significantly as compared to control (stressed) animals.

Actophotometer was used to assess the spontaneous motor activity. Increase in count was regarded as central nervous system stimulant activity and decrease in count was regarded as central nervous system depressant activity [13]. In the present study, the stress control animals reduced the actophotometer score, indicating depressant action. Animals treated with extracts of *C. longa* rhizomes (CEE and BPO) increased the actophotometer score (spontaneous motor activity) and significantly reversed stress-induced depression in a dose dependent fashion.

Studies have shown reduced brain levels of adrenaline and nor-adrenaline in animals exposed to stress such as the swimming test and immobilization stress [14]. Under stressful conditions, utilization and synthesis of these amines are increased in various regions of the brain. However, if the stress persists and becomes uncontrollable, the utilization of the amines exceeds synthesis thereby resulting in their depletion [15]. In the present study, pretreatment with extracts of *C. longa* rhizomes (CEE and BPO) was found to prevent the stress induced depletion of norepinephrine and dopamine levels thus helping the animals to cope up better during stress.

Serotonin is widely distributed monoamine in brain and involved in mood and impulse control. Different stressors like immobilization or restrain stress, foot shock lead to increased synthesis of serotonin in limbic regions [16]. Pretreatment with extracts of *C. longa* rhizomes (CEE and BPO) was found to be significantly reduced the stress induced rise in brain serotonin level by preventing the alarm reaction, thereby arresting the genesis of stress related disorders. With the preliminary HPLC analysis of *C. longa* rhizomes (CEE and BPO) wherein three peaks detected in the UV spectra (420 nm) represents the presence of curcuminoids as one of the active principles.

To conclude, in the present study the extracts of *C. longa* rhizomes (CEE and BPO) exhibited protective effect against physical stress model followed by post-swimming muscle co-ordination and spontaneous motor activity, which could be ascribed to its curcuminoids content. Further studies may be carried out to identify the presence of other constituents responsible for the activity.

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