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Research Article

EFFECTS OF AQUEOUS EXTRACT OF PURIFIED *CURCUMA LONGA* ON MOTOR PERFORMANCE IN MICE

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ABSTRACT

Objective: This study was performed to see the effects of aqueous extract of purified *Curcuma longa* (CL) on motor performance of albino mice using rota test.

Methods: CL at 50mg/kg body weight (b.w.) (CL50), CL at 100 mg/kg b.w. (CL100), and CL at 200 mg/kg b.w. (CL200) with negative and positive controls were used. The experimental results were represented as mean±2 standard deviation, p was set at < 0.05. Statistical differences between the test drug and control groups as well as within the test drug groups were calculated using Mann–Whitney U-test.

Results: As compared to CL200, DW (distilled water 10 ml/kg p.o.) group fall off time was significantly less (p=0.004). Diazepam (5 mg/kg i.p.) group stayed for lesser time on the rotating rod than CL50, CL100, and CL200 groups (p=0.004). Fall off time in CL100 group was significantly lesser than CL 200 group (p=0.019).

Conclusion: This study showed that CL possesses motor performance increasing effects at 200 mg.

Keywords: *Curcuma longa*, Aqueous extract, Motor performance.

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INTRODUCTION

Volatile oil is the main component of the root and contains turmerone and curcuminoids. Natural antioxidants such as curcumin demethoxycurcumin, 5'-methoxycurcumin, and dihydrocurcumin are the constituents of Curcuminoids found in the root of *Curcuma longa* (CL) [1,2]. While performing the nutritional analysis of the 100g of turmeric roots have been shown to contain 390 kcal, 10 g total fat, 3 g saturated fat, 0 mg cholesterol, 0.2 g calcium, 0.26 g phosphorous, 10 mg sodium, 2500 mg potassium, 47.5 mg iron, 0.9 mg thiamine, 0.19 mg riboflavin, 4.8 mg niacin, 50 mg ascorbic acid, 69.9 g total carbohydrates, 21 g dietary fiber, 3 g sugars, and 8 g protein [3]. Large amounts of ω-3 fatty acid and α-linolenic acid (2.5%) are also found in turmeric [4]. Dried powdered rhizome of (CL) (turmeric) consist of moisture (>9%), curcumin (5–6.6%), extraneous matter (<0.5% by weight), mold (<3%), and volatile oils (<3.5%). Volatile oils include d-α-phellandrene, d-sabinene, cinol, borneol, zingiberene, and sesquiterpenes [5]. The rhizomes are also reported to contain four new polysaccharides-ukonans along with stigmasterole, β-sitosterole, cholesterol, and 2-hydroxymethyl anthraquinone [6,7]. Mitochondria in is an essential organelle for energy production in the muscle cells and its number decreases progressively as the organism ages [8].Thus, preventing the decrease in mitochondrial count might prevent muscle loss [9]. CL helps in the induction of mitochondrial biosynthesis in muscle, thereby promoting smooth muscle differentiation [10,11]. This study is an attempt to study the muscle regulatory action of aqueous extract of purified *CL* and find novel drugs for increasing muscle performance.

METHODS

Design of the study
This study was quantitative experimental study in mice and rats

Setting
This study was Laboratory of Department of Clinical Pharmacology and Therapeutics, BPKIHS, Nepal.

Duration of the study
This study was 1 year

Drugs and chemicals
1. Purified CL (the Himalaya Drug Company, India).
2. Diazepam (Neon laboratories ltd, India).

Plant material
Purified CL was obtained from the Himalaya Drug Company, India.

Extract preparation of the plant
The purified CL were obtained from the Himalaya drug company in the form of coarse powder. Then, 25 g of this powder was subjected to Soxhlet extraction in 150 ml distilled water for 12 h at 100°C. The crude extract thus obtained was first subjected to filtration with Whatman filter paper no 1 and then concentrated to dryness at room temperature to yield 257.3 mg brown/black viscous residue, this is the aqueous extract of purified CL. The above procedure was repeated several times to yield 5.10 g of CL. CL thus obtained was then utilized for the experiments by suspending in distilled water.

Animals
The experiments were performed on adult albino mice of either sex weighing 20–35 g. The animals were bred in the animal house of the Department of Clinical Pharmacology and Therapeutics. They were maintained under controlled room temperature (25±2°C), and light and dark (12:12 h) conditions. The animals were given food pellets and water *ad libitum* but fasted overnight before the experiment. Before conducting the experiment, ethical clearance was obtained from the Local Ethical Committee on Animal Research, BPKIHS, Dharan. The ethical guidelines for investigations were followed in accordance with Indian National Science Academy (INSA) [12].

Experimental design
All animals were randomly divided into five groups. Each group consisted of six animals. Group 1 was vehicle control animals (DW)

used to estimate the baseline values of the parameters studied. Group 2 were standard control animals (Diazepam) which were given standard drugs. Group 3, 4, and 5 animals were given three different doses of the test, that is, aqueous extract of purified CL. The test drugs and vehicle (distilled water) were given through oral route with the help of orogastric tube. Intraperitoneal route was used for the standard control drug (Diazepam). The test drug was administered orally in doses of 50, 100, and 200 mg/kg b.w. to the Groups 3, 4, and 5, respectively, once daily for 21 consecutive days in the morning. The vehicle (distilled water) was administered orally to the Group 1 in a dose of 10 ml/kg b.w. daily for 21 days. The doses of the test drug were chosen according to the study done by Baxla *et al*. [13] (rats), Kumar *et al*. [14] (mice), and volume guidelines for compound administration [14]. All the oral drugs were administered 60 min before the experiment and the intraperitoneal drug was administered 30 min before the experiment. The experiments in test drug and vehicle treated groups were conducted on day 21 and 60 min after the last dose administration. Aqueous extract of purified CL and Diazepam were dissolved in distilled water. Only the freshly prepared drug solutions were used. Distilled water (10 ml/kg p.o.) was used as vehicle control in the experiment. Diazepam 5 mg/kg i.p. was the standard control for motor coordination in rota rod test.

The different groups received drugs and vehicles as follows:

- Group 1 (vehicle control 10 mg/kg b.w.);
- Group 2 (standard control);
- Group 3 (CL 50 mg/kg b.w.);
- Group 4 (CL100 mg/kg b.w.); and
- Group 5 (CL200mg/kg b.w.).

Experimental model

Motor performance (Rota rod) test

Loss of motor performance is one of the pharmacological effects of many drugs including anxiolytic drugs [15]. The effect of the plant extract on coordinated motor movement was assessed using rota rod test. The method described by Kalakonda and Kadiri was used with slight modifications [16]. A rota rod (Techno, India) biological research apparatus was used for the evaluation of motor incoordination. It consists of ahorizontal metal rod attached to a motor with the speed adjusted to 25 rotations/min (25 RPM). The rod is divided into five sections by plastic discs, thereby allowing the simultaneous testing of five mice. The rod is in a height of about 30 cm above the table top to discourage the animals from jumping off the roller (Fig. 1). To avoid a bias due to inability not related to drug treatment, on the previous day, animals were evaluated and those that showed the ability of walking on the bar for at least 1 min were selected for the experiment. After the administration of drugs, each mouse was placed on the rotating rod. The latency (in seconds) to fall off the rota rod was recorded up to a limit of 90 s. Diazepam 5 mg/kg i.p. was used as standard control, administered 30 min before the test [16].

Fig. 1: Albino mice in rota rod test

Statistical analysis

All data were presented as mean±2 standard deviation. Median and standard error of mean (SEM) were also calculated. Statistical differences between the test drug and control groups as well as within the test drug groups were calculated using Mann–Whitney U-test.

A probability (p-value) level <0.05 was considered significant.

RESULTS

Motor performance and effects of aqueous extract of CL in three graded doses 50, 100, and 200 mg/kg b.w. were evaluated in this study and the effects were compared with vehicle control and standard control. The aqueous extract of CL was given daily for 21 days and the experiments were performed on the 21st day. As compared to DW, CL at all the three doses (50 mg/kg, 100 mg/kg, and 200 mg/kg) increased fall of time hence endurance and motor performance in mice, although the this difference is significant only at 200 mg/kg dose of CL. CL at all the three doses significantly increased the fall off time when compared with Diazepam. Fall off time in CL 200 group was higher than CL 50 and CL 100, but this difference is significant only when compared to CL 100 (Tables 1 and 2, Fig. 2).

Table 1: Rota rod

Drug	Fall off time (mean±2 SD)	Median	SEM
Distilled water	62.833±4.458	63	±0.910
Diazepam	12.833±9.24	12.5	±1.887
CL 50mg/kg	70.330±18.096	67	±3.694
CL 100mg/kg	65.667±15.882	66	±3.242
CL 200mg/kg	77.667±10.856	77	±2.216

SD: Standard deviation, SEM: Standard error of mean, CL: *Curcuma longa*

Table 2: Rota rod test

Comparisons between groups	p
Group I	
Group III	0.077
Group IV	0.293
Group V	0.004*
Group II	
Group I	0.004*
Group III	0.004*
Group IV	0.004*
Group V	0.004*
Group III	
Group IV	0.467
Group V	0.146
Group IV	
Group V	0.019*

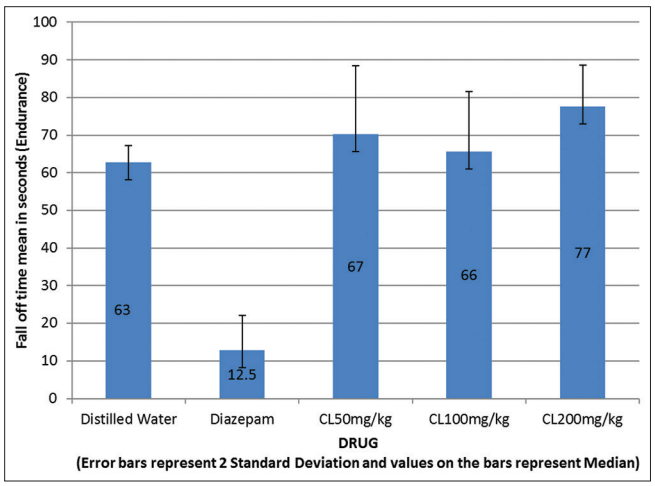

Fig. 2: Rota rod test mean values

DISCUSSION

In CL has been used by various tribes as a medicine for treatment of different diseases from ages. For this reasons, various CNS effects of aqueous extract of purified form of this plant have been evaluated in this experimental study. The CL was given daily for twenty one days in three graded doses of 50, 100, and 200 mg/kg in mice and rats. The experiments were performed on the 21st day.

MuRF-1 is an E3 ubiquitin ligase which breaks down myosin heavy chains leading to myopathy, whereas Atrogin-1 is a protein that constitutes E3 ubiquitin ligase which causes proteasomal degradation of the target protein [17,18]. Both MuRF-1 and Atrogin-1 are activated by myostatin [19]. Myostatin is one of the myokines responsible for inhibiting muscle cell growth and differentiation. Aqueous extract of curcuma supplementation has been shown to decrease the expression of myostatin, thereby reducing the expression of MuRF-1 and Atrogin-1 by Shintae Kim *et al*. [20]. In the present study, this inhibition of myosin mediated expression of MuRF-1 and atrogin-1 might have led to increased muscle cell growth and differentiation in the mice which have improved their performance in the rota rod test. It is also been shown that aqueous extract of CL decreases reactive oxygen species (ROS) and MDA levels along with increase in the antioxidant enzyme activities by Shintae Kim *et al*. Quercetin is abundant in aqueous extract of *Curcuma longa* L. which is responsible for this anti-oxidant property of aqueous extract of CL [21,22]. Quercetin is a bioactive polyphenolic flavonoid compound found abundantly in *Curcuma longa* L. [20]. Due to its high solubility and bioavailability, it exhibits strong antioxidant activity after forming a complex. Oxygen free radicals are generated in the body, this O_2-is quickly captured by superoxide dismutase (SOD). SOD then transforms O_2-transforms into H_2O_2. This enzyme further catalyzes the decomposition of H_2O_2 to the non-toxic H_2O. This reaction requires GSH as a hydrogen donor. Animal and cell studies found that quercetin induces GSH synthesis [23,24]. Quercetin increases the body's antioxidant capacity by regulating levels of GSH. Thus, decreases in the reactive oxygen species (ROS) and MDA levels along with increase in the antioxidant enzyme activities and suppression of the expression of atrogenes by quercetin might have led to increase in the endurance and muscle performance of the mice in the rota rod test in this study [25].

CONCLUSION

Aqueous extract of purified *CL* adds endurance to motor performance in mice at 200 mg/kg dose. This observed effect is probably due to and anti-oxidant activity of quercetin and decrease in the expression of myostatin. Further, research is imperative to validate these mechanisms for this observation.

AUTHORS' CONTRIBUTIONS

Ashish Sharma: Principal Investigator, performed all the experiments, compiling of data and its analysis, literature study, referencing, and writing of the finished article including discussion writing. Arnav Sharma: Assisted in analysis of data, search and study of literature for referencing of discussion, proof reading, searching, and marketing of the chemicals involved in the study.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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