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Original Research Article

Anticonvulsive Effects of Purified Curcuma Longa in Mice

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ABSTRACT

Background: *Curcuma longa* has been used as a medicine for centuries although many of its medicinal properties still need to be scientifically proven.
Methods: It was a quantitative experimental study done in the laboratory setting of the department of Clinical Pharmacology and Therapeutics, BP Koirala Institute of Medical Sciences, Dharan, Nepal (BPKIHS). Maximum electroshock seizure test (MES) and Pentylenetetrazole (PTZ) induced seizures were used for evaluating the anticonvulsive effects in albino mice. The animals were divided into five groups of six each, group I as control (vehicle), group II as standard control whereas groups III, IV and V as test groups (three doses) .Control and the three test drug doses were given for 21 days. Data were presented as mean ±Standard Error of Mean. 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 level less than 0.05 ($p < 0.05$) was considered significant.
Results: Aqueous extract of purified *Curcuma longa* (CL) showed significant anticonvulsive effects in comparison to vehicle at 100 mg/kg and 200mg /kg. No significant effect of CL (as compared to vehicle control) was observed at 50mg/kg.
Conclusion: This study showed that CL possesses anticonvulsive effect at 100 and 200 mg/kg doses. The mechanism(s) and active principle(s) behind the effects of CL could not be established.

Key words: *Curcuma longa*, Maximum Electro Shock Seizure (MES), Pentylenetetrazole (PTZ), Anticonvulsive effect.

INTRODUCTION

Curcuma longa plant is known by many names in India and Nepal like Haldi, Haridra, Besar etc and is being used as a medicine since centuries. It is a rhizomatous herbaceous perennial plant of the ginger family Zingiberaceae. [1] When not used fresh, the rhizomes are boiled for several hours and then dried in hot ovens, after which they are ground into a deep orange-yellow powder (turmeric) commonly used as a spice in curries and other South Asian and Middle Eastern cuisine, for dyeing, and to impart color to mustard condiment.

More than 100 components have been isolated from root of *Curcuma longa*. The main component of the root is a volatile oil, containing turmerone, and there are other coloring agents called curcuminoids also. Curcumin demethoxycurcumin, 5'-methoxycurcumin, and dihydrocurcumin, are the natural antioxidants found in curcuminoids. [2,3] Dried powdered rhizome of *Curcuma longa* (turmeric) contains moisture (>9%), Curcumin (5-6.6%), extraneous matter (<0.5% by weight),

mould (<3%), and volatile oils (<3.5%). d-α-phellandrene, d-sabinene, cinol, borneol, zingiberene, and sesquiterpenes are the known volatile oils found in rhizomes of *Curcuma longa*. [4] The rhizomes are also reported to contain four new polysaccharides-ukonans along with stigmasterol, β-sitosterol, cholesterol, and 2-hydroxymethyl anthraquinone. [5,6] Furthermore nutritional analysis showed that 100 g of turmeric contains 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. [7] It is also a good source of the ω-3 fatty acid and α-linolenic acid (2.5%). [8]

Medicinal properties of *Curcuma longa* and its constituents

Curcumin has potential for increasing brain monoamine levels in discrete regions of the brain. [9] It inhibits cytochrome P450 (CYP1A2) and enhances CYP2A6 activity. [10]

The bioavailability of curcumin is low but because of its low molecular weight and lipophilic nature it is absorbed well and reaches central nervous system by crossing the blood brain barrier. [11] Sharma et al. [12] reported curcumin to be an effective agent inhibiting lipid peroxide formation in liver during inflammation and attributed this effect to an antioxidant property. Curcumin also inhibited the lipid peroxide formation in vitro. [13] Rat experiments also have shown that Curcumin can be tried concurrently with conventional epileptic drugs for lowering their doses. [14] These observed properties of *Curcuma longa* and its constituents may be used for treating convulsive disorders.

As the existing antiepileptic drugs have disturbing adverse effects like impairment of memory, [15] there is need of a novel anticonvulsant which can lower such unwanted effects which is the reason for this study. According to several publications about 70% of the people with epilepsy are lacking proper treatment and increased incidence of recurrence with a high prevalence of about 0.8 % in children below the age of 7 years. [16] *Curcuma longa* can be used to lower this burden.

RESEARCH DESIGN AND METHODOLOGY

Design of the study: Quantitative experimental study in mice.

Setting: Laboratory of Department of Clinical Pharmacology and Therapeutics, BP Koirala Institute of Health Sciences, Dharan, Nepal (BPKIHS).

Duration of the study: One year

Drugs and chemicals

1. Purified *Curcuma longa* (The Himalaya Drug Company, India)
2. Phenytoin (M-Toin, Medopharm, India);
3. Diazepam (Neon laboratories ltd, India);
4. Pentylenetetrazole (Sigma Chemicals, USA);

Plant material

Purified *Curcuma longa* was obtained from The Himalaya Drug Company, India.

Extract preparation of the plant

The purified Curcuma longa were obtained from the Himalaya drug company in the form of coarse powder. Then 25g of this powder was subjected to Soxhletextraction in 150 ml distilled water for 12 hours at 100°C. The crude extract thus obtained was first subjected to filtration with What man filter paper no 1 and then concentrated to dryness at room temperature to yield 257.3mg brown / black viscous residue, this is the aqueous extract of purified *Curcuma longa* (CL).The above procedure was repeated several times to yield 5.10g 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 20-35g.

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 hour) 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). [17]

Experimental design

All animals were randomly divided into five groups. Each group consisted of six animals. Group 1 was vehicle control animals used to estimate the baseline values of the parameters studied. Group 2 were standard control animals which were given standard drugs. Group 3, 4 and 5 animals were given three different doses of the test, i.e. aqueous extract of Purified *Curcuma longa* (CL). The test drugs, and vehicle (distilled water) were given through oral route with the help of orogastrictube. Either oral or intra peritoneal route was used for standard control drugs. The test drug was administered in doses of 50, 100 and 200 mg/kg b. w. (body weight) to the groups 3, 4 and 5 respectively once daily for 21 consecutive days in the morning. The vehicle (distilled water) was administered to the group I 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, Kumar et al [18] and Volume Guidelines for Compound Administration. [19]All the oral drugs were administered 60 min prior to the experiment and all the intraperitoneal drugs were administered 30 minutes prior to the experiment. The experiments in test drug and vehicle treated groups were conducted on day 21, 60 minutes after the last dose administration. Aqueous extract of purified *Curcuma longa* (CL), Pentylenetetrazole (PTZ), Phenytoin 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 both the experiments.

The standard controls for anticonvulsant effect were Phenytoin 20mg/kg i.p. in Maximal electroshock seizure test and Diazepam 4mg/kg i.p. in Pentylenetetrazole induced seizure test.

The different groups received drugs and vehicles as follows:

Group I (vehicle control 10 mg/kg b. w.);
Group II (standard control);
Group III (CL50 mg/kg b. w.);
Group IV (CL100 mg/kg b. w.) and
Group V (CL200mg/kg b. w.).

Experimental models:

Maximal Electroshock Seizure (MES) test

The maximal electroshock seizure pattern was induced in animals by using a convulsion meter (Techno, India) to give an alternating current of 150 mA for 0.2sec. After 60 minutes of post dosing with the test drugs, mice were subjected to MES of 150 mA of alternating current from the convulsion meter for 0.2 sec through a pair of electrodes attached to each ear. [20] Seizures were manifested as tonic hindlimbextension (THLE). The duration of the tonic hind limb extensor phase and the number of animals protected from convulsions were noted. The ability to prevent this feature or prolong the latency and/or onset of the THLE was considered as an indication of anticonvulsant activity. [21,22] Phenytoin in dose of 20mg/kg [23] i.p. was used as standard control, administered 30 minutes prior to the experiment.

Pentylenetetrazole (PTZ) induced seizures:

This assay was used to evaluate antiepileptic drugs. PTZ was used in a dose of 80mg/kg [24] i. p.

This is the dose that produces clonic seizures in all the animals without mortality. The test drugs were administered 60 minutes prior to PTZ administration. The latency to first convulsion and the no. of mice which exhibited seizure were observed immediately after the PTZ injection for a period of 30minute. [24,25] Diazepam 4mg/kg i.p. was used as standard control,

administered 30minutes prior to the experiment. [24]

RESULTS

Anticonvulsant effects of aqueous extract of *Curcuma longa* 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 *Curcuma longa* was given daily for twenty one days and the experiments were performed on the 21st day.

In Maximal Electroshock Seizure (MES) test, pretreatment with phenytoin 20mg/kg and CL 200mg/kg b. w. significantly ($p<0.05$) protected animals from convulsion as compared to that of vehicle treated mice. CL 50mg/kg and CL100mg/kg treated groups did not show significant decrease in the duration of convulsion in mice as compared to that of vehicle treated mice. In this test phenytoin significantly ($p<0.05$) decreased the duration of convulsion in comparison to all the three test groups but there was no significant difference within the three test groups. (Table1)

Table1: Maximum Electroshock Seizure Test

Drug	Duration of tonic hind limb extension in sec (Mean ± SEM)	Median	Standard deviation	P value
I .Distilled water	15.667±2.060	16	5.046	III 0.435 IV 0.069 V 0.013*
II. Phenytoin	0.5±0.500	5	1.224	I 0.003* III 0.011* IV 0.006* V 0.046*
III.CL50mg/kg	12.667±3.383	16	5.859	IV 0.372 V 0.053
IV.CL 100mg/kg	9.25±1.652	9	3.304	V 0.065
V.CL200mg/kg	4±1.871	3	4.183	

* Significant ($p < 0.05$), SEM: Standard error of mean.

Table 2: Pentylenetetrazole-Induced Seizure Test

Drug	Latency to convulsion in sec (Mean ± SEM)	Median	Standard Deviation	P value
I.Distilled Water	65.667±0.955	66	2.338	III 0.054 IV 0.043* V 0.006*
II.Diazepam	181±4.662	184.5	11.419	I 0.004* III 0.522 IV 0.337 V 0.314
III.CL 50mg/kg	280.8±103.860	187	232.238	IV 0.522 V 0.175
IV.CL100mg/kg	133.83±70.917	125.5	173.71	V 0.522
V.CL 200mg/kg	170±11.811	180	26.41	

In Pentylenetetrazole (PTZ) induced seizure test CL 50mg/kg followed by diazepam 4mg/kg, CL200mg/kg and CL 100mg/kg increased the latency to convulsion as compared to the vehicle treated group. Only CL 100mg/kg and CL200mg/kg showed significant ($p<0.05$) increase in latency when compared to the vehicle. There was no significant ($p>0.05$) difference seen within the CL50, 100,200mg/kg and diazepam treated groups (Table2)

DISCUSSION

Indian indigenous and complementary systems of medicine have been claiming the beneficial effects of *Curcuma longa* in innumerable diseases. This study has been carried out to verify some of these claims. The CL was given daily for twenty one days in three graded doses of 50, 100 and 200 mg/kg in mice. The experiments were performed on the twenty first day. In the present study, the CL was studied in two experimental models: Maximum Electroshock Seizure Test and Pentylenetetrazole-Induced Seizure Test to

investigate its possible anticonvulsive effects in mice. These tests are classical models for screening anticonvulsive actions.

Although alcoholic extract of *Curcuma longa* has been studied in great detail by a number of workers, very less work has been done to evaluate the anticonvulsive effect of its aqueous extract. The present study indicates that the CL possesses anti-convulsive effects.

At 100 and 200mg/kg, CL delayed onset of convulsion in PTZ test whereas at 50 mg/kg, CL decreased the duration of convulsion in the MES model. It is to be noted that although Phenytoin was successful in preventing the occurrence in almost all the mice in the MES test, CL pretreatment did not protect the mice from the occurrence of convulsion in any of the models.

From this study, it is revealed that CL has probable anticonvulsant effect although this effect is lesser than that of the known anticonvulsives.

Similar results were obtained by Mehla and coworkers [26] with Curcumin (alcoholic extract of *Curcuma longa*).They found out that pretreatment with Curcumin ameliorates seizures, oxidative stress and cognitive impairment in PTZ induced kindling in rats. Curcumin showed dose-dependent anti-seizure effect. Curcumin (300mg/kg) significantly increased the latency to myoclonic jerks, clonic seizures as well as generalized tonic-clonic seizures, improved the seizure score and decreased the number of myoclonic jerks.

Chimakurthy et al. [27] also showed a potentiating effect of Curcumin on sub therapeutic doses of Phenytoin and sodium valproate against Generalised tonic clonic seizures.

CONCLUSION

This study showed that aqueous extract of purified *Curcuma longa* possesses anticonvulsive effects at 100 and 200 mg /kg. Since not much work has been done on the aqueous extract of purified *Curcuma longa*, there is a need for more precise studies to isolate the active constituents and to elucidate the mechanism of action .Hence a lot of work is needed to know the mechanism(s) behind the anticonvulsive effects.

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