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PHYTOCHEMICAL ANALYSIS AND ANTICONVULSANT ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *CURCUMA CAESIA*

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ABSTRACT

Bio-efficacy of plants and their derivatives have been reemphasized in recent times. *Curcuma caesia* Roxb (*C. caesia* 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 the treatment of various ailments and metabolic disorders like leukoderma, asthma, tumours, piles, bronchitis, etc. Considering the importance of natural products in modern phytomedicine, the aim of the present study is to examine hydroalcoholic extract of *C. caesia* rhizomes for qualitative and qualitative phytochemical analysis and the anticonvulsant activity (100 and 200mg/kg, p.o.) in mice by using maximum electroshock seizure (MES) test and Pentylenetetrazole (PTZ) test. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Phytochemical screening of the extract showed the presence some common compounds like terpenoids, flavonoids, carbohydrate etc. The total flavonoids content of extract was found to be (0.897mg /100mg). The hydroalcoholic extract of *C. caesia* rhizomes significantly reduced the duration of seizures induced by MES. The hydroalcoholic extract in doses of 100 and 200 mg/kg conferred protection (17and50%, respectively) on the mice. The same doses also protected animals from PTZ-induced tonic seizures. The hydroalcoholic extract of *C. caesia* possess anticonvulsant activity since it reduced the duration of seizures produced by maximal electroshock and delayed the latency of seizures produced by PTZ.

Key words: *Curcuma caesia* Roxb, Qualitative and qualitative phytochemical, Anticonvulsant, Pentylenetetrazole, Maximal electroshock.

INTRODUCTION:

Epilepsy is a common and frequently devastating disorder affecting an estimated 7 million people in India and 50 million people worldwide. Approximately 40% of them are women. The estimated incidence rate ranges from 40 to 60 per 1,000,000 population/year. The WHO estimated that approximately 80% patients with epilepsy live in developing countries and most of them do not get adequate medical treatment. Unfortunately, currently used antiepileptic drugs cause side effects which vary in severity from minimal impairment of the central nervous system to aplastic anemia or hepatic failure. Moreover, approximately 30% of people with epilepsy have intractable seizures that do not respond to even the best available treatment (Reddy,

2005). Approximately 30% of the patients continue to have seizures with current antiepileptic therapy (Mattson, 1995). This fact demands new safer antiepileptic drugs for better and effective control of epilepsy. Since plants have provided many drugs in the past and they remain a rich source of novel compounds based on nature's combinatorial natural products chemistry during millions of years of evolution, they should be continuously investigated as a source of novel therapeutic agents (Phillipson, 2003). The Ayurvedic system of medicine has a quite sophistical classification of medicinal plants as per the dominant pharmacological/therapeutic activity of mental functions (Vaidhya, 1997). Ethnopharmacological approaches have provided leads to identify potential new drugs from plant sources, including targets for neuronal disorders (Howes and Houghton, 2003). Numerous studies point to medicinal plants as an interesting source of novel antiepileptic drugs (Schechter, 2009; Nsour et al., 2000). One interesting example in this regard is losigamone derived from the kava kava plant and originally used by traditional healers in the South Pacific as an anxiolytic, which is now in early clinical development as novel antiepileptic drugs (Malawska, 2005; Willmore, 2001). Likewise, *Curcuma caesia* Roxb (Zingiberaceae) commonly known as kali haldi is a perennial herb belonging to Genus curcuma. The plant is distributed throughout tropical and subtropical regions of the world. In India it is found in North-East and Central part and also sparsely found in Papi Hills of East Godavari, West Godavari and Khammam Districts of Andhra Pradesh (Das et al., 2013). The rhizomes are traditionally used in the treatment of hemorrhoids, leprosy, asthma, cancer, fever, wounds, vomiting, menstrual disorder, anthelmentic, aphrodisiac, gonorrheal discharges and inflammation (Amalraj et al., 1989; Singh and Jain, 2003). The rhizome paste is applied on bruises, contusions and rheumatic pains. The rhizome is also used in dysentery, diarrhea and cough as an aromatic and as a source of D-camphor (Craker and Simon, 1996; Sarangthem and Haokip, 2010). Previous bioactivity studies have been revealed that plant rhizome possesses antifungal activity (Banerjee and Nigam, 1976), anxiolytic and CNS depressant activities (Karmakar et al., 2011a), neuropharmacological assessment (Karmakar et al., 2011c), anti-asthmatic, smooth muscle relaxant activity [Arulmozhi et al., 2006] and free radical scavenging activity against reactive oxygen and nitrogen species [Karmakar et al., 2011b]. Presence of curcuminoids, 1,8-cineole, camphor, ar-turmeone, phenolics, flavonoids, protein and alkaloids were reported in the rhizomes of *C. caesia* [Sarangthem and Haokip, 2010; Paliwal et al., 2011]. However, anticonvulsant activity of its rhizomes has not yet been scientifically explored; therefore the main objective of present study is to evaluate anticonvulsant status of the hydroalcoholic extract of *C. caesia* on (MES) test and (PTZ) test in Swiss albino mice.

MATERIALS AND METHODS

Plant materials

The rhizomes of *C. caesia* were purchased from local market of Bhopal (M.P.).

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Extraction Procedure

Defatting of plant material

Powdered Plant material (rhizomes) *C. caesia* was shade dried at room temperature. The shade dried material was coarsely

powdered and subjected to extraction with petroleum ether (60-80°C) in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place.

Extraction

Dried powdered of rhizomes of *C. caesia* has been extracted with hydroalcoholic solvent (70:30) using hot continuous percolation process for 48hrs and dried using vacuum evaporator at 40^0C and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts (Mukherjee, 2007).

Qualitative phytochemical analysis of plant extract

The *C. caesia* rhizomes extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate (Khandelwal, 2005; Kokate, 1994). he extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavanoids, glycosides, saponins, alkaloids, fats or fixed oils, protein, amino acid and tannins.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Extracts obtained from rhizomes of *C. caesia* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

Total phenolics content

The total phenolics content of *C. caesia* was estimated using Folin-Ciocalteau reagent by the method of *Olufunmiso et al* (Olufunmiso and Afolayan, 2011). A volume of 2 ml of *C. caesia* rhizomes extracts or standard was mixed with 1ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (75g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min at 40°C for colour development. The absorbance was measured at 765nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/gm).

Total flavonoids content

The total flavonoids content was estimated using the procedure described by *Olufunmiso et al* [Olufunmiso and Afolayan, 2011]. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/gm).

Animals

Swiss albino male mice (20-25 gm) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2°C,55-65%). Mice received standard rodent chow and water *ad libitum*. Mice were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00hr. Separate group (n=6) of mice was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity

Acute toxicity study of the prepared rhizomes extracts of *C. caesia* was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines-423 [OECD Guideline No. 423] the animals were fasted for 4 hr, but allowed free access to water throughout. As per the

OECD recommendations, the starting dose level should be that which is most likely to produce mortality in some of the dosed animals; and when there is no information available on a substance to be tested in this regard; for animal welfare reasons, The dose level to be used as the starting dose is selected from one of three fixed levels 50, 100, 300 and 2000 mg/kg body weight. Acute toxicity was determined as per reported method (Jonsson et al., 2013).

Maximal electrical shock (MES)-induced seizures

The animals were divided in four groups (n=6). Group I served as vehicle control group. Groups III, and IV served as test groups treated with the extract (100 and 200 mg/kg, p.o., 60min), respectively, and group II served as reference standard group received phenytoin (25 mg/kg, i.p., 20 min), prior to the induction of convulsion. The number of animals protected from hind limb tonic extension seizure (HLTE) and the time spent in this position were determined for each dose group (Hegde et al., 2009).

Pentylenetetrazole (PTZ)-induced seizures

The animals were divided in four groups (n=6). Group I served as vehicle control group. Groups III, and IV served as test groups treated with the extract 100 and 200 mg/kg, p.o. The extract was administered 60 min before the subcutaneous injection of PTZ (80 mg/kg). Group II received diazepam (2.0 mg/kg, i.p.) as a reference standard. The animals were observed for onset of convulsion upto 30 min after PTZ. Hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period were noted. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity (Shirish et al., 2002; Amabeoku et al., 1993).

RESULTS

The crude extracts so obtained after the hot continuous percolation process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of *C. caesia* extracts was 8.9%w/w. Preliminary phytochemical screening of *C. caesia* hydroalcoholic rhizomes extracts revealed the presence of various components such as phenolic compounds, carbohydrates, flavonoids, saponins and diterpins among which flavones were the most prominent ones and the results are summarized in table 1.

Table Result of phytochemical screening of *Curcuma caesia*

S. No.	Constituents	*Curcuma caesia*
1.	**Alkaloids** Hager's test	 -ve
2.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve
3.	**Phenolics** $Fecl_3$	 -ve
4.	**Proteins And Amino acids** Xanthoproteic test	 -ve
5.	**Carbohydrates** Fehling's test	 +ve
6.	**Saponins** Foam test	 +ve
7.	**Diterpins** Copper acetate test	 +ve

The content of total flavonoid (TFC) was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.06X+0.019$, $R^2= 0.999$,** where X is the quercetin equivalent (QE) and Y is the absorbance Table 2& Fig 1. Total phenolic content was absent in extract.

Table 2 Total flavonoid content of hydroalcoholic extract

S. No.	Plant	Total flavonoid (QE) (mg/100mg)
1.	*Curcuma caesia*	0.897

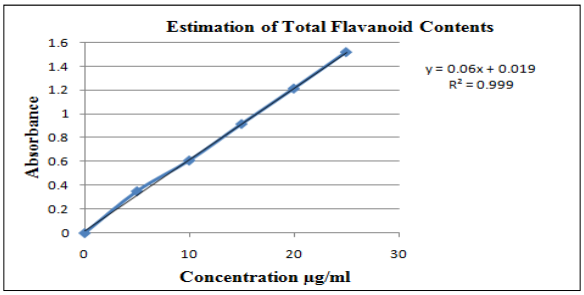

Fig.1 Graph of estimation of total flavonoid content

Maximal electroshock produced hind limb tonic extension seizures (HLTE) in all the animals. The vehicle-treated mice showed tonic hind limb extension for duration of 15.23±0.30sec. The hydroalcoholic extract at doses of 100 and 200mg/kg, respectively, protected 17% and 50% of mice and significantly reduced the duration of the seizures. However, phenytoin completely abolished the MES-induced tonic seizures in the entire animals table 3. Pentylenetetrazole produced tonic seizures in all the animals used. The hydroalcoholic extract, in doses of 100 and 200mg/kg, respectively, protected 33% and 83% of mice against seizures and significantly delayed the latency of the seizures. The standard antiepileptic drug, diazepam inhibited seizures completely table 4.

Table 3 Effect of hydroalcoholic extract of the rhizomes **of *C. caesia* on maximal electroshock induced seizures**

Treatment	Dose	No. of animal convulsed/No. of animal used	% Protection	Duration of HLTE (sec) Mean ± SEM
Control	----	6/6	00	15.23 ± 0.30
Standard (Phenytoin)	25 mg/kg, .i.p.	0/6	100	-----
Low dose of *C. caesia*	100 mg/kg, p.o	5/6	17	13.49** ± 0.46
High dose of *C. caesia*	200 mg/kg, p.o	3/6	50	9.23** ± 0.19

Results are expressed as Mean ± SEM; (n=6). Significance at P<0.05, P<0.01**as compared to control

Table 4 Effect of hydroalcoholic extract of the rhizomes **of *C. caesia* on Pentylenetetrazole induced seizures**

Treatment	Dose	No. of animal convulsed/No. of animal used	% Protection	Latency of tonic convulsion (min) Mean ± SEM
Control	----	6/6	00	5.53 ± 0.40
Standard (diazepam)	2.0 mg/kg, .i.p	0/6	100	----
Low dose of *C. caesia*	100 mg/kg, p.o.	4/6	33	12.80** ± 0.47
High dose of *C. caesia*	200 mg/kg, p.o.	1/6	83	15.33** ± 0.31

Results are expressed as Mean ± SEM; (n=6). Significance at P<0.05, P<0.01**as compared to control

Discussion

PTZ and MES are the most commonly used preliminary tests for screening of potential anticonvulsant drugs. The MES test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures whereas the PTZ test represents a valid model for human generalized myoclonic and also absence seizures [Loscher and Schmidt, 1988]. The observation of present study indicates that hydroalcoholic extract of *C. caesia* possesses anticonvulsant activity in mice. GABA is the major inhibitory neurotransmitter in the brain while glutamic acid is an excitatory neurotransmitter in the brain. The inhibition of GABA neurotransmitter and the enhancement of the action of glutamic acid have been shown to be the underlying factors in epilepsy. The present study shows that the hydroalcoholic extract of *C. caesia* protected some of the animals against seizures induced by maximal electroshock. Antiepileptic drugs which inhibit voltage-dependent Na^+ channels such as phenytoin can prevent MES-induced tonic extension (Westmoreland et al., 1994; Rang et al., 2005). Pentylenetetrazole may elicit seizures by blocking GABA/Cl^-channel complex. Picrotoxin induces seizure, by blocking the chloride channels linked to GABA-A receptor. Diazepam, a standard antiepileptic drug is believed to produce their effects by enhancing GABA mediated opening of chloride channel on GABA-A receptor leading to more chloride ion entering the neuron which in turn decreases the neuronal activity in the brain (McDonald and Kelly, 1993). In the present study diazepam shown to antagonize the seizure induced pentylenetetrazole. The extract was also shown to delay the latency of pentylenetetrazole induced seizures, suggesting that the extract exhibiting anticonvulsant affect, probably by opening the chloride channels associated with GABA receptors. Therefore, in conclusion, hydroalcoholic extract of *C. caesia* possesses anticonvulsant property against the MES and PTZ induced seizures. From the above result, further investigation is warranted in order to isolate and identify the specific molecules which are responsible for the anticonvulsant activity.

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