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EXPERIMENTAL EVALUATION OF ETHANOL EXTRACT OF *CURCUMA CAESIA* ROXB. ON LOCOMOTOR AND LEARNING BEHAVIOUR

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ABSTRACT

Curcuma caesia Roxb. belongs to the family *Zingiberaceae*. Fresh and dried *Curcuma caesia* rhizomes in traditional system of medicine are used in treating wound healing, infections, leukoderma, fever, asthma, cancer, piles, bronchitis, ulcer, bruises etc. In current study c*urcuma caesia* was evaluated for locomotor, memory and learning behavioral activities in mice. The effects of the drug were assessed on its exploratory behaviour using head dip apparatus. Learning behavioral activity was performed using stationary rod. Plant extract in different doses (150 and 300mg/kg) was administered at 07, 15 and 30 days orally, and observed the effects regarding its antidepressant and anxiolytic activities. In this study, it is considered *curcuma caesia* is a valuable source of natural products of potential medicinal value. It requires further studies.

KEYWORDS:- Antidepressant, Cage crossing activity, *Curcuma caesia*, Head dip test, Mood elevation, Stationary rod test.

INRTODUCTION

Curcuma caesia (Roxb.) or black turmeric (Kali haldi) is a perennial herb with bluish-black rhizome, belonging to the family *Zingiberaceae*. Plant has large tuberous rhizome which is underground and ovoid about 2–6 cm in diameter and covered with adventitious roots (Panchol *et al.*, 2003). Plant rhizomes have aromatic nature. Color of inner portion of the c*urcuma caesia* rhizome is bluish-black that produces a sweet smell that is indicating the presence of essential oil (Pandey and Chowdhary, 2003).

Volatile oil of rhizome contains 30 constituents that is representing the oil (97.48%) with (*Z*)-ocimene (8.2%), camphor (14%–28.3%), curcumene (2.82%), ar-curcumene (6.8%), borneol (4.4%), 1, 8-cineole (5.3%), 1, 8-cineole (27%–48%), ar-turmeone (12.3%), elemene (4.8%), bornyl acetate (3.3%) (Pandey and Chowdhary, 2003) as well as ocimine (15.66%), zingiberol (12.60), total phenol, flavonoids, alkaloids and soluble protein (Sarangthem and Haokip, 2010), alpha-pinene, isofuranodienone, zingiberene, starch and furanodienone.

Medicinal uses of the c*urcuma caesia* arise from the bioactive constituents such as curcuminoids exhibit free radical scavenging property (Song *et al*., 2001), flavonoids and phenolic compounds are responsible for multiple biological effects including anti-inflammatory, anti-carcinogenic etc (Miller, 1996), oleoresins from these species (most of which are unutilized) would have good potential of anti-bacterial agent (Rajamma *et al*., 2012), curcuminoids are responsible for wound healing, hypoglycemic, anti-microbial activities (Chatopadhyay *et al*., 2004) and essential oil of plant rhizome has been known for its antifungal activity (Banerjee and Nigam, 1976).

It has anti-oxidant (Chirangini *et al*., 2004; Jayaprakasha *et al*., 2010), anti-leprosy (Israr *et al*., 2012), analgesic and antipyretic (Kaur *et al*., 2013), anti-coagulant (Trivedi, 2003), anti-cancer (Syamkumar and Sasikumar, 2007), anti-asthmatic (Paliwal *et al*., 2011), anti-emetic (Ravindran *et al*., 2007), muscle relaxant property (Karmakar *et al*., 2011) and anti-ulcer properties (Das *et al*., 2012).

MATERIALS AND METHODS

Extract preparation: The rhizome of *Curcuma caesia* was collected for soaking in ethanol for ten days and ethanol was filtered. Extract of *Curcuma caesia* was collected by means of evaporation of the mixture and thick concentrated mass was achieved. The concentrated mass of c*urcuma caesia* was used for locomotor and learning behavioral activities.

Animals: Healthy adult albino mice weighing 25-30 g were used in present study. Animals divided into three groups each containing six animals. Group-I: treated with 0.1ml (p.o) normal saline served as control. Group-II: treated with 150 mg/kg (p.o) plant extract served as treated group. Group-III: treated with 300 mg/kg (p.o) plant extract served as treated.

Housing: The mice were housed two per cage under an alternate 12 h light: dark cycle at temperature (25 ± 2°C) and humidity (45 to 55%) controlled environment at the department of Pharmacology, University of Karachi with free access to food and water.

Methods

Locomotor activity by cage crossing method: Locomotor activity of plant extract was determined by using cage crossing test in mice (Nuzhat and Rahila, 2012). The animals were divided into three groups each containing six mice. One served as control group, two groups for extract at doses 150 and 300 mg/kg.

Cage crossing activity: Cage crossing activity (Prut and Belzung, 2003) was used for the locomotor activity in mice. It consisted of a transparent cage measuring about 26cm (h) x26cm (w) x 26cm (l) and by the surrounded walls escaping of the animals was prevented. Albino mice control and other two treated groups were placed in the centre of the cage separately for the period of 10 minutes and count the number of cage crossing (Tahira *et al.,* 2006) after administration of extract of c*urcuma caesia* at 07, 15 and 30 days of dosing.

Memory and learning behavioral methods: Memory and learning behavior of plant extract were determined by using head dip test and stationary rod test in mice (Nuzhat and Rahila, 2012). The animals were divided into three groups each containing six mice. One served as control group, two groups for extract at doses 150 and 300 mg/kg.

Head dip test: For learning ability and exploration and of the animals, head dip test was used for measuring head-dipping (Kliethermes and Crabbe, 2006). Exploratory box is consisted a wooden board measuring about 35cm x 45cm x 45cm with 10 holes of 2.5cm diameter which are evenly spaced. Albino mice control and treated groups of two different doses were placed separately in an exploratory box for head dipping for 10 minutes. The number of head dips was counted after the administration of extract of c*urcuma caesia* at 07, 15 and 30 days of dosing at150 and 300 mg/kg.

Stationary rod test: Stationary rod test provides a unique opportunity to exploration, general locomotor activity, and provide an initial screen for anxiety-related behavior in rodents. For the learning ability, the stationary rod test (Kishioka *et al.,* 2009) was used. It has stainless steel rods with two platforms in both ends. Before start of the experiment mice were given a brief training. Before administration of drug albino mice control and treated groups were placed in the centre of the rod and allowed to walk separately one by one and note the time of crossing to reach the another platform and repeat the procedure after dosing of extract of *Curcuma caesia* at 07, 15 and 30 days of dosing.

Statistical calculations: All results were expressed as average value ± standard deviation. The significance of difference between averages was determined by Newman (1939) and Keuls (1952) test and data obtained from current study was analyzed for P-value <0.01 which was considered significant and P-value <0.001 was considered highly significant, following the one way ANOVA.

RESULTS

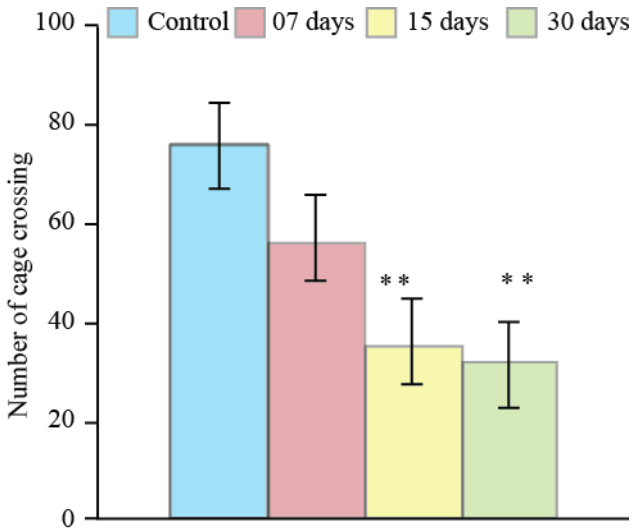
Effect on cage crossing activity: Post-hoc analysis by Newman-Keuls test showed that animals at 15 and 30 days of dosing of extract of c*urcuma caesia* (300 mg/kg) showed highly significant decrease in number of crossing of cage, i.e., 35 ± 9.71 and 32 ± 8.23 respectively in comparison to control animals group, i.e., 76 ± 8.36 (Fig. 1). Animals after dosing of extract of c*urcuma caesia* (300 mg/kg) at 07 days showed non-significant decrease in number of crossing of cage, i.e., 56 ± 9.45 in comparison to control animals.

Result showed that animals at 15 and 30 days of dosing of extract of c*urcuma caesia* (150 mg/kg) showed significant decrease in number of crossing of cage, i.e., 45 ± 7.74 and 36 ± 2.33 respectively in comparison to control animals group, i.e., 76 ± 8.36. Animals after dosing of extract of c*urcuma caesia* (150 mg/kg) at 07 days showed non-significant decrease in no. of crossing of cage, i.e., 68 ± 7.42 in comparison to control animals (Fig. 2).

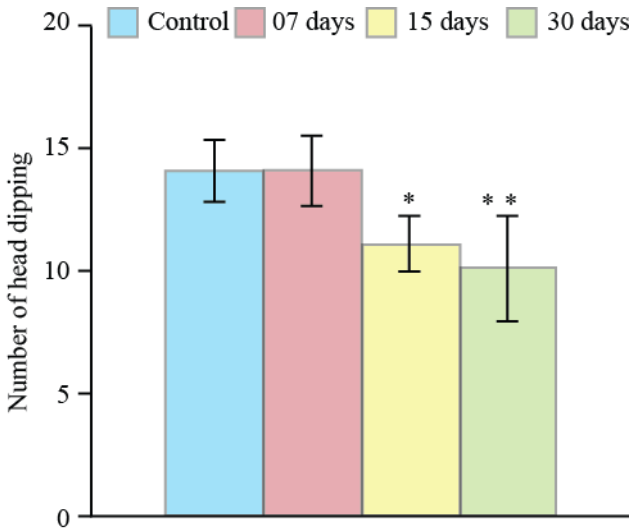
Results showed that the number of crossings of cage due to the extract of c*urcuma caesia* (300 mg/kg) group after 15 and 30 days of dosing was significantly not lower than 150 mg/kg of dosing.

Effect on head dip test: Results showed that animals after dosing of extract of c*urcuma caesia* (300 mg/kg) at 30 days of dosing showed significant decrease in number of head dips, i.e., 21 ± 4.23 in comparison to control animals group, i.e., 43 ± 2.11. Animals at 15 days of dosing of extract of c*urcuma caesia* (300 mg/kg) showed significant decrease in number of head dips, i.e., 27 ± 3.43 in comparison to control animals. Animals after dosing of extract of c*urcuma caesia* (300 mg/kg) at 07 days showed non-significant decrease in number of head dips, i.e. 36±5.23 in comparison to control animals (Fig. 3).

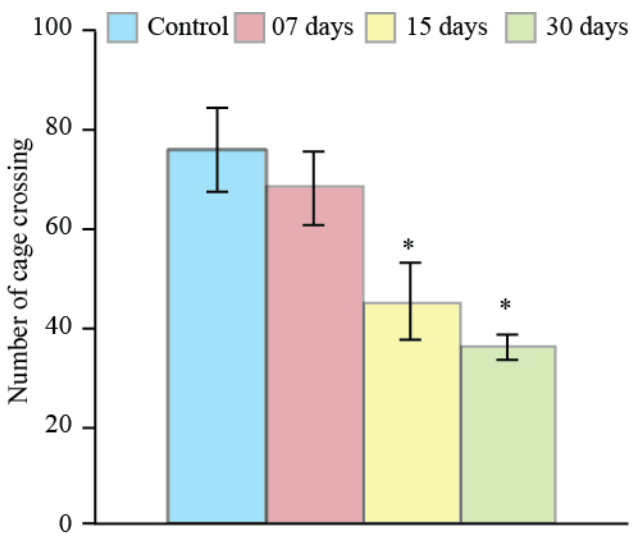
Post-hoc analysis by Newman-Keuls test shows that animals after dosing of extract of c*urcuma caesia* (150 mg/kg) at 15 and 30 days of dosing showed significant decrease in number of head dips, i.e., 29 ± 1.24 and 25 ± 3.63 respectively in comparison to control animals group, i.e. 43 ± 2.11. Animals after dosing of extract of c*urcuma caesia* (150 mg/kg) at 07 days showed non-significant decrease in no. of head dips, i.e., 40 ± 2.26 in comparison to control animals group, i.e., 43 ± 2.11 (Fig. 4).



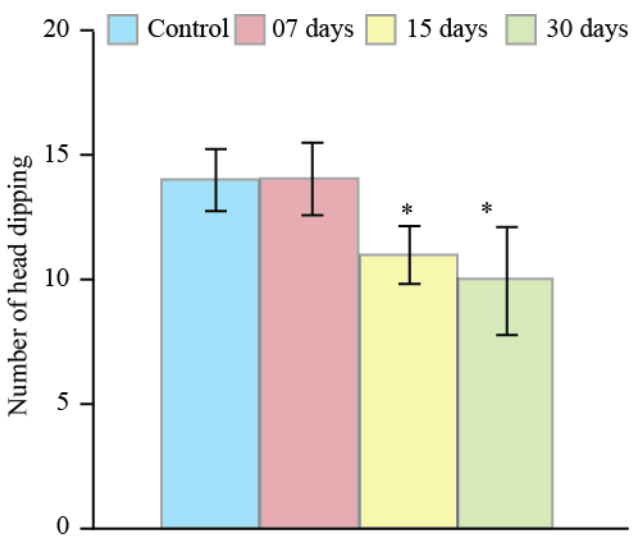
n = 6
Average value ± St. Dev.
**p < 0.001 is highly significant as compared to control
Fig. 1. Effect of *Curcuma caesia* on cage crossing activity of mice at the dosage of 300mg/kg for various duration.



n = 6
Average value ± St. Dev
*p<0.01 is significant
**p<0.001 is highly significant as compared to control
Fig. 3. Effect of *Curcuma caesia* on head dip at the dosage of 300mg/kg for various duration.



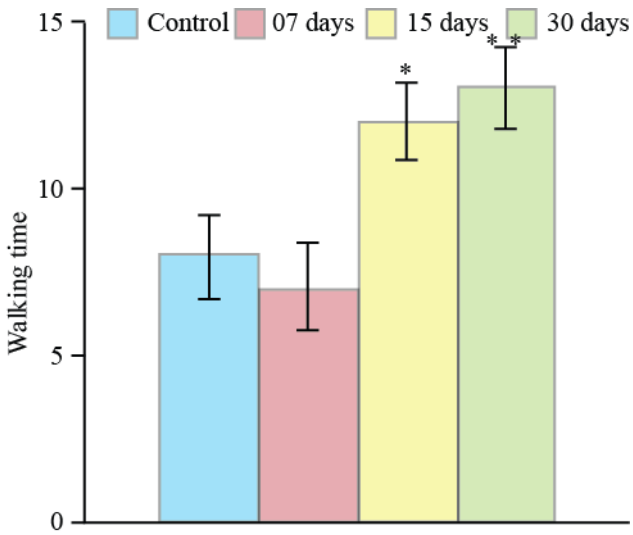
n = 6
Average value ± St.Dev.
*p < 0.01 is significant as compared to control
Fig. 2. Effect of *Curcuma caesia* on cage crossing activity of mice at the dosage of 150mg/kg for various duration.



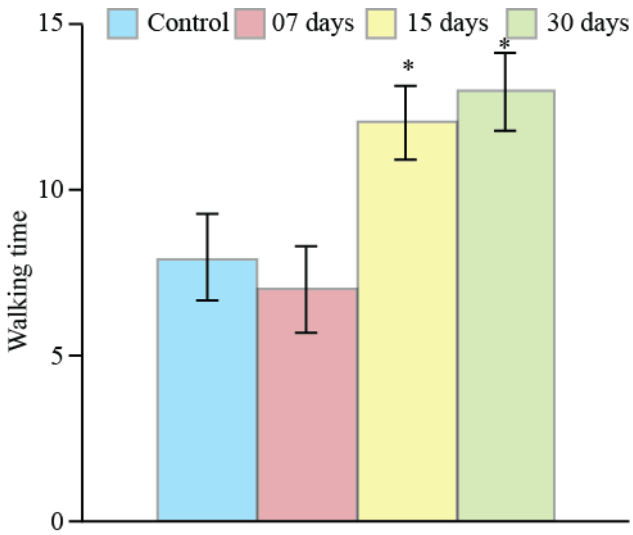
n = 6
Average value ± St. Dev
*p<0.01 is significant as compared to control
Fig. 4. Effect of *Curcuma caesia* on head dip at the dosage of 150mg/kg for various duration.

Effect on stationary test: Post-hoc analysis by Newman-Keuls test shows that animals at 30 days of dosing of extract of c*urcuma caesia* (300 mg/kg) showed highly significant increase in time taken to cross the rod, i.e., 13 ± 2.15 (sec) in comparison to control animals group, i.e., 8 ± 1.25 (sec). Animals at 15 days of dosing of extract of c*urcuma caesia* (300 mg/kg) showed significant decrease in time taken to cross the rod, i.e., 11 ± 1.21 (sec) in comparison to control animals group, i.e., 8 ± 1.25 (sec). Animals after dosing of extract of c*urcuma caesia* (300 mg/kg) at 07 days showed non-significant decrease in time taken to cross the rod, i.e., 8 ± 1.32 (sec) in comparison to control animals group, i.e., 8 ± 1.25 (sec) (Fig. 5).

Post-hoc analysis by Newman- Keuls test shows that animals at 15 and 30 days of dosing of extract of c*urcuma caesia* (150 mg/kg) showed significant decrease in time taken to cross the rod, i.e., 12 ± 1.14 and 13 ± 1.15 (sec) in comparison to control animals group, i.e., 8 ± 1.25 (sec). Animals after dosing of extract of *Curcuma caesia* at 07 days showed non-significant decrease in time taken to cross the rod, i.e., 7 ± 1.32 (sec) in comparison to control animals group, i.e., 8 ± 1.25 (sec) (Fig. 6).



n = 6
Average value ± St.Dev
*p<0.01 is significant
**p<0.001 is highly significant as compared to control
Fig. 5. Effect of *Curcuma caesia* on stationary rod at the dosage of 300mg/kg for various duration.



n = 6
Average value ± St. Dev
*p<0.01 is significant as compared to control
Fig. 6. Effect of *Curcuma caesia* on stationary rod at the dosage of 150mg/kg for various duration.

DISCUSSION

Most of the pharmaceutical modern medicines have side and toxic effects and they are expensive. Traditional medicines are potent, effective, and inexpensive and possess lesser side effects (Kumar *et al.*, 2010). *Curcuma caesia* appears to have a wide spectrum of activities against several pathological conditions.

In current study exploratory and locomotor activities were measured by the head dip test. Such experiments are used in the field of behavioral pharmacology. The spontaneous activity of the mice was decreased after long-term dosing of *curcuma caesia*. Decrease number of head dips indicates the reduction locomotion and exploratory behavior in animals. After observing the behavior and results, it suggests that plant has anxiolytic activity because mice is not showing the exploratory activity.

Antipsychotic drugs including typical and atypical decreased the locomotor activity. Brain region amygdala is involved in anxiety and fear responses and also involved in other several neurological disorders like acute state of anxiety (Moya *et al.*, 2011). By the tyrosine modulation or changes in dopamine psychological stress can be reduced and concentrations of nor adrenaline may improve stress (Chen *et al.*, 2009).

For the muscular coordination and locomotor activity, stationary rod test was performed (Griffey *et al.*, 2006). Dopamine plays an important role in movement disorders such as Parkinson disease (Leng *et al*., 2004). In stationary rod activity experiment animals were passive after long-term dosing of *curcuma caesia*, it could be due to the effect of anxiolytic and it is in accordance to Niimia *et al*., (2008). In current study there is reduction in muscular coordination and locomotor activity which is responsible the passive behavior and not allowing the animals to reach the other plateform of the stationary rod and it is probably due to low level of 5Hydroxytryptophan (5HT). Viggiano *et al*., (2003), reported that the brain regions striatum and cerebellum are related to the muscular coordination and locomotor activity. Galina and Pavlova (2001) reported that dopamine and 5Hydroxytryptophan are also involved in muscular coordination and locomotor control but the mechanism and effects of both are different in different species of animals as well as increase level of 5Hydroxytryptophan increase the locomotor but since current study shows reduction in locomotor activity it could be due to the decrease level of 5Hydroxytryptophan produced by *Curcuma caesia*.

In cage crossing test, animals were passive as compared to the treated animal group. Muscles relaxant activity of the animals was also observed after administration of *Curcuma caesia*. According to the Radhakrishnan *et al*., (2001), the muscles strength reduction is due to sedative action of the plant extract and anxiolytic activity which was observed is due to decreased level of 5Hydroxytryptophan. The current study indicates that the decrease level of 5Hydroxytryptophan and increase level of dopamine could be responsible for the reduction in muscles strength. *Curcuma caesia* anxiolytic activity requires further research and investigations so as confirm its use as anxiolytic agent.

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