ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 15, Issue 8, 2022

Online - 2455-3891
Print - 0974-2441

Research Article

# EFFECTS OF AQUEOUS EXTRACT OF PURIFIED *CURCUMA LONGA* ON ANXIETY LEVELS IN SWISS ALBINO MICE

**ASHISH SHARMA***

**Department of Pharmacology, Chirayu Medical College and Hospital, Bhopal 462 030, Madhya Pradesh, India.**
**Email: drsharma450@gmail.com**

***Received: 01 April 2022, Revised and Accepted: 02 June 2022***

**ABSTRACT**

**Objectives:** This study was performed to see the effects of aqueous extract of purified *Curcuma longa* (CL) on anxiety levels of Swiss albino mice using open-field test.

**Methods:** CL at 50 mg/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±standard deviation, $p<0.05$ was considered. 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:** The number of squares crossed in 5 min was least in distilled water (DW) as compared to all other groups (CL50, CL100, and CL200 [p=0.002], diazepam [p=0.002]).Time spent in the central square was lesser in the DW group than CL50 (p=0.045), CL200 (p=0.005), and DP (p=0.004). More time was spent by DP in the central square than CL50 (p=0.045) and CL100 (p=0.037) groups. The number of rearing was lesser in DW group as compared to CL50 (p=0.030), CL100 (p=0.006), and CL 200 (p=0.006) as well as DP. The number of rearing was less in CL50 than CL200 (p=0.045) group.

**Conclusion:** This study showed that CL possesses anxiolytic effect.

**Keywords:** *Curcuma longa*, Aqueous extract, Anxiolytic.

 DOI: http://dx.doi.org/10.22159/ajpcr.2022v15i8.44810. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

## INTRODUCTION

Our indigenous system has been using plants and plant products for the alleviation of human sufferings from the ancient times and this has been reported in the vast array of Materia Medica [1]. Many of these plants have shown central nervous system activities and this study is a humble effort to utilize these activities for treating nervous system disorders effectively. Turmeric (*Curcuma longa* [CL]) is a rhizomatous plant produced in India and Pakistan in large quantities [2]. This perennial plant belongs to the ginger family, *Zingiberaceae* [3]. Volatile oil containing turmerone is the main component of the root of this plant. It also contains other coloring agents called curcuminoids which consist of curcumindemethoxycurcumin, 5'-methoxycurcumin, and dihydrocurcumin. These constituents of curcuminoids have been found to be natural antioxidants [4], [5]. Recently, several studies have shown that curcumin has anticonvulsant effects against seizures induced by kainic acid in rats [6]. Studies have also shown anticonvulsive effects of curcumin in FeCl3 induced seizure in rats [7]. Previously, it was shown that high doses (100 and 300 mg/kg i.p.) of curcumin inhibited amygdala-kindled seizures in rats [8]. Recent researches have shown that curcumin exerts anticonvulsant effect against acute generalized seizures induced by maximal electroshock and delays the development of amygdala kindling [9]. Some of the physiological effects seen in animals are also attributed to curcumin and has activity against a range of neurological diseases, including Alzheimer's disease (AD), in animal models [10]. Curcumin is active against multiple sclerosis, Parkinson's disease as well as it has effect on age-associated neurodegeneration [11], [12], [13] Studies have shown its effect on schizophrenia and depression [14], [15]. In animals, curcumin is also associated with the prevention of cognitive deficits [16]. It has ability to improve learning and memory in mouse models of AD and reverse scopolamine-induced amnesia in rats [17]. There is evidence of better cognitive performance in frequent or occasional curry consuming Asians compared with non-consumers or rare consumers of curry [18]. Curcumin is currently the subject of a wide range of ongoing clinical trials. These include assessments of its efficacy in the treatment of AD as a monotherapy and in combination with GuillainBarre Sydrome [19].

## METHODS

### Design of the study

This was a quantitative experimental study in mice and rats.

### Setting

- Laboratory of Department of Clinical Pharmacology and TherapeuticsBP Koirala Institute of Health Sciences
- Dharan, Nepal. (BPKIHS).

### 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 was obtained from the Himalaya drug company in the form of coarse powder. Then, 25g of this powder was subjected to soxhlet extraction in 150 ml distilled water (DW) for 12 h at 100°C. The crude extract thus obtained was first subjected to filtration with Whatman filter paper number 1 and then concentrated to dryness at room temperature to yield 257.3mg 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 DW.

**Animals**

*Inclusion criteria*

- Swiss albino mice of either sex were used
- Mice weighing 20–35g were used.

**Exclusion criteria**

Apparently, free of any disease or handicap was excluded from the study.

The animals were bred in the animal house of the Department of Clinical Pharmacology and Therapeutics, BPKIHS, Dharan, Nepal. 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.

*Ethical clearance*

Ethical clearance was obtained from the Local Ethical Committee on Animal Research, BPKIHS, Dharan, Nepal before conducting the experiment.

**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 was standard control animals 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 (DW) were given through oral route with the help of orogastrictube. Intraperitoneal route was used for standard control drugs. The test drug was administered in doses of 50, 100, and 200 mg/kg bw. to the Groups 3, 4, and 5, respectively, once daily for 21 consecutive days in the morning. The vehicle (DW) was administered 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 Kumar *et al.* and Volume Guidelines for Compound Administration [20,21]. All the oral drugs were administered 60 min before the experiment, the intraperitoneal diazepam was administered 30 min before the experiment. The experiments in test drug and vehicle treated groups were conducted on day 21, 60 min after the last dose administration. Aqueous extract of purified CL and Diazepam was dissolved in DW. Only the freshly prepared drug solutions were used. DW (10 ml/kg p.o.) was used as vehicle control in all the experiments. Diazepam 1 mg/kg i.p. was the standard control for Open-field test.

The different groups received drugs and vehicles as shown in Table 1.

**Experimental models**

*Open field test*

This is an experimental model for assessment of anxiogenic activity and loco motor activity [22]. In the open-field test (OFT), confrontation with the situation induces anxiety in rodents. The anxiety is triggered by two factors, that is, individual testing (the animal was separated from its social group) and agoraphobia (as the arena is very large, relative to the animals breeding or the natural environment). In such situations, rodents show thigmotaxic behavior identified by spontaneous preference to the periphery of the apparatus and reduced ambulation. The OFT consisted of a wooden box (40 cm × 40 cm with 30 cm high walls) with painted black floor, subdivided into nine equal fields by white lines. The experimental room is a sound attenuated dark room. The OFT, illuminated with a 40W bulb, focusing on the field from a height of about 50 cm, was placed in the experimental room, a picture of OFT is shown in Fig. 1. After 60 min of test drug treatment, the mice were placed individually in a corner square of the OFT and the ambulation (number of squares crossed at periphery), total locomotion activity in the center (number of central squares crossed), and rearing (number of times the animal stands on the rear paws) were recorded for 5 min [23]. Rearing reflects an exploratory tendency of the animal that can be reduced due to a high level of fear [24]. Enhanced peripheral, central, and total number of squares crossed are taken as increased loco motor activity. In addition, increased rearing, number of inner squares crossed and time spent in them reflect enhanced exploratory activity and reduced fear [25]. All the above parameters are inversely proportional to the level of anxiety. The observation was made on a closed circuit TV as shown in Fig. 2. Diazepam 1 mg/kg i.p. was used as standard control, administered 30 min before the experiment [26].

**Table 1: Drugs used in Open-field tests**

| |
|---|
| Open-field test |
| Group 1 (vehicle control 10 mg/kg b.w.) |
| Group 2 (Diazepam 1 mg/kg b.w.) |
| Group 3 (CL 50 mg/kg b.w.) |
| Group 4 (CL 100 mg/kg b.w.) |
| Group 5 (CL 200 mg/kg b.w.) |

**Statistical analysis**

All 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 ($p<0.05$)* was considered significant.

**RESULTS**

**Effect of CL on anxiety in open field test**

The rodents are always flitting freely and there is a conflict between the exploration of a new environment and the aversion to open spaces from which escape is prevented by a surrounding wall. The stimulus of the novel environment may simultaneously induce anxiety and exploratory

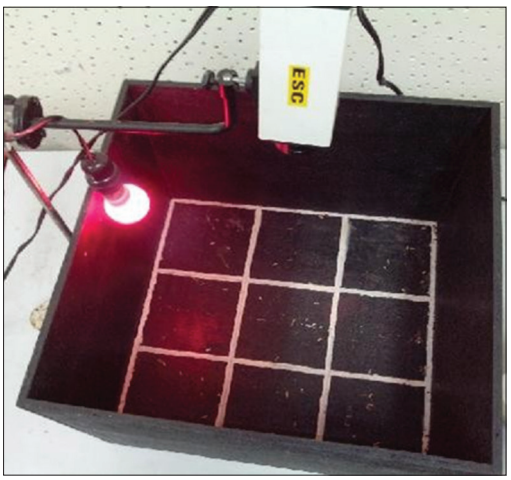

**Fig. 1: Apparatus for open-field test**

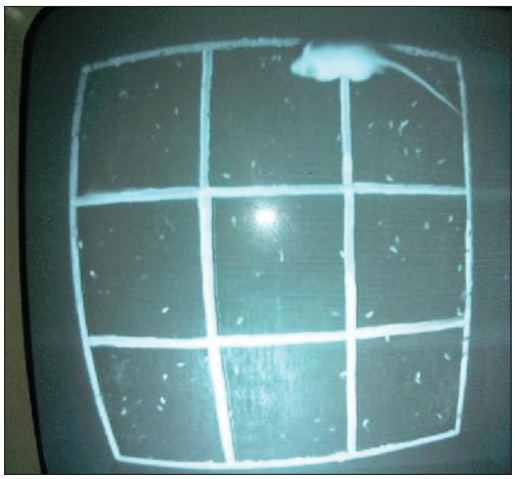

**Fig. 2: The mouse in action being observed in closed circuit TV for Open-field test**

behavior in them. This behavior is the basis of the OFT which was used in this experiment to study the anxiolytic properties of CL in this study [27].

**Number of squares crossed in five minutes**

Diazepam caused significant ($p<0.05$) increase in the number of squares crossed in 5 min with respect to the vehicle treated group. This increase was significantly ($p<0.05$) more than that caused by CL at 50 mg/kg and 100 mg/kg. There was no significant ($p>0.05$) difference between Diazepam and CL 200 mg/kg treated groups. All the CL treated groups showed dose dependent and significant ($p<0.05$) increase in the number of squares crossed when compared with the vehicle.

At 50 mg/kg, the number of squares crossed in 5 min was significantly ($p<0.05$) lesser than CL 100 mg/kg, CL 200 mg/kg and the Diazepam treated groups. There was no significant ($p>0.05$) difference between CL 100 mg/kg and CL 200 mg/kg treated groups (Tables 2 and 3, Fig. 3).

**Time spent in the central square**

Although the time spent in the central square was maximum in the Diazepam treated group but this difference was significant ($p<0.05$) only in comparison with the CL 50 mg/kg, CL 100 mg/kg, and the vehicle treated groups. CL at 50 mg/kg and CL 200 mg/kg caused significant ($p<0.05$) increase in the time spent in the central square when compared with the vehicle. No significant ($p>0.05$) effect on the time spent in the central square was seen in the CL 100 mg/kg group when compared to the vehicle. There was no significant ($p>0.05$) difference within the three CL treated groups (Tables 4 and 5, Fig. 4).

**Number of rearing**

Significant ($p<0.05$) increase was seen in Diazepam treated group with respect to the vehicle treated group. Dose dependent and significant ($p<0.05$) increase were seen in all the three CL treated groups with respect to the vehicle treated group. Maximum number of writhes was seen in the CL200mg/kg group. The number of writhes was significantly ($p<0.05$) more in 200 mg/kg group when compared to CL 50 mg/kg group. All other findings were not found to be statistically significant (Tables 6 and 7, Fig. 5).

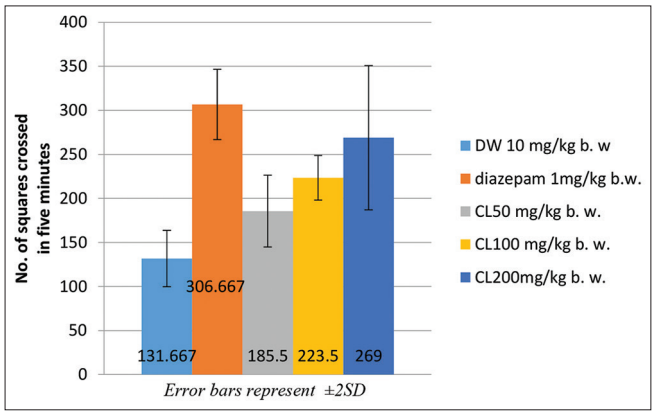


**Fig. 3: Number of square crossed in 5 min. DW: Distilled water, CL: *Curcuma longa***

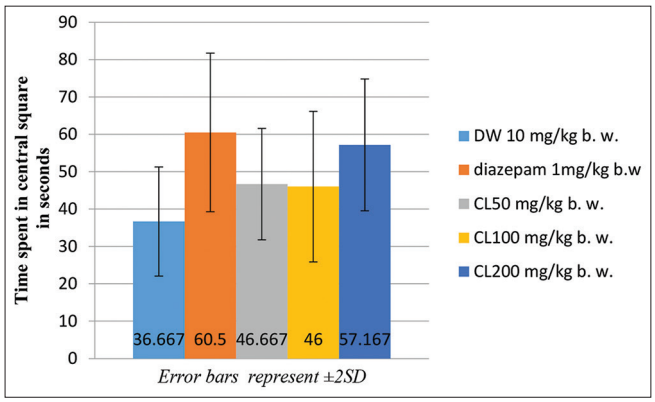


**Fig. 4: Time spent in central square. DW: Distilled water, CL: *Curcuma longa***

**Table 2: Number of squares crossed in 5 min**

| Drug | Number of squares crossed in 5 min (mean±SD) | Median±SEM |
|---|---|---|
| DW | 131.667±15.983 | 134±6.525 |
| Diazepam | 306.667±19.957 | 302.5±8.147 |
| CL 50 mg/kg | 185.5±20.374 | 187±8.318 |
| CL 100 mg/kg | 223.5±12.65 | 223±5.162 |
| CL 200 mg/kg | 269±40.963 | 281±16.723 |

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

**Table 3: *P* values for number of squares crossed in 5 min**

| Comparisions between groups | *P* value number of squares crossed in 5 min |
|---|---|
| Group I | |
| Group III | 0.004* |
| Group IV | 0.004* |
| Group V | 0.004* |
| Group II | |
| Group I | 0.002* |
| Group III | 0.004* |
| Group IV | 0.004* |
| Group V | 0.150 |
| Group III | |
| Group IV | 0.006* |
| Group V | 0.004* |
| Group IV | |
| Group V | 0.078 |

Significant values are star marked ( p>0.05)

**Table 4: Time spent in the central square**

| Drug | Time spent in central square (mean±SD) | Median±SEM |
|---|---|---|
| DW | 36.667±7.312 | 37±2.985 |
| Diazepam | 60.5±10.597 | 62.5±4.326 |
| CL 50 mg/kg | 46.667±7.448 | 47±3.040 |
| CL 100 mg/kg | 46±10.06 | 44.5±4.107 |
| CL 200 mg/kg | 57.167±8.819 | 58.5±3.600 |

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

**Table 5: *P* values for time spent in the central square**

| Comparisions between groups | *P* value time spent in central square |
|---|---|
| Group I | |
| Group III | 0.045* |
| Group IV | 0.108 |
| Group V | 0.005* |
| Group II | |
| Group I | 0.004* |
| Group III | 0.045* |
| Group IV | 0.037* |
| Group V | 0.522 |
| Group III | |
| Group IV | 0.748 |
| Group V | 0.077 |
| Group IV | |
| Group V | 0.054 |

Significant values are star marked ( p>0.05)

**Table 6: Number of rearing**

| Drug | Number of rearing (mean±SD) | Median±SEM |
|---|---|---|
| DW | 24.5±10.483 | 22±4.280 |
| Diazepam | 49.667±7.312 | 49±2.999 |
| CL 50 mg/kg | 41.667±6.713 | 41.5±2.740 |
| CL 100 mg/kg | 55.167±10.998 | 60±4.490 |
| CL 200 mg/kg | 55.833±11.215 | 60.5±4.578 |

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

**Table 7: *P* values for number of rearing**

| Comparison between groups | *P* value number of rearing |
|---|---|
| Group I | |
| Group III | 0.003* |
| Group IV | 0.006* |
| Group V | 0.006* |
| Group II | |
| Group I | 0.009* |
| Group III | 0.063 |
| Group IV | 0.258 |
| Group V | 0.296 |
| Group III | |
| Group IV | 0.054 |
| Group V | 0.045* |
| Group IV | |
| Group V | 0.810 |

Significant values are star marked ( p>0.05)

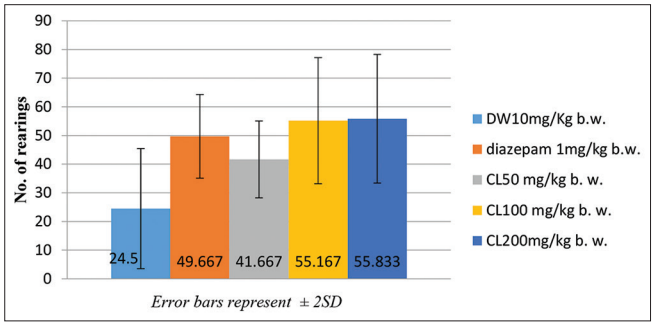


**Fig. 5: Number of rearings. DW: Distilled water, CL: *Curcuma longa***

In our study, the loco motor activity increased significantly as indicated by the enhanced total number squares crossed. Increase in loco motor activity reveals anxiolytic activity of the CL. There was significant increase in the number of rearing in all the three dosage groups of CL. In addition, there was significant increase in the time spent in the central square in the 50 mg/kg and 200 mg/kg groups which reflects enhanced exploratory activity and reduced fear [25].

## DISCUSSION

Effect of CL on anxiety was screened using OFT. This test is used mainly to assess the anxiety level of animals. In our study, the loco motor activity increased significantly as indicated by the enhanced total number squares crossed. Increase in loco motor activity reveals anxiolytic activity of the CL. There was significant increase in the number of rearing in all the three dosage groups of CL. In addition, there was significant increase in the time spent in the central square in the 50 mg/kg and 200 mg/kg groups which reflects enhanced exploratory activity and reduced fear. Twenty-five monoamine oxidize A (MAO) is involved in the metabolism of in a vast variety of monoamine neurotransmitters such as noradrenaline, dopamine, and 5-hydroxytryptamine. There are two forms of MAO, A and B. In a similar study performed in male ICR mice by Yu, Kong and Chen using oral doses of aqueous extract of CL in Male ICR mice [28]. Aqueous extract of CL doses from 140 to 560 mg/kg for 14 days showed dose-dependent relation of immobility reduction in the tail suspension test and the forced swimming test in mice at a dose-dependent manner. This extract, at the dose of 140 mg/kg or above for 14 days, significantly inhibited the MAO activity in mouse whole brain at a dose-dependent manner. In addition to the above study, Wouters and Knoll also have stated that MAO A inhibition can be used to treat depression and anxiety [29]. Thus, in the present study, this MAO A inhibition property of aqueous extract of CL might have caused decreased anxiety levels in the subjects which is reflected as significant increase in the number of rearing and time spent in the central square in the OFT at 50 mg/kg and 200 mg/kg doses.

## CONCLUSION

Aqueous extract of purified CL at 50, 100, and 200 mg/kg doses decreases anxiety levels in subjects. Further research done to identify the active principles responsible for the above effect will pave a way for discovery of novel neuropsychiatric drugs.

## AUTHOR CONTRIBUTION

Ashish Sharma-Corresponding author and Principal Researcher.

## AUTHORS FUNDING

None.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## REFERENCES

1. Md S, Kumar SMS, Narasu ML. NeuropharamcologicalProfile of Trans-01 a polyherbal formulation in mice. Pharmacologonline 2007;1:146-51.
2. Tahira JJ. Weed flora of *Curcuma* longa. Pak J Weed Sci Res 2010;16:241-6.
3. Chan EW, Lim YY, Wong SK, Lim KK, Tan SP, Lianto FS, *et al*. Effects of different drying methods on the antioxidant properties of Leaves and tea of ginger species. Food Chem 2009;113:166-72. doi: 10.1016/j.foodchem.2008.07.090
4. Ruby AJ, Kuttan G, Babu KD, Rajasekharan KN, Kuttan R. Anti-tumour and antioxidant activity of natural curcuminoids. Cancer Lett 1995;94:79-83. doi: 10.1016/0304-3835(95)03827-j, PMID 7621448
5. Selvam R, Subramanian L, Gayathri R, Angayarkanni N. The anti-oxidant activity of turmeric (*Curcuma longa*). J Ethnopharmacol 1995;47:59-67. doi: 10.1016/0378-8741(95)01250-h, PMID 7500637
6. Gupta YK, Briyal S, Sharma M. Protective effect of curcumin against kainic acid seizures and oxidative stress in rats. Indian J Physiol Pharmacol 2009;53:39-46. PMID 19810575
7. Jyoti A, Sethi P, Sharma D. Curcumin protects against electrobehavioral progression of seizures in the iron induced experimental model ofepileptogenesis. Epilepsy Behav 2009;14:300-8. doi: 10.1016/j.yebeh.2008.11.011, PMID 19100339
8. Du P, Li X, Lin HJ, Peng WF, Liu JY, Ma Y, *et al*. Curcumin inhibits amygdaloid kindled seizures in rats. Chin Med J (Engl) 2009;122:1435-8. PMID 19567167
9. Bharal N, Sahaya K, Jain S, Mediratta PK, Sharma KK. Curcumin has anticonvulsant activity on increasing current electroshock seizures in mice. Phytother Res 2008;22:1660-4. doi: 10.1002/ptr.2551, PMID 18661468
10. Kaur C, Ling EA. Blood brain barrier in hypoxic-ischemic conditions. Curr Neurovasc Res 2008;5:71-81. doi: 10.2174/156720208783565645, PMID 18289024
11. Zbarsky V, Datla KP, Parkar S, Rai DK, Aruoma OI, Dexter DT. Neuroprotective properties the natural phenolic antioxidants curcumin and naringenin but not quercetin and fisetin in a 6-OHDA model of Parkinson's disease. Free Radic Res 2005;39:1119-25. doi: 10.1080/10715760500233113, PMID 16298737
12. Natarajan C, Bright JJ. Curcumin inhibits experimental allergic encephalomyelitis by blocking IL-12 signaling through Janus kinase STAT pathway in T lymphocytes. J Immunol 2002;168:6506-13.

doi: 10.4049/jimmunol.168.12.6506, PMID 12055272

13. Calabrese V, Scapagnini G, Colombrita C, Ravagna A, Pennisi G, Giuffrida Stella AM, *et al*. Redox regulation of heat shock protein expression in aging and neurodegenerative disorders associated with oxidative stress: A nutritional approach. Amino Acids 2003;25:437-44. doi: 10.1007/s00726-003-0048-2, PMID 14661103
14. Bishnoi M, Chopra K, Kulkarni SK. Protective effect of curcumin, the active principle of turmeric (*Curcuma longa*) in haloperidol-induced orofacial dyskinesia and associated behavioural, biochemical and neurochemical changes in rat brain. Pharmacol Biochem Behav 2008;88:511-22. doi: 10.1016/j.pbb.2007.10.009, PMID 18022680
15. Xu Y, Ku BS, Yao HY, Lin YH, Ma X, Zhang YH, *et al*. The effects of curcumin on depressive-like behaviors in mice. Eur J Pharmacol 2005;518:40-6. doi: 10.1016/j.ejphar.2005.06.002, PMID 15987635
16. Ishrat T, Hoda MN, Khan MB, Yousuf S, Ahmad M, Khan MM, *et al*. Amelioration of cognitive deficits and neurodegeneration by curcumin in rat model of sporadic dementia of Alzheimer's type (SDAT). Eur Neuropsychopharmacol 2009;19:636-47. doi: 10.1016/j.euroneuro.2009.02.002, PMID 19329286
17. Ahmed T, Gilani AH. Inhibitory effect of curcuminoids on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia may explain medicinal use of turmeric in Alzhiemers disease. Pharmacol Biochem Behav 2009;91:554-9. doi: 10.1016/j.pbb.2008.09.010
18. Ng TP, Chiam PC, Lee T, Chua HC, Lim L, Kua EH. Curry consumption and cognitive function in the elderly. Am J Epidemiol 2006;164:898-906. doi: 10.1093/aje/kwj267, PMID 16870699
19. Hatcher H, Planalp R, Cho J, Torti FM, Torti SV. Curcumin: from ancient medicine to current clinical trials. Cell Mol Life Sci 2008;65:1631-52. doi: 10.1007/s00018-008-7452-4, PMID 18324353
20. KumarR, Gupta D, Mukul S, Singh AK, Kumar A, Ali MD, *et al*. Effect of Curcuma longa on Ovary of Endosulfan Exposed Mice. IJPBA 2012;3:617-21.
21. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, *et al*. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol 2001;21:15-23. doi: 10.1002/jat.727, PMID 11180276
22. Sonavane G, Sarveiya V, Kasture V, Kasture SB. Behavioural actions of *Myristica fragrans* seeds. Indian J Pharmacol 2001;33:417-24.
23. Bhattacharya SK, Satyan KS. Experimental methods for evaluation of psycho tropic agents in rodents: I- Antianxiety agents. Indian J Exp Biol 1997;35:565-75. PMID 9357158
24. Van der poel AM. A note on "stretched attention", a behavioral element indicative of an approach-avoidance conflict in rats. Anim Behav 1979;27:446-50. doi: 10.1016/0003-3472(79)90181-7
25. Denenberg VH. Open field behaviour in the rats: What does it mean? Ann N Y Acad Sci 1969;159:852-9. doi: 10.1111/j.1749-6632.1969.tb12983.x, PMID 5260302
26. Yadav AV, Kawale LA, Nade VS. Effect of *Morus alba* L. (mulberry) leaves on anxiety in mice. Indian J Pharmacol 2008;40:32-6. doi: 10.4103/0253-7613.40487, PMID 21264159
27. Kopniczky Z, Dochnal R, Mácsai M, Pál A, Kiss G, Mihály A, *et al*. Alterations of behavior and spatial learning after unilateral entorhinal ablation of rats. Life Sci 2006;78:2683-8. doi: 10.1016/j.lfs.2005.10.014, PMID 16313927
28. Yu ZF, Kong LD, Chen Y. Antidepressant activity of aqueous extracts of *Curcuma longa* in mice. J Ethnopharmacol 2002;83:161-5. doi: 10.1016/s0378-8741(02)00211-8, PMID 12413724
29. Wouters J. Structural aspects of monoamine oxidase and its reversible inhibition. Curr Med Chem 1998;5:137-62. doi: 10.2174/0929867305666220314202430, PMID 9481038