Research Article

Assessment of The Anxiolytic Potential of Ethanolic Extract of *Curcuma Longa* Leaves in Experimental Animal Models

Ms. Utkarsha Prabhakar Gaikwad[1]*, Dr. Shirish Sidgonda Patil[1], Dr. Padma Laxmikant Ladda[1], Dr. Sourabh Subhash Malabade[2]

[1]Department of Pharmacology, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India – 416416
[2]Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Palus, Maharashtra, India – 416310
Email: utkarshagaikwad2310@gmail.com

ABSTRACT

Objective: To study the anxiolytic activity of ethanolic extract of *Curcuma longa* leaf in experimental animals.
Methods: Phytochemical screening and acute oral toxicity test were carried out. Behavioral tests included the Elevated Plus Maze, Hole-Board, Staircase, Open-Field and Light-Dark tests. Biochemical and histological examination were also performed.
Results: Phytochemical screening showed the presence of alkaloids, flavonoids, glycosides, coumarins, tannins, phenolics and diterpenoids. No mortality or toxicity occurred with the acute oral toxicity test at 2000 mg/kg, indicating that the median lethal dose is greater than 2000 mg/kg. The extract showed dose-dependent anxiolytic effects, with the highest effects at 400 mg/kg, as well as increased brain serotonin and dopamine levels and improved tissue architecture.
Conclusion: Ethanolic extract of *Curcuma longa* leaf exhibited potential anxiolytic activity suggesting further studies on its therapeutic potential.

Keywords: Anxiety, *Curcuma longa*, Phytochemical Screening, Neurotransmitters, Anxiolytic Activity.

INTRODUCTION

Modern lifestyles frequently involve stress and worry, which manifests as symptoms including exhaustion, palpitations, nausea, and dyspnoea [1]. Specific phobias, panic disorder, social anxiety disorder, and generalized anxiety disorder are all included in the category of anxiety disorders [2]. Benzodiazepines are highly prescribed class of drugs for treating anxiety despite major adverse effects such as drowsiness, muscle relaxation, and impaired coordination (ataxia) and tolerance [3]. Similarly, one of the currently approved medications for PTSD, GAD, and SAD, is Serotonin Selective Reuptake Inhibitors (SSRIs) [4].

Curcuma longa L. belong to family Zingiberaceae it grows in Northern Australia, Thailand, the Archipelago, and India. Turmeric helps digestion, infections, diarrhoea, arthritis, jaundice, and other fever conditions [5]. Bioactive compounds possessing antioxidant, anti-inflammatory, anticancer, antibacterial, antidiabetic qualities are found in the leaves and stems [6,7]. Phytochemical studies have revealed a variety of secondary metabolites in Curcuma longa leaves, including steroids, saponins, phenolics, flavonoids, alkaloids, and glycosides [8].

MATERIAL AND METHODS

Collection Authentication and Extraction of Leaves: A botanist at Willingdon College verified the authenticity of *Curcuma longa* leaves that was collected from Sangli in November 2025. The leaves were then shade-dried, crushed, and extracted using 95% ethanol using a Soxhlet extraction technique and extract was concentrated at room temperature.

Percentage Yield of *Curcuma longa* Leaves:

%Yield of Extract = weight of extract /weight of powder X 100
%yield of Extract = 14.08 /100 x 100 = 14.08 % W/W

Phytochemical Screening: The extract was first screened for several phytochemical constituents, including alkaloids, flavonoids, glycosides, coumarins, tannins, phenolic compounds, diterpenoids, and triterpenoids [9].

LD50 Study: In accordance with OECD Guideline 423, acute oral toxicity was assessed in healthy adult female Wistar rats. Animals were given a single oral dose of 2000 mg/kg by gavage, and toxicity was assessed every day for 14 days. Physical, neurological, and behavioral alterations were noted.

Experimental Animals

Wistar rats (180–230 g) were used after approval from the Institutional Animal Ethics Committee, A.B.C.P., Sangli (Approval No. IAEC/ABCP/04/2025–26). Animals had free access to food and water and were kept in regular laboratory environment. They were randomly divided into five groups of six animals each and treated for 14 consecutive days.

Table .1 Experimental Design

Groups	Treatment
1	Vehicle – 0.5% CMC P. O
2	Standard drug- Diazepam (1mg/kg P.O) for 14 days
3	Extract of C. longa leaves (100mg/kg P. O) for 14 days
4	Extract of C. longa leaves (200mg/kg P. O) for 14 days
5	Extract of C. longa leaves (400mg/kg P. O) for 14 days

Experimental Models

1. **Elevated Plus Maze:** It consists of Two open & closed arms made up the device. For five minutes, each rat was positioned separately in middle of the maze, facing an open arm. Open and closed arm entries and Time spent were noted. [10].
2. **Light and Dark:** The device consists of two chambers, one dark and one lighted, joined by a floor-level hole. For 5 minutes, each rat was placed separately in the middle of the light room. duration spent in the light chamber and the quantity of entries into both chambers were noted [11].
3. **Staircase:** It contained 5 identical steps in the device. The number of steps climbed, and rearing episodes were noted while each rat was observed for 5 minutes. When all four paws were on it, a step was counted [12].
4. **Hole Board:** The device was a board with 16 evenly spaced holes that was raised 15 cm above the table. For 5 minutes, each rat was positioned separately in the middle of the board. The duration, frequency, and delay to the first head dip were noted [13].
5. **Open Field:** The equipment comprised of square arena (68 x 68 × 30 cm) with 16 outside squares along the walls and 9 core squares arranged in a 5 × 5 grid [14]. Entries and time spent in the center was observed [15].

Neurotransmitter Estimation

Serotonin Estimation: To estimate serotonin, 0.2 mL of aqueous extract and 0.25 mL of o-phthalaldehyde reagent were combined, heated for 10 minutes at 100°C, and then cooled to room temperature. At wavelengths of 470 nm for emission and 360 nm for excitation, fluorescence was detected [16].

Dopamine Estimation: 100 mg activated alumina was added and the brain homogenate (1 mL) was adjusted to pH 8.4 with tris buffer. Dopamine was eluted with 0.5 ml of 0.4M perchloric acid. The eluate (0.5 mL) was mixed with sodium acetate buffer (0.5 mL) and potassium ferricyanide (0.1 mL) for 2 minutes. Then, 2 mL of ethylenediamine reagent was added, and the mixture was heated to 60 °C for 30 min. After cooling, fluorescence was measured with excitation at 330 nm and emission at 375 nm. [17,18].

Statistical Analysis

Data are expressed as mean ± SEM and were analyzed by one-way ANOVA with Dunnett's test (GraphPad Prism 8.0.1)

Histopathological Studies

Brain tissues were processed using standard fixation, embedding, microtome sectioning, and staining, the tissues were examined under a high-resolution digital microscope.

RESULT

Extract Characteristics and Yield: Brownish-black, sticky ethanolic extract had a distinctively aromatic smell. 14.08% w/w was the average extraction yield.

Phytochemical Investigation

Alkaloids (Dragendroff's and Wagner's tests), flavonoids (lead acetate and ferric chloride tests), coumarins (NaOH test), tannins (Braymer's, lead subacetate, and bromine water tests), phenolic compounds (iodine and ferric chloride tests), and diterpenoids were all found by phytochemical screening. These phytoconstituents might have CNS-modulatory and neuroprotective qualities.

Acute Oral Toxicity: The acute oral toxicity study was performed following OECD Guideline 423. 2000 mg/kg body weight of ethanolic *Curcuma longa* leaf extract did not show any mortality, toxicity, clinical signs or abnormal behavioral changes. The LD50 was therefore estimated to be greater than 2000 mg/kg indicating a favorable acute oral safety profile.

Behavioral Models and Biochemical Estimation:

Table. 2 Elevated Plus Maze (a) Open and Closed Arms Entries

Treatment	No. of entries in open arm	No. of entries in closed arm
Vehicle (0.5% CMC)	3.5 ±0.4282	18.00 ± 0.4472
Diazepam (1mg/kg)	12.83 ± 0.6009 **** (266.67% ↑)	7.000 ± 0.2582**** (61.11% ↓)
C. longa leaf extract (100 mg/kg)	6.000 ± 0.3651 ** (71.43 % ↑)	16.17 ± 0.3073** (10.19% ↓)
C. longa leaf extract (200 mg/kg)	7.167 ± 0.4773 *** (104.76 % ↑)	14.67± 0.3333**** (18.52% ↓)
C. longa leaf extract (400 mg/kg)	10.83 ± 0.6009**** (209.52% ↑)	9.833± 0.3073**** (45.37 % ↓)

Table. 2 Elevated Plus Maze (b) Time Spends in Open and Closed Arms (sec)

Treatment	Time spends in open arms (sec)	Time spends in closed arms (sec)
Vehicle (0.5% CMC)	48.00 ± 4.502	241.3± 5.863
Diazepam (1mg/kg)	126.0 ± 1.932**** (162.50 % ↑)	118.8 ± 1.447**** (50.76% ↓)
C. longa leaf extract (100 mg/kg)	68.67 ± 3.180** (43.06% ↑)	220.8 ± 3.591** (8.49% ↓)
C. longa leaf extract (200 mg/kg)	72.17 ± 3.673*** (50.35 % ↑)	185.8 ± 5.069**** (22.99% ↓)
C. longa leaf extract (400 mg/kg)	105.0 ± 5.073**** (118.75 % ↑)	139.3 ± 2.894**** (42.27% ↓)

Table 2. (c) Light–Dark Box Test

Treatment	Number of entries in bright chamber	Number of entries in dark chamber	Time spends in bright chamber (sec)
Vehicle (0.5% CMC)	3.333 ± 0.3333	10.00 ± 0.5774	78.17 ± 1.869

Diazepam (1mg/kg)	10.00 ± 0.6831**** (160.88% ↑)	5.333± 0.4944**** (53.63 % ↓)	148.0 ± 2.366**** (89.34 % ↑)
C. longa leaf extract (100 mg/kg)	5.167 ± 0.3073* (58.96 % ↑)	7.833 ± 0.8333* (19.65 % ↓)	87.17 ± 3.497* (11.51 % ↑)
C. longa leaf extract (200 mg/kg)	5.667 ± 0.4216** (81.32 % ↑)	6.833± 0.3073** (27.11 % ↓)	90.67 ± 1.944** (15.99 % ↑)
C. longa leaf extract (400 mg/kg)	6.167 ± 0.4773*** (102.72 % ↑)	6.000± 0.3651**** (34.24 % ↓)	142.5 ± 1.232**** (82.30 % ↑)

Table 2. (d) Hole - Board Test

Treatment	Time for first head dip	Duration of head dip	Number of head dips
Vehicle (0.5% CMC)	25.17 ± 0.7923	59.33 ± 4.185	9.833 ± 0.6009
Diazepam (1mg/kg)	11.00 ± 0.5164**** (56.29% ↓)	122.7±2.319**** (106.74 % ↑)	22.50 ± 0.7638**** (128.81% ↑)
C. longa leaf extract (100 mg/kg)	21.00±0.8165** (16.56% ↓)	73.00 ±1.528** (23.03 % ↑)	13.00 ± 0.8165* (32.20 % ↑)
C. longa leaf extract (200 mg/kg)	20.00 ± 0.6831*** (20.53 %↓)	77.00 ± 2.221*** (29.78 % ↑)	14.50 ± 0.8466*** (47.46 % ↑)
C. longa leaf extract (400 mg/kg)	12.00 ±0.8563**** (52.32% ↓)	119.3 ± 2.654**** (101.12 % ↑)	21.50 ± 0.7638**** (118.64 % ↑)

Table. 2 (e) Open Field Test

Treatment	Number of entries in centre	Time spends in centre
Vehicle (0.5% CMC)	4.167 ±0.4773	21.83 ±1.740
Diazepam (1mg/kg)	15.83 ±0.6009**** (87.16 % ↑)	69.17 ±1.887**** (216.79 % ↑)
C. longa leaf extract (100 mg/kg)	7.000 ±0.5774** (38.47 % ↑)	28.33 ±1.626* (29.77 % ↑)
C. longa leaf extract (200 mg/kg)	7.500±0.5627*** (38.73 % ↑)	33.17 ±1.621*** (51.91 % ↑)
C. longa leaf extract (400 mg/kg)	14.83 ±0.3073**** (82.13%↑)	65.00 ±1.571**** (197.71 %↑)

Table. 2 (f) Staircase Test

Treatment	Number of steps climbed	Number of Rearings
Vehicle (0.5% CMC)	9.000 ± 0.5774	16.83 ± 1.078
Diazepam (1mg/kg)	19.50 ± 0.7638**** (116.67 % ↑)	8.833 ± 0.6009**** (47.52 % ↓)

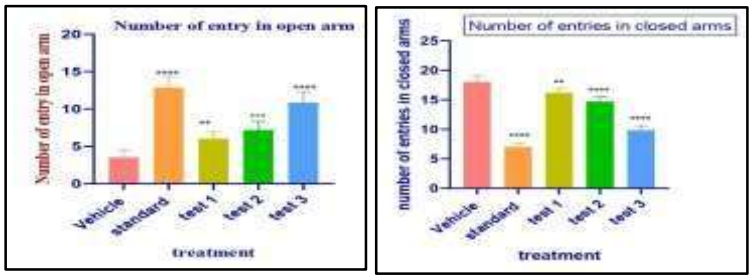
C. longa leaf extract (100 mg/kg)	12.00 ±0.3651* (33.33 %↑)	13.33 ± 1.145* (20.79 % ↓)
C. longa leaf extract (200 mg/kg)	13.17 ± 1.138** (46.30 % ↑)	11.00 ± 0.3651*** (34.65 % ↓)
C. longa leaf extract (400 mg/kg)	18.17 ±0.6009**** (101.85 % ↑)	10.00 ± 0.5774**** (40.59% ↓)

Table. 2 (g) Serotonin and Dopamine Levels

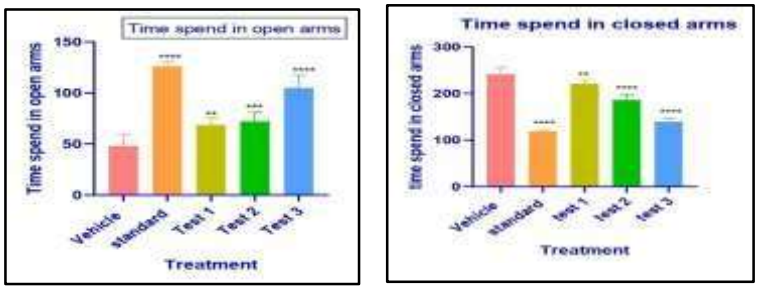
Treatment	Serotonin (µg/ml)	Dopamine (µg/ml)
Vehicle (0.5% CMC)	1.850±0.02236	2.107 ±0.01687
Diazepam (1mg/kg)	3.223±0.01745****	3.473±0.01333****
C. longa leaf extract (100 mg/kg)	1.967 ±0.008433**	2.227 ±0.02777**
C. longa leaf extract (200 mg/kg)	2.005±0.006708***	2.252 ±0.01621***
C. longa leaf extract (400 mg/kg)	3.118±0.03719****	2.595±0.03766****

Data are reported as mean ± SEM for six animals per group. Dunnett's test after one-way ANOVA. Significance levels are indicated as $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$; ns indicates a non-significant. (Compared to control)

a. Elevated Plus Maze

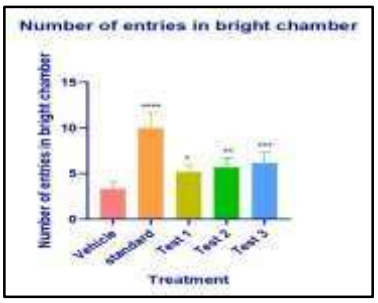


(A)

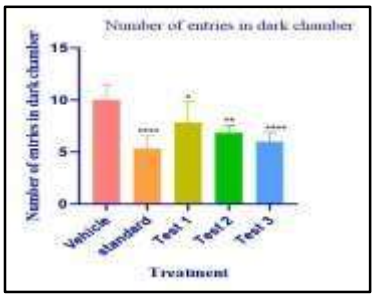


(B)

b. Light–Dark Box Test



(A)



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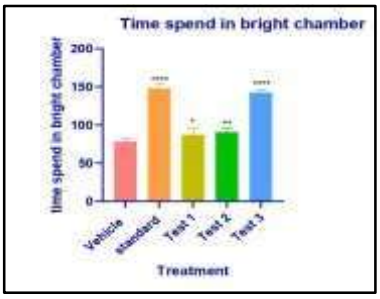
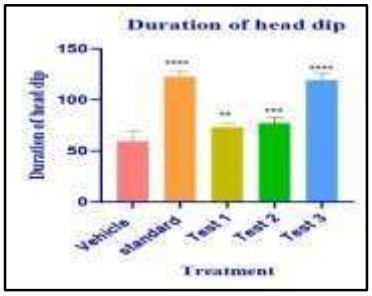


(C)

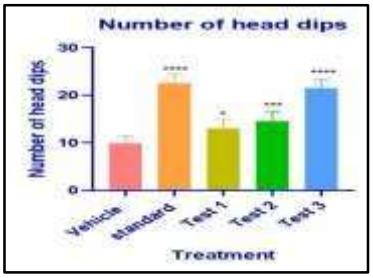
c. Hole-Board Test



(A)

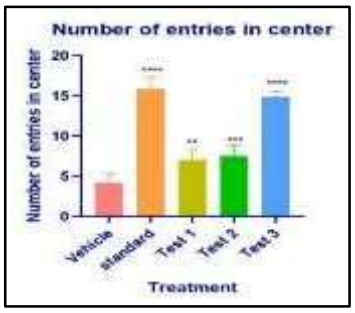


(B)

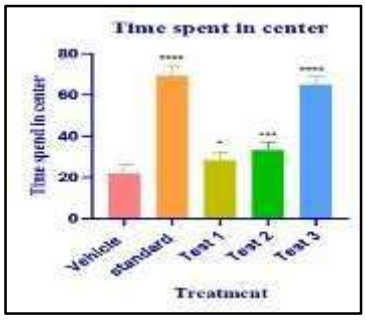


(C)

d. Open Field Test

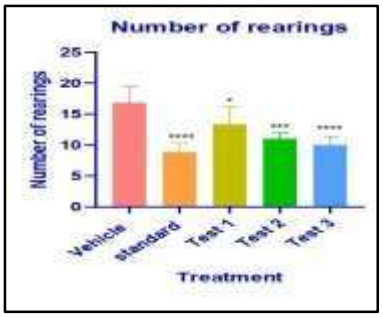


(A)

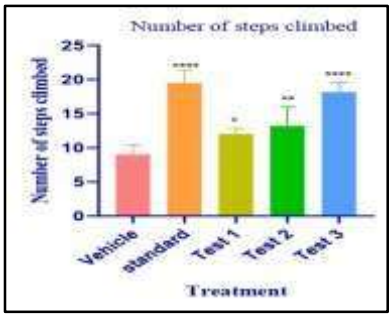


(B)

e. Staircase Test



(A)

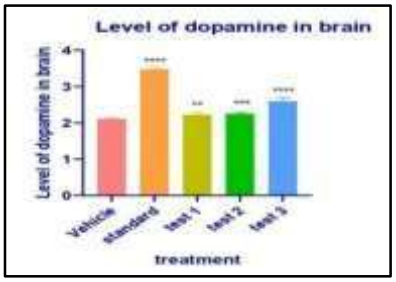


(B)

f. Biochemical Estimation



(A)



(B)

Fig. 1. Effects of *Curcuma longa* leaf extract on behavioral and biochemical parameters: (a) Elevated Plus Maze — (A) No. of entries in open and closed arms (B) time spent in open and closed arms; (b) Light–Dark — (A) bright chamber entries (B) dark chamber entries (C) bright chamber time ; (c)Hole - Board Test — (A) first head dip latency, (B) head dipping duration (C) head dips number ; (**d**) Open field Test — (A) number of entries in center, (B) Time spend in center ; and (e) Staircase Test — (A) Number of Rearing's (B) number of steps climbed; (f) Biochemical Estimation — (A) serotonin (B) dopamine

Histopathological Examination

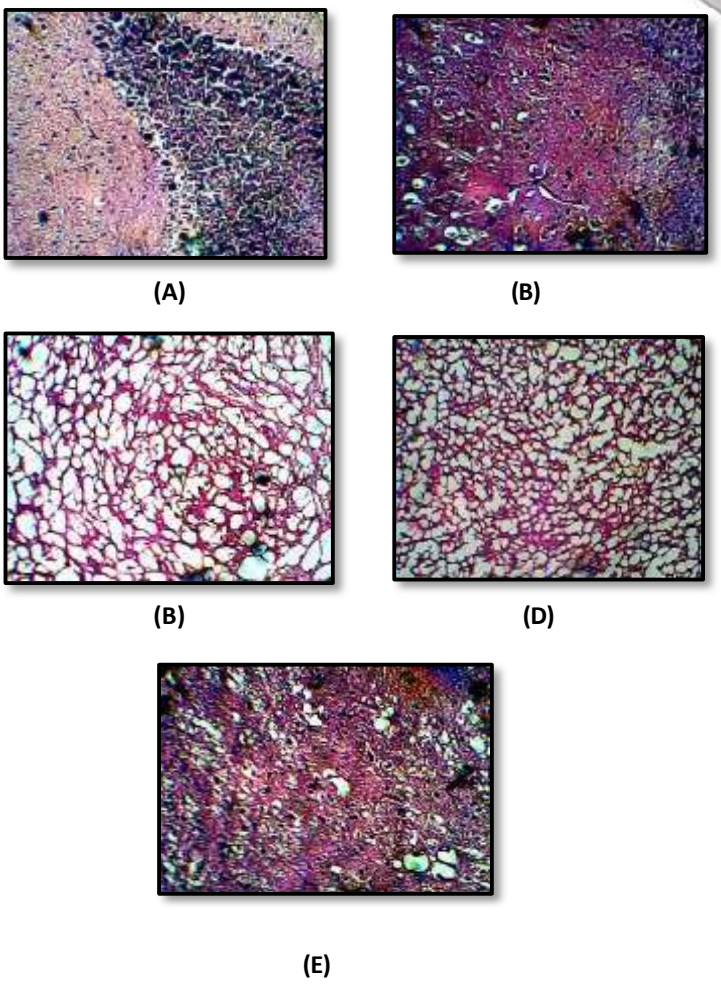


Fig. 2. H&E-stained whole-brain sections: (A) Vehicle (0.5% CMC), (B) Diazepam (1 mg/kg), *Curcuma longa* extract at (C) 100, (D) 200, and (E) 400 mg/kg.

Histopathological examination of whole brain sections revealed significant neuropil disorganization and increased vacuolation in the vehicle group. *Curcuma longa* leaves extract decreased vacuolation and increased neuronal density at 200 mg/kg. The highest improvement of cellular structure and neuronal density was found at 400 mg/kg. (Fig. 2).

DISCUSSION

The current study used various behavioral models in rats to assess the ethanolic *Curcuma longa* leaf extract's anxiolytic-like capabilities. The results showed reductions in a number of behavioral indicators associated to anxiety, with the strongest effects being seen at 400 mg/kg. The number of entries into the open arms in the Elevated Plus Maze model was increased by 71.43 %, 104.76 %, 209.52 % at 100, 200, 400 mg/kg, while the time spent in the open arms was increased by 43.06 %, 50.35 %, 118.75 %, compared to the control group. More significant effect was observed at 400 mg/kg, indicating a dose-dependent increase in activity compared with the lower doses [Table 2 (a-b), Fig 1(a)]. At dosages of 100, 200, 400 mg/kg, bright-chamber entry improved by 58.96%, 81.32%, and 102.72% in the light-dark test, while bright-chamber time increased by 11.51%, 15.99%, 82.30% [Table .2 (c), Fig .1 (b)]. The hole-board test revealed dose-dependent anxiolytic-like action of ethanolic *Curcuma longa* leaf extract, with head-

dip number increasing by 32.20%, 47.46%, 118.64% head dipping duration increasing by 23.03%, 29.78%, 101.12% at 100, 200, 400 mg/kg [Table .2 (d), Fig .1 (c)]. Open field test showed increases in central-area entrances of 38.47%, 38.73%, 82.13%, and increases in time spent in the central area of 29.77%, 51.91%, 197.71% at dosages of 100, 200, 400 mg/kg [Table 2 (e), Fig. 1 (d)]. The number of steps climbed in the staircase test increased by 33.33%, 46.30%, 101.85% at dosages of 100, 200, 400 mg/kg. At 400 mg/kg, the most impact was seen as compared to control group [Table 2 (f), Fig. 1 (e)]. Analysis of brain homogenate revealed increased levels of serotonin and dopamine post administration of ethanolic *Curcuma longa* leaf extract at 200 and 400 mg/kg than the control group. Serotonin and dopamine are involved in the regulation of mood, motivation and exploratory behavior, therefore alterations in their levels may be related to the behavioral effects observed in the present study [Table 2 (g), Fig. 1 (f)]. In histopathological analysis highest improvement of cellular structure and neuronal density was found at 400 mg/kg (Fig. 2).

CONCLUSION

The study showed anxiolytic-like effects of ethanolic *Curcuma longa* leaf extract in experimental animal models in a dose-dependent manner. Phytochemical screening revealed the presence of several bioactive constituents which may be responsible for these effects. These findings indicate the potential of *C. longa* leaves as a natural source for the development of anxiolytic drugs and support further studies to identify active constituents, to understand molecular mechanisms involved and to evaluate safety and efficacy in humans.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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