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Evaluation of effects of *Curcuma amada* extracts on 6-OHDA-induced behavioral alterations

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Abstract**—** *In the current work, 6-OHDA injection intrastriatally severely reduced motor function in rats. Similar to previous research showing that motor deficits in Parkinsonian rats can be mitigated by antioxidant supplementation (Zafar et al., 2003a), we found that Curcuma amada significantly and dose-dependently restored locomotor deficits in 6-OHDA-lesioned rats.*

Keywords**— *Curcuma amada, 6-OHDA, Parkinson, Ethanolic extract, Methanolic extract, Aqueous extract.***

I. INTRODUCTION

Parkinson's disease is a recognisable clinical syndrome with a range of causes and clinical presentations. Parkinson's disease represents a fast-growing neurodegenerative condition; the rising prevalence worldwide resembles the many characteristics typically observed during a pandemic, except for an infectious cause. In most populations, 3–5% of Parkinson's disease is explained by genetic causes linked to known Parkinson's disease genes, thus representing monogenic Parkinson's disease, whereas 90 genetic risk variants collectively explain 16–36% of the heritable risk of non-monogenic Parkinson's disease. (Bloem BR et al., 2021) This research aims to show that *Curcuma amada* has a protective effect against 6-OHDA-induced lesioning in rats.

II. MATERIALS AND METHOD

6-hydroxydopamine (6-OHDA) from Sigma. GSH, glutathione oxidized (GSSG), glutathione reductase (GR), nicotinamide adenine dinucleotide phosphate reduced (NADPH), 1-chloro- 2,4-dinitrobenzene (CDNB), 5-5′-dithio-bis-2-nitrobenzoic acid (DTNB), bovine serum albumin (BSA), thiobarbituric acid, ethylene diamine tetra acetic acid (EDTA) was purchased from Sisco Research Laboratories (SRL). Homovannilic acid (HVA) and 3, 4-dihydroxy phenyl acetic acid (DOPAC) were acquired from Sigma Aldrich. Analytical-grade substances were also utilized.

Collection of plant material and extraction procedure

The botanical samples were gathered in and around alwar and verified by botany department in Sunrise university, Alwar, Rajasthan India. Extractions were made using several solvents from the 1 kilogram of shade-dried, coarsely powdered leaves. To get the green syrupy substance, the extracts were filtered and distilled in a water bath. It was then vacuum-dried to a total weight of 52g. Finally, a suspension containing dissolved extracts of Curcuma amada was administered orally.

Extraction of dried leaves by using various solvents of increasing polarity

The extraction procedure was finished by obtaining, processing, and milling into powder Curcuma amada leaves. 500 grams of the powdered material were layered uniformly within the Soxhlet apparatus. After that, other solvents, ranging from nonpolar to polar, such ethanol, chloroform, acetone, and petroleum ether, were used to extract it. The solvents were sterilized and decontaminated before use. The

extraction method included three days of continuous hot percolation using a succession of solvents. Cold maceration was utilized to complete the aqueous extraction procedure. To remove the residual solvent, we transferred the extracts to a 100 mL beaker and heated the beaker in a water bath. The extracts were concentrated using vacuum distillation, decreasing their volume by a factor of 10. After being brought down to room temperature, they were dried out in a desiccator. The concentrated extracts were stored in airtight containers for further use in research. (Kokate et al. 2008)

Experimental animals

For this experiment, we utilized 250-300g male albino Wistar rats in good health. When sourcing animals, we made sure to contact. The animals slept on husk bedding and had access to food and drink in their roomy enclosure. The animal enclosure was aired and kept on a light/dark cycle of 12 hours for the duration of the experiment. Commercial rat feed marketed as "Amrut rat feed" was used to sustain the rodents. A sufficient quantity of vitamins and minerals are included in the feed's 5% fat, 21% protein, 55% nitrogen-free, 4% fiber (wt/wt) composition. The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment & Forests (Animal Welfare Division), Government of India mandated that all animal experiments be conducted in accordance with university and institutional regulations.

Acute Toxicity Research

The Organization for Economic Co-operation and Development (OECD) has issued recommendations for studies of oral acute toxicity. To lessen the distress caused to test subjects by acute toxicity experiments, this group operates on a global scale (OECD, 1996). OECD suggestions fall under the following broad groups.

5 animals were used in a fixed-dosage study (in accordance with Guideline 420). Acutely hazardous substances. (Use of 3 Animals, under Directive 423)

• The Up-and-Down Method (Rule 425, 1 Animal)

Acute toxic class. (guideline 423, 3 animals used)

Test Compounds

Methanol extract of Curcuma amada (MECA)

Ethanol extract of Curcuma amada (EECA)

Aqueous extract Curcuma amada (AECA)

Principle

When determining a substance's acute oral toxicity, the acute toxic category technique is used. The purpose of this strategy is to pinpoint the lethal dosage.

Unrestrained young adult rats are tested by orally ingesting test compounds. Body weights and necropsies are recorded for the mice for up to 15 days after they are given the test compounds. This method employs predetermined, uniform dosages of the chemicals under scrutiny. In each case, the administered dose was 5 milligrams per kilogram, 50 milligrams per kilogram, 300 milligrams per kilogram, 2000 milligrams per kilogram, or 5000 milligrams per kilogram. These lethal levels were shown to have a direct impact on mortality rates. In this investigation, female rats were employed at a higher rate than males, and the optimal number of animals per treatment group was three.

Animals

Female Wistar albino rats weighing between 150 and 200 milligrams were used in the acute toxicity studies. These rodents came from the Central Drug Research Institute in Lucknow. A regular mouse pellet diet (Hindustan Lever Limited, Bangalore) was provided, and they had free access to water. They lived in cases made of polypropylene. The rats were put through a cycle of 12 hours of darkness followed by 12 hours of light. The experimental methods were reviewed and authorized by an institution's animal ethics committee, and the rats fasted for at least twelve hours beforehand. The committee gave its OK to the trial, as well. All experiments were performed in the morning to comply with the CPCSEA regulations (CPCSEA, 2003) for the care of laboratory animals and the ethical guideline for studies of experimental pain in conscious animals. When dosing rats, the standard orogastric cannula was employed for oral delivery.

Procedure

Selecting and weighing fasting female rats was essential to the method. A systematic procedure was used to determine the appropriate dosage for each extract. Animals were monitored for two weeks following injection, and the starting dosage was determined based on the time before adverse effects were seen. The previous explanation provides the basis for calculating lethal dosages.

Experimental design

Experimental strategies were used in this investigation.

Eight groups of six rats each participated in the experiments.

Table no 1 : Experimental strategies

Group I	Vehicle treated, control group received 2µl of vehicle (0.1% ascorbic acid-saline) intracranially.
Group II	Vehicle treated, lesioned with 6 hydroxy dopamine on 22nd day.
Group III	Rats pretreated with methanol extract of *Curcuma amada (MECA) (250mg/kg,bw)* orally for 21 days; on 22nd day single dose of 6-hydroxydopamine (12 µg of 6-OHDA/2µl in 0.1% ascorbic acid- saline) injected into right striatum.
Group IV	Rats pretreated with methanol extract *Curcuma amada (MECA) (500mg/kg,bw)* orally for 21 days; on 22nd day single dose of 6-hydroxydopamine (12 µg of 6-OHDA/2µl in 0.1% ascorbic acid- saline) injected into right striatum.
Group V	Rats pretreated with ethanol extract of *Curcuma amada (EECA) (250mg/kg,bw)* orally for 21 days; on 22nd day single dose of 6-hydroxydopamine (12 µg of 6-OHDA/2µl in 0.1% ascorbic acid- saline) injected into right striatum.
Group VI	Rats pretreated with ethanol extract *Curcuma amada (EECA) (500mg/kg,bw)* orally for 21 days; on 22nd day single dose of 6-hydroxydopamine (12 µg of 6-OHDA/2µl in 0.1% ascorbic acid-saline) injected into right striatum.
Group VII	Rats pretreated with aqueous extract *Curcuma amada (AECA) (250mg/kg,bw)* orally for 21 days; on 22nd day single dose of 6-hydroxydopamine (12 µg of 6-OHDA/2µl in 0.1% ascorbic acid-saline) injected into right striatum.
Group VIII	Rats pretreated with aqueous extract *Curcuma amada (AECA) (500mg/kg,bw)* orally for 21 days; on 22nd day single dose of 6-hydroxydopamine (12 µg of 6-OHDA/2µl in 0.1% ascorbic acid-saline) injected into right striatum.

Behavioral Studies

All of the behavioral tests were conducted in a quiet, temperature-controlled area with no outside noise or distractions. From 10 a.m. until 6 p.m., all of the tests were run.

Locomotor activity

On day 36, we checked in with the animals to see how active they were. By activating the camera, we were able to observe the animal's locomotor activities in a chamber of 50 by 50 by 35 centimeters. To provide a clear image on the monitor, the activity chamber was outfitted with black paper. Three 5-minute sessions were conducted for each animal to evaluate their mobility. Time spent in each of the following states was counted: wall clinging (min), walking (sec), running (sec), sitting (sec), standing (sec), rearing (sec), displaying stereotypical behavior (number), turning (clockwise, counterclockwise, and no direction), and moving (cm). To prevent contamination from animal scents, the exercise chamber was wiped off after each animal with 10% alcohol.

III. RESULTS AND DISCUSSION

Extractive Values of *Curcuma amada* leaves is given below.

Table no. 2: Extractive Values of Curcuma amada leaves

Solvent	***Curcuma amada***
Pet. Ether	4.34%
Chloroform	6.22%
Acetone	13.20%
Methanol	19.50%
Hydroethanol	25.01%
Aqueous	29.72%

Acute Toxicity Study of *Curcuma amada* leaves

Acute toxicity studies are performed to ascertain a drug's safety In vivo and establish its therapeutic index. The LD50 value is often determined by acute toxicity tests in experimental animals.In order to conduct acute toxicity tests in accordance with OECD Guideline 423, we selected an extract from the leaves of *Curcuma amada* based on our phytochemical results. The results showed that no animals died in any of the dosage groups, and that even at a dose of 5000 mg/kg, there were no signs of toxicity or changes in behavior. All of the extracts were deemed Category 5 (>5000) safe for use with rats, indicating their nontoxicity.The results are shown in Table 3

Table no. 3: Acute Toxicity Studies of Curcuma amada leaves

No. of	Extract	Dose (mg/kg)	Results
3	*MECA, EECA, AECA*	5	No death
3		50	No death
3		300	No death
3		2000	No death
3		5000	No death

Table no. 4: Effect of extracts on Locomotor activity

Groups	Treatment	No. of Sq. crossed	Rearing	Grooming
Group I vehicle, p.o	Control	22.66±1.35	10.33±2.12	**7.83±1.19**
Group II 6-OHDA	Negative Control	6.16 ±0.70a***	4.86±0.74a***	**2.50±1.08a*** **
Group III 250mg/kg,bw	6-OHDA + (*MECA*)	10.16±1.07b*	9.02±2.25b*	**3.83±1.49b***
Group IV 500mg/kg,bw	6-OHDA + (*MECA*)	11.33±1.54b**	9.00±1.67b**	**6.00±1.26b** **
Group V 250mg/kg,bw	6-OHDA + (*EECA*)	8.01±1.36b*	6.33±1.43b*	**5.00±0.77b***
Group VI 500mg/kg,bw	6-OHDA+ (*EECA*)	13.33±1.35b**	8.16±1.57b**	**6.33±1.28b** **
Group VII 250mg/kg,bw	6-OHDA + (*AECA*)	11.16±1.07b*	9.52±2.15b*	**3.731±1.29b***
Group VIII 500mg/kg,bw	**6-OHDA + (*AECA*)**	**12.23±2.54b****	**10.10±1.21b****	**4.52±1.01b** **

IV. CONCLUSION

The biological activity and active components of several therapeutic plants have been studied. Traditional Ayurvedic claims that the medicinal plants *Curcuma amada* can improve behavior are supported by scientific studies. These plants are used to stimulate muscular movement, learning, and concentration. *Curcuma amada* and its active ingredient have not been the subject of much research into their impact on 6-OHDA-induced lesioning. Therefore, the primary goal of this research was to determine whether or not *Curcuma amada* could prevent rats from developing 6-OHDA-induced Parkinsonism.

This research aims to show that *Curcuma amada* has a protective effect against 6-OHDA-induced lesioning in rats. In Ayurvedic medicine, *Curcuma amada* is used to cure anxiety, boost brainpower, and enhance memory in both young and old (Singh and Dhawan, 1997). In addition, it is used to treat a wide range of neurological conditions (Russo and Borrelli, 2005). As a result of its widespread use, several over-the-counter preparations of *Curcuma amada* is marketed as effective ways to improve memory in people of all ages. Motor impairments and aberrant involuntary movements, comparable to those reported in the 6-OHDA rat model of PD, are clearly shown in rats with injuries to the nigrostriatal pathway. These changes in behavior are more similar to those seen in individuals with clinical characteristics and drug-induced aberrant movements.

In the current work, 6-OHDA injection intrastriatally severely reduced motor function in rats. Similar to previous research showing that motor deficits in Parkinsonian rats can be mitigated by antioxidant supplementation (Zafar et al., 2003a), we found that *Curcuma amada* significantly and dose-dependently restored locomotor deficits in 6-OHDA-lesioned rats.

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