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Evaluation of neuroprotective activity of *Tagetes erecta on* 6-hydroxy dopamine induced neurotoxicity

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Abstract:

The present study evaluates the neuroprotective potential of *Tagetes erecta* extracts against 6-hydroxydopamine (6-OHDA)-induced neurotoxicity in Wistar rats. Methanolic (METE), ethanolic (EETE), and aqueous (AETE) extracts were administered orally at doses of 250 and 500 mg/kg for 21 days prior to induction of neurotoxicity. Behavioral assessments including locomotor activity and motor coordination (rota rod test) were conducted. 6-OHDA administration significantly impaired locomotor functions and muscle coordination, as evidenced by reduced square crossings, rearing, grooming, and retention time. Pretreatment with *Tagetes erecta* extracts significantly and dose-dependently improved these behavioral parameters. Among all treatments, higher doses (500 mg/kg) showed more pronounced neuroprotection. The findings suggest that *Tagetes erecta* possesses significant neuroprotective activity, possibly due to its antioxidant properties, and may be beneficial in managing neurodegenerative disorders.

Keywords: *Tagetes erecta* , Neuroprotection, 6-Hydroxydopamine (6-OHDA), Locomotor activity, Rota rod test

Introduction:

Neurodegenerative disorders such as Parkinson's disease are characterized by progressive loss of dopaminergic neurons, leading to impaired motor and cognitive functions. Experimental models using 6-hydroxydopamine (6-OHDA) are widely employed to mimic Parkinsonian neurodegeneration due to their ability to induce oxidative stress and selective neuronal damage.

Natural products have gained increasing attention for their therapeutic potential in neuroprotection due to their antioxidant and anti-inflammatory properties. *Tagetes erecta* (family Asteraceae), commonly known as marigold, is traditionally used for its medicinal properties, including anti-inflammatory, antimicrobial, and antioxidant effects. Phytochemical studies reveal the presence of flavonoids, carotenoids, and phenolic compounds, which are known to combat oxidative stress.

The present study aims to investigate the neuroprotective effects of methanolic, ethanolic, and aqueous extracts of *Tagetes erecta* against 6-OHDA-induced neurotoxicity in rats using behavioral models such as locomotor activity and motor coordination tests.

Materials and method

The plant *Tagetes erecta* were gathered in and around alwar and verified by botany department in Sunrise university, Alwar, Rajasthan India. Extractions were made using several solvents (Methanol, ethanol and water) 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 *Tagetes erecta* was administered orally.

Experimental animals

For this experiment, we utilized 250-300g male albino Wistar rats in

Table 1:

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 Tagetes erecta (METE) (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 Tagetes erecta (METE) (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 Tagetes erecta (EETE) (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 Tagetes erecta (EETE) (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 Tagetes erecta (AETE) (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 Tagetes erecta (AETE) (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.

Methanol extract of *Tagetes erecta (METE),* Ethanol extract of *Tagetes erecta (EETE),* Aqueous extract *Tagetes erecta (AETE)*

Lesioning

All animals in both the experimental and sham-operated groups were given ketamine and xylazine intraperitoneally (i.p.) after 3 weeks of therapy. The skulls of the animals were exposed by cutting the skin overlying them, and then the stereotaxic stands were used to precisely measure the striatum's (Paxinos and Watson, 1982) coordinates (antero-posterior 0.5mm, lateral 2.5mm, dorso-ventral 4.5mm relative to bregma and ventral from dura) with the tooth bar set at 0mm. Afterward, 12g 6-OHDA/2l in 0.1% ascorbic acid-saline was injected into the right striatum of all rats in the experimental groups, whereas the sham-operated group received 2.0l of the vehicle. In both groups, burr holes were drilled into the skull surface,

and injections were given manually using a Hamilton syringe. Injections were given at a rate of 1.0 l/min, and the needle was left in place for an extra minute before being gradually withdrawn. All procedures used in the trials were approved by the Institutional Animal Ethics Committee (IEAC).

Post operative care

Anesthesia recovery time was about 4–5 hours. The rats were housed in groups of four per cage until they reached full awareness, at which point they were returned to a well-ventilated room where the temperature was maintained at 253°C. For the first week, we provided the animals' dietary needs by keeping food and drink inside their cages, where they could get it without risk of physical damage from reaching too high. The animals were then given the standard care that is expected of them, including fresh water, food, and cage bedding every week.

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.

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.

Rota rod (muscular coordination)

On day 40, the subject's muscle coordination was assessed using a Rota rod (Instruments and Chemicals, Ambala, New Delhi). The device consists of a revolving rod (75 mm in diameter) with four parallel chambers, allowing for the testing of four rats simultaneously. When the rodents tumble down the revolving shaft, the equipment instantly registers the 0.1-second interval. The timer was set for 180 seconds at a speed of 10 revolutions per minute. Animals that had never been exposed to drugs were taught to balance on the rod for as long as possible.

Results and discussion

Neuroprotective Effect

Neurobehavioral tests were performed on both the control and experimental rats. 6-OHDA induction resulted in a variety of behavioral alterations, including a reduction in locomotor activity and rota rod , muscle coordination, stereotypic behavior, distance traveled, rearing, grip strength, balance and working capacity, as well as an impairment of short-term memory, elevated anxiety, and depression. *Tagetes erecta* extract substantially and dose-dependently reversed the impairments in behavioral activity caused by 6-OHDA lesioning.

Tagetes erecta was investigated for their potential to counteract the metabolic alterations brought on by 6-OHDA lesioning. *Tagetes erecta* extract effectively and dose-dependently restored the reduced antioxidant state.

Behavioral Studies

All of the experiments on behavior were conducted at ambient temperature in a quiet, undisturbed environment. The research was conducted from 10 a.m. to 6 p.m.

Locomotor activity

All the animals' mobility was evaluated on day 36. The open field test is a paradigm for gauging the degree of nerve excitability and assessing the impact of medicines on locomotor activity. Summaries of Findings Table The number of square crossed,

groomed, and raising (Locomotor) animals that had been treated with 6-OHDA decreased significantly ($p<0.001$). The number of square crosses increased significantly ($p<0.01$) after extract administration, and at the same dosage, the animals exhibited an increase in grooming and rearing behavior that was statistically significant ($p<0.001$) although less so ($p<0.05$). Number of squares traversed, grooming, and rearing all improved significantly ($p<0.01$) in animals given second extract. There was also a statistically significant increase in the amount of squares crossed after taking the std medication ($p<0.001$). A rise in square crossings may reflect more vigorous motor activity.

Table 1: Results of effect of *Tagetes erecta* extract on Locomotor activity

Group	Treatment	No. of Squares Crossed	Rearing	Grooming
Group I	Control (vehicle, p.o)	22.66 ± 1.35	10.33 ± 2.12	7.83 ± 1.19
Group II	Negative Control (6-OHDA)	6.16 ± 0.70^{a}***	4.86 ± 0.74^{a}***	2.50 ± 1.08^{a}***
Group III	6-OHDA + (METE) (250 mg/kg, bw)	10.16 ± 1.07^{b}*	9.02 ± 2.25^{b}*	3.83 ± 1.49^{b}*
Group IV	6-OHDA + (METE) (500 mg/kg, bw)	11.33 ± 1.54^{b}**	9.00 ± 1.67^{b}**	6.00 ± 1.26^{b}**
Group V	6-OHDA + (EETE) (250 mg/kg, bw)	8.01 ± 1.36^{b}*	6.33 ± 1.43^{b}*	5.00 ± 0.77^{b}*
Group VI	6-OHDA + (EETE) (500 mg/kg, bw)	13.33 ± 1.35^{b}**	8.16 ± 1.57^{b}**	6.33 ± 1.28^{b}**
Group VII	6-OHDA + (AETE) (250 mg/kg, bw)	11.16 ± 1.07^{b}*	9.52 ± 2.15^{b}*	3.73 ± 1.29^{b}*
Group VIII	6-OHDA + (AETE) (500 mg/kg, bw)	12.23 ± 2.54^{b}**	10.10 ± 1.21^{b}**	4.52 ± 1.01^{b}**

* Comparisons were made between

^{a} Control Vs Negative control ; ^{b}Negative control Vs Treatment groups

* Values are expressed as mean ± SEM of 6 animals.

* Symbols represent statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001.

* One-way analysis of variance (ANOVA) and Dunnett's t-test were used to determine statistical significance.

Rota rod (muscular coordination)

On day 40, we tested the subjects' muscle coordination using a Rota rod (Instruments and Chemicals, Ambala, New Delhi). The rota rod test data for motor coordination is shown in the table below. Animals not given 6-OHDA had much better motor coordination than those given the drug. The motor coordination is significantly enhanced by 500mg/kg of *Tagetes erecta* extract ($p<0.01$). The std medication has also resulted in markedly enhanced motor coordination ($p<0.001$). Muscle coordination and other functions may have been affected by the restoration of dopamine levels.

Table 2: Results of effect of *Tagetes erecta* extract on muscle coordination

Group	Treatment	Retention Time (Sec)
Group I	Control (vehicle, p.o)	319.2 ± 40.30
Group II	Negative Control (6-OHDA)	11.07 ± 1.49***
Group III	6-OHDA + (METE) (250 mg/kg, bw)	165.93 ± 4.70^{b}***
Group IV	6-OHDA + (METE) (500 mg/kg, bw)	234.70 ± 26.96^{b}***
Group V	6-OHDA + (EETE) (250 mg/kg, bw)	175.17 ± 6.01^{b}***
Group VI	6-OHDA + (EETE) (500 mg/kg, bw)	241.2 ± 20.75^{b}***
Group VII	6-OHDA + (AETE) (250 mg/kg, bw)	187.21 ± 3.12^{b}***
Group VIII	6-OHDA + (AETE) (500 mg/kg, bw)	197.21 ± 6.31^{b}***

* Comparisons were made between aControl Vs Negative control ; bNegative control Vs Treatment groups
* Values are expressed as mean ± SEM of 6 animals.
* Each symbol denotes a level of statistical significance: *P 0.05, **P 0.01, ***P 0.001. One-way analysis of variance (ANOVA) and Dunnett's t-test were used to determine statistical significance between the groups.

Conclusion

The study demonstrates that *Tagetes erecta* extracts exhibit significant neuroprotective effects against 6-OHDA-induced neurotoxicity. Pretreatment with METE, EETE, and AETE improved locomotor activity and motor coordination in a dose-dependent manner, with 500 mg/kg showing superior efficacy. These effects may be attributed to the antioxidant properties of the plant, which help mitigate oxidative stress-induced neuronal damage. The findings support the potential use of *Tagetes erecta* as a natural therapeutic agent for managing neurodegenerative disorders such as Parkinson's disease. Further studies involving biochemical and histopathological evaluations are recommended to elucidate the exact mechanisms of action.

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