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ANTI-PARKINSONIAN ACTIVITY OF METHANOLIC EXTRACT OF *ACORUS CALAMUS* RHIZOMES ON PARAQUAT INDUCED PARKINSONISM IN RATS.

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Abstract:

Objective: The goal of the current study was to assess the effects of paraquat (PQ)-induced Parkinsonism in rats and the methanolic extract of rhizomes of Acorus calamus (MEAC) on that condition.

Methods: In this study, three doses of MEAC (100, 200 and 400 mg/kg p.o) and standard Madopar (10mg/kg p.o)were daily administered to rat for consecutive 28 days and Paraquat was administered once a week by intraperitoneal route for 4 consecutive weeks .The effect of extract was monitored using various behavioural ,anti-oxidant and histopathological assessments . Researchers examined how the activity of various biomarkers of oxidative stress and brain aging changed over time.

Results: The optimum anti-parkinsonian activity was observed at 400mg/kg group of animals. All the behavioural parameters like Catalepsy, Akinesia, No. of falls, immobile period, tremors were significantly elevated and rearing behaviour is declined in PQ treated animals. However, a dose-dependent protection of the activities was seen in the rats after pre-treatment with MEAC.

When PQ was chronically administered, the levels of reduced glutathione, glutathione reductase, glutathione peroxidase, and catalase, which are indicators of oxidative stress, considerably dropped and lipid peroxidation significantly increased, according to biochemical analyses of the brain tissue. MEAC treatment improved various biochemical indicators and reduced lipid peroxidation. Following pre-treatment of rats with MEAC, a dose-dependent protection of activities was observed.

Conclusion: The In vivo studies showed a dose dependent increase in neuroprotective activity against Paraquat induced Parkinsonism. It was established that MEAC therapy effectively reduced motor deficits and protected the brain from oxidative stress.

Keywords: Parkinson's disease (PD), Oxidative stress, Lipid peroxidation, Catalase, *Acorus calamus*, Behavioural parameters, MEAC, Biochemical Parameters.

Introduction:

Parkinson's disease is largely caused by the loss of dopaminergic neurons in the substantia nigra pars compact (SNpc), which lowers striatal dopamine (DA) and resulting in the development of intracellular Lewy bodies[1]. Neurotransmitters including acetylcholine and DA become imbalanced in the striatum when dopaminergic supply is cut off.

Numerous regions in Europe, Asia, and North America are home to the annual, semi-aquatic *Acorus calamus* Linn[2]. In India, it is frequently referred to as a "sweet flag." The leaves, roots, and rhizomes of this plant have been utilized in Indian Traditional Medicine for more than a century[3]. According to reports, AC has antispasmodic, carminative, anthelmintic, aromatic, expectorant, nauseate, nervine, sedative, and stimulant effects. It is also used to treat epilepsy, mental disorders, chronic diarrhoea, dysentery, intermittent fevers, and abdominal and glandular cancers.

Methods:

Selection of plant species:

The plant *Acorus calamus* was chosen and the methanolic extracts from its rhizomes were used for complete work, which was authenticated by Dr.K.Madavachetty , Plant taxonomist, Assistant professor, Department of botany, Sri Venkateswara university, Tirupathi , AP, India

Preparation of plant Extract:

The rhizomes of *Acorus calamus* were collected and powered into fine particles. Weigh 50gms of rhizome powder and mix it with 50ml methanol and is taken into round bottomed flask. The round bottomed flask was fixed to the Soxhlet apparatus and heating mantle is placed under the Soxhlet, temperature was maintained at 10^0c. After few days the extract was collected and concentrated. Before usage, the crude extract (MEAC) was harvested and kept in an airtight glass container at a temperature of 4–80 c. The analysis of phytochemical components was completed.

Animal Selection:

Thirty male Wistar rats weighing 150g to 250g were provided by San zyme Bio-Analytical Laboratory, Plot No.SY.NO.542, Kolthur(V), Shameerpet(M), R.R Dist, Biotech Park Phase-II, Hyderabad. The animals were kept in polypropylene cages at 24°C/2°C with a 12-hour light/dark cycle. They were given a typical pellet diet, and water was available at all times. The Malla Reddy College of Pharmacy's Institutional Animal Ethics Committee in Maisammaguda, Secunderabad, approved the method for animal experiments.

.Selection of Doses for Study:

Since the safety of MEAC up to 2000 mg/kg had already been confirmed in a previous study, no acute toxicity investigations on this substance were carried out. Based on earlier studies that showed superior response at dosages over 400 mg/kg in terms of neuroprotective effect, the three doses of 100,200, and 400 mg/kg were chosen.

Experimental design:

For the experiment, 36 Wistar albino rats weighing between 150 and 250 grams were employed. Six groups of six animals each were formed from the animal population.

Group 1: Distilled water was given to the control group.

Group 2: This group was given paraquat (10 mg/kg, i.p.).

Group 3: This group was given the drug madopar (10 mg/kg, p.o.) and paraquat 10mg/kg, i.p.

Group 4: Test dose treated group received MEAC and paraquat (100mg/kg, p.o.+ 10mg/kg, .i.p.)

Group 5: Test dose treated group received MEAC and paraquat (200mg/kg, p.o+ 10mg/kg, i.p.)

Group 6: Test dose treated group received MEAC and paraquat (400mg/kg, p.o+ 10mg/kg, .i.p.)

Behavioral models:

- **Catalepsy**:

The rats were positioned on a wooden box with their forelimbs raised to a height of 9 cm, and it was recorded how long it took each rat to withdraw its paw from the box[4].

Rearing Behaviour:

Rats are kept in a 20-cm-diameter, clear plexiglass cylinder for five minutes to count the number of rears. Based on the no. of rearing, rearing score is given to each animal.

- **Rota rod test**: In this test, mice are required to balance on a spinning rod for 5 minutes, with their latency to fall being recorded as the endpoint measure.
- **Tremors**: Following the administration of the paraquat dosage, tremors were observed. Using a modified intensity score on a range of 0 to 5, tremor was quantified.
- **Akinesia**:

The rat was held with one paw on the table, and the experimenter slowly moved the animal sideways (5 sets for 0.9 m), first in the forehand direction and then in the backhand direction. The steps of adjustment in the forehand and backhand directions were included for both paws. Prior to testing the left paw forehand and backhand, the right paw forehand and backhand were tested.

- **Open field test**:

Before the test, rats were used to the open field apparatus, and at the conclusion of the investigation, behaviour was observed for any alterations. Estimates were made for variables such total immobility time and total incidents.

Rats were put to death by carbon dioxide inhalation on the 28th day, immediately following behavioural evaluation, in order to collect blood samples for biochemical analysis.

Anti-Oxidant Parameters:

Lipid Peroxidation:

- The fundamental theory is dependent on polyunsaturated fatty acids from lipid cell membranes on lipid peroxidation caused by reactive oxygen species that are then converted to thiobarbituric reactive chemicals like MDA**.**

Reduced Glutathione:

- There are two states of glutathione: reduced (GSH) and oxidized (GSSG). The thiol group of cysteine can give another stable molecule, like reactive oxygen species, a reducing equivalent (H++e-) when it is in the reduced state. Glutathione becomes reactive when it is oxidized, but it also interacts easily with other reactive glutathione to create glutathione disulfide (GSSG).

Catalase:

- The enzyme known as catalase is responsible for converting H_2O_2 into water and oxygen. To measure catalase activity, the rate of hydrogen peroxide breakdown at 240 nm was utilized. The catalase activity is measured by the variation in absorbance (E 240) over time.

Glutathione Reductase:

The tissue's glutathione reductase activity was assessed by monitoring the decline in absorbance brought on by NADPH oxidation at 340 nm.

- **Glutathione peroxidises**:

The rate of glutathione oxidation at 420 nm was used to calculate the total amount of glutathione peroxidase.

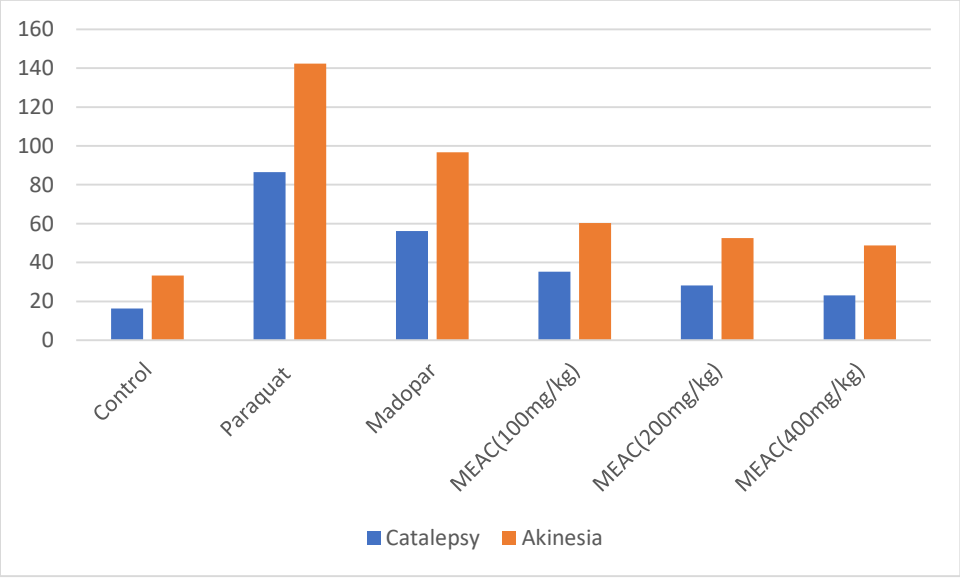
 The animals were put to death by breathing CO2 in a chamber used for euthanasia. The brains were removed and treated in 10% v/v formalin. Sections were excised from the tissue after processing. Photomicrographs were taken as the slides were prepared, stained with haematoxylin and eosin, then viewed with a high power microscope at 100x and 400x magnification. The results were expressed using mean SEM. Data were analysed using one-way analysis of variance (ANOVA).

Results And Discussion:

- The Phytochemical investigation of MEAC rhizomes revealed the presence of Alkaloids, glycosides, flavonoids, amino acids, saponins.
- Tremors were brought on by the prolonged injection of paraquat in rats. The MEAC-treated group also experienced tremors, albeit to a lesser degree.
- Progressive akinesia was brought on by paraquat. When compared to the Madopar and Paraquat treated groups, the MEAC treated group demonstrated superior performance in the form of significant delay intervals. The paraquat-treated animals showed signs of catalepsy. The MEAC group demonstrated higher performance by removing the bar in a noticeably shorter amount of time. The number of rears was significantly reduced in Paraquat-treated animals, but the number of rears in the MEAC group was significantly higher than that of Paraquat- and Madopar-treated animals (p0.001). Rats' ability to balance was considerably impaired (p=0.0001) by paraquat, as seen by an increase in falls over a 5-minute period. Increased balance behaviour in the MEAC-treated group was demonstrated by a reduction in falls.

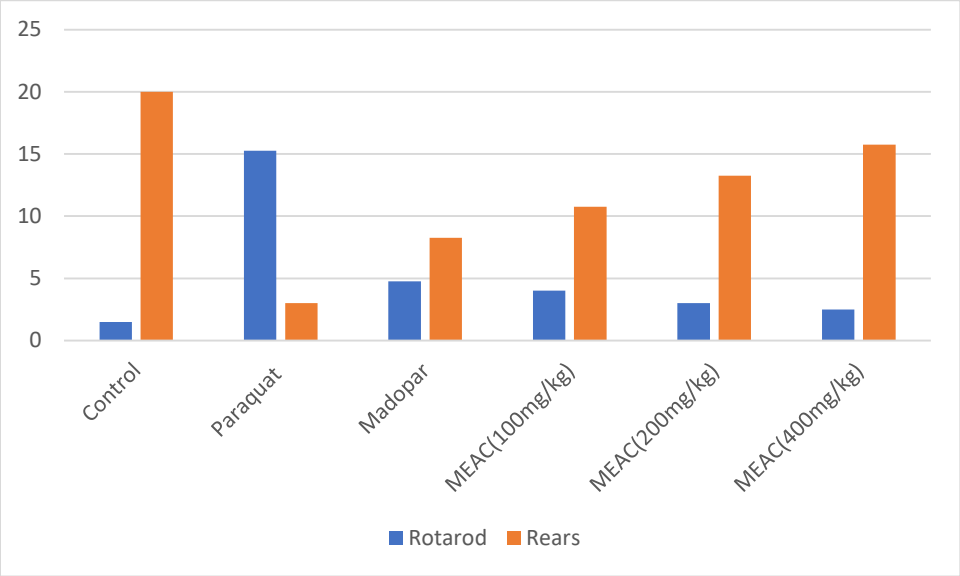
- Prior to Paraquat intoxication, rats treated with MEAC showed a dose-dependent and significant (p=0.0001) decrease in MDA levels compared to rats in the toxin group. Reduced glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPX), and catalase concentration were observed to be markedly (p=0.0001) decreased in the toxin group. There was a dose-dependent and significant (p=0.0001) normalization of their concentrations in brain tissues when comparing MEAC pre-administration groups to animals in the toxin group, as well as a recovery of their activities similar to normal levels**.**

Figure-1: Effect of MEAC on rat parkinsonism brought on by paraquat: catalepsy and akinesia



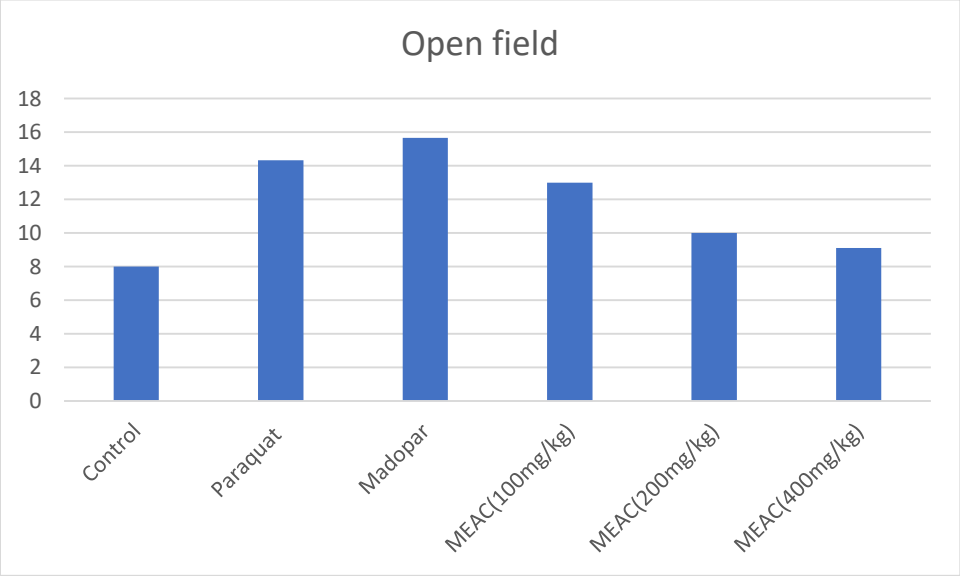
Values are presented as the mean SEM, with n=6. GraphPad Prism 6.0 software was used to conduct the t-test and analyse the data using one-way analysis of variance (ANOVA). * (p=0.0001) in comparison with the control group, ** (p=0.0001) and # (p=0.0007) when compared with animals treated with paraquat.

Figure-2: Effect of MEAC on rota rod test and rearing behaviour in paraquat induced parkinsonism in rats



Values are presented as the mean SEM, with n=6. Data were evaluated using one way analysis of variance (ANOVA), with * (p=0.0001) in comparison with control and ** (p=0.0001), # (p=0.0002) in comparison with toxin treated animals, utilizing the t-test to determine the intergroup variation between distinct groups.

Figure-3: MEAC's impact on a rat open field test for Parkinsonism brought on by paraquat

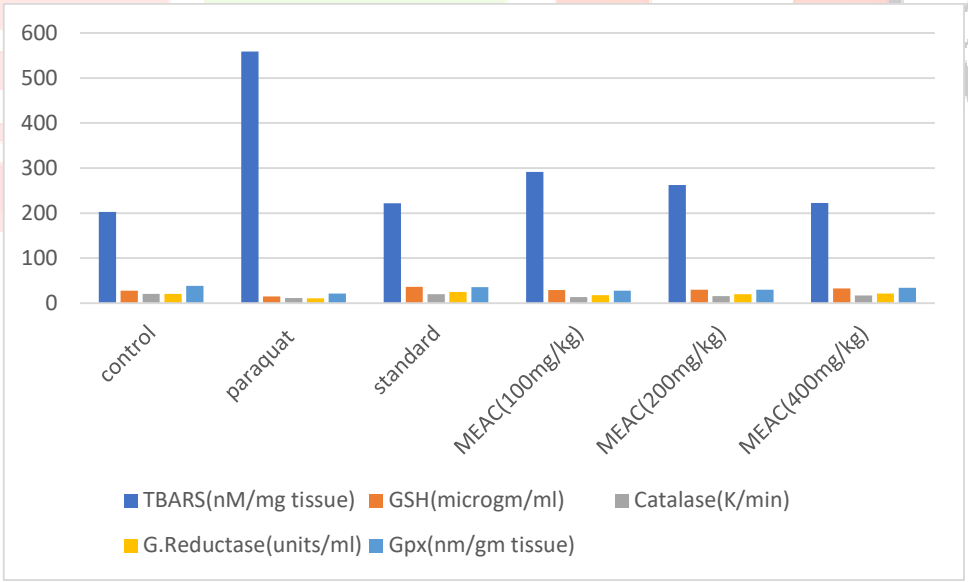


Values are presented as the mean SEM, with n=6. With the use of the graph pad Prism 6.0 software and the t-test, the data were analysed using one-way analysis of variance (ANOVA). * (p=0.0001) in comparison to the control group and ** (p=0.001) in comparison to the group that had been treated with the toxin.

Table-1 : Effect of MEAC *on* Tremors in Paraquat induced Parkinsonism in rats.

S.NO	GROUP	Score	Activity
1	Control (saline)oral	0	No tremor
2	Paraquat(10mg/kg) i.p	4	Severe tremor
3	Madopar(10mg/kg) p.o+ paraquat (10mg/kg) i.p	0	No tremor
4	MEAC(100mg/kg) p.o+ paraquat (10mg/kg) i.p	3	Moderate tremor
5	MEAC(200mg/kg) p.o+ paraquat (10mg/kg) i.p	2	Moderate intermittent
6	MEAC (400mg/kg) p.o + paraquat (10mg/kg i.p	1	Mild tremor

Figure:4. Effect of MEAC on Lipid peroxidation, Reduced Glutathione, Catalase and Glutathione Reductase, Glutathione peroxidase in Paraquat induced Parkinsonism in rats.



Values are presented as the mean SEM, with n=6. Data were evaluated using one way analysis of variance (ANOVA) and the t-test to determine the intergroup variation across distinct groups. *, **, #(p=0.0001) in comparison to the control group and **(p=0.001) in comparison to the animals that had been given a toxin were used to indicate significant differences.

CONCLUSION:

- Through its antioxidant action, MEAC rhizomes appear to shield the rat brain from Parkinsonism brought on by paraquat.
- A specific therapeutic dose of sweet flag may be effective against the neurotoxicity caused by paraquat. It could equally well find a home in complementary medicine or integrative medical procedures. No information regarding *Acorus Calamus* side effects has been reported as of yet. Accordingly, we may draw the conclusion from this research that methanolic extract of *Acorus calamus* rhizome is a potent neuroprotective agent and can be utilized as a successful preventative measure against Parkinsonism caused by paraquat.

- • The extract may operate as a ROS scavenger, but it's also possible that the antioxidant activity is mediated via modulating antioxidant enzymes. To find the active principle (s) contained in the plant's leaves and to clarify its potential mode of action, more research is required. The findings provided here could have therapeutic implications in the future, especially in regions where people are exposed to paraquat on a regular basis through their jobs or the environment.
- It may also serve as a possible nutritional intervention and the extract itself or fractions obtained there from may be used also as a future nutritional supplement to combat oxidative stress-induced tissue damage in the people exposed to paraquat.

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