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Evaluation of Antiparkinsonism Activity of Musa Paradisiaca Unripe Fruit Juice against Haloperidolinduced Parkinsonism in Swiss Albino Mice

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ABSTRACT:
In the present antiparkinsonism study was assessed by two following experimental models, In Haloperidol model the animal's swiss albino mice were divided into 5 (n=6). Group I received Vehicle, group II received Haloperidol 1mg/kg, group III received L-dopa 30mg/kg, group IV and V received low dose 137mg/kg and high dose 205mg/kg of Musa Paradisiacaunripe fruit juice along with haloperidol for 7days.Musa Paradisiacaunripe fruit juice was found to be significantly increase in the hanging time in hang test, and significantly increases in the fall of time in horizontal bar test, significantly increases in the number of squares travelled in open field test and significantly increases in the number of rotations in despair swim test respectively as compared to the disease control group.In-vivo lipid peroxidation study revealed disease control treated group showed increase in the MDA level when compared to the control group. Where L-dopa and high dose groups were able to prevent the rise in MDA levels where as low dose is significant as compared to disease control treated group, and increase in the activity of reduced glutathione and catalase when compared to disease control group.

Key words: antiparkinsonism, Musa Paradisiaca, lipid peroxidation,

I. INTRODUCTION:

Medicinal plants are a great source of economic value all over the world. Nature has given us a very rich botanical wealth and large number of diverse types of plants grows in different parts of the country. Ayurveda, Unani, and Siddha are systematically used nearly 1500 plants in indigenous system of medicine. Medicinal plants are the oldest existing complete medical system in the world. However use of herbal medicines in Asia represents a long history of human interaction with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as communicable diseases. Many plants are used as powerfull drugs medicinally in different countries. The World Health Organization (WHO) reveals that 80% of the world's population relies on traditional medicine for their primary health needs. Medicinal plants contain large varieties of chemical substances which possess important therapeutic properties that can be utilized in the treatment of humandiseases.[1]

Parkinson's disease (PD) is a slowly progressive neurodegenerative disease caused when a small group of brain cells that control body movements die. This disease was first described by James Parkinson in 1817. Pathological features of PD include loss of dopamine neurons in substantial nigra and the presence of Lewy bodies in surviving dopamine neurons. The disease occurs in about 1% of the people over the age of 65years. [2]

It is characterised by pill rolling tremors, Akathisia (inability to sit still), Rigidity, Kinesias (akinesia, bradykinesia), instable (stooped) posture, No arm swinging in rhythm with legs, sialorrhea, Oculogyric crisis (eyes are held fixed for a variable length of time), Nervous depression, Involuntary tremors, Seborrhea and masked facial expression. The term parkinsonism applies for a disease state having such common characteristics while parkinson's disease (paralysis agitans) is restricted to the primary or idiopathic parkinsonism. Most patients suffer from this type of parkinsonism and intriguingly the cause remain unknown or are multifactorial, e.g., genetic predisposition and aging of brain due to free radical damage to dopaminergic neurons in basal ganglia. The clinical symptoms of Parkinson's disease appear at about 80% loss in dopaminergic neurotransmission which gradually worsens with the age of the patient.Prognosis, even after drug therapy is, therefore, poor.

Secondary parkinsonism occurs from known cause and many of these are curable.It may result after prolonged use of antipsychotic drugs (D_2 receptor antagonists). Unlike idiopathic

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Parkinsonism (parkinson's disease), the striatal contents of DA are not reduced by administration of these drugs. They rather decrease the dopaminergic activity by blocking the post synaptic D2 receptors.Parkinsonism may also result by poisoning due to manganese, carbon monoxide or MPTP[3].The disease affects 2% of the world wide population and 1.5 million Americans aged 65 and older with the incidence increasing significantly with age. [4]

Parkinson disease also shows non-motoric symptoms such as sensoric disturbances, neurobehavioral disturbances (depression, anxiety, and psychosis), and decreased ability to remember. Recently, pharmacological treatment for Parkinson disease has use synthetic L-Dopa[5]. Levodopa, a dopamine structural analog which improves the level of dopamine in brain, is the best presently available medication for the treatment of Parkinson's disease.[6]

There is no effective cure for PD since currently available drugs can't stop and reverse the progression of the disorders and produce more side effects such as depression, confusion, hallucination, insomnia, anxiety, myocardial infractions and hepatotoxicity. Therefore the recent research mainly focused on searching newer drugs from plants having effective therapeutic properties against progression of disorder with less side effects.[7]

II. MATERIALS AND METHODS:

Plant material:[8]

The fresh unripe fruits of Musa Paradisiacawere collected in chitradurga, kaarnataka,India the plant was identified and authenticated by Prof. N. Shankaramma, Dpt of botany, Govt science college, chitradurga.

Preparation of unripe fruit juice:[8]

The juice was prepared by blending chopped banana pulp with distilled water to come up with two concentrations: 1.0g/ml and 1.5g/ml. The mixture was filtered thrice using cheese cloth, to remove starch from the juice. The juice was placed in 30ml amber bottles and was stored in the freezer until the day of experimentation

Experimental Animals:

Healthy young adult swiss albino mice (25-30g)of either sex were used for the study. Animals were procured from Animal house, SJM college of pharmacy, chitradurga, Karnataka and were housed under controlled temperature provided with food and water ad libitum. The protocol was approved by institutional animal ethical committee. Experiments were carried out as per CPCSEA guidelines (SJMCP/IAEC/2016-2017).

Haloperidol induced Antiparkinsonism activity:[9]

Group 1 Vehicle (orally, once/day ×1 week).
Group 2 - Haloperidol (1 mg/kg, once/day i.p ×1 week).
Group 3 - Levodopa (30 mg/kg, i.p. once/day ×1 week) along with haloperidol
Group 4- Low dose of Musa Paradisiacaunripefruit juice 137mg/kg orally, respectively, 1 week along with haloperidol.

Group 5 - High dose of Musa paradisiacaunripe fruit juice205mg/kgorally,
respectively 1 week along with haloperidol

BehaviouralParamater:

1. Hang test:[10]

Neuromuscular strength was determined in the grid hang test. Mice were lifted by their tail and slowly placed on a horizontal grid and supported until they grabbed the gird with both their fore and hind paws. The grid was then inverted so that the mice were allowed to hang upside down. The grid was mounted 20 cm above a hard surface, to discourage falling but not leading to injury in case of animal fall. Start a stop clock and note the time when the mice fall off or remove it. When the criterion time of 30 seconds is reached.

2. Horizontal bar test:

Hold the mouse by the tail; place it on the bench in front of the apparatus. Slide it quickly backwards about 20cms, rapidly raise it and let it grasp the horizontal bar at the central point with its fore paws only and release the tail simultaneously starting the stop clock. The criterion point is either a fall from the bar before the mouse reaches one of the end columns of the bar, or the time till one forepaw touches a column Maximum cut off time is 30 seconds.

3. Open field test: [11]

The open field apparatus consists of a big square area 76 × 76 with walls 42 cm high. The floor was divided into 25 equal squares. To determine activity, an animal was placed at the corner of a square of the open field and immediately after the placement the number of squares crossed was scored for 5 min.

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4. Despair swim test:

Each animal was introduced into a pool (45 cm long; 22 cm wide diameter, and 20 cm high) filled with 10 cm deep water. The animals were allowed to make rotations. The number of rotations made per 3 min was recorded.

Brain tissue collection:

After the treatment period, animals were sacrificed by over dose of anaesthetic ether and the whole brain was immediately dissected out and washed in ice - cold saline to remove all traces of blood. The brains were weighed and a 10% tissue homogenate was prepared in 0.1 M Potassium Phosphate pH 8 for the activities Lipid per oxidation, Glutathione level determination, Catalase activity.

Biochemical Analysis:

1. Lipid peroxidation (LPO) [12]

The amount of Malondialdehyde (MDA), was used as an indirect measure of lipid peroxidation and was determined by reaction with thiobarbituric acid (TBA). Briefly, 1ml of aliquots of supernatant was placed in test tubes and added to 3ml of TBA reagent: TBA 0.38% (w/w), 0.25M hydrochloric acid (Hcl),andtrichloroacetic acid (TCA 15%). The solution was shaken and placed for 15min,followed by cooling in an ice bath.After cooling, solution was centrifuged to 3500 for 10min.The upper layer was collected and assessed with a spectrophotometer at 532nm. Results were expressed as nanomoles per mg of protein. The concentration of MDA was calculated using formula.

$$\text{Conc of MDA} = \frac{\text{Abs}_{532} \times 100 \times V}{(1.56\times10^{5}) \times W_T \times W_V}$$

Where,
Abs_{532} is absorbance
V_T is total volume of mixture(4ml)
1.56×105 is molar extinction co-efficient
W_T is weight of dissected brain
V_U is aliquot volume (1 ml)

2. Glutathione level determination (GSH):

For the estimation of reduced glutathione, 1ml of tissue homogenate was precipitated with 1ml of 10% TCA. To an aliquot of the supernatant, 4ml of phosphate solution and 0.5ml of 5,5-dithiobis 2-nitrobenzoic acid (DTNB) reagent were added and absorbance was taken at 412nm.The values were expressed as nM of reduced glutathione per mg of protein.

$$\text{GSH level} = \frac{Y - 0.00314}{0.0314} \times \frac{DF}{BT\times VU}$$

Where,
Y is Abs412 of tissue homogenate
D_F is dilution factor (1)
B_T is brain tissue homogenate (1)
VU is aliquot volume (1ml)

3. Catalase activity:[13]

Principle: In U.V range H_2O_2 shows a continual increase in absorbance with decreasing wavelength. Catalase catalyses the rapid decomposition of hydrogen peroxide to water. The decomposition of hydrogen peroxide to water. Then of H_2O_2 can be followed directly by the decrease in absorbance at 240nm. The difference in absorbance per unit is a measure of catalyse activity.

Procedure:

1. Preparation of Hydrogen peroxide solution (7.5 mM):

1.043ml of 30% w/w H_2O_2 was made upto 100ml with sodium chloride and EDTA solution (9g of NaCl and 29.22 mg of EDTA dissolved in 1 litre distilled water)

2. Preparation of potassium phosphate buffer (65mM,pH 7.8):

2.2 g potassium dihydrogen phosphate and 11.332 g of di-potassium hydrogen phosphate were dissolved in 250ml and 1litre of distilled water respectively, and mixed together. The pH was adjusted to 7.8 with KH_2PO_4

3. Preparation of sucrose solution:

10.95g of sucrose was dissolved in 100ml of distilled water

4. To 2.25ml of potassium phosphate buffer 100 µl of the tissue homogenate was added and incubated at 25^0C for 30min.

5. For blank, sucrose solution was used instead of tissue homogenate

6. Then 0.65ml of H_2O_2 was added to initiate the reaction.

7. The change in absorbance of the reaction mixture at 240nm was measured for 2-3min

8.dy/dx for 1min for each assay was calculated and the results are expressed at cat units/mg of tissue.

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Calculation:

$$\text{Cat (U)/ 100 µl of Sample} = \frac{Dy/dx \times 0.003}{38.3956 \times 10^{-6}}$$

Where ,
Dy/dx – Change in absorbance

38.3956×10^{-6} – molar extinction co-efficient of H_2O_2 at 240nm

Statistical Analysis:

Values were expressed as Mean±SEM(n=6), and the difference between means was analyzed by one way ANOVA followed by tukey's multiple comparision test

III. RESULTS

Behavioural parameters

Effect of Musa Paradisiaca unripe fruit juice on hang test in mice.

Groups	Treatment and dose (mg/kg b.w.)	Hanging time (sec)
I.	Normal	15.2 ±0.794***
I.	Disease control	6.09 ± 0.327***
I.	L-dopa +Haloperidol	11.2±0.216***
V.	MPLD + Haloperidol	8.23 ± 0.312*
V.	MPHD + Haloperidol	8.98 ± 0.446**

Effect of Musa Paradisiaca unripe fruit juice on horizontal bar test in mice.

Groups	Treatment and Dose (mg/kg b.w.)	Time (sec)
I.	Normal	14.0 ± 0.357
II.	Disease control	6.42 ±0.337***
III.	L-dopa +Haloperidol	11.2 ±0.464***
IV.	MPLD +Haloperidol	8.22 ±0.209**
V.	MPHD+Haloperidol	9.32 ±0.180***

Effect of Musa Paradisiaca Unripe fruit juice on open field test in mice.

Groups	Treatment and dose (mg/kg b.w.)	Number of squares Crossed/5min
I.	Normal	21.2±0.060
I.	Haloperidol	9.52±0.231***
I.	L-dopa +Haloperidol	15.7±0.357***
V.	MPLD+Haloperidol	11.0±0.334*
V.	MPHD+Haloperidol	14.9±0.267**

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Effect of Musa Paradisiaca Unripe fruit juice on despair swim test in mice.

Groups	Treatment and Dose (mg/kg b.w.)	Number of rotations/3min
I	Normal	7.43±0.225
II	Haloperidol	4.12±0.108***
III	L-dopa +Haloperidol	5.92±0.206***
IV	MPLD+Haloperidol	5.00±0.171*
V	MPHD+Haloperidol	5.43±0.294**

Biochemical parameters:

Groups	Treatment and dose (mg/kg b.w.)	LPO (nM/ mg of protein)	GSH nM/mg of protein	Catalase (Unit/mg tissue)
I	Normal	165±0.198	262±13.7	5.86±0.542
II	Haloperidol	363±48.8***	127±0.211***	1.96±0.181***
III	L-dopa +Haloperidol	214±2.88***	238±3.21***	4.98±0.577***
IV	MPLD+Haloperidol	262±0.403*	156±1.46*	3.83±0.289*
V	MPHD+Haloperidol	240± 0.409**	206±1.09***	4.71±0.400**

Note: Data was analysed by using one way ANOVA followed by tukey's multiple comparision test. Values are expressed as Mean ± SEM. n=6, ***p<0.001, *P<0.01, *P<0.05

Musa Paradisiacaunripe fruit juice was found to be significantly increase in the hanging time in hang test, and significantly increases in the fall of time in horizontal bar test, significantly increases in the number of squares travelled in open field test and significantly increases in the number of rotations in despair swim test respectively as compared to the disease control group.In-vivo lipid peroxidation study revealed disease control treated group showed increase in the MDA level when compared to the control group. Where L-dopa and high dose groups were able to prevent the rise in MDA levels where as low dose is significant as compared to disease control treated group, and increase in the activity of reduced glutathione and catalase when compared to disease control group.

IV. DISCUSSION:

Musa Paradisiacaunripe fruit juice was found to be significantly increase in the hanging time in hang test, and significantly increases in the fall of time in horizontal bar test, significantly increases in the number of squares travelled in open field test and significantly increases in the number of rotations in despair swim test respectively as compared to the disease control group.

There are several pathological mechanisms proposed for PD. These include oxidative stress, accumulation of α-synuclein protein within neurons inside lewy bodies, neuro inflammation and microglia induced inflammation, mitochondrial dysfunction particularly reduced mitochondrial complex I activity, apoptosis and neuronal excitotoxicity. Among all, oxidative stress is also considered as an important pathological mechanism for PD. Free radical susceptible polyunsaturated fatty acid rich neuronal membrane, high oxygen consumption and low levels of glutathione of brain makes neurons more vulnerable to oxidative stress. Oxidative stress is mainly cause by over production of free radicals such as hydroxyl and super oxide anion-radical and non radicals such as nitric oxide and hydrogen peroxide as an enzymatic and non enzymatic oxidation of dopamine. SNpc is more vulnerable to reactive oxygen species as it is contains more amount of dopamine.[14]

Haloperidol a neuroleptic drug,induces catalepsy which is due to a blocking of post synaptic striatal dopamine D2 receptors and many studies have shown reactive oxygen species as a cause of haloperidol induced toxicity.Drugs which attenuate haloperidol-induced motor disorders might reduce the extrapyramidal signs of PD.[15]

The oxidative stress was measured through determination of levels of TBARS or (MDA), reduced glutathione and catalase. The extent of lipid peroxidation was estimated by measuring the levels of thiobarbituric acid, a product of lipid peroxidation. Lipid peroxidation is

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the process of oxidative degradation of polyunsaturated and its occurrence in biological membranes causes impaired membrane function,impaired structural integrity,drcreased fluidity and inactivation of number of membrane bound enzymes. There is substantial evidence of oxidative damage in the brains of PD patients. Increased levels of lipid peroxidation product, thiobarbituric acid have been found in the substantianigra of PD patients. Similar results were observed in the brain homogenates of Haloperidol and Chlorpromazine treated animals

A defect in one or more of the naturally occurring antioxidant defences particularly GSH is an important factor in etiology of PD. A reduction in GSH levels may impair H2O2 clearance and promote hydroxyl radical formation leading to the generation of pro-oxidant milieu.[16]

Increase is the level of lipid peroxides in the brain reflects the neuronal damage. The depletion of antioxidant defences and or rise in free radical production deteriorates the pro-oxidant antioxidant balance, leading to oxidative stress and cell death. Chlorpromazine and haloperidol induced oxidative stress has been associated with increased amount of lipid peroxidation. Indeed, Musa paradisiaca unripe fruit juice in our study was potentially effective in blunting lipid peroxidation suggesting that Musa Paradisiaca unripe fruit juice has anti-parkinsonism activity to reduce oxidative stress induced membrane lipid peroxidation.

Our study also revealed that the oxidative stress is induced by the administration of haloperidol and chlorpromazine in mice depletes the Glutathione and Catalase.

V. CONCLUSION:

Among the neurological disorders Parkinsonism disease was found to have increased incidence rate in coming years.Oxidative stress was found to be one of the major causes for PD.The flavonoids having free radical scavenging and antioxidant ability can be the possible solution for PD.Further studies of identification,characterization of the active constituents of the unripe fruit juice may give a new pathway for the treatment options of PD. The preclinical study has proved that, Musa Paradisiaca has got a therapeutic role in parkinsonism. The active constituent responsible for its anticataleptic action has to be identified. This will help in explaining the exact mechanism of its antiparkinsonian activity.

Hence the present study concludes that the effect of Musa Paradisiaca unripe fruit juice exhibited significant actions on Antiparkinsonism activity.

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