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Combination of Honey and L-Dopa Protected the Dopaminergic Neurons against MPTP Induced Parkinsonism in Adult Male Swiss Mice

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Abstract—Parkinson's disease (PD) is a progressive condition that is the second most common neurodegenerative disease worldwide that is characterized by gradual loss of dopaminergic neurons of the substantia nigra pars compacta (SNPc) thereby leading to bradykinesia, rigidity, tremor and postural instability. Approximately 5.25 million Nigerians are above 65 years and are therefore at risk for developing PD with approximately 10-249 cases per 100,000 people per year, with the mean age of onset close to 60 years. Oxidative stress plays an important role in the pathogenesis and progression of PD Diagnosis of this condition is usually late because, before the manifestation of the movement disorders, about 75% of these neurons are already lost, in fact, one of the differential diagnoses of PD is its response to levodopa treatment which does not seem to positively affect the progression of the condition. This explains why there is a need for a neuroprotective therapy that will prevent the death of these neurons and/ or halt the progression of this condition. Honey has been observed by various studies to be a radical scavenging gel as well as an enhancer of the antioxidant defense system. In this study, there was a combination of Honey with L-Dopa to ascertain if the combination will cause neuroprotection to the dopaminergic neurons of the substantia nigra in adult male Swiss mice via the mediation of the antioxidant system. 40 animals used for this study were divided into the control and parkinsonism groups of 20 animals each. 10 of the control animals received PBS while the others received 1.5ml/kg body weight of honey (HON) and 80mg/ kg body weight of L-Dopa (L-Dopa) for 21days. However, 10 of the parkinsonism group were pretreated with HON and L-Dopa before parkinsonism induction. Behavioral studies were conducted 2 days after the induction while the animals were sacrificed 7 days after the induction. after which some brain samples were prepared for biochemical analysis and the others were used for histological and immune histo chemical staining. The footprint behavioral test showed that the Honey + L-Dopa group had less abnormalities in gait when compared to the MPTP group. Immuno histochemical analysis showed significantly reduced number of Nissl substance, pyktotic and pale stained cells in the MPTP group, while the Honey + Ldopa before MPTP group had relatively increased number of Nissl substance and deeply stained cells when stained with Cresyl fast Violet (CFV) stain. Combination of L-DOPA and Honey helped in the reduction of MPTP-induced dopaminergic neuron loss as well as improvement of motor-related symptoms of MPTP induced Parkinsonism in adult male albino Swiss mice.

Keywords:- *Parkinson's disease; Dopaminergic neurons; MPTP; L-DOPA; Honey.*

I. INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide. PD is known to be a progressive, neurological disease involving motor (e.g., bradykinesia, tremor, rigidity, and postural impairment) and nonmotor (e.g., depression, anxiety, apathy, psychosis, and problems with impulse control)(1)(2)(3). The symptom profile and progression of the disease differ between individuals. The core pathology said to cause the main motor symptoms is a striatal dopamine deficit due to progressive loss of nigrostriatal dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the Midbrain(4)(5).

Various studies have repeatedly shown how persons with PD experience various disease aspects, such as loss of motor control, walking difficulties, environmental influences, cognitive and executive dysfunctions, emotional reactions, sleep disorders, difficulties in managing activities of daily living, neuropsychiatric reactions, social withdrawal and isolation, communication difficulties, and loss of physical and psychosocial competence.(9)(10)(11)(7)(8)

Nonmotor difficulties can be as, if not more, challenging as motor difficulties to people with PD and their caregivers and are a major contributor to patient perceptions of quality of life (1).

Parkinson's disease (PD) affects 1-2% of individuals above 60 years amounting to over 7 million people worldwide. Thus, PD has become an important contributor to the neurological disease burden (12). Worldwide, approximately 2% of people above the age of 60 and 4% above the age of 80 years are affected with Parkinson disease (PD) (13). Nigeria is the most populous country in Africa, and alarmingly, approximately 5.25 million Nigerians are above 65 years and are therefore at risk for developing PD with approximately 10-249 cases per 100,000 people per year, with the mean age of onset close to 60 years (12), an important caveat associated with these numbers is that they do not reflect undiagnosed cases which means the disease is more rampant than documented.

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1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a potent and selective nigrostriatal dopaminergic neurotoxicant that can induce parkinsonism when administered to rodents (14) showing evidence of oxidative stress in these models(15)(16)(17)

L-3,4-dihyroxyphenylalanine (L-DOPA), the naturally occurring isomer of aromatic amino acid 3,4-dihydroxyphenylalanine (DOPA), is a classic example of neurotransmitter replacement therapy in the brain. As a precursor of dopamine, L-DOPA is the mainstay of treatment in PD (18). Unlike dopamine, L-DOPA is transported into the brain by the large neutral amino acid transport system (19). L-DOPA is converted by neuronal aromatic L-amino acid decarboxylase into DA, hence restoring DA levels in surviving neurons, but not halting neuronal death (20)(18).

Nevertheless, it is well known that chronic use of L-DOPA, especially in patients in advanced stages of the disease, leads to the development of motor complications that are very resistant to therapy, which aggravates disability in PD patients due to L-DOPA autoxidation which gives rise to radical species(21)(22)(23).

Honey, a natural food product, is a sweet, viscous substance that is formed from the nectar of flowers by honeybees (*Apis mellifera*; Family: Apidae) (24). Honey has been utilized by humans since prehistoric times before civilization appeared approximately 5,500 years ago. Most ancient civilizations, such as the Egyptians, Greeks, Chinese, Mayans, Romans, and Babylonians, used honey both for nutritional purposes and for its medicinal properties (25); Taher *et al.*, 2022). Honey is the only insect-derived natural product, and it has therapeutic, religious, nutritional, cosmetic, industrial, and traditional value (24).

The traditional knowledge of honey and modern science are merged in the word ‘apitherapy’, which denotes medical use of honey bee products (27). Honey has been used as an antioxidant to protect various organs including the brain from oxidative damages(28)(29)(30)(31). Honey contains significant antioxidant activities as well as choline and acetylcholine which are essential for brain function and as neurotransmitters (26).

Due to the hypothesis that the reduction in L-DOPA response is due to oxidative injury, the inclusion of an antioxidant like Honey with L-DOPA may mitigate the decline in response to L-DOPA. This study was done to ascertain if the combination of Honey with L-Dopa will improve the longevity of L-DOPA treatment and cause neuroprotection of the dopaminergic neurons of the substantia nigra.

II. MATEIALS AND METHODS

A. *Animal care and Ethical Approval*

40 male albino Swiss mice ranging from 25-30g were used in the present study. The mice were purchased from TOSAB laboratories limited Ogbomosho, Oyo state, were housed in the animal house of the Central Research Lab, University of Ilorin and were placed randomly in six groups. The cages under hygienic controlled laboratory conditions (reversed light/dark cycle, temperature 25 ± 3°C and 55% relative humidity) and acclimatized for 14 days prior to start of the experiment.

Water and food pellets were provided *ad libitum.*

The experimental protocol was approved by the Department of Anatomy Departmental Research and Ethical Review Committee, University of Ilorin.

B. *Chemicals and Drugs*

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine HCl (Med Chem Express, 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ, USA) powder was dissolved in PBS (Sigma-Aldrich, St. Louis, MO, USA). L-DOPA (Sinemet, C-14, S.I.T.E, Karachi -75700, Pakistan) was dissolved in PBS, Honey (A & Shine Int’l LTD) was dissolved in PBS.

III. EXPERIMENTAL DESIGN

Male Swiss Mice were randomly divided into 4 groups

S/N	Groups	Number of Mice	Administered Drugs
1	Control	10	0.2ml Phosphate Buffer Saline (PBS) given for 21days
2	L-dopa + Honey only	10	80mg/kg of L-DOPA + 1.5ml/kg of Honey was given for 21days
3	MPTP only	10	After 21days of O.2ml of PBS, 20mg/kg of MPTP given intraperitoneally (I.P), 4 times in one day at 2hours interval
4	L-dopa + Honey before MPTP	10	80mg/kg of L-DOPA + 1.5ml/kg of Honey was given for 21days. On 22nd day, 20mg/kg of MPTP given intraperitoneally (I.P), 4 times in one day at 2hours interval

Table 1: Experimental Design

- Shows the grouping and treatment of experimental mice. At the end of all the groups administration (21 days), 6 mice were selected at random from each group and were tested for their locomotor activity and then, sacrificed using chloroform.

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IV. FUNCTIONAL ASSESSMENT

Each assessment was carried out 3 times at 48hours interval.

A. Footprint Analysis

This is used to analyze gait. Both fore and hind paws of each mouse was painted using poster color, each mouse was the placed on a white sheet and then allowed to walk. The sheets were studied and analyzed. The steps that are consistently spaced with clear non smudged footprints were used for scoring.

V. HISTOLOGICAL TECHNIQUES

A. Cresyl Violet (CFV)

The tissue sections were;

- Deparaffinized and hydrated to distilled water.
- Cresyl violet, two minutes.
- Wash in distilled water.
- Dehydrated, cleared in xylene, coverslip.
  - Cresyl violet Stock solution:
- 0.2 g cresyl violet acetate in 150 ml distilled water. Mix with a stir bar for at least 20 minutes.
- Buffer solution pH 3.5:
  - 282 ml of 0.1 M acetic acid (6 ml of concentrated acetic acid per 1000 ml distilled water) and add to
  - 18 ml of 0.1 M sodium acetate (13.6 g in 1000 ml of distilled water)

Take 30 ml of the cresyl violet stock solution and add 300 ml of buffer. Mix for at least 30 minutes.

VI. RESULTS

During this research work, behavioral, morphological, biochemical, and histological effects of MPTP, L-DOPA, Honey were observed.

A. General Observation

All the experimental mice were active, agile and apparently healthy prior to administration, this was also observed all through the period of administration in the control group which was given 0.2ml of phosphate buffered saline (PBS) for 21 days. The MPTP treated group given 20mg/kg/2h MPTP after 21 days of 0.2ml of PBS were weak, had posture disturbance, became rigid and repeated tremor occured. The Honey +L-Dopa group treated with 1.5ml/kg of honey and 80mg/kg L-Dopa for 21 days were agile and active similar to the control group. The Honey + L-Dopa group given 20mg/kg/2h of MPTP on the 22nd day after 1.5ml/kg of honey and 80mg/kg L-Dopa for 21 days showed improved behavior after MPTP administration with progressively agile movements & less tremor until normal movements observed.

B. Incidental Findings

Mortality was experienced in the MPTP group, but it was less than the expected 50% mortality rate.

C. Quantitative Analysis

a) Footprint Test Analysis

To characterize more quantitatively differences in gait, footprint test was used to compare difference in gait between groups. Stride length of MPTP group higher than other groups due to gait abnormalities of the Parkinson-like effect of MPTP, and it is slightly reduced in Honey + L-Dopa before MPTP group due to the neuroprotective effect of Honey + L-Dopa as seen in Figure 1 and Figure 2.

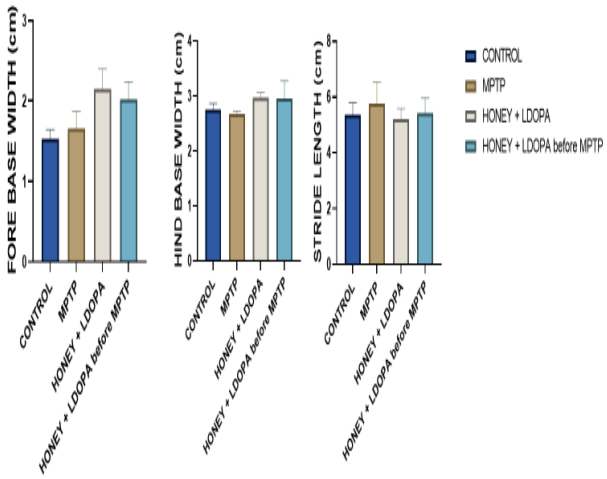

Fig. 1: Showing the Values, Mean and Standard Error of Mean (SEM) of Fore base width, hind base width and stride length of footprint test showing the abnormalities in gait with comparison among groups

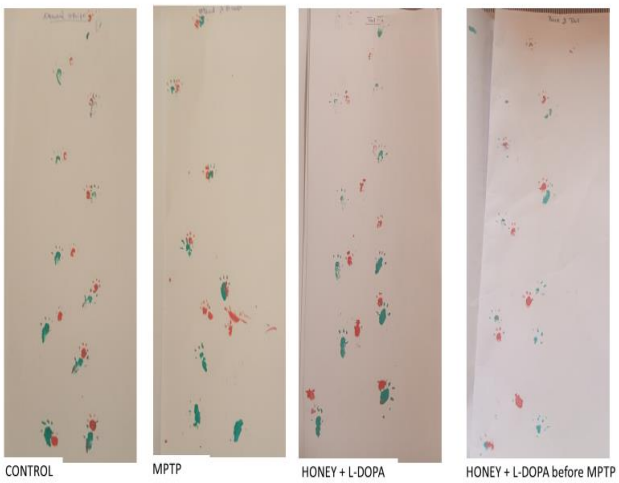

Fig 2: showing footprint test of treatment groups

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D. Qualitative Analysis

Representative photomicrograph of the histological sections of the Midbrain of the experimental animals are shown below.

a) Histoarchitecture of Nissl Substance in the Midbrain of the experimental animals

The Midbrain of the experimental animals given 0.2 ml of phosphate buffered saline (PBS) for 21 days (Control group) depict abundant and more deeply stained Nissl Substance as observed from the photomicrograph of CFV stain (Figure 3). The Midbrain of the group given 20mg/kg/2h MPTP after 21 days of 0.2ml of PBS (MPTP group) was seen to have considerably reduced Nissl substance with pyknotic cells and pale stained Nissl substance due to brain insult caused by MPTP which will lead to oxidative stress in comparison to the control group (Figure 3) The Midbrain of the group given 1.5ml/kg of honey and 80mg/kg L-Dopa for 21 days (Honey + L-Dopa group) was seen to have deeply stained and plentiful Nissl Substance similar to that of the control group (Figure 3). The Midbrain of the group given 1.5ml/kg of honey and 80mg/kg L-Dopa for 21 days and on the 22nd day 20mg/kg/2h of MPTP (Honey + L-Dopa before MPTP group) more Nissl Substance and fewer pale stained cells compared to the MPTP (Figure 3).

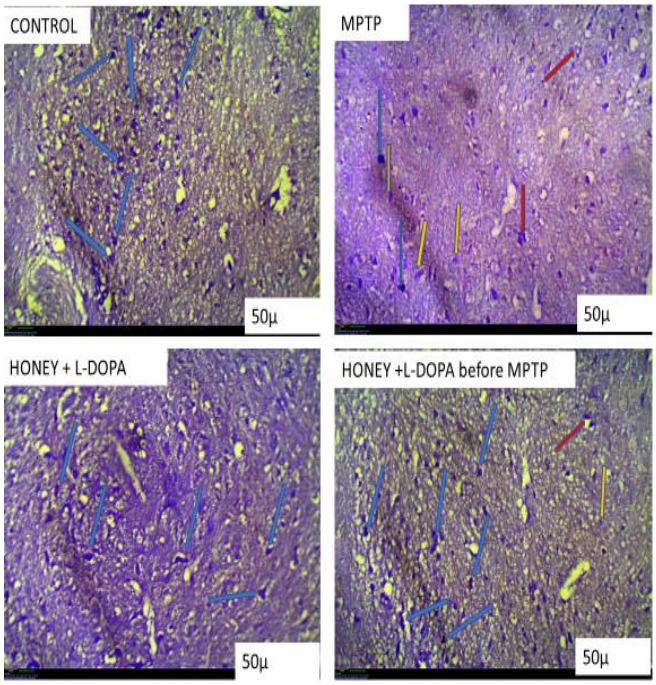

Fig. 3: Representative Photomicrograph of midbrain of adult albino Swiss mice stained with cresyl fast violet for the Nissl substance

Red arrow = pyknotic cells, **yellow arrow** = Pale/ghost like cells, **blue arrow =** Deeply stained cells (Figure 3). The neurons of the control group are deeply stained, and abundant Nissl substance was present in the midbrain**.** Most neurons in the MPTP group are palely stained and pyknotic indicating that the Nissl substance of the neurons are lost. The neurons of the Honey + L-Dopa group are deeply stained, and abundant Nissl substance was present in the midbrain similar to the control group. Most of the neurons in the Honey + L-Dopa before MPTP group are deeply stained, with little pale cells compared to the MPTP group indicating neuroprotection by the Honey and L-Dopa.

VII. DISCUSSION

Our study has reported the neuro protective effects of the combination of Honey with L-Dopa by effectively determining the motor related effects and histo architecture of structures in the midbrain in MPTP induced Parkinsonism in adult albino mice. Oxidative stress and inflammation in neurons have been reported to play critical role in PD pathogenesis (6). Considerable experimental evidence suggests that a significant contributor to dopaminergic neuronal loss in the PD brain is ROS, which result from dopamine metabolism, low glutathione (GSH), and high levels of iron and calcium in the SNpc(7)(8).

When researchers discovered the L-Dopa could treat immobile people who had Parkinson's disease, it was a significant breakthrough, patients began moving and talking just like their old selves. But as every patient knows, this turned out to be short-lived. After a couple of years, instead of helping patients regain normal function, L-Dopa triggers gross involuntary movements. And once the condition sets in, there is no way forward unless L-Dopa is stopped, this is due to the autoxidation of L-DOPA giving rise to radical species (32)(23)(18).

Honey, a natural food product, is a sweet, viscous substance that is formed from the nectar of flowers by honeybees (*Apis mellifera*; Family: Apidae) (24). Honey possesses non-enzymatic antioxidant activity such as scavenging free radicals, and enzymatic antioxidant activity such as increasing protein level of antioxidant enzymes. Studies also observed that these compounds could alleviate oxidative stress via reducing the formation of MDA and ROS; and enhance antioxidant defense via increasing GSH retention and restoring the activity of three antioxidant enzymes as well as up-regulating cardiac mRNA expression of these antioxidant enzymes (24)(27)(33)(26).

This research was done to study the effect of the combination of Honey & L-DOPA on MPTP induced Parkinsonism in adult albino mice.

A. Influence of Honey + L-Dopa on the motor related/Parkinsonian effect of MPTP

The control group given 0.2ml of PBS and the group given honey +L-dopa for 21 days appeared normal and active all through the experiment, while the groups given MPTP four times with two hours' interval for a day were generally weak and tremor was observed after MPTP administration. However, the groups given honey +L-dopa before MPTP are more active and littler to no tremor was observed compared to MPTP group depicting that honey +L-dopa has neuroprotective effect that help to reduce the motor related complication related to Parkinsonism. Parkinsonism was induced by administering 20mg/kg of MPTP for a day, four times with two hours' interval between each dose. The four cardinal motor symptoms in Parkinson's disease are; tremor, bradykinesia, rigidity and

postural instability (4)(34)(35). The motor-related behaviors were assessed using the footprint test used to observe the differences in gait of the treatment groups. The comparison between the groups were not statistically significant ($p>0.05$).

a) Footprint test:

The Parkinsonian effect observed here was the differences in gait of the treatment groups using the footprint test. Stride length of MPTP group are higher than other groups due to gait abnormalities of the Parkinson-like effect of MPTP(36)(37), and it is slightly reduced in Honey + L-Dopa before MPTP group, this is attributed to the neuroprotective effect of Honey + L-Dopa which would then mitigate postural/ gait abnormalities (36)(24)(33)as opposed to having L-Dopa alone (Figure 1&2)

B. Assessment of Histoarchitecture of Nissl Substance in the Midbrain of the experimental animals

MPTP mouse studies have shown nigrostriatal damage associated with gliosis similar to Parkinson's disease(37). MPTP group given 20mg/kg/2h have pale stained, pyknotic cells and reduced Nissl substance due to neurodegeneration that occurred in the mice midbrain. comparing the Honey (1.5ml/kg) + L-Dopa (80mg/kg) group to the MPTP group to study if Honey + L-Dopa had a neuroprotective effect by improving Nissl substance, the group had less pale stained cells and plentiful Nissl substance showing its neuroprotective effect(38)(33). All the data was compared to the control using the control group as the standard (Figure 3).

VIII. CONCLUSION

It was demonstrated from this study that Honey when combined with L-Dopa protected the Nissl Substance, and improved the gait abnormality after inducing MPTP induced Parkinsonism in Swiss Albino adult Mice.

IX. RECOMMENDATION

- Honey should be incorporated in diets of PD patients and people prone to PD (elderly)
- A longitudinal study should be done to examine if the long-term combination of Honey with L-Dopa will alleviate the dyskinesia effect of L-Dopa after long term use

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