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Research Article

NEUROPROTECTIVE EFFECT OF POLYHERBAL FORMULATION IN PARKINSON'S ANIMAL MODEL

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ABSTRACT

Objective: The study is aimed to evaluate the antiparkinsonian effect of polyherbal formulation containing methanolic extract of *Prunus amygdalus*, *Arachis hypogaea,* and *Citrullus lanatus* (MEPAC) in chlorpromazine (CPZ)-induced Parkinson's disease in Wistar albino rats.

Methods: The antiparkinsonian activity of polyherbal formulation was studied in CPZ (3 mg/kg i.p.) induced Parkinson rat model. Rats were subjected to treatment with MEPAC and standard drug for a period of 21 days. The behavioral assessments, i.e., catalepsy and locomotor activity were assessed during the treatment period. Then animals were sacrificed, brains were isolated and homogenized for the estimation of biochemical parameters such as dopamine (DA), lipid peroxidation (LPO), glutathione (GSH), and superoxide dismutase (SOD). Histopathology of the brains was also done.

Results: The cataleptic score of MEPAC (200 mg/kg and 400 mg/kg) treated rats was significantly reduced. On the other hand, there was improved in the locomotor activity. MEPAC (200 mg/kg and 400 mg/kg) treated rats showed increase in the level of DA, reduced GSH, SOD, and decreased LPO significantly.

Conclusion: It may be concluded that methanolic extract of polyherbal formulation consisting of *P. amygdalus*, *A. hypogaea,* and *C. lanatus* showed a good antioxidant and neuroprotective effect in CPZ-induced Parkinson rats.

Keywords: Polyherbal formulation, *Prunus amygdalus*, *Arachis hypogaea*, *Citrullus lanatus*, Parkinson's disease, Dopamine, Antioxidants.

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INTRODUCTION

Neurodegenerative diseases (NDDs) are defined as debilitating disorders that cause progressive neuronal loss in the brain which results in impaired mental functioning and difficulty in movement. Alzheimer's disease and Parkinson's disease (PD) constitute the common neurological disorders with loss or death of neurons of the brain [1]. PD being a common neurological disorder affects at any time 1–2/1000 of the population [2]. The incidence and prevalence of PD increases along with increasing age and affects 1% of people over the age of 65 years [3]. It is a movement disorder of the central nervous system which occurs due to the loss of dopamine (DA) producing cells in the brain. This loss transpires in substantia nigra pars compacta of midbrain which depletes the DA in the striatum. The other remaining cells show intracytoplasmic "Lewy bodies" [4-6]. Oxidative stress and free radical formation are presumed to play a major part in the progression of PD [7]. The four main symptoms of PD are tremors at rest, bradykinesia, stiffness or rigidity, and postural instability [8]. The present treatments for NDDs are limited to providing symptomatic relief to disease but do not alleviate damage to the neurons [9].

Since there has been an increase in evidence suggests that oxidative stress may be one of the underlying mechanisms involved in the development of PD, the plants possessing antioxidant properties may be selected to study their neuroprotective effects and potentially their applicability in PD. In the present study, seeds of *Prunus amygdalus* (Common name: Almond), *Arachis hypogaea* (Common name: Peanut), and *Citrullus lanatus* (Common name: Watermelon) were used. *P. amygdalus* (Family: Rosaceae) and *A. hypogaea* (Family: Fabaceae) have been reported to possess antioxidant activity [10,11]. *C. lanatus* (Family: Cucurbitaceae) has shown an ameliorative effect with caffeine to increase DA levels in rats and also shown to possess antioxidant activity [12,13].

METHODS

Collection, extraction, and phytochemical screening of plant materials

The seeds of *P. amygdalus*, *A. hypogaea,* and *C. lanatus* were collected and authenticated by Dr. Shaik Mohammed Aliuddin, Secretary, Hyderabad Unani Research Foundation, Hyderabad, Telangana. The procured samples were ground and the resultant coarse powder was taken for extraction with methanol through Soxhlet apparatus. Methanolic extract of *Prunus amygdalus*, *Arachis hypogaea,* and *Citrullus lanatus* (MEPAC) was subjected to preliminary phytochemical screening for the identification of the type of phytoconstituents present [14].

Experimental animals

Wistar albino rats of either sex were used in the present study. The rats weighing about 100–150 g were procured from Sainath Animal Agency, Musheerabad, Hyderabad, India. They were maintained under standard well-controlled conditions throughout the experimental duration. The temperature was maintained at 22°C (±3°C) and relative humidity, 50–60%. They were fed pellet diet and water *ad libitum*. The experimental protocol was given approval by the Institutional Animal Ethical Committee No: IAEC/SUCP/2019/09.

Experimental design

Thirty experimental rats of either sex were randomly divided into five groups each consisting of six and treated as follows:

1. Normal control group: This group was given 1% w/v gum acacia vehicle p.o. and normal pellet diet.
2. Disease control group: This group was treated with chlorpromazine (CPZ) (3 mg/kg) dissolved in 1% w/v gum acacia i.p.
3. Standard treatment group: This group was treated with standard tab. Syndopa (10 mg/kg) in 1% w/v gum acacia i.p. + CPZ (3 mg/kg) dissolved in 1% w/v gum acacia i.p.
4. MEPAC (200 mg/kg): This group was treated with MEPAC 200 mg/kg p.o. + CPZ (3 mg/kg) dissolved in 1% w/v gum acacia i.p.

5. MEPAC (400 mg/kg): This group was treated with MEPAC 400 mg/kg p.o. + CPZ (3 mg/kg) in 1% gum acacia i.p.

Thirty minutes before the administration of the standard and test drugs, CPZ (3 mg/kg, i.p.) suspended in 1% w/v gum acacia was administered and this treatment was carried out for 21 days [15].

Behavioral assessments

Catalepsy by block method

The effect of standard drugs and MEPAC on cataleptic behavior of CPZ-treated rats was done using a wooden block method. The following steps were involved in this method: First step: The rats were placed on the table and pushed or gently touched on back. On the failure of the rat to move, a cataleptic score of 0.5 was provided. Second step: Alternatively, the rat's front paws were placed on a wooden block of 3 cm height. Then, a score of 0.5 for each paw was provided and added it to the 1st step's score if rats failed to correct their posture in 15 s. Third step: Alternatively, the rat's front paws were placed on wooden block of 9 cm height. Then, a cataleptic score of 1 for each paw was provided and added it to the 1st and 2nd step's score if rats failed to correct their posture in 15 s. The cutoff score of any animal was 3.5 that indicated total catalepsy [16].

Locomotor activity by actophotometer

An actophotometer contains an infrared beam. This beam is digitally displayed after being recorded. It works on counter connected photoelectric cells. When the rat cuts off an infrared beam falling on photocell, a count is recorded. This was given an estimate of total locomotor activity of an animal. The assessment of locomotor activity of CPZ-treated rats was done with actophotometer. The locomotor activity was recorded as a total counts/5 min per rat [17].

Dissection and homogenization of rat brains

After 21 days of treatment, the experimental rats were sacrificed by the overdose of anesthetic ether. The brains were isolated and homogenized with 10% v/w phosphate buffer (0.1 M, pH 8). This brain extract was subjected to the following parameters estimation.

Determination of antioxidant parameters

Lipid peroxidation (LPO)

Up to 5 ml solution was made using 1 ml of the tissue homogenate, 0.2 ml of solution of sodium lauryl sulfate, and 1.5 ml each of thiobarbituric acid and acetic acid (20%). This was incubated for couple of min and then heated for 30 min in a water bath. n-Butanol-pyridine mixture was utilized to extract the chromogen and centrifuged for 10 min at 4000 rpm. At 532 nm, the absorbance of the organic layer was assessed. Concentration of the malondialdehyde (MDA) is expressed as nano mol/mg of protein [18].

Reduced glutathione (GSH)

To the tissue homogenate, 20% trichloroacetic acid in equal volume containing 1 mM ethylenediaminetetraacetic acid was added and made to stand for 5 min. This was centrifuged at 2000 rpm for 10 min and 200 µl of supernatant was taken. A 1.8 ml of Ellman's reagent (0.1 mM) was made with 0.3 M phosphate buffer and 1% sodium citrate solution up to a volume of 2 ml. The solution was assessed at 412 nm against blank after the end of the reaction [19].

Superoxide dismutase (SOD)

The supernatant (0.1 ml) was added to carbonate bicarbonate buffer (pH 9.7). To this, epinephrine (1 ml) was added and absorbance was measured at 480 nm for 2 min [20].

Estimation of DA

The rat brain tissue was homogenized in HCl-butanol (1:10 for 1 min). The homogenate was subjected to centrifugation at 3000 rpm for 10 min. To the centrifuge tube containing hexane (2.5 ml) and 0.1 M HCl (0.3 ml), 1 ml of aliquot supernatant was added. The assay of DA was carried out using the aqueous phase (0.2 ml) at 0°C. To the aqueous phase (0.2 ml), 0.4 M HCl (0.05 ml) and sodium acetate buffer, pH 6.9, were added which was followed by the addition of 0.1 M in ethanol-iodine solution (0.1 ml) for oxidation. Sodium sulfite solution (0.1 ml) was added after 2 min for stopping the reaction followed by the addition of acetic acid (0.1 ml) after 90 s. The solution was heated till 100°C for 6 min. The excitations and emission spectra were read at 330–375 nm from the spectrofluorimeter. With the addition of oxidation step's reagents in reverse order, (iodine after sodium sulfite), the tissue blanks were prepared [21].

Histopathological studies

The brains of the experimental rats were dissected and kept in 10% formalin soln. and taken for carrying the histopathological studies.

Statistical significance

The statistical analysis was performed using GraphPad Prism 8.2.0 software. $p<0.05$ was considered to be statistically significant. The results were given as graphs. The comparisons between different groups were carried out using one-way analysis of variance to determine the significance.

RESULTS

Phytochemical screening

The preliminary phytochemical analysis of MEPAC extract confirmed the presence of flavonoids, phenols, amino acids, steroids, and alkaloids.

Effect of MEPAC on CPZ-induced catalepsy in rats screened by wooden block

The cataleptic score of the experimental rats was investigated on days 1, 7, 14, and 21 of the experimental duration. The normal control group did not show any signs of catalepsy. Administration of CPZ (3 mg/kg, ip) induced catalepsy significantly and the cataleptic score was significantly higher in disease control group when compared to the control group. On treatment with MEPAC (200 mg/kg and 400 mg/kg), the cataleptic score was significantly reduced when compared with disease control group which was shown in Fig. 1.

Effect of MEPAC on locomotor activity in rats using actophotometer

The locomotor activity of the experimental rats was investigated on days 1, 7, 14, and 21 of the experimental duration employing an actophotometer. The disease control CPZ group revealed a significant lowering in locomotor activity when collating with normal control group throughout the study. On treatment with MEPAC (200 mg/kg

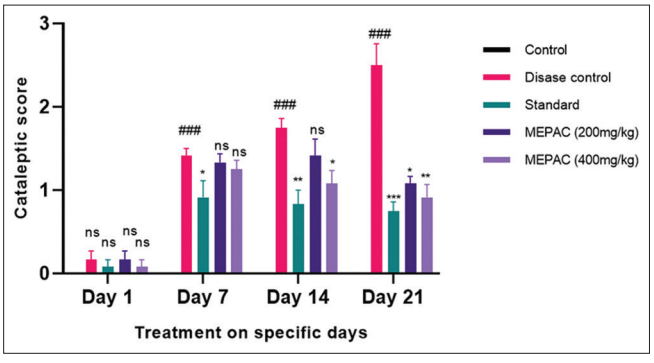

Fig. 1: Effect of methanolic extract of *Prunus amygdalus*, *Arachis hypogaea*, and *Citrullus lanatus* on catalepsy of different experimental groups. All values were expressed as mean±standard error of the mean (n=6). One-way analysis of variance was carried out followed by Dunnett's multiple comparison test. ***p<0.001, **p<0.01, and *p<0.05 when compared with CPZ-treated group. ###p<0.001 when compared with vehicle control group. ns: Not significant

and 400 mg/kg), the locomotor activity was increased significantly on collating with disease control CPZ group and MEPAC 400 mg/kg revealed an increase in locomotor activity nearing the standard Syndopa group which was shown in Fig. 2.

Effect of MEPAC on the levels of DA in rat brain

CPZ (3 mg/kg, ip) decreased significantly, the levels of brain DA in disease control CPZ group when compared with normal control group. MEPAC (200 mg/kg and 400 mg/kg) treated groups showed a significant increase in the levels of DA when compared with disease control group. MEPAC (400 mg/kg) revealed DA levels nearing the standard Syndopa group which was shown in Fig. 3a.

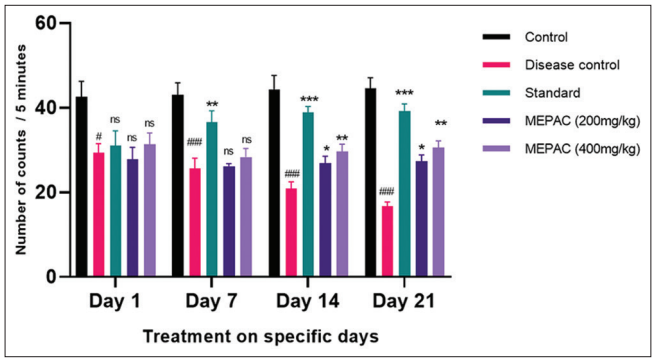

Fig. 2: Effect of methanolic extract of *Prunus amygdalus*, *Arachis hypogaea,* and *Citrullus lanatus* on locomotor activity of different experimental groups. All values are expressed as mean±standard error of the mean (n=6). One-way analysis of variance was carried out followed by Dunnett's multiple comparison test. ***p<0.001, **p<0.01, and *p<0.05 when compared with CPZ-treated group. ###p<0.001 when compared with vehicle control group. ns: Not significant

Effect of MEPAC on the levels of LPO in rat brain

CPZ (3 mg/kg, ip) increased significantly, the levels of MDA in disease control CPZ group when compared with normal control group. On treatment with MEPAC (200 mg/kg and 400 mg/kg), the MDA levels were significantly lowered when compared with disease control CPZ group. MEPAC (400 mg) revealed MDA levels nearing the standard Syndopa group, as shown in Fig. 3b.

Effect of MEPAC on the levels of GSH in rat brain

CPZ (3 mg/kg, ip) decreased significantly, the levels of GSH in disease control CPZ group on comparison with control group. On treatment with MEPAC (200 mg/kg), the change in levels of GSH was not significant. However, MEPAC (400 mg/kg) significantly increased the GSH levels when compared with disease control CPZ group. MEPAC (400 mg) revealed GSH levels nearing the standard Syndopa group, as shown in Fig. 3c.

Effect of MEPAC on the levels of SOD in rat brain

CPZ (3 mg/kg, ip) decreased significantly, the levels of SOD in disease control CPZ group on comparison with normal control group. On treatment with MEPAC (200 mg/kg and 400 mg/kg), the SOD levels were significantly increased when compared with disease control CPZ group. MEPAC (400 mg) revealed SOD levels similar to the standard Syndopa group, as shown in Fig. 3d.

Effect of MEPAC on the cerebral cortex and hippocampus of rat brain – histopathological study

Effect of MEPAC was studied on the cerebral cortex and the hippocampus of the rat brain. Lymphocyte infiltration and multifocal inflammation in the cerebral cortex were detected in CPZ control group while MEPAC (200 mg/kg) and MEPAC (400 mg/kg) did not indicate any signs of apoptosis in the cerebral cortex in compliance with the standard group, as shown in Fig. 4. The hippocampus revealed apoptotic neurons in the rats belonging to the CPZ control group, whereas treatment with MEPAC (200 mg/kg) and MEPAC (400 mg/kg) showed results similar to standard group, the

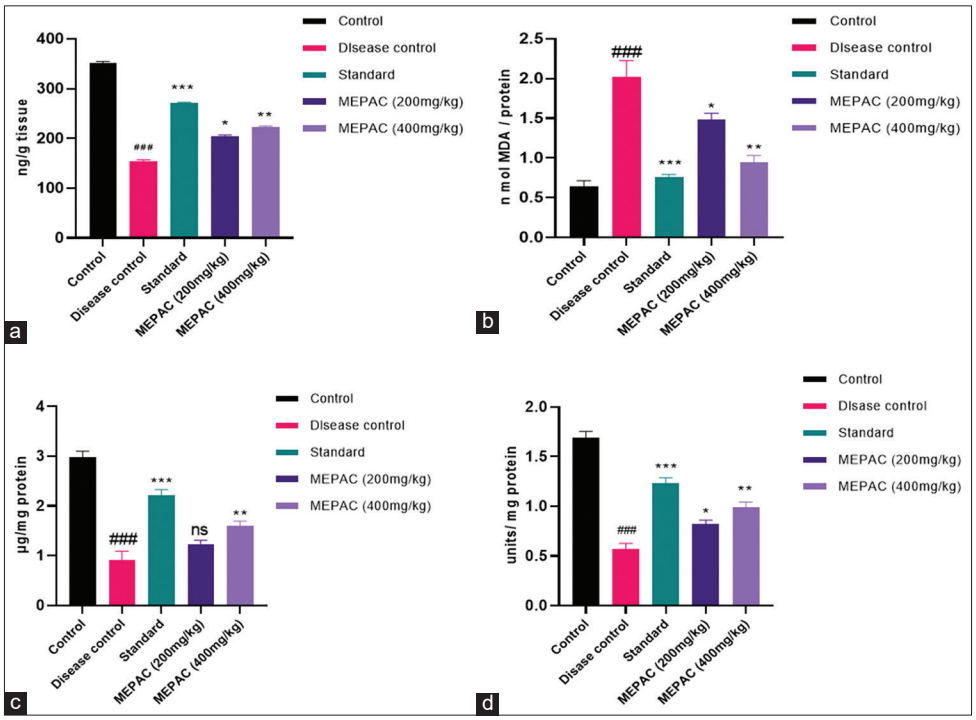

Fig. 3: Effect of methanolic extract of *Prunus amygdalus*, *Arachis hypogaea,* and *Citrullus lanatus* on dopamine (a), lipid peroxidation (b), glutathione (c), and superoxide dismutase (d) levels of different experimental groups. All values are expressed as mean±standard error of the mean (n=6). One-way analysis of variance was carried out followed by Dunnett's multiple comparison test. ***p<0.001, **p<0.01, and *p<0.05 when compared with CPZ-treated group. ###p<0.001 when compared with vehicle control group. ns: Not significant

hippocampus appeared normal and also showed mild proliferation in the region of hippocampus, as shown in Fig. 5.

DISCUSSION

Typical antipsychotics cause free radical-mediated damage in rats and oxidative stress by varying the levels of antioxidant enzymes [22]. CPZ when administered chronically, caused a decrease in the extracellular levels of DA, increased the hyperkinetic movements, and developed tardive dyskinesia [23].

In the present study, CPZ (3 mg/kg body weight, i.p.) administered for 21 days was found to induce catalepsy to a greater extent in rats since the animals belonging to CPZ-treated disease control group had a higher cataleptic score when measured on days 1, 7, 14, and 21. Treatment with MEPAC (200 mg/kg) and MEPAC (400 mg/kg) gave a reduced cataleptic score on the 21st day. These findings were similar to the previous study [24]. CPZ-treated disease control group showed a decrease in the locomotor activity. On treatment with MEPAC (200 mg/kg) and MEPAC (400 mg/kg), the locomotor activity was found to be reversed which was measured on days 1, 7, 14, and 21 on collation with disease control CPZ group. These results were in accordance with the previous reported study [25].

On the 21st day after the behavioral assessments, rats were sacrificed and brains were isolated and homogenized, rat brain homogenates were prepared. Neuronal damaged brain contains reduced levels of DA andthere is a direct relationship between motor dysfunction and loss of DA [26,27]. The rats belonging to MEPAC (200 mg/kg) and MEPAC (400 mg/kg) groups showed a protective effect toward increase in the levels of DA in rats. This was found to be similar with the previous scientific report [28].

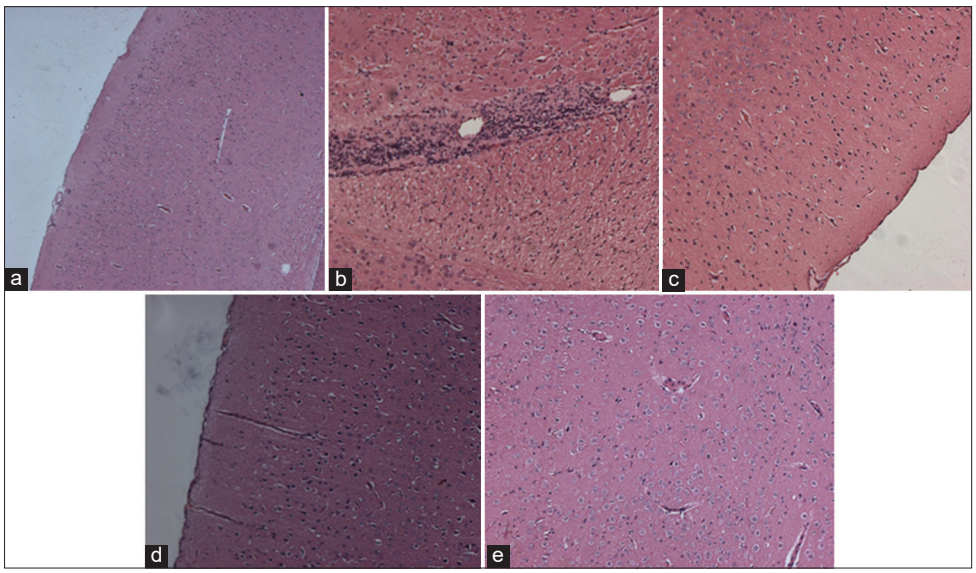

Fig. 4: Cerebral cortex of rat brain. (a) Normal control; (b) chlorpromazine (CPZ) 3 mg/kg; (c) CPZ 3 mg/kg + Syndopa 10 mg/kg; (d) CPZ 3 mg/kg + methanolic extract of *Prunus amygdalus*, *Arachis hypogaea,* and *Citrullus lanatus* (MEPAC) 200 mg/kg; (e) CPZ 3 mg/kg + MEPAC 400 mg/kg

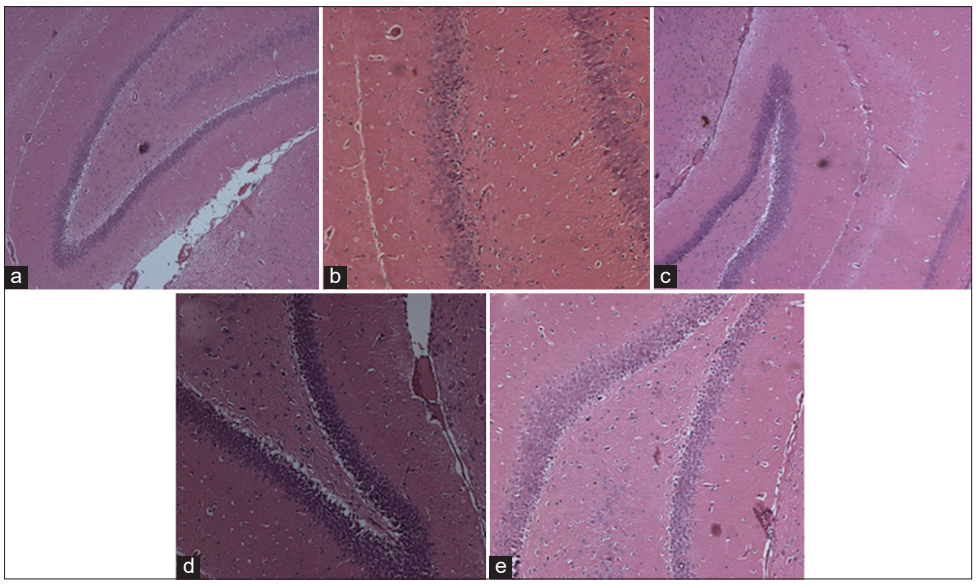

Fig. 5: Hippocampus of rat brain. (a) Normal control; (b) chlorpromazine (CPZ) 3 mg/kg; (c) CPZ 3 mg/kg + Syndopa 10 mg/kg; (d) CPZ 3 mg/kg + methanolic extract of *Prunus amygdalus*, *Arachis hypogaea,* and *Citrullus lanatus* (MEPAC) 200 mg/kg; (e) CPZ 3 mg/kg + MEPAC 400 mg/kg

Oxidative stress may be one of the underlying mechanisms of PD. Hence, the estimation of antioxidant markers was carried after the treatment period. On treatment with MEPAC (200 mg/kg) and MEPAC (400 mg/kg) groups, the MDA levels were found to be reduced which falls in line with the previously conducted study [29]. On treatment with MEPAC (200 mg/kg) and MEPAC (400 mg/kg), the levels of GSH and SOD were found to be increased. These results were in conformity with previously conducted studies [30,31]. The histopathological reports show that treatment with MEPAC (200 mg/kg) and MEPAC (400 mg/kg) showed results similar to standard group where the cerebral cortex and hippocampus appeared normal and showed mild proliferation in the region of hippocampus.

These results indicate the potential neuroprotective effect of the polyherbal formulation consisting of *P. amygdalus*, *A. hypogaea,* and *C. lanatus*.

CONCLUSION

The methanolic extract of polyherbal formulation containing *P. amygdalus*, *A. hypogaea,* and *C. lanatus* showed a significant antioxidant and neuroprotective activity. However, further extensive studies on the isolation of phytochemical constituents of *P. amygdalus*, *A. hypogaea,* and *C. lanatus* are needed to explore the detailed mechanism for the prevention and treatment of PD.

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AUTHORS' CONTRIBUTIONS

Both the authors have equally contributed in making this article successful.

CONFLICTS OF INTEREST

There are no conflicts of interest among the authors.

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