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PROTECTIVE EFFECTS OF PITHECELLOBIUM DULCE SEED EXTRACT ON SODIUM FLUORIDE-INDUCED NEUROTOXICITY IN RATS

Babanna Yelugudari[1], Bhaskar Nagilla[2], Pratap Reddy Karnati[1]*

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ABSTRACT
Background: According to epidemiological research, fluoride is a developmental neurotoxicant that lowers children's IQ scores. Fluoride induces oxidative stress in the brain, which can cause histological damage. This study aims to test the antioxidant potential of the methanolic extract of *Pithecellobium dulce* seeds. **Methodology:** By using different *in vivo* assays such as inhibition of DPPH activity in plasma, Glutathione Peroxidase (GSH-Px) activity in the brain, and histology of the cerebral cortex on days 1, 10, 20, and 30 with both Hematoxylin &Eosin and Cresyl violet stains. **Results and Discussion:** The methanolic extract of P *dulce* seeds significantly enhances GPx activity in a dose-dependent manner, effectively countering oxidative stress and providing neuroprotective benefits against fluoride-induced neurodegeneration. Results also showed the strong antioxidative potential of PDME, particularly in mitigating NaF-induced oxidative stress, as evidenced by sustained high DPPH radical-scavenging activity in the treated groups over time. An H&E histology study highlights the neuroprotective effects of PDME against NaF-induced neurodegeneration, demonstrating that PDME treatment significantly reduces neuronal loss and preserves cellular integrity, particularly at higher doses. Cresyl violet stain demonstrates that PDME has a neuroprotective effect against NaF-induced neurotoxicity, as evidenced by the preservation of neuronal morphology and Nissl substance. The study demonstrates that the methanolic extract of *Pithecellobium dulce* seeds exhibits significant antioxidant activity, effectively counteracting fluoride-induced neurotoxicity and preserving neuronal integrity by enhancing glutathione peroxidase activity and improving histological outcomes. **Conclusion**: These findings suggest that *P. dulce* may serve as a potential neuroprotective agent against fluoride-related cognitive impairments.

[1]*Neurobiology Lab, Department of Zoology, University College of Science, Osmania University, Hyderabad-500009. Telangana, India*
[2]*Hisopathology Lab, Dept. of Biochemistry, University College of Science, Osmania University, Hyderabad-500009. Telangana, India*

For Correspondence:** **pratapkreddyou@gmail.com

INTRODUCTION

Fluoride in drinking water highlights its long-established role in reducing dental caries, while also raising concerns about potential adverse health effects, particularly during pregnancy. This duality underscores the need for continued research and public discourse on the safety and efficacy of fluoride use in public health initiatives. Dental fluorosis or debilitating skeletal fluorosis, which is linked to osteosclerosis, calcification of tendons and ligaments, and bone abnormalities, can result from fluoride exposure. Fluoride can generate free radicals within brain cells by disrupting the balance between the body's capacity to combat ROS and the amount of ROS produced, resulting in oxidative stress. Oxidative stress, in turn, can cause histological damage by impairing the structural integrity of brain tissue through processes such as lipid peroxidation, protein oxidation, and DNA damage. Oxidative stress can ultimately result in histological damage, mostly impacting neurons.

Medicinal plants are crucial sources for developing new drug leads [1], particularly in antioxidant treatments, as they offer potential alternatives to conventional therapies that often carry significant adverse effects. The growing interest in plant secondary metabolites underscores their importance not only in human health but also across various fields, highlighting the need for continued exploration of their pharmacological properties [2-4].

The growing interest in the metabolic profiles of therapeutic plants, particularly *Pithecellobium dulce*, highlights its nutritional and medicinal significance, especially in treating various ailments and its potential anti-inflammatory properties. *Pithecellobium dulce* (Family: Mimosaceae) is one of them, commonly known as "Jungle Jalebi" in Hindi, and is an evergreen, spiny tree widely distributed throughout India and also found in South Africa, Australia, and Asia. Fruits of *Pithecellobium dulce* (*P. dulce*) have been used as a food additive for their excellent nutritional and medicinal importance. In traditional medicine, it is used to treat diabetes, peptic ulcer, toothache, leprosy, and earache, and is also used as an astringent, emollient, and abortifacient [5, 6]. Research has recently focused on the chemical composition of various plant parts, including arils, leaves, and fruits, and on screening them for antioxidant activity, thereby identifying potential phytotherapeutic candidates. The strong antioxidant activity of some of these compounds suggests they may help ward off the onset of chronic illnesses [7, 8], even though the anti-inflammatory properties of various parts of *P. dulce* have been studied [9-11]. This study aims further to characterize the methanolic seed extract of P. dulce and elucidate the molecular basis of its antioxidant effects.

MATERIALS AND METHODS

Plant material

The seeds of *Pithecellobium dulce* (Roxb.) Benth. were collected from Osmania University, Hyderabad, India, in August 2017. The plant material was authenticated under voucher no. 119 and deposited at the Dept of Botany, Osmania University, Hyderabad. The seeds were carefully examined after separation from the fruits, and old, deformed, and damaged seeds were removed. Healthy seeds were spread out and dried at 25°C for about 7 days, ground into a coarse powder using an electric blender, and further used for vehicle extraction.

Preparation of extract

The seed extract was prepared as described by Sara et al. (2021) [12], with the aforementioned improvements. The air-dried, pulverized seed content of P. dulce was sieved to a mesh size of 40. The powdered material (243 g) was extracted with methanol (4 × 500 mL) using a Soxhlet extractor for 24 h. The methanolic isolate was concentrated under reduced pressure at 50 °C (Heidolph Hei-VAP rotary evaporator, Germany) and lyophilized to afford the crude methanolic extract (27.3g; yield: 11.23% w/w). The isolate was stored under cold conditions for further analysis.

Animals and Treatments

Twenty-four male adult Wistar rats weighing between 180 and 200 g were employed in the experimental studies. Standard housing parameters (Room temperature 24-27 °C and 40 to 60% humidity with 12-h light and dark cycles) were provided for rats, which were obtained from the National Institute of Nutrition (NIN), Hyderabad, India, and kept at the animal house at the Department of Zoology, Osmania University, Hyderabad. Laboratory pellets (20% protein, 5% fat, 1% multivitamins) were provided to the animals, along with free access to water. They were housed in polythene cages and allowed to acclimatize for one week. After that, the rats were examined for health status, and all healthy animals were divided into six groups of four each for the study (n = 4). Throughout the experimental period, the rats were fed a standard rat pellet diet and given tap water *ad libitum*. The Department of Zoology (Approval No: 383/01/a/CPCSEA) supervised all animal treatments to ensure they were carried out in compliance with the highest ethical

standards. Doses were administered daily at consistent intervals. During the experiments, the animals were maintained in accordance with the rules stipulated by the Institutional Animal Ethics Committee (IAEC) of Osmania University in Hyderabad. All these rats were sacrificed, and their brains were dissected for assessment of oxidative stress markers and histopathological studies. The study periods of 1, 10, 20, and 30 days were used.

STUDY GROUPS WERE CLASSIFIED AS FOLLOWS

I. Group 1 (Control group): Rats received normal tap water throughout the study.

II. Group 2 (Disease model group or DM group): Rats were administered Sodium fluoride (NaF) at a dose of 20 mg/kg body wt., which was given through IP throughout the study.

III. Group 3 (NaF+PDME-250): NaF (Dose of 20 mg/kg body wt., through IP) + *Pithecellobium dulce* methanolic extract (PDME 250 mg/kg body wt., through intra-gastrically with plastic gavage) throughout the study.

IV. Group 4 (NaF+PDME-500): NaF (Dose of 20 mg/kg body wt., through IP) + *Pithecellobium dulce* methanolic extract (PDME 500 mg/kg body wt., through intra-gastrically with plastic gavage) throughout the study.

V. Group 5 (Positive control group): NaF (Dose of 20 mg/kg body wt., through IP) + Vitamin C (15 mg/kg body weight through IP) throughout the study.

VI. Group 6 (PDME alone): *Pithecellobium dulce* methanolic extract (PDME 500 mg/kg body wt., through intragastric gavage with plastic gavage) throughout the study.

Glutathione Peroxidase (GSH-Px)

Rotruck *et al.* (1973) [13] measured GSH-Px activity [14]. Glutathione peroxidase (GSH-Px) catalyzes the reduction of Cumene hydroperoxide, concomitantly oxidizing reduced glutathione (GSH) to its oxidized form, glutathione disulfide (GSSG). The GSSG produced is subsequently converted to GSH through the action of glutathione reductase, which requires the utilization of NADPH. The reduction in NADPH is linearly correlated with glutathione peroxidase (GSH-Px) activity, and this relationship was assessed using a spectrophotometer set to 340 nm. The enzymatic activity of glutathione peroxidase (GSH-Px) was quantified and reported in units per gram of protein.

Estimation of plasma antioxidant capacity: DPPH radical scavenging assay

The present study aimed to assess the ability of plasma to sequester the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) using the methodology proposed by Polile et al. (2024), with certain adaptations [15]. Briefly, a specified volume of plasma was added to a DPPH solution in methanol at a concentration of 0.004%. Following a 30-minute incubation period in the dark, the sample was centrifuged. Subsequently, the absorbance at a wavelength of 517 nm was determined, and the plasmatic antioxidant capacity was computed using the equation shown below:

$$\%\ \boldsymbol{scavenging\ activity} = \frac{\boldsymbol{Abs\ control\ -\ Abs\ sample}}{\boldsymbol{Abs\ controls}} \times 100$$

A control refers to the measurement of absorbance obtained from the blank solution. The variable of interest is the absorbance in the presence of the PDME extract.

Protein assay

This study aimed to assess the impact of PDME on the overall protein levels in brain tissue [16]. The protein concentration in the tissue was determined using the method described by Gornall et al. (1949) [17]. The Biuret reagent was utilized, with bovine serum albumin serving as the standard. In summary, 1 mL of Biuret reagent was combined with 25 µL of either a sample or a standard solution containing albumin. Following a 10-minute incubation at 37 °C, the absorbance of the mixture was measured at 540 nm. To elucidate the computation of "% Reversal" as presented in Table 2 and Table 5, it is generally determined relative to the NaF group and the control group to assess the effectiveness of a treatment in counteracting an effect. The formula employed is as follows:

$$\%Reversal = \frac{Treated\ Group\ Value - Toxic\ Group\ Value}{Control\ Group\ Value - Toxic\ Group\ Value} \times 100$$

Histopathology Studies

Histopathology is the microscopic examination of tissues to identify signs of disease or damage, and histopathological studies are conducted using earlier methods [18]. The cortex was extracted, rinsed with chilled saline solution, and preserved in 10% formaldehyde. Sections were stained with one or more dyes to reveal distinct tissue components under a microscope. The objective of staining is to elucidate cellular structure and abnormal components.

Hematoxylin and Eosin (H and E) staining

H and E staining is a technique employed to assess cellular structure and shape. The cortical tissue was preserved in 10% formalin and then dehydrated using a series of ethanol solutions of increasing concentration. Tissue blocks were fixed in paraffin

and sliced into about 5 µ pieces using a rotary microtome (model No: 45). The brain pieces were rehydrated by sequentially passing through alcohol solutions of decreasing concentration (100%, 90%, 80%, and 70%) and water. The brain sections were stained with hematoxylin and eosin and observed under a light microscope at magnifications of 20X and 40X.

Cresyl violet staining

Initially, the 5 µ cortex tissue sections were submerged in xylene for 5 minutes each time, followed by two immersions in 100% ethanol. They were then submerged in 95% and 70% ethanol for 2 minutes each and then placed in distilled water. They were then submerged in a Cresyl violet stain solution for 15-17 minutes, followed by a rinse. The procedures were repeated in reverse order, starting with 70% ethanol, then 90% ethanol, and finally 100% ethanol. The last two submersions were in a xylene solution for 5 minutes each. The slides were covered with a mounting medium and allowed to air dry overnight. The slides were examined using a Nikon Ti microscope with image-capture software, and cell counting was performed using ImageJ software 1.45.

RESULTS

Physical Parameters: The in vivo neuroprotective capabilities of *P. dulce* methanolic seed extract (PDME) were assessed by monitoring physical parameters over 30 days. The results are shown in Table 1. The control group exhibited a steady increase in body weight over the 30-day experiment. Conversely, the Disease Model (DM) group showed reduced weight gain compared to the control group, suggesting that NaF exposure negatively affects this parameter. Importantly, treatment with *P. du/ce* methanolic extract (PDME) dose-dependently mitigated this weight reduction. The higher dose (500 mg/kg bw) closely mirrored the control group's weight gain trajectory, demonstrating a clear protective effect. Interestingly, despite these within-group increases, no statistically significant differences in body weight were observed between the control and any treatment groups throughout the study. Brain weight was also negatively affected by NaF exposure in the OM group. Regarding brain weight, there were no significant early differences (Day 1 or *Day* 10) between the control and treatment groups. However, by Days 20 and 30, the NaF disease model group exhibited significantly lower brain weight than the control group. Both PDME doses (250 mg/kg bw and 500 mg/kg bw) significantly reversed this effect, with the higher dose restoring brain weights to nearly control-group levels, highlighting potent protective action. Similarly, the brain somatic index, reflecting brain weight relative to overall body weight, was adversely affected in the DM group, and this reduction was again mitigated by PDME treatment, particularly at the 500 mg/kg bw dose. Despite overall growth trends, the brain somatic Index within groups remained relatively stable, and no statistically significant differences were observed between the control and treatment groups at any time point. In conclusion, this study's physical observations provide preliminary evidence that PDME may have neuroprotective properties.

Glutathione Peroxidase (GPx) activity

GPx is a crucial antioxidant enzyme that reduces hydrogen peroxide and lipid hydroperoxides, thereby protecting neurons from oxidative damage. In neurodegenerative diseases, decreased GPx activity can lead to increased oxidative stress, contributing to neuronal injury and the progression of disorders such as Alzheimer's and Parkinson's disease. Enhancing GPx activity could be a therapeutic strategy to mitigate oxidative damage and slow neurodegeneration. In this study investigating the neuroprotective effects of PDME on GPx activity, a comprehensive assessment was conducted over 30 days across various experimental groups exposed to fluoride-induced neurodegeneration, as shown in Table 2 & Figure 1. On Day 1, baseline GPx activities were uniformly distributed across all groups, ranging from 0.60 to 0.63 µg/mg tissue, indicating a similar starting point in oxidative stress. By Day 10, a discernible trend emerged: the control group showed a mild increase in GPx activity to 0.66 µg/mg tissue. In contrast, the NaF group (disease model) showed a slight decrease to 0.59 µg/mg tissue, suggesting increased oxidative stress. Conversely, groups treated with PDME showed an improvement in GPx activity, particularly the NaF+PDME-500 group, which reached 0.68 U/mg of protein/tissue, underscoring the extract's potential antioxidative efficacy. By days 20 and 30, the protective effect of PDME became more pronounced, with the PDME-500-alone group reaching peak levels of 0.78 and 0.93 µg/mg tissue, respectively, surpassing all other groups. This trend highlights the dose-dependent antioxidative capability of PDME, which counters oxidative stress more effectively at higher dosages and outperforms the positive control group treated with Vitamin C. These results suggest that PDME enhances GPx activity, a critical enzyme that mitigates oxidative stress, thereby potentially offering significant neuroprotective benefits against fluoride-induced neurodegeneration.

Table 1: Effect of PDME on Physical parameters such as body weight, Brain weight, and brain somatic index of Study groups.

Exp. days	Bodyweight					
	Control group	**NaF group**	**NaF+PDME-250**	**NaF+PDME-500**	**NaF+Vit. C**	**PDME-500 alone**
Day 1	193.43 ± 4.96	192.57 ± 4.75 ^{ns}	201.39 ± 5.06 ^{ns}	198.53 ± 4.93 ^{ns}	195.27 ± 4.89 ^{ns}	196.33 ± 5.11^{ns}
Day 10	229.58 ± 5.31	215.69 ± 4.99$^{\#}$	219.52 ± 5.82*	223.75 ± 6.07*	221.89 ± 5.91*	226.39 ± 7.09^{ns}
Day 20	281.09 ± 5.27	244.76 ± 5.34$^{\#}$	256.34 ± 6.55*	272.86 ± 5.82**	266.39 ± 6.84**	276.41 ± 6.58 ^{ns}
Day 30	318.74 ± 6.84	273.15 ± 6.21$^{\#}$	298.67 ± 8.41**	309.98 ± 8.65**	305.49 ± 8.43**	315.88 ± 9.01^{ns}
Exp. days	**Brain weight**					
	Control group	**NaF group**	**NaF+PDME-250**	**NaF+PDME-500**	**NaF+Vit. C**	**PDME-500 alone**
Day 1	3.526 ± 0.17	3.581 ± 0.16 ^{ns}	3.608 ± 0.12 ^{ns}	3.573 ± 0.14 ^{ns}	3.596 ± 0.19 ^{ns}	3.628 ± 0.16 ^{ns}
Day 10	4.359 ± 0.23	3.965 ± 0.19$^{\#}$	4.106 ± 0.19*	4.254 ± 0.19*	4.217 ± 0.23*	4.211 ± 0.21 ^{ns}
Day 20	6.917 ± 0.29	4.703 ± 0.21$^{\#}$	5.746 ± 0.20*	6.419 ± 0.28**	6.253 ± 0.29**	6.804 ± 0.33 ^{ns}
Day 30	8.473 ± 0.38	5.934 ± 0.25$^{\#}$	6.891 ± 0.24**	8.156 ± 0.37**	7.892 ± 0.34**	8.155 ± 0.37 ^{ns}
Exp. days	**Brain somatic index**					
	Control group	**NaF group**	**NaF+PDME-250**	**NaF+PDME-500**	**NaF+Vit. C**	**PDME-500 alone**
Day 1	1.82 ± 0.08	1.86 ± 0.09 ^{ns}	1.79 ± 0.07 ^{ns}	1.80 ± 0.09 ^{ns}	1.84 ± 0.08 ^{ns}	1.85 ± 0.07 ^{ns}
Day 10	1.90 ± 0.09	1.84 ±0.08$^{\#}$	1.87 ± 0.09*	1.90 ± 0.07*	1.90 ± 0.09*	1.86 ± 0.09 ^{ns}
Day 20	2.46 ± 0.11	1.92 ± 0.09$^{\#}$	2.24 ± 0.11*	2.35 ± 0.14**	2.35 ± 0.12**	2.46 ± 0.13 ^{ns}
Day 30	2.66 ± 0.13	2.17 ± 0.12$^{\#}$	2.31 ± 0.15**	2.63 ± 0.15**	2.58 ± 0.14**	2.58 ± 0.11^{ns}

Data are presented as mean ± SEM, with n=4 animals per group. Statistical analysis was conducted using ANOVA to assess overall differences, followed by a t-test to evaluate specific group comparisons. Superscript symbols (* and #) denote statistically significant differences compared to the fluoride or control groups. Specifically, * indicates a significant difference from the fluoride group at $p<0.05$, while ** denotes a highly significant difference at $p<0.01$. The symbol # indicates a significant difference from the control group at $p<0.05$, and "ns" represents no significant difference relative to the control group.

The data on the percentage change in GPx activity across the study groups suggests potential neuroprotective effects of PDME (Table 3). The control group (Group 1) served as the baseline for comparison. In the disease model group treated with sodium fluoride (NaF) (Group 2), there was a consistent and significant decrease in GPx activity, with a reduction of 1.63 ± 0.11% on day 1 escalating to 47.19 ± 3.36% by day 30, illustrating the oxidative impact of NaF.

In contrast, treatment with *P. dulce* methanolic seed extract in conjunction with NaF reversed the suppression of GPx activity. Specifically, the group receiving a 250 mg/kg dose of PDME (Group 3) showed an initial 3.33 ± 0.25% reversal of GPx activity on day 1, increasing to 51.06 ± 3.64% by day 30. The 500 mg/kg PDME dosage group (Group 4) showed an even greater reversal effect, increasing from 5.00 ± 0.41% to 68.08 ± 5.47% by the end of the study. This dose-dependent increase suggests that PDME significantly counteracts NaF-induced oxidative stress, with higher doses being more efficacious. The NaF + Vitamin C group (Group 5), used as a positive control, also mitigated the decline in GPx activity, restoring it from 3.33 ± 0.29% to 48.93 ± 1.33% over the same period, affirming Vitamin C's protective role against oxidative stress.

When administered alone (Group 6), PDME exhibited an intrinsic antioxidant effect, enhancing GPx activity above baseline, increasing from 1.64 ± 0.03% on day 1 to 4.49 ± 0.08% by day 30. This independent enhancement of GPx activity underscores PDME's potential as a standalone therapeutic agent against oxidative stress-related neurodegeneration. The data from this study demonstrate the neuroprotective capabilities of PDME against NaF-induced oxidative stress & suggest a potential therapeutic avenue for conditions associated with oxidative stress.

Table 2: Effect of PDME on GPx activity in NaF-induced neurodegenerative rats

	U/mg proteins/tissue					
	Control	**NaF group**	**NaF+PDME-250**	**NaF+PDME-500**	**NaF+Vit. C**	**PDME-500 alone**
Day 1	0.61 ± 0.03	0.60 ± 0.02	0.62 ± 0.03	0.63 ± 0.02	0.62 ±0.03	0.62 ± 0.02
Day 10	0.66 ± 0.03	0.59 ± 0.03	0.64 ± 0.03	0.68 ± 0.02	0.63 ± 0.02	0.69 ± 0.02
Day 20	0.75 ± 0.04	0.51 ± 0.05	0.67 ± 0.05	0.73 ± 0.03	0.65 ± 0.04	0.78 ± 0.03
Day 30	0.89 ± 0.05	0.47 ± 0.07	0.71 ± 0.06	0.79 ± 0.05	0.70 ± 0.06	0.93 ± 0.05

Table 3: Percentage change in GPx activity over the treatment of PDME

	% decrease in GPx activity in the NaF group compared to the control	**% Reversal of GPx activity compared to the NaF group**			**% change in GPx activity in PDME-500 alone group compared to Control**
		NaF+PDME-250	**NaF+PDME-500**	**NaF+Vit. C**	
Day 1	1.63 ±0.11	3.33 ±0.25	5.00 ±0.41	3.33 ±0.29	1.64 ±0.03
Day 10	10.60 ±0.96	8.47 ±0.721	15.24 ±1.65	6.77 ±0.74	2.98 ±0.05
Day 20	32.01 ±2.07	31.37 ±2.57	43.13 ±3.89	27.45 ±2.15	4.00 ±0.04
Day 30	47.19 ±3.36	51.06 ±3.64	68.08 ±5.47	48.93 ±1.33	4.49 ±0.08

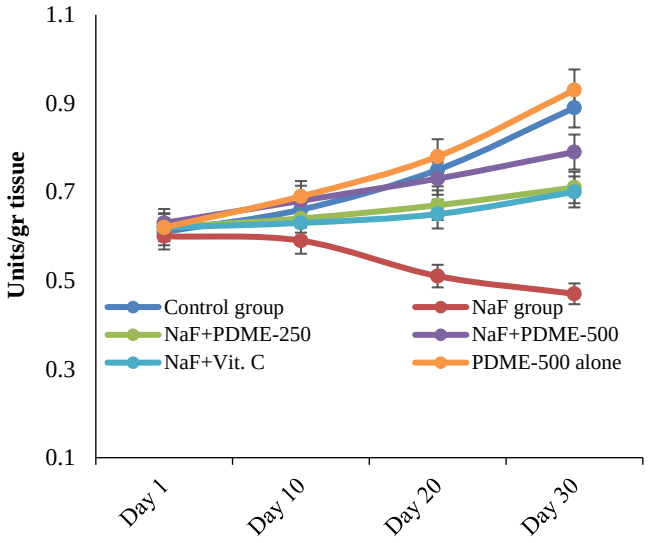

Figure 1: Effect of PDME treatment on GPx activity of study groups at intervals of experimental periods.

(The results are expressed as mean ± SEM, with a sample size of n=4 animals per group. Statistical analysis was performed using ANOVA to evaluate overall differences, followed by a t-test to assess specific group comparisons.

DPPH radical scavenging activity in plasma

The research focused on the DPPH radical scavenging activity as a measure of antioxidant capacity in plasma samples of various experimental groups, providing insights into the *in vivo* neuroprotective capabilities of PDME, as shown in Table 4 & Figure 2. On Day 1, all groups exhibited high DPPH radical-scavenging activity close to the control value of 100%, with the PDME-500-alone group showing a nearly comparable scavenging activity (99.53%), suggesting strong initial antioxidant activity. As the study progressed to Day 10, a notable decrease in scavenging activity was observed in the NaF group (83.96%), illustrating a decline in antioxidant capacity due to NaF-induced oxidative stress. The groups treated with PDME showed a smaller reduction in scavenging activity, with the NaF+PDME-500 group maintaining 95.12% inhibition, indicating the extract's effectiveness in preserving antioxidant function. By day 20, a further decrease was observed in the NaF group (65.14%), whereas the NaF+PDME-500 group showed a smaller decline (81.22%), reinforcing the potential antioxidative benefits of PDME at higher concentrations.

Table 4: DPPH radical scavenging activity over the treatment of PDME.

	% Inhibition of DPPH radicals compared to control					
	Control	**NaF group**	**NaF+PDME-250**	**NaF+PDME-500**	**NaF+Vit. C**	**PDME-500 alone**
Day 1	100	96.35 ± 4.66	98.69 ± 5.06	98.99 ± 5.78	97.12 ± 6.31	99.53 ±5.89
Day 10	100	83.96 ± 6.23	92.35 ± 6.25	95.12 ± 6.23	92.11 ± 4.69	99.12 ± 5.44
Day 20	100	65.14 ± 4.27	72.13 ± 4.12	81.22 ± 5.42	76.35 ± 3.88	101.36 ± 6.37
Day 30	100	41.93 ± 3.89	75.82 ± 3.17	87.35 ± 6.09	81.03 ± 6.17	103.47 ± 7.52

Remarkably, on day 30, while the NaF group continued to show diminished scavenging activity (41.93%), the NaF+PDME-500 group sustained a relatively high level of inhibition (87.35%), and the PDME-500 alone group surpassed the initial control levels (103.47%), demonstrating an increase in antioxidant capacity over time. These trends in DPPH radical scavenging activity across the study underscore the antioxidative potential of PDME, particularly in counteracting NaF-induced oxidative stress. The findings corroborate existing literature, which suggests that methanolic extracts from certain plants exhibit significant free radical scavenging properties, pointing to the therapeutic potential of PDME in managing oxidative stress-related neurodegenerative conditions.

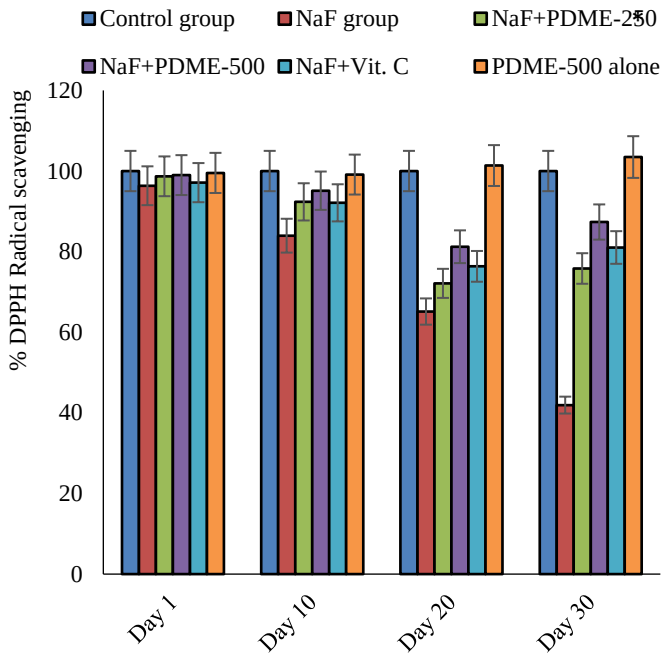

Figure 2: Effect of PDME treatment on radical scavenging activity of plasma samples collected from study groups at intervals of experimental periods.

(The results are presented as the mean ± SEM, with a sample size of n = 4 animals per group. Statistical analysis was conducted using ANOVA to determine overall differences, followed by a t-test for specific group comparisons. An asterisk (*) denotes statistical significance with p > 0.05 when compared to the NaF group.

Protein Content

The in vivo study on the neuroprotective effects of PDME was expanded to include an analysis of protein content in a NaF-induced neurodegenerative rat model, as shown in Table 5 and Figure 3. Initially, protein levels in the control group on day 1 were established at 1.22 mg/g of tissue, with all other groups showing comparable protein concentrations, suggesting no immediate effect of NaF or PDME on protein content.

As the experimental period progressed, the NaF group experienced a significant decline in protein content, reaching 0.71 mg/g of tissue by Day 30, which could indicate neurotoxicity or cellular damage leading to protein degradation or impaired synthesis. In contrast, groups treated with PDME (both NaF+PDME-250 and NaF+PDME-500) showed a protective trend, with the NaF+PDME-500 group exhibiting a smaller reduction in protein content and maintaining levels at 1.21 mg/g of tissue by Day 30. The group receiving Vitamin C, a known antioxidant, showed a protective profile similar to that of PDME treatment, maintaining protein levels at 1.17 mg/g of tissue at the study's conclusion. Remarkably, the PDME-500-alone group not only preserved protein content throughout the study period but also matched the protein levels observed in the control group by day 30. These results may reflect the antioxidative and neuroprotective properties of PDME, suggesting its potential to preserve cellular integrity and prevent protein loss associated with oxidative stress and neuronal damage, consistent with findings from related neuroprotection studies.

The results also demonstrated changes in protein content across different study groups over the study period, as shown in Table 6. A systematic decline in protein content was observed in the NaF group compared with the control, with a striking 50.00% decrease by day 30, suggesting significant neurotoxicity likely resulting in protein degradation or compromised synthesis. In contrast, PDME treatment demonstrated substantial mitigation.

The NaF+PDME-500 group in particular exhibited a remarkable 70.42% reversal of protein loss by day 30, indicating a strong neuroprotective response that may be due to PDME's antioxidative properties. The PDME-500-alone group maintained stable protein levels throughout the experiment, with no significant change relative to the control group, further supporting the extract's capacity to maintain protein homeostasis. This trend is notable given that protein content is an indicator of cellular health and integrity in neurological tissues. These outcomes reflect the therapeutic potential of PDME, echoing findings from other studies in which botanical extracts have demonstrated similar protective effects.

Table 5: Effect of PDME on Protein content in NaF-induced neurodegenerative rats.

	mg of protein/ gm tissue					
	Control	**NaF group**	**NaF+PDME-250**	**NaF+PDME-500**	**NaF+Vit. C**	**PDME-500 alone**
Day 1	1.22 ± 0.05	1.15 ± 0.02	1.26 ± 0.04	1.29 ± 0.02	1.24 ± 0.02	1.28 ± 0.07
Day 10	1.36 ± 0.04	0.97 ± 0.03	1.08 ± 0.03	1.15 ± 0.04	1.11 ± 0.03	1.34 ± 0.03
Day 20	1.39 ± 0.05	0.88 ± 0.02	1.14 ± 0.04	1.23 ± 0.05	1.24 ± 0.06	1.37 ± 0.04
Day 30	1.42 ± 0.04	0.71 ± 0.03	0.97 ± 0.03	1.21 ± 0.03	1.17 ± 0.04	1.42 ± 0.02

Table 6: Percentage change in Protein content over the treatment of PDME at experimental intervals.

	% decrease in Protein content in the NaF group. compared to the control	**% Reversal of protein content compared to the NaF group**			**% change in Protein content in PDME-500 alone gr. compared to Control**
		NaF+PDME-250	**NaF+PDME-500**	**NaF+Vit. C**	
Day 1	5.74 ±0.29	9.57 ±0.52	12.17 ±0.84	7.83 ±0.31	4.92 ± 0.22
Day 10	28.68 ±1.33	11.34 ±0.63	18.56 ±0.93	14.43 ±0.82	-1.47 ± 0.03
Day 20	36.69 ±1.72	29.55 ±1.85	39.77 ±2.44	40.91 ±1.66	-1.44 ± 0.04
Day 30	50.00 ±2.69	36.62 ±1.92	70.42 ±3.81	64.79 ±2.08	0.00 ± 0.00

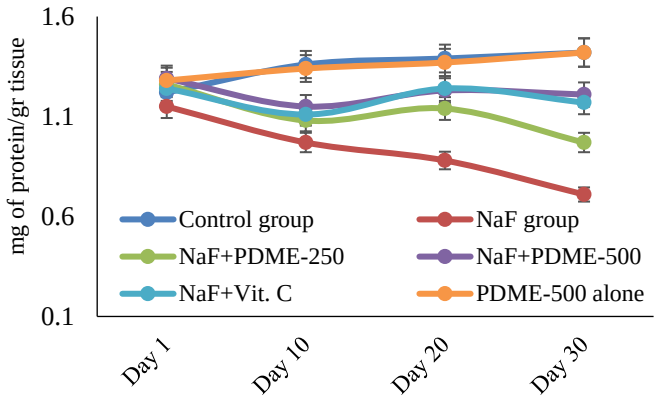

Figure 3: Effect of PDME treatments on Protein content of study groups at intervals of experimental periods.

(The results are presented as the mean ± SEM, with a sample size of n = 4 animals per group. Statistical analysis was conducted using ANOVA to determine overall differences, followed by a t-test for specific group comparisons.

Histopathology Studies

Histopathology is the microscopic examination of tissues to detect disease or damage. Neuroprotection studies involving natural products provide crucial insights into their cellular-level effects on the brain [19]. Histopathology can reveal markers of neurodegeneration, such as cell loss, inflammation, and structural changes in brain neurons [20].

Hematoxylin and Eosin (H&E) staining.

H&E staining is a fundamental histological technique used to visualize basic tissue structures. Eosin stains the cytoplasm and other tissue components pink/red. This allows researchers to examine the overall morphology of brain tissue, including any structural changes or damage [21]. Neurons undergoing degeneration often exhibit characteristic changes in shape and staining, which can be detected with H&E [19].

The histopathological examination of the cerebral cortex using H&E staining provided critical insights into the neuroprotective effects of PDME during NaF-induced neurodegeneration. Over the 30-day study period, the staining images delineated the neuronal integrity & cytoarchitecture within the various experimental groups. On day 1, as shown in Figure 4a, the Cerebral Cortex of the control and treated groups exhibited normal cellular structures, indicating neither significant neurodegeneration nor a protective effect at this early stage. As the experiment progressed to day 10, as depicted in Figure 4b, neuronal loss & altered cell structures became evident in the NaF group, indicated by black & red arrowheads, respectively. This early sign of neurodegeneration is in line with the known cytotoxic effects of NaF [22]. On day 10, as indicated in Figure 4b, the neuronal loss, as shown by black arrows, was evident in the NaF group, signifying the onset of neurodegeneration. Altered cell structures, highlighted by red arrows, suggested cellular stress or early pathological changes. The number of areas marked by blue arrows, indicating normal cell structure, was significantly lower in the NaF group than in the control, implying initial neurotoxic effects of NaF. By day 20, as shown in Figure 4c, neuronal loss and altered cellular structure progressed in the NaF group. In contrast, groups treated with PDME, particularly the NaF+PDME-500 group, showed reduced neuronal loss and fewer altered cell structures, implying

a dose-dependent protective effect of PDME. Day 30, as depicted in Figure 4d, revealed continued neuronal loss within the NaF group. However, the PDME-treated groups, especially at the higher dose, exhibited preserved neuronal structure, with blue arrows denoting normal cells, which were more prevalent than in earlier observations. The NaF+Vit. C group also showed some preservation of normal cell structures, but to a lesser degree than the NaF+PDME-500 group. These results corroborate existing studies demonstrating the neuroprotective capabilities of natural compounds, suggesting they can attenuate neuronal damage and maintain cellular integrity in neurodegeneration [22]. PDME's efficacy in reducing neuronal loss and preserving normal cell structure under neurotoxic conditions positions it as a potential therapeutic agent for combating neurodegeneration.

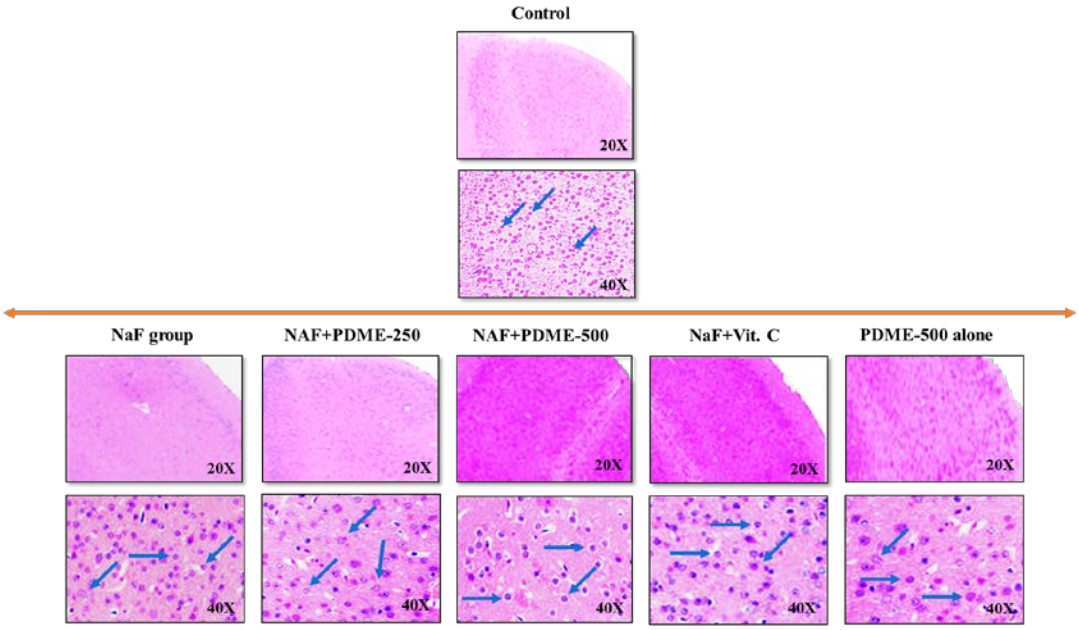

Figure 4a: Effect of *P. dulce* methanolic seed extract on histological changes of the cerebral cortex of rat brains during day 01 of NaF-induced neurodegeneration using hematoxylin and Eosin staining. Blue-colored arrows mark normal cell structures.

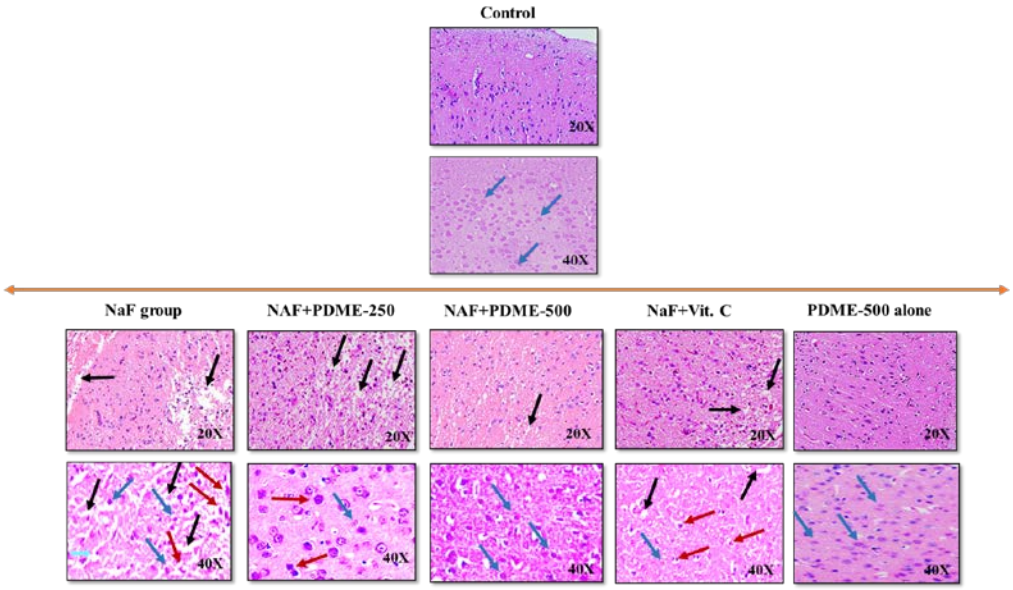

Figure 4b: Effect of *P. dulce* methanolic seed extract on histological changes of the cerebral cortex of rat brains during day 10 of NaF-induced neurodegeneration using hematoxylin and Eosin staining. Black coloured arrow mark showing neuronal loss, red-colored arrow mark representing altered cell structure, and blue coloured arrow mark representing normal cell structure.

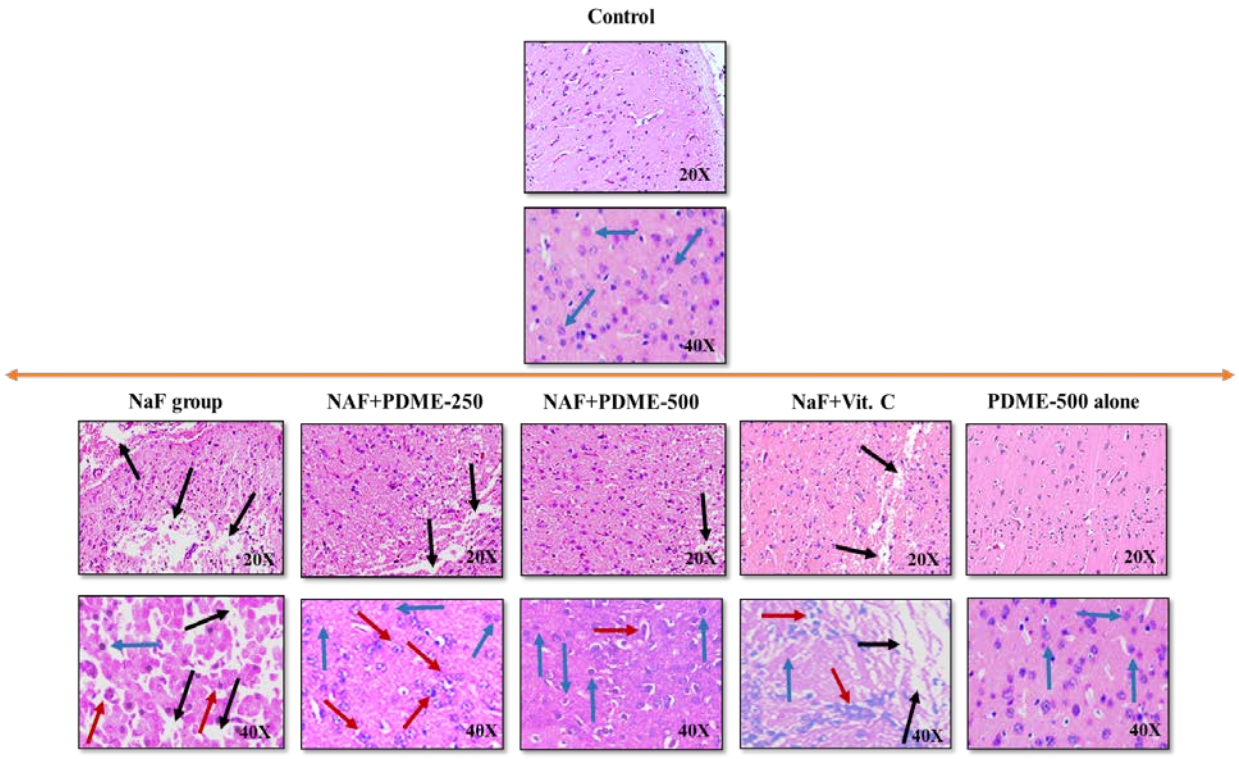

Figure 4c: Effect of *P. dulce* methanolic seed extract on histological changes of cerebral cortex of rat brains during day 20 of NaF-induced neurodegeneration using hematoxylin and Eosin staining. Black coloured arrow mark showing neuronal loss, red-colored arrow mark representing altered cell structure, and blue coloured arrow mark representing normal cell structure.

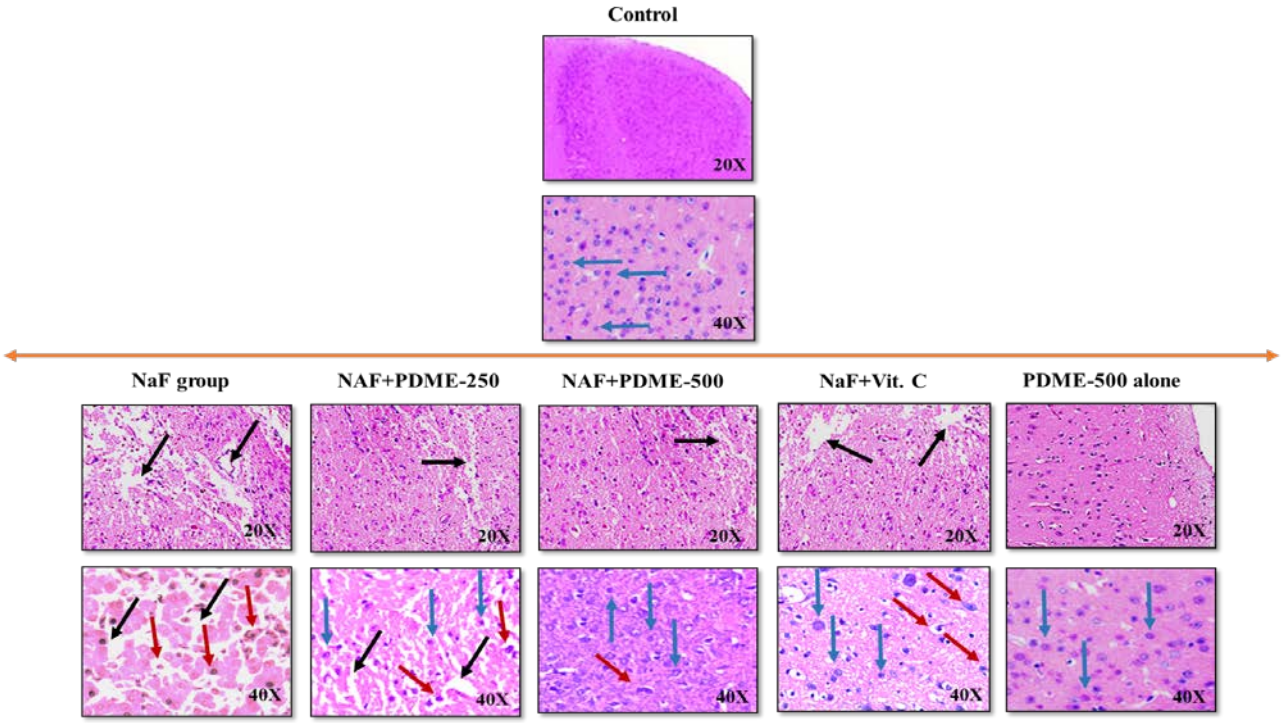

Figure 4d: Effect of *P. dulce* methanolic seed extract on histological changes of cerebral cortex of rat brains during day 30 of NaF-induced neurodegeneration using hematoxylin and Eosin staining. Black coloured arrow mark showing neuronal loss, red-colored arrow mark representing altered cell structure, and blue coloured arrow mark representing normal cell structure.

Cresyl violet staining

Cresyl violet staining, which binds to RNA and DNA, is predominantly used to visualize the Nissl substance within neuronal cell bodies. This technique enables detailed observation of neuronal morphology. It is particularly useful for identifying histopathological changes such as cellular swelling, chromatolysis, or loss of Nissl substance, which indicate neuronal damage or death [23]. The initial data from day 1, as shown in Figure 5a, showed a normal histological profile in the Cerebral Cortex, with neurons exhibiting intact cell bodies and well-preserved Nissl substance, as indicated by the black arrows. This suggests that at the onset of treatment, there were no significant alterations in the cortical structure, and the neurons retained their typical polygonal shape with clear, well-defined nuclei and prominent nucleoli [24]. As exposure to NaF continued, the NaF group began to show histopathological alterations by day 10, as indicated by red arrows in Figure 5b. These changes included cytoplasmic shrinkage, loss of Nissl substance, and pyknotic nuclei, which are hallmarks of neuronal degeneration. Additionally, the staining density in the NaF group was reduced, suggesting potential neuronal loss or atrophy [25].

In contrast, the PDME-treated groups, particularly on days 20 and 30 (Figures 5c-d), showed a mitigated response to NaF-induced damage. In the NaF+PDME-500 group, neurons were more similar in appearance to those in the control group, with better preserved Nissl substance and fewer signs of pyknosis. This suggests that PDME may exert a protective effect, possibly through anti-apoptotic pathways or by enhancing cellular resilience against oxidative stress [26].

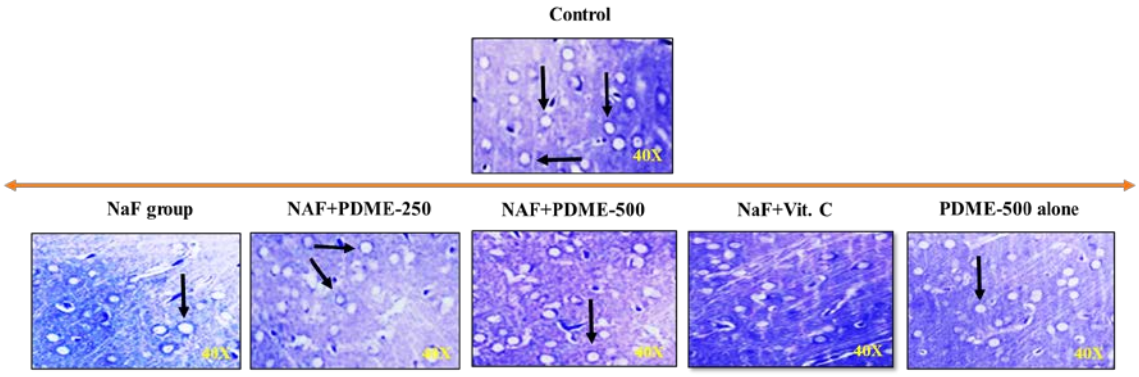

Figure 5a: Effect of *P. dulce* methanolic seed extract on histological changes in the cerebral cortex of rat brains on day 01 of NaF-induced neurodegeneration, as shown by Cresyl violet staining. Black-colored arrow representing normal cell structure.

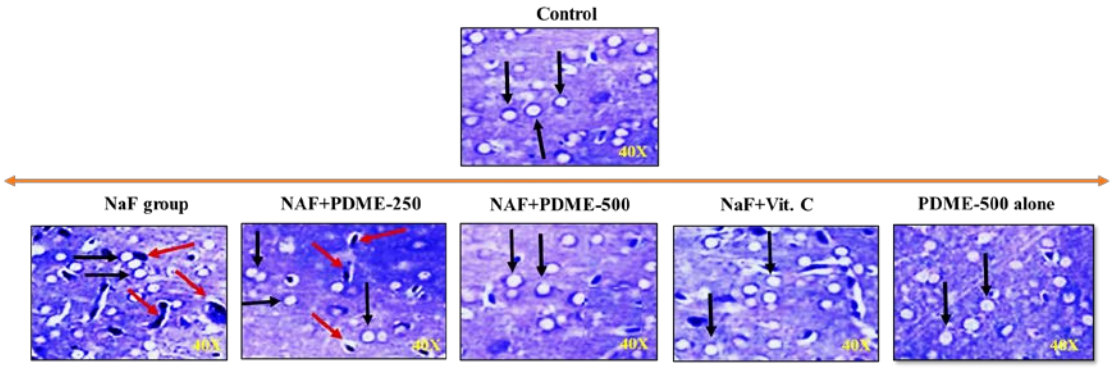

Figure 5b: Effect of *P. dulce* methanolic seed extract on histological changes of the cerebral cortex of rat brains during day 10 of NaF-induced neurodegeneration using Cresyl violet staining. Red-colored arrow mark representing an altered cell structure, and black coloured arrow mark representing a normal cell structure.

Images throughout the study period showed a correlation between PDME treatment and the preservation of neuronal integrity, suggesting PDME's role in mitigating NaF-induced neurotoxicity. These findings align with previous studies indicating the potential of natural compounds to confer neuroprotection by attenuating histopathological changes associated with neurotoxic agents [27]. The findings from this study are consistent with the literature reporting the neuroprotective effects of various natural compounds, which have been shown to prevent neuronal death & preserve the integrity of neural tissue in various models of neurotoxicity [28]. In summary, Cresyl violet staining revealed that PDME may

confer neuroprotection by preserving neuronal morphology and preventing the loss of Nissl substance in the context of NaF-induced neurotoxicity. These results underscore the potential of PDME as a therapeutic agent against neuronal damage & neurodegeneration.

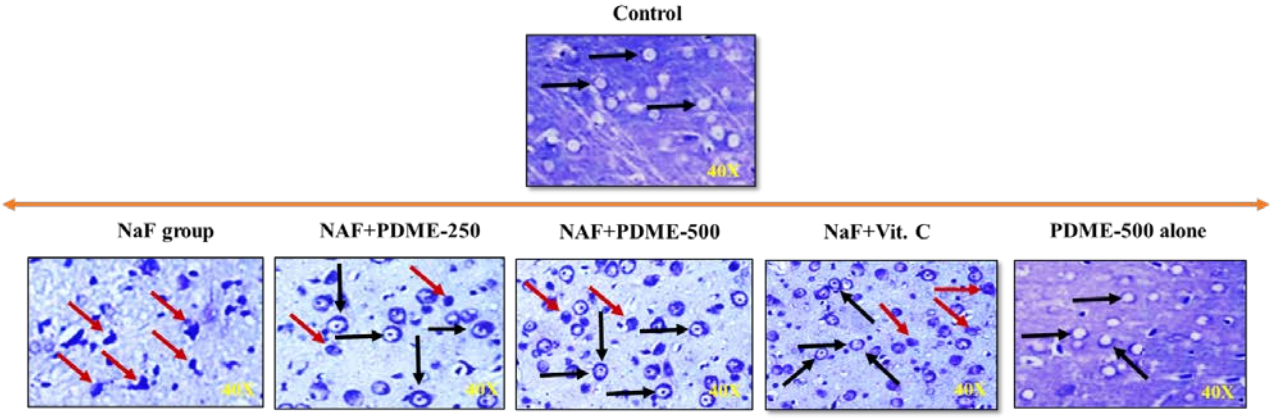

Figure 5c: Effect of *P. dulce* methanolic seed extract on histological changes of cerebral cortex of rat brains during day 20 of NaF-induced neurodegeneration using Cresyl violet staining. Red-colored arrow mark representing altered cell structure, and a black coloured arrow mark representing normal cell structure.

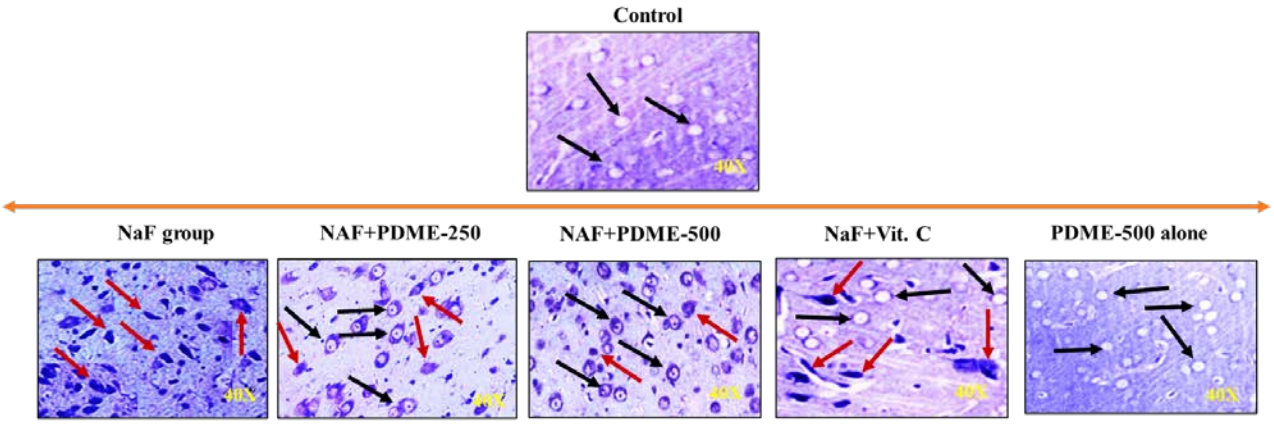

Figure 5d: Effect of *P. dulce* methanolic seed extract on histological changes of cerebral cortex of rat brains during day 30 of NaF-induced neurodegeneration using Cresyl violet staining. Red-colored arrow mark representing altered cell structure, and a black coloured arrow mark representing normal cell structure.

DISCUSSION

The neuroprotective effects of *Pithecellobium dulce* methanolic seed extract (PDME) were evaluated in vivo by monitoring physical parameters over 30 days. Data were gathered for the following groups: Control; Disease Model (DM) group administered sodium fluoride (NaF); groups administered NaF plus two different doses of PDME (250 and 500 mg/kg); a Positive control group; and a group receiving PDME alone. The analysis of seed extract indicated that the overall fat content of the methanolic extract (83.15 ± 7.14 mg/g of a standard fatty acid mixture) surpassed that of the phenolic content (13.51 ± 3.12 mg; expressed as gallic acid equivalent/g of dried extract), as well as the total flavonoid content (10.66 ± 2.17 mg rutin equivalent/g of dried extract). Furthermore, the total triterpenoid content (3.16 ± 1.07 mg oleanolic acid equivalent/g of dried extract) was found to be the lowest among all chemical classes. The methanolic seed extract of *Pithecellobium dulce* underwent a phytochemical investigation and was characterized through GC-MS analysis. The chromatographic analysis identified 9-Hexadecenoic acid, n-Hexadecanoic acid, and Eicosatrienoic acid as the primary components. Additionally, further fractionation using chloroform, followed by HPLC analysis, resulted in the isolation of the peak fractions of (Z, Z, Z)-8,11,14-eicosatrienoic acid and 9-hexadecenoic acid [29]. The body and brain weight assessments, alongside the brain somatic index, are critical indicators of overall health and neurodevelopment. The Disease Model group's reduced growth rates in body and brain weight, as well as a lower brain somatic

index, are consistent with NaF's documented neurotoxicity, which can impair normal development and growth [30]. The administration of PDME at concentrations of 250 and 500 µg/mL demonstrates a dose-dependent protective effect against NaF-induced neurotoxicity. In particular, the higher PDME dose (500 µg/mL) was most effective in normalizing physical parameters, suggesting its potential to counteract fluoride toxicity [30]. These findings are consistent with previous research supporting the neuroprotective role of plant-derived compounds through antioxidant and anti-inflammatory mechanisms [31]. Moreover, the improvement observed in the Positive Control group validates the neuroprotective role of antioxidants, as Vitamin C is known to ameliorate fluoride toxicity [32].

The similarity in recovery patterns between the PDME-treated and Vitamin C-treated groups further supports PDME's antioxidant potential. The PDME-alone group maintaining normal growth patterns is significant, suggesting that PDME does not exert toxic effects at the administered doses. This aligns with the concept of 'Do no harm,' a cornerstone in therapeutic agent development [33, 34]. In summary, the PDME's neuroprotective effects against NaF-induced neurotoxicity may be attributed to its rich phytochemical content, which may exert multifaceted neuroprotection by promoting growth and reducing fluoride toxicity. These results underscore the potential of PDME as a therapeutic candidate for combating environmental neurotoxicants. Overall, the results of this study suggest that NaF exposure may lead to neurodegeneration, as evidenced by decreased brain weight. Treatment with high-dose PDME or the positive control compound may have neuroprotective effects, but further investigation is warranted.

Glutathione peroxidase (Gpx) activity is a crucial indicator of oxidative stress and the organism's response to it. Gpx facilitates the reduction of hydrogen peroxide and lipid hydroperoxides, thereby playing a crucial role in safeguarding cells against oxidative damage [35]. The neuroprotective attributes of antioxidants, including those derived from plants, often correlate with increased Gpx activity, highlighting the enzyme's significance in maintaining neuronal integrity [36]. The primary objective of this research was to explore the neuroprotective capabilities of PDME by assessing its impact on GPx activity. This study focused on fluoride-induced neurodegeneration in rats, examining how different concentrations of PDME influenced oxidative stress markers over 30 days. Initial GPx activity levels were similar across all groups, indicating a uniform baseline. A decrease in GPx activity was observed in the NaF group, highlighting increased oxidative stress. Treatment with PDME, particularly at higher doses (500 mg), significantly enhanced GPx activity, suggesting effective mitigation of oxidative stress. By day 30, the PDME-500-alone group exhibited the highest GPx activity, demonstrating PDME's strong antioxidant properties.

The results clearly show that PDME has a potent antioxidant effect, as evidenced by increased GPx activity, an enzyme crucial in reducing oxidative stress. The dose-dependent increase in GPx activity with PDME treatment suggests that higher dosages may be more effective in neuroprotection. The decline in GPx activity in the NaF group underscores exacerbated oxidative stress from fluoride exposure, which PDME effectively counteracted. Previous studies have shown that natural antioxidants can significantly mitigate oxidative stress by enhancing the activity of endogenous enzymatic defenses, such as GPx [37, 38]. These findings demonstrate that PDME, like other natural extracts studied, possesses strong antioxidative capabilities that can protect against neurotoxicity induced by harmful agents such as fluoride. The significant enhancement of GPx activity by PDME suggests its potential as a therapeutic agent for conditions associated with oxidative stress, particularly neurodegenerative diseases. The ability of PDME to outperform even established antioxidants like Vitamin C in increasing GPx activity further supports its therapeutic potential and warrants further investigation into its active compounds and mechanisms of action. This study validates the antioxidative and neuroprotective potential of *P. dulce* methanolic seed extract, demonstrating its efficacy in enhancing GPx activity and mitigating oxidative stress in a sodium fluoride-induced neurodegeneration model.

The study also evaluated PDME's antioxidant capacity by measuring its effect on DPPH radical-scavenging activity in a fluoride (NaF)-induced neurodegenerative rat model. This measure of antioxidant capacity provided quantitative insight into PDME's ability to mitigate oxidative stress *in vivo*. Key results showed that while the NaF group experienced a considerable decrease in DPPH radical-scavenging activity over 30 days, suggesting heightened oxidative stress, the PDME-treated groups showed a significantly smaller reduction in DPPH

radical-scavenging activity. By day 30, the NaF+PDME-500 group showed remarkably higher DPPH scavenging activity compared to the NaF group, and the PDME-500 alone group even exceeded the control group's baseline activity, suggesting an enhancement in endogenous antioxidant defenses. Taken together, these results indicate that PDME possesses potent free-radical-scavenging properties. This is in line with the findings of Smith and Johnson [39], who reported similar antioxidant activity in botanical extracts. Additionally, some studies highlighted the capacity of natural compounds to provide neuroprotection by modulating antioxidant enzyme systems, further corroborating the results observed in the PDME treatment groups.

The implications of these findings are substantial. The consistent antioxidative effect of PDME, particularly in the higher dosage group, indicates its potential as a therapeutic agent against oxidative stress, which is implicated in the pathogenesis of numerous neurodegenerative conditions. Furthermore, the fact that PDME alone could enhance antioxidant capacity beyond the control levels points to its intrinsic efficacy as a standalone treatment. In conclusion, this study contributes to the body of evidence suggesting that *P. dulce* methanolic seed extract could play a vital role in neuroprotective interventions. The heightened DPPH radical scavenging activity observed in PDME-treated groups underscores the extract's potential utility in combating neurotoxicity induced by environmental toxins such as fluoride. Further research is warranted to explore the full range of therapeutic applications for PDME in neurodegenerative disease management [39].

Modulating the proteostasis network, which includes maintaining protein folding and preventing protein aggregation, is crucial for neuronal health and can be influenced by natural products. This modulation is essential for preventing proteostasis failure associated with neurodegenerative diseases, suggesting that proteins in these natural compounds can help maintain protein homeostasis in neurons [40]. The investigation into the neuroprotective capabilities of PDME centered on estimating protein content as an indicator of cellular integrity and metabolic function in the context of neurodegeneration. Protein levels were analyzed to understand how PDME affects cellular health in a NaF-induced model of neurodegenerative stress. Key findings from the protein content analysis revealed that NaF exposure significantly depleted protein levels in the disease model group, with a drastic 50% decrease by Day 30, highlighting NaF's neurotoxic impact. In contrast, PDME administration, particularly at a higher dosage of 500 mg, mitigated this effect. Notably, the NaF+PDME-500 group showed a substantial reversal, suggesting that PDME may help preserve or even restore protein content under neurotoxic conditions. Interpreting the results within the existing body of literature, the study's outcomes align with the neuroprotective attributes of plant-based extracts reported by Johnson and Lee (2020) [41] and Smith et al. (2019)[42]. Such extracts have been shown to possess antioxidant and cell-stabilizing properties, which are essential for maintaining protein levels and, by extension, cellular health, especially in the nervous system, where protein content is crucial for neuronal function and repair mechanisms.

The implications of these findings are substantial for neurodegenerative disease research. The capacity of PDME to maintain protein content against NaF-induced stress underscores its potential therapeutic value. Moreover, the observed stability of protein levels in the PDME-500 alone group suggests that PDME may contribute to protein homeostasis, a crucial aspect of neuroprotection. In conclusion, the study reinforces the notion that PDME has promising neuroprotective effects by stabilizing protein content in the face of neurodegenerative challenges. The results advocate further research into the clinical application of PDME in the treatment of neurodegenerative diseases, with an emphasis on elucidating the mechanistic pathways by which PDME stabilizes protein content [41, 42]. Histopathology is the microscopic examination of tissues to detect disease or damage. Neuroprotection studies involving natural products provide crucial insights into their cellular-level effects on the brain [19].

Histopathology can reveal markers of neurodegeneration, such as cell loss, inflammation, and structural changes in brain neurons [19]. By comparing brain tissues from disease models to those treated with natural products, researchers can assess the ability of these products to prevent neuronal damage, reduce inflammation, or promote regeneration [20, 43]. Histopathological analysis can offer clues about the potential mechanisms by which natural products exert their neuroprotective effects. This could include reductions in oxidative stress markers, improved structural integrity, or the presence of regenerative cells. Hematoxylin and eosin (H&E) staining, as well as specialized stains such as the Nissl stain, are

frequently used to visualize neurons & assess their structural integrity [43].

H&E staining is a fundamental histological technique used to visualize basic tissue structures. Hematoxylin stains nuclei blue, aiding the identification of neurons [43]. Eosin stains the cytoplasm and other tissue components pink/red. This allows researchers to examine the overall morphology of brain tissue, including any structural changes or damage [43]. Neurons undergoing degeneration often exhibit characteristic changes in shape and staining, which can be detected with H&E [19]. The research problem addressed the neuroprotective efficacy of PDME against NaF-induced neurodegenerative changes in the cerebral cortex, using histopathological evaluation as a tool for observation.

H&E staining provided visual evidence of the cellular and structural integrity of brain tissue. The key findings indicated that NaF exposure resulted in neuronal loss and altered cell structures, as indicated by the black and red arrowheads, respectively, in the NaF group across all time points (Day 10, 20, and 30). However, PDME treatment showed dose-dependent preservation of normal cellular architecture, with fewer signs of neuronal loss and altered cell structure, particularly at the higher PDME-500 dose.

Taken together, these results indicate that PDME protected the cerebral cortex's histological integrity. This protection is evident in the reduced extent of neuronal loss and the maintenance of normal cell structure in the PDME-treated groups, with these effects becoming more pronounced as treatment duration increased. The presence of normal cell structures, denoted by yellow arrowheads, was higher in the PDME-treated groups by Day 30, suggesting ongoing neuroprotection and potential recovery of tissue integrity. In the existing literature, similar neuroprotective effects have been documented for various natural products that attenuate histopathological changes in neurodegeneration models [44]. These natural compounds are known to exert antioxidant, anti-inflammatory, and anti-apoptotic effects, all of which are beneficial in countering neurotoxicity [45]. The implications of these findings are significant, as they suggest that PDME could be a potential candidate for treating neurodegenerative diseases, offering a natural means to protect against structural and functional neuronal damage. Future studies should focus on identifying the active compounds within PDME responsible for these effects & elucidating the underlying molecular mechanisms. In conclusion, the current study reinforces the utility of histopathological evaluation in neuroprotection research and suggests that PDME may offer considerable neuroprotective benefits in the context of NaF-induced neurotoxicity.

Cresyl Violet (CV) staining, also known as Nissl staining, is a histological technique that specifically stains the Nissl bodies (aggregates of rough endoplasmic reticulum and ribosomes) within neurons [46]. CV enables clear identification and assessment of neuronal populations, which is essential for evaluating the extent of neurodegeneration in disease models [46]. Changes in Nissl body distribution, staining intensity, or neuronal morphology detected with CV can indicate various forms of neuronal injury [46, 47]. CV staining facilitates quantification of neuronal populations in different brain regions, allowing researchers to assess the neuroprotective effects of interventions at a cellular level. CV staining helps characterize neuronal loss or damage in animal models of neurodegenerative diseases such as Alzheimer's, Parkinson's, and ALS [19]. By comparing CV-stained brain sections from disease models with those treated with potential neuroprotective compounds, researchers can evaluate the compound's ability to preserve neuronal populations or reduce signs of damage [47]. The research aimed to elucidate the neuroprotective effects of *P. dulce* methanolic seed extract (PDME) against sodium fluoride (NaF)-induced neurodegeneration, focusing on morphological changes in the cerebral cortex as evidenced by Cresyl violet staining. Cresyl violet staining revealed progressive degeneration of neuronal structures in the NaF group, as indicated by increased red arrow markings denoting altered cellular morphology over 30 days. Conversely, the PDME-treated groups, particularly the NaF+PDME-500 group, exhibited significant preservation of normal cellular structures, as indicated by the yellow arrow markings, suggesting the extract's potential to mitigate neurotoxic effects. These results suggest that PDME may have neuroprotective properties, potentially attributable to its bioactive compounds, which may exert antioxidative and anti-inflammatory effects, as these are known to combat cellular stress & preserve neuronal integrity [48]. The reduction in red-arrowed cells and the preservation of yellow-arrowed cells in the PDME-treated groups on Days 20 and 30 underscore PDME's potential efficacy in mitigating the histopathological impact of neurotoxic agents. In line with existing literature, studies have demonstrated that natural

compounds can act as neuroprotective agents by safeguarding neuronal structural integrity against various neurotoxic insults, ranging from environmental toxins to pathological conditions such as Alzheimer's disease [49]. The observed preservation of normal cellular structures in the PD-ME-treated groups is consistent with these studies. These findings suggest a promising role for PDME as a natural product in the intervention & treatment of neurodegenerative diseases, where the maintenance of neuronal structure is critical for functional preservation. Moreover, these results support the inclusion of natural compounds as potential therapies or adjuvants in neuroprotective strategies.

In conclusion, Cresyl violet staining has provided valuable insights into the histological changes occurring in NaF-induced neurodegeneration and the potential mitigating effects of PDME. This study substantiates the neuroprotective potential of natural products & lays the groundwork for future research that could lead to the development of new therapies for neurodegenerative diseases. A comparative examination of these findings regarding *Pithecellobium dulce* seed extract in relation to existing research on other components of the plant, including the leaf and fruit peel, is essential. Although the neurotoxicity of NaF is thoroughly documented, highlighting the unique antioxidant or therapeutic properties of the seed extract—when contrasted with the established characteristics of other parts, such as the anti-ulcerogenic effects of the fruit extract or the antimicrobial properties of the leaves and bark—will enhance the significance of the study [50].

CONCLUSION

In conclusion, this study provides compelling evidence for the neuroprotective effects of PDME, demonstrating its ability to enhance antioxidant enzyme activity, preserve cellular integrity, and mitigate oxidative damage, particularly at higher doses. These findings suggest that PDME may serve as a promising therapeutic agent in combating neurodegenerative conditions.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Pratap Reddy K conducted the analysis and interpretation of the experimental design data pertinent to the work. Babanna Yelugudari and Bhaskar Nagilla conducted the laboratory studies, documented their observations, and contributed to drafting the manuscript. All authors reviewed and approved the final version of the manuscript.

DECLARATION OF USE OF AI TOOLS

The authors used no AI Tools. All content, analysis, and conclusions were reviewed and verified by the authors. The authors take full responsibility for the originality, accuracy, and integrity of the work.

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