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Evaluation of the Mitigatory Potential of Nigella sativa Oil on Cisplatin-Induced Toxicity in the Prefrontal Cortex of Adult Wistar Rats

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Abstract*— Cisplatin remains a mainstay of modern cancer chemotherapy, but its clinical utility is constrained by dose-limiting toxicities, including neurotoxicity affecting the prefrontal cortex (PFC), a brain region central to executive function, working memory, and emotional regulation. This toxicity is driven by oxidative stress, neuroinflammation, and mitochondrial dysfunction, for which effective, well-tolerated adjunctive agents remain scarce. This study evaluated the mitigatory potential of Nigella sativa oil (NSO) against cisplatin-induced toxicity in the PFC of adult Wistar rats. Thirty adult male Wistar rats were randomly allocated into five groups of six: control, cisplatin-only (10 mg/kg, single intraperitoneal dose), NSO pretreatment plus cisplatin, cisplatin plus NSO post-treatment, and NSO-only (2 ml/kg orally, daily for 28 days). Body and brain weights were recorded, and prefrontal cortical tissue was analysed for oxidative stress markers (superoxide dismutase, catalase, lipid peroxidation), the pro-inflammatory cytokine tumour necrosis factor-alpha (TNF-α), and the mitochondrial enzymes lactate dehydrogenase and succinate dehydrogenase, alongside Haematoxylin and Eosin and Cresyl violet staining. Cisplatin produced a progressive decline in body weight, elevated lipid peroxidation, TNF-α and lactate dehydrogenase, reduced succinate dehydrogenase activity, and marked pyknosis, vacuolation and loss of Nissl substance in prefrontal cortical neurons. NSO administration, whether before or after cisplatin exposure, significantly attenuated these derangements, most consistently for the inflammatory, mitochondrial and histomorphological parameters, with a more modest effect on lipid peroxidation and no significant change in superoxide dismutase or catalase activity. These findings indicate that Nigella sativa oil possesses genuine, timing-independent neuroprotective potential against cisplatin-induced prefrontal cortical toxicity, supporting further molecular, behavioural and clinical evaluation of its candidacy as an adjunctive agent in platinum-based chemotherapy regimens.

***Keywords*— Prefrontal Cortex; Cisplatin; Nigella sativa Oil; Neurotoxicity; Neuroprotection; Wistar Rats**

I. INTRODUCTION

Cancer remains a leading cause of morbidity and mortality worldwide, placing a substantial burden on healthcare systems and families [1]. Among chemotherapeutic agents, cisplatin (cis-diamminedichloroplatinum II) has been a cornerstone of treatment for testicular, ovarian, lung, bladder, cervical, and head-and-neck cancers since its approval for clinical use in 1978 [2], [3]. Its cytotoxicity derives principally from the formation of intrastrand and interstrand DNA cross-links, which disrupt replication and transcription and drive rapidly dividing tumour cells toward apoptosis [2].

Despite this efficacy, cisplatin's clinical utility is limited by dose-dependent toxicities, including nephrotoxicity, ototoxicity, gastrointestinal injury, myelosuppression, and neurotoxicity [4]. Central neurotoxicity, once regarded as a rare complication, is increasingly recognised as an important contributor to cisplatin-induced cognitive impairment, colloquially termed "chemo-brain," characterised by deficits in memory, attention, and mood [4], [5]. Cisplatin is known to compromise blood–brain barrier integrity and, once within neural tissue, triggers oxidative stress, mitochondrial dysfunction, neuroinflammation, and apoptosis across multiple brain regions [5], [6].

The prefrontal cortex (PFC) governs executive function, working memory, decision-making, and emotional regulation through dense reciprocal connections with the thalamus, hippocampus, amygdala, and basal ganglia [7]. Its high synaptic density and metabolic demand render it disproportionately dependent on mitochondrial oxidative phosphorylation, and therefore particularly

vulnerable to oxidative and metabolic insult [7], [8]. Experimental evidence confirms that cisplatin increases lipid peroxidation, suppresses antioxidant enzyme activity, and promotes apoptotic gene expression within the PFC, with associated anxiety-like behaviour that is attenuated by antioxidant co-treatment [9].

Assessment of cisplatin-induced neurotoxicity typically combines biochemical and histological endpoints. Superoxide dismutase (SOD) and catalase (CAT) constitute the primary enzymatic antioxidant defence, while lipid peroxidation, assessed as malondialdehyde (MDA), indicates oxidative membrane injury [10]. Tumour necrosis factor-alpha (TNF-α) reflects glial-driven neuroinflammation, whereas lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) respectively index cellular membrane integrity and mitochondrial respiratory competence [11], [12]. Haematoxylin and Eosin (H&E) and Cresyl violet (Nissl) staining complement these biomarkers by revealing structural neuronal injury and loss of Nissl substance, a hallmark of impaired neuronal protein-synthetic capacity [13].

Natural antioxidant phytochemicals have attracted growing interest as candidate neuroprotective adjuncts to chemotherapy. Nigella sativa L. (black seed), a medicinal herb with a long history of traditional use across the Middle East, South Asia, and the Mediterranean, is rich in thymoquinone, its principal bioactive constituent, which exhibits potent antioxidant, anti-inflammatory, anti-apoptotic, and neuroprotective actions mediated in part through modulation of NF-κB, MAPK, and Nrf2 signalling [14]–[16].

Although Nigella sativa oil (NSO) has shown protective effects in several models of chemically induced neurotoxicity, direct evidence for its efficacy against cisplatin-induced injury of the prefrontal cortex specifically remains limited. A hydro-alcoholic extract of Nigella sativa reduced whole-brain MDA levels and restored SOD activity in cisplatin-exposed rats, with corresponding improvement in spatial memory [17], while NSO itself was recently reported to mitigate cisplatin-induced cognitive impairment, anxiety- and depressive-like behaviour, oxidative stress, neuroinflammation, and apoptosis, again at the level of the whole brain and without a dedicated oil-only comparator or region-specific analysis of the PFC [18]. Neuroprotective efficacy against cisplatin cannot, however, be assumed across phytochemicals or brain regions: the flavonoid quercetin restored cerebellar neuromorphology in one cisplatin model [19], yet failed to prevent, and in some respects aggravated, cisplatin-induced damage to the hippocampus, prefrontal cortex, and cerebellum in another [20]. This divergence underscores the necessity of direct, region- and agent-specific experimental evaluation rather than inference from mechanistically adjacent literature.

This study therefore evaluated the mitigatory potential of Nigella sativa oil, administered either before or after cisplatin exposure, against cisplatin-induced toxicity in the prefrontal cortex of adult Wistar rats, using an integrated panel of oxidative stress (SOD, CAT, MDA), inflammatory (TNF-α), mitochondrial (SDH), cellular-injury (LDH), and dual histological (H&E and Cresyl violet) endpoints.

II. MATERIALS AND METHODS

A. Ethical Approval

Ethical approval for this study was obtained from the Departmental Committee on Animal Research, Department of Anatomy, Lead City University, Ibadan, Nigeria. All experimental procedures complied with institutional guidelines and the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals [21]. Measures were taken throughout to minimise pain, distress, and discomfort, particularly during drug administration and sacrifice.

B. Experimental Animals and Housing

Thirty adult male Wistar rats (150–170 g) were obtained from the animal house of the Department of Anatomy, Lead City University. Male rats were used exclusively to eliminate the confounding influence of oestrous-cycle hormonal fluctuations. Only clinically healthy, active animals were selected. Animals were housed in wire-mesh cages under standard laboratory conditions (24–28 °C; 12 h light/12 h dark cycle) with free access to standard rat pellets and clean water ad libitum, following a two-week acclimatisation period.

C. Experimental Design

After acclimatisation, the animals were randomly allocated into five groups of six (n = 6), consistent with a previously published cisplatin-neurotoxicity

protocol in rats [9]. Group allocation and treatment protocols are summarised in Table I.

Group	Treatment & Route	n	Duration
1. Control	Normal saline, orally	6	Daily for 28 days
2. Cisplatin-only	Cisplatin 10 mg/kg, i.p.	6	Single dose, Day 1
3. NSO pretreatment + Cisplatin	NSO 2 ml/kg orally + Cisplatin 10 mg/kg i.p.	6	NSO Days 1–27; Cisplatin Day 28
4. Cisplatin + NSO post-treatment	Cisplatin 10 mg/kg i.p. + NSO 2 ml/kg orally	6	Cisplatin Day 1; NSO Days 2–28
5. NSO-only	NSO 2 ml/kg, orally	6	Daily for 28 days

TABLE I. Experimental Groups and Treatment Protocol

D. Drug Preparation and Administration

Cisplatin injection (Salplatin® 50; 50 mg/50 mL), a ready-to-use sterile aqueous solution, was obtained from the Oncology Pharmacy Unit, Lagos State University Teaching Hospital (LASUTH), Ikeja, Nigeria, and dosed by body weight. Nigella sativa oil (Al-Zayah Store, Sagamu, Nigeria) was stored in sealed, light-protected containers at room temperature and administered orally by oropharyngeal gavage at 2 ml/kg body weight, consistent with previously established protocols [14]. Cisplatin was given as a single intraperitoneal injection of 10 mg/kg body weight [19]. Body weights were recorded before each administration to guide dose adjustment.

E. Tissue Collection

Body weights were recorded at 3-day intervals throughout treatment. At the end of the 28-day protocol, animals were humanely sacrificed by cervical dislocation. Whole brains were rapidly excised and weighed to determine absolute brain weight; relative brain weight was calculated as a percentage of terminal body weight. For histology, brains were fixed in 10% formocalcium. For biochemistry, the brain was sectioned coronally on a chilled matrix and the prefrontal cortex dissected rostral to the genu of the corpus callosum using stereotaxic anatomical landmarks. Isolated tissue was homogenised in cold phosphate-buffered saline (PBS) using a glass-Teflon homogeniser, centrifuged, and the supernatant stored at –20 °C pending assay.

F. Histological Analysis

Paraffin-embedded, 5-µm-thick prefrontal cortical sections were deparaffinised, rehydrated, and stained with Haematoxylin and Eosin to assess general cytoarchitecture, laminar organisation, and pyramidal-neuron morphology, and with Cresyl violet to evaluate Nissl substance distribution as an index of neuronal metabolic and protein-synthetic integrity, using standard histological protocols [22]. Stained sections were examined under a light microscope at ×40 and ×400 magnification.

G. Biochemical Assays

Superoxide dismutase (SOD) activity was determined from inhibition of pyrogallol autoxidation (SOD Assay Kit). Catalase (CAT) activity was assayed colorimetrically from hydrogen-peroxide decomposition (Elabscience, Cat. No. E-BC-K031-S; 405 nm). Lipid peroxidation was quantified as malondialdehyde (MDA) by the thiobarbituric-acid method (Elabscience, Cat. No. E-BC-K025-M; 532 nm) [23]. TNF-α concentration was measured by sandwich ELISA (Elabscience Rat TNF-α Kit, Cat. No. E-EL-R2856; 450 nm). LDH activity was determined from the rate of NADH oxidation during pyruvate-to-lactate conversion (Biorex Diagnostics, Product Code BXC0242; 340 nm) [24]. SDH activity was assessed colorimetrically from the succinate-to-fumarate conversion rate (Abcam, Cat. No. ab228560; 600 nm). All assays were performed on prefrontal cortical tissue homogenates according to the respective manufacturers' instructions.

H. Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM) and were analysed in GraphPad Prism

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v10 using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple-comparison test. Statistical significance was set at $p < 0.05$, indicated in figures as *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

III. RESULTS

Relative organ weight was calculated as (organ weight / final body weight) × 100. Group differences were assessed by one-way ANOVA followed by Tukey's post hoc test; significance levels are indicated on the figures as described in Section II-H.

A. Body, Brain, and Relative Brain Weight

All groups except the cisplatin-only group showed a gradual increase in body weight over the 28-day period; the NSO pretreatment group gained the most weight, followed by the NSO-only and post-treatment groups, whereas the cisplatin-only group showed a progressive decline (Fig. 1). Absolute brain weight did not differ significantly among groups ($p = 0.0933$), despite a slightly higher mean in the pretreatment group (Fig. 2). Relative brain weight, expressed as a percentage of terminal body weight, differed significantly among groups ($p < 0.0001$); the control group recorded the highest value, while the cisplatin-only and all NSO-treated groups recorded significantly lower values (Fig. 3). Because absolute brain weight did not differ significantly among groups, this pattern in relative brain weight is attributable principally to the greater body-weight gain of the treated groups rather than to a genuine structural effect on brain mass.

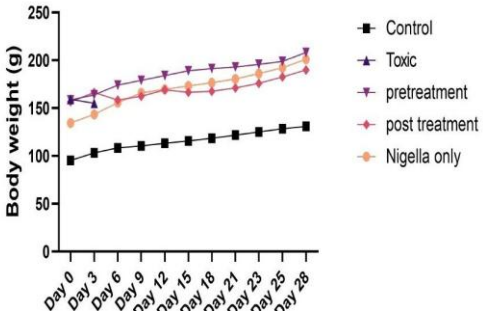

Fig. 1. Body weight changes over the 28-day treatment period across experimental groups.

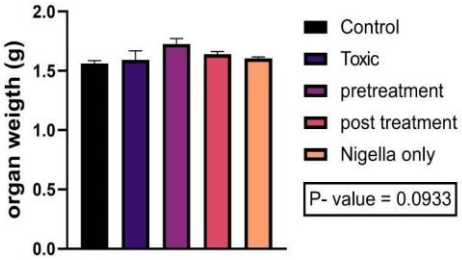

Fig. 2. Absolute brain weight across experimental groups (one-way ANOVA, $p = 0.0933$).

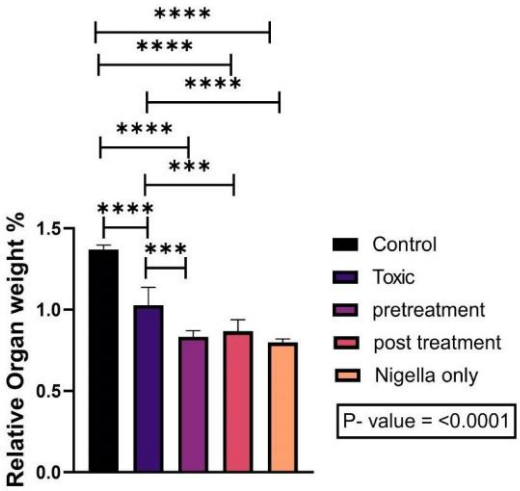

Fig. 3. Relative brain weight (% of terminal body weight) across experimental groups ($p < 0.0001$).

B. Oxidative Stress and Inflammatory Markers

MDA concentration differed significantly among groups ($p = 0.0247$), with the post hoc contrast reaching significance only between the cisplatin-only and NSO-only groups (Fig. 4). SOD ($p = 0.0788$; Fig. 5) and CAT ($p = 0.3629$; Fig. 6) activities showed no statistically significant differences among groups, although both were numerically lowest in the cisplatin-only group and highest in the control group. TNF-α concentration differed significantly among groups ($p = 0.0035$); cisplatin markedly elevated TNF-α relative to control ($p < 0.01$), while both NSO pretreatment and post-treatment significantly suppressed this elevation relative to the cisplatin-only group ($p < 0.01$ and $p < 0.05$, respectively), as did the NSO-only group ($p < 0.05$) (Fig. 7).

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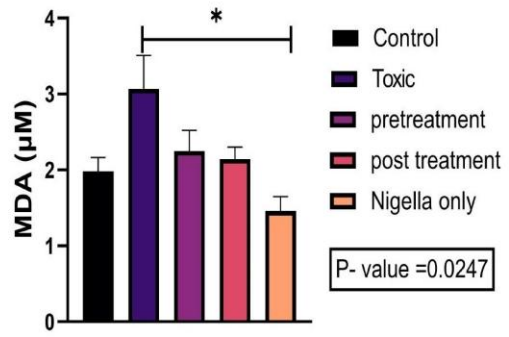

Fig. 4. Effect of Nigella sativa oil on prefrontal cortical malondialdehyde (MDA) concentration (p = 0.0247).

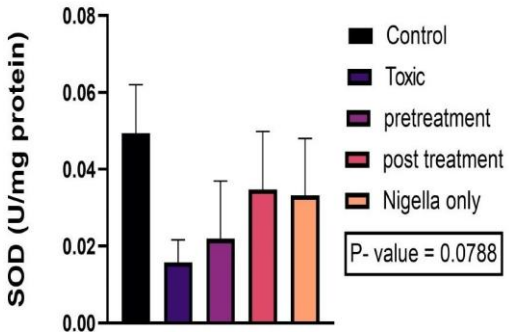

Fig. 5. Effect of Nigella sativa oil on prefrontal cortical superoxide dismutase (SOD) activity (p = 0.0788).

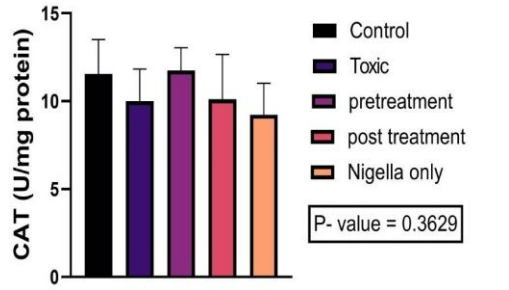

Fig. 6. Effect of Nigella sativa oil on prefrontal cortical catalase (CAT) activity (p = 0.3629).

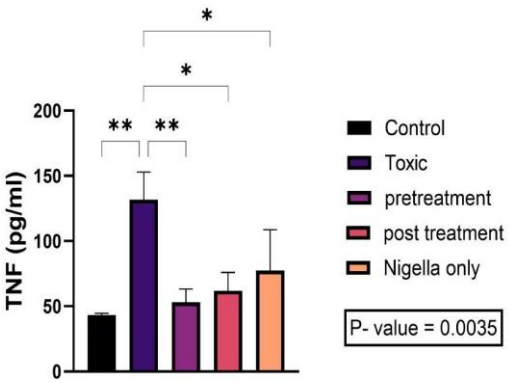

Fig. 7. Effect of Nigella sativa oil on prefrontal cortical TNF-α concentration (p = 0.0035).

C. Mitochondrial and Cellular-Injury Markers

SDH activity differed significantly among groups (p = 0.0022); cisplatin significantly reduced SDH relative to control, whereas both the pretreatment and post-treatment groups showed significantly higher SDH activity than the cisplatin-only group, and the NSO-only group maintained activity comparable to control (Fig. 8). LDH activity also differed significantly among groups (p = 0.0370), with the cisplatin-only group showing the highest activity, consistent with increased cellular injury and membrane damage; a statistically significant reduction was confirmed between the cisplatin-only and NSO-only groups, with numerically lower LDH activity also evident in the pretreatment and post-treatment groups relative to the cisplatin-only group (Fig. 9).

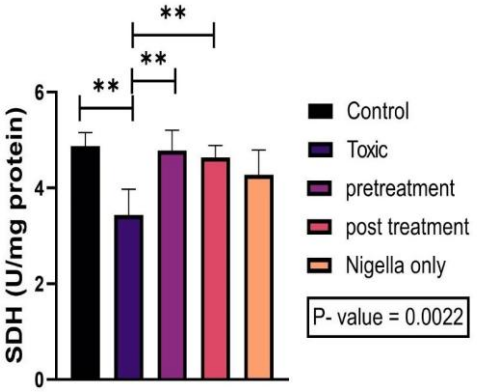

Fig. 8. Effect of Nigella sativa oil on prefrontal cortical succinate dehydrogenase (SDH) activity (p = 0.0022).

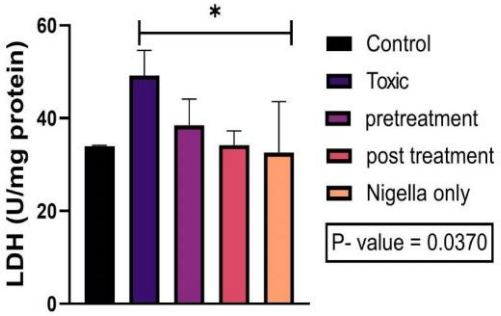

Fig. 9. Effect of Nigella sativa oil on prefrontal cortical lactate dehydrogenase (LDH) activity (p = 0.0370).

D. Histomorphology

Haematoxylin and Eosin staining showed normal cytoarchitecture, healthy pyramidal and stellate neurons, and an absence of vacuolation or gliosis in the control group. The cisplatin-only group displayed mild-to-moderate vacuolation, disrupted neuropil architecture, and numerous pyknotic neurons with hyperchromatic, shrunken nuclei, although a few

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healthy pyramidal neurons remained visible and no gliosis was observed. Both the pretreatment and post-treatment groups showed a reduced number of pyknotic neurons and only mild vacuolation, with abundant healthy pyramidal and stellate neurons, indicating substantial preservation of neuronal morphology relative to the cisplatin-only group. The NSO-only group showed the most favourable histological picture among the treated groups, with numerous healthy neurons, well-defined axonal projections, and only minimal vacuolation (Fig. 10).

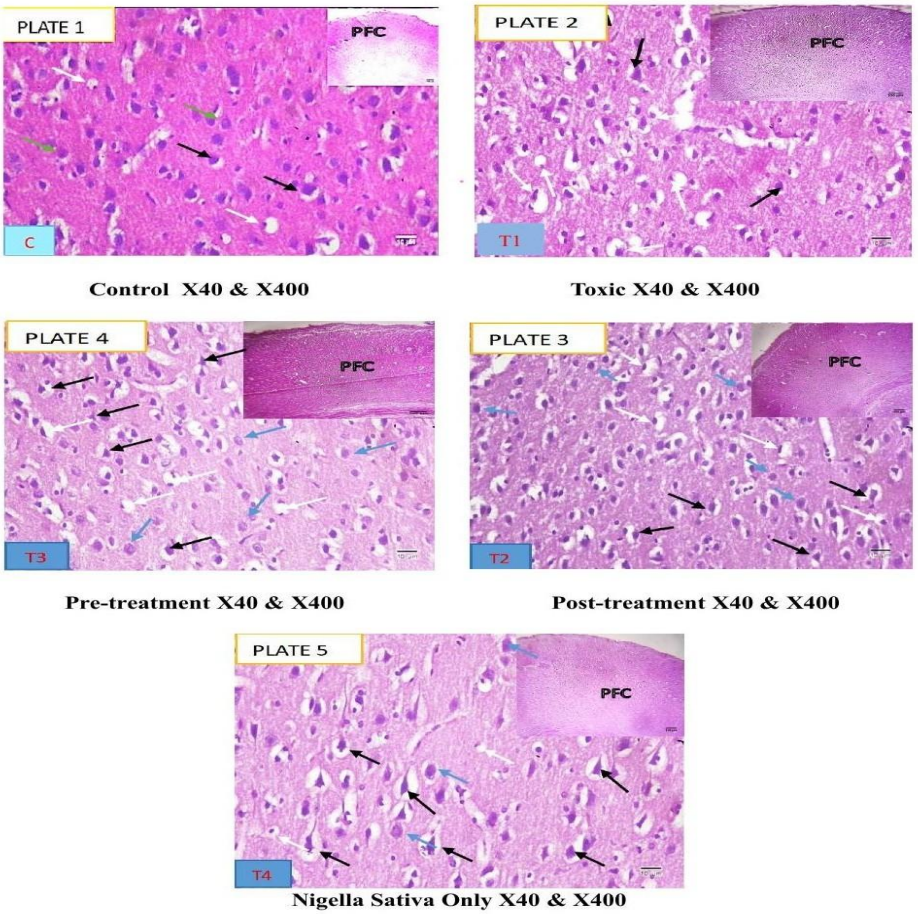

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Fig. 10. Photomicrographs of the prefrontal cortex stained with Haematoxylin and Eosin (×40 inset, ×400 main) for the Control, Cisplatin-only, Pretreatment, Post-treatment, and NSO-only groups. Black arrows: healthy pyramidal neurons; white arrows: pyknotic neurons; blue/green arrows: healthy stellate neurons.

Cresyl violet staining paralleled these findings. Control tissue showed densely and uniformly stained Nissl bodies within intact pyramidal and stellate neurons. The cisplatin-only group showed chromatolysis and reduced Nissl staining, with numerous pyknotic, hyperchromatic neuronal profiles and mild vacuolation, although some healthy neurons remained. The pretreatment group showed numerous healthy pyramidal neurons with preserved Nissl substance alongside a considerable number of residual pyknotic neurons, indicating partial preservation, while the post-treatment group showed more complete preservation, with markedly fewer pyknotic neurons than the cisplatin-only group. The NSO-only group exhibited predominantly healthy, intact pyramidal neurons, with preserved cortical architecture and only occasional pyknotic profiles (Fig. 11).

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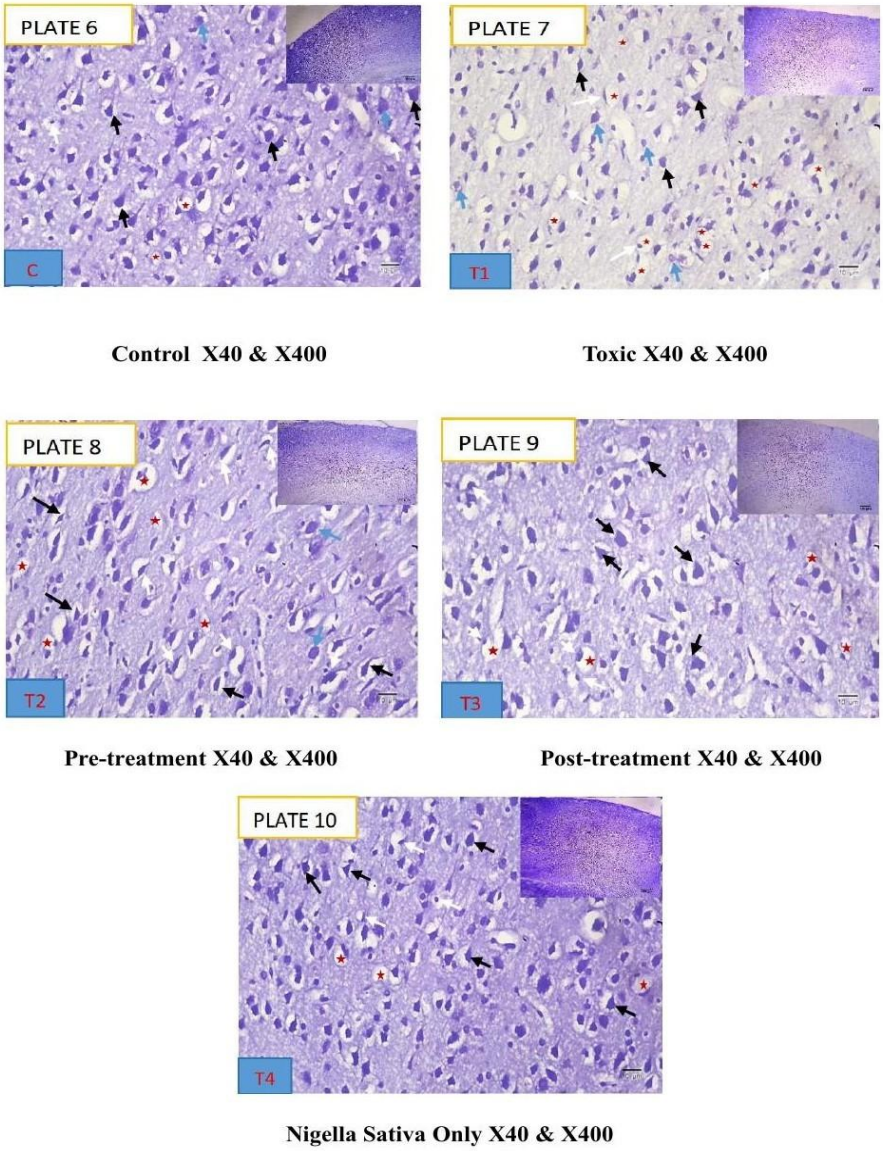

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Fig. 11. Photomicrographs of the prefrontal cortex stained with Cresyl violet (×40 inset, ×400 main) for the Control, Cisplatin-only, Pretreatment, Post-treatment, and NSO-only groups.

IV. DISCUSSION

This study evaluated the mitigatory potential of Nigella sativa oil (NSO) against cisplatin-induced toxicity in the prefrontal cortex (PFC) of adult Wistar rats across body and brain weight, oxidative stress, neuroinflammatory, mitochondrial, and dual histological endpoints. Overall, the results indicate a biomarker-dependent pattern of protection: body weight, TNF-α, SDH, and LDH showed statistically significant evidence of NSO-mediated mitigation; MDA differed significantly among groups, with the significant post hoc contrast confined to the cisplatin-only versus NSO-only comparison; and SOD, CAT, and absolute brain weight showed favourable but non-significant trends. Histomorphological (H&E) and histochemical (Cresyl violet) evaluation demonstrated a consistent gradient of structural preservation that broadly paralleled the biochemical findings.

The progressive decline in body weight observed in the cisplatin-only group is consistent with cisplatin-induced anorexia and negative energy balance, a well-documented systemic effect mediated partly through peripheral serotonergic signalling that

suppresses food intake [25], compounded by cisplatin's broader propensity to disturb metabolic and visceral organ function [26]. The comparatively preserved weight trajectories of the NSO-treated groups are consistent with the antioxidant, anti-inflammatory, and appetite-supportive properties reported for Nigella sativa and related phytotherapeutic agents in cisplatin models [27], [28], although the specific mechanism underlying this pattern was not directly investigated here.

The absence of a significant difference in absolute brain weight, despite clear biochemical and histological derangement in the cisplatin-only group, is consistent with reports that the earliest and most consistently detectable abnormalities in cisplatin neurotoxicity are synaptic, biochemical, and behavioural rather than grossly morphometric, with detectable mass change typically requiring more prolonged or higher-dose exposure than employed here [29], [30]. The significant differences observed in relative brain weight are more parsimoniously explained by differential body-weight gain across groups than by a genuine structural effect of either cisplatin or NSO on brain mass, illustrating why relative organ weight should not be interpreted in isolation from its absolute components [31].

Malondialdehyde, the principal stable end-product of membrane lipid peroxidation, was selectively and significantly elevated in the cisplatin-only group relative to the NSO-only group, consistent with cisplatin-induced reactive oxygen species overwhelming endogenous lipid-protective antioxidant capacity, while the comparatively low MDA in animals receiving NSO alone reflects the intrinsic radical-scavenging activity attributed to thymoquinone and related constituents [32], [33]. This pattern is broadly consistent with prior reports that Nigella sativa preparations reduce cisplatin-associated brain MDA [17], [18]; however, in the present dataset the post hoc comparisons did not identify a statistically significant reduction in MDA in the pretreatment or post-treatment groups relative to the cisplatin-only group, a genuine divergence from stronger effects reported elsewhere that may reflect differences in oil dose, formulation, or treatment duration.

SOD and CAT activities showed directionally favourable but statistically non-significant responses. The consistent, if modest, suppression of SOD in the cisplatin-only group is directionally consistent with cisplatin-mediated inactivation and depletion of this primary antioxidant enzyme [34], [35], although the failure to reach significance contrasts with reports of significant SOD suppression and restoration in other cisplatin-NSO models [17] and may reflect a smaller effect size, greater inter-animal variability, or a genuinely more modest impact of the present dosing regimen. Catalase activity was comparatively resilient to both cisplatin insult and NSO intervention, consistent with reports that individual antioxidant enzyme responses are not uniform across brain regions, exposure durations, or dosing protocols [35], [36]. Taken together, these findings identify SOD and CAT as comparatively less sensitive biomarkers of cisplatin-induced oxidative injury in the PFC within this model, reinforcing the value of a multi-marker biochemical panel rather than reliance on any single oxidative index.

TNF-α showed the clearest, most statistically robust, and most timing-independent response of all biochemical markers assessed. Cisplatin produced a marked pro-inflammatory surge, consistent with oxidative and mitochondrial stress activating NF-κB-dependent glial cytokine release that amplifies neuronal injury through downstream JNK and JAK/STAT signalling [37]. The robust suppression of TNF-α by NSO, evident in the pretreatment, post-treatment, and NSO-only groups alike, corroborates reports that Nigella sativa oil mitigates cisplatin-associated neuroinflammation [18] and possesses potent immunomodulatory, anti-neuroinflammatory properties in other models of central nervous system inflammation [38], [39]. This suggests that suppression of neuroinflammation, rather than restoration of the primary enzymatic antioxidant defences alone, may be a principal and more readily demonstrable mechanism underlying NSO's protective action in this model.

SDH, a component of both the tricarboxylic acid cycle and the electron transport chain, is highly sensitive to oxidative and structural mitochondrial injury owing to its dependence on intact iron–sulphur clusters [11]. Its significant reduction in the cisplatin-only group provides direct biochemical evidence of mitochondrial dysfunction in the PFC, consistent with cisplatin-induced neurotoxicity reported in other CNS regions [30], while its significant restoration in both the pretreatment and post-treatment groups indicates that NSO protects mitochondrial respiratory

capacity regardless of the timing of administration relative to cisplatin exposure—a finding of direct relevance to the clinical scenario in which adjunctive protection is typically introduced only after chemotherapy has commenced. The concurrent elevation of LDH, a cytosolic enzyme released upon membrane compromise [12], and its attenuation by NSO corroborates this mitochondrial and membrane-level protection, strengthening the internal coherence of the biochemical dataset: cisplatin produces impaired mitochondrial enzyme activity accompanied by increased membrane leakage, and NSO measurably counters both dimensions of this injury.

The histomorphological and histochemical findings provide structural corroboration of the biochemical dataset. Pyknosis reflects irreversible chromatin condensation associated with apoptotic and necrotic injury, while vacuolation reflects osmotic and organellar swelling driven by oxidative and metabolic stress; both features represent the structural correlate of the elevated MDA and LDH, suppressed SDH, and markedly elevated TNF-α documented in the cisplatin-only group. The graded reduction in pyknosis and vacuolation across the pretreatment, post-treatment, and NSO-only groups closely parallels the graded biochemical improvement in these same markers, consistent with cisplatin-induced pyknosis, vacuolation, and neuropil disruption reported elsewhere in the rodent PFC, hippocampus, and cerebellum [30]. That gliosis was not detected in any group, including the cisplatin-only group, suggests that the dose and 28-day duration employed produced an early-to-moderate degenerative phenotype dominated by neuronal pyknosis and vacuolation without progression to overt reactive astrogliosis, a finding that may reflect the relatively short exposure window rather than a true absence of glial involvement.

Considered jointly, the biochemical markers showing the strongest, most timing-independent responses to NSO—TNF-α, SDH, and LDH—correspond closely to the histological gradient of neuronal preservation (control > NSO-only ≈ post-treatment > pretreatment > cisplatin-only), more closely than the more equivocal SOD/CAT findings. This convergence suggests that suppression of TNF-α–driven neuroinflammation and preservation of mitochondrial respiratory and membrane integrity are the more proximate and readily demonstrable correlates of NSO's structural neuroprotection in this model, while restoration of the classical enzymatic antioxidant defences may require a longer treatment duration, a higher dose, or more sensitive detection to be fully established. The contrasting cerebellar findings reported for quercetin, protective in one study [19] but ineffective or deleterious in another [20], reinforce that the neuroprotective efficacy demonstrated here for NSO against cisplatin-induced PFC injury cannot be assumed to generalise to structurally related phytochemicals or other brain regions without direct experimental confirmation.

This study has limitations that should inform its interpretation. The model was restricted to adult male Wistar rats over a single 28-day protocol and dosing regimen, precluding assessment of sex-related variation or longer-term, more clinically representative dosing. Functional correlates of the observed biochemical and structural protection—behavioural and cognitive performance—were not assessed, and pathway-specific molecular markers (e.g., Nrf2/HO-1, Bax/Bcl-2, caspase-3, GFAP) were not evaluated, limiting mechanistic resolution beyond the biomarker panel employed. Whether NSO co-administration compromises the antitumour efficacy of cisplatin was also outside the scope of this study and remains an essential question for translational consideration.

V. CONCLUSION

Nigella sativa oil demonstrated significant and biologically coherent neuroprotective effects against cisplatin-induced prefrontal cortical toxicity in adult Wistar rats, most decisively for TNF-α, SDH, LDH, and histomorphological/histochemical integrity, and to a more circumscribed extent for MDA, while effects on SOD, CAT, and absolute brain weight, though favourable in direction, did not reach statistical significance. These findings support rejection of the null hypothesis for the inflammatory, mitochondrial, lipid-peroxidation, and body-weight parameters, for which NSO produced statistically significant, biologically coherent mitigatory effects, while the null hypothesis cannot be rejected for the enzymatic antioxidant markers SOD and catalase. Protection was evident whether NSO was administered before or after cisplatin exposure, a timing-independence with direct relevance to its potential application as an adjunct introduced either prophylactically or once chemotherapy has already begun.

Future work should incorporate additional molecular markers (Nrf2/HO-1, Bax/Bcl-2, caspase-3), behavioural and cognitive assessments, and dose-response and longer-duration protocols to establish the optimal regimen for consistent protection across all biomarkers, including SOD and CAT. Critically, the pharmacokinetic interaction between Nigella sativa oil and cisplatin, and in particular whether co-administration compromises cisplatin's antitumour efficacy, must be resolved before any translational application can be responsibly considered. Overall, Nigella sativa oil can be reasonably concluded to possess mitigatory potential against cisplatin-induced prefrontal cortical toxicity, supporting its further investigation as a candidate adjunctive neuroprotective agent in platinum-based chemotherapy regimens.

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Conflict of Interest
The authors declare no conflict of interest.

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