ISRG JOURNAL OF CLINICAL MEDICINE AND MEDICAL RESEARCH [ISRGJCMMR]

ISRG PUBLISHERS
Abbreviated Key Title: ISRG J Clinic.Medici.Medica.Res.
ISSN: 3048-8850 (Online)
Journal homepage: https://isrgpublishers.com/cmmr/
Volume – III, Issue - VI (November-December) 2026
Frequency: Bimonthly



Assessment of the Neuroprotective Effect of Rutin Hydrate Against Cisplatin-Induced Neurotoxicity on the Hippocampus of Adult Wistar Rats

Joshua A. Ajao[1], Ponle B. Fakunle[2], Ridwan Hammed Ajao[1], Ovioke David Temidayo[1], and Adeniji Adeola Boluwatife[1]

[1] Department of Human Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University (Shagamu Campus) Ago Iwoye, Ogun State, Nigeria.

[2] Olabisi Onabanjo University.

| **Received:** 13.10.2026 | **Accepted:** 17.10.2026 | **Published:** 11.11.2026

***Corresponding author:** Joshua A. Ajao

Abstract

AIM: *To assess the effects of rutin hydrate on cisplatin-associated biochemical and histological alterations in the hippocampus of adult Wistar rats, with particular attention to the time-matched pre-treatment comparison.*

METHODS: *Thirty adult male Wistar rats were allocated to five treatment groups. The study included a vehicle control, cisplatin-only group, post-cisplatin rutin group, pre-treatment rutin-plus-cisplatin group, and rutin-only group. Hippocampal tissues were examined by haematoxylin and eosin and Cresyl Violet staining, and SOD, MDA, SDH and LDH activities were assessed.*

RESULTS: *The reported analyses showed significant between-group differences in SOD, MDA, SDH and LDH (all $p < 0.0001$) and absolute brain weight ($p = 0.0011$), whereas relative brain weight was not significantly different ($p = 0.2544$). Cisplatin-only animals showed lower SOD and SDH and higher MDA and LDH than controls. In the time-matched comparison, the pre-treatment group had higher SOD and SDH and lower MDA and LDH than cisplatin alone. The post-cisplatin group was sacrificed 15 days after cisplatin, whereas the cisplatin-only group was assessed 24 h after dosing; therefore, that comparison cannot establish post-treatment protection.*

CONCLUSION: *The findings indicate that rutin hydrate pre-treatment was associated with attenuation of several cisplatin-related biochemical alterations. The experiment does not provide a time-matched test of post-cisplatin protection, and the adverse oxidative-stress profile observed in the rutin-only group requires further verification.*

Keywords: *cisplatin; rutin hydrate; hippocampus; neurotoxicity; oxidative stress; Wistar rats; mitochondrial enzyme activity.*

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DOI: 10.5281/zenodo.24126589

Introduction

Cisplatin (cis-diamminedichloroplatinum[II]) remains an important platinum-based chemotherapeutic agent used against several solid tumours. Its clinical utility is accompanied by toxic effects in multiple organs, including the nervous system. Although cisplatin-associated neurotoxicity is classically recognized as peripheral sensory neuropathy, experimental evidence also indicates effects within the central nervous system and hippocampus, including cognitive and biochemical disturbances.[1,2,3,15]

The hippocampus is a highly organized brain region involved in learning, memory and spatial processing, making it a useful structure for investigating chemotherapy-associated neural injury.[4,5,6] Oxidative stress is an important component of cisplatin-related neural injury, with experimental studies reporting increased reactive oxygen species and lipid peroxidation together with impaired antioxidant defence and mitochondrial enzyme activity.[2,3,7]

Superoxide dismutase (SOD) represents an endogenous antioxidant defence, whereas malondialdehyde (MDA) is commonly used as an index of lipid peroxidation. Succinate dehydrogenase (SDH) provides information on mitochondrial enzyme activity. In contrast, tissue lactate dehydrogenase (LDH) activity reflects enzyme activity within the homogenate and should not, by itself, be interpreted as direct evidence of membrane leakage or cell injury.

Rutin hydrate is a flavonoid glycoside with reported antioxidant, anti-inflammatory and neuroprotective properties. Experimental work has shown that rutin can improve glutamate uptake and reduce glutamate excitotoxicity in rat brain slices, while other studies have described effects on neuroinflammatory pathways.[8,9] Rutin has also been investigated in cisplatin-related toxicity in peripheral nerve, heart, testis and cochlear models.[10–13]

An earlier rat study investigated rutin in a cisplatin neurotoxicity model using 30 mg/kg rutin and 5 mg/kg cisplatin.[14] The present study used 100 mg/kg rutin hydrate and 2.5 mg/kg cisplatin and included both post-cisplatin and pre-cisplatin schedules.

This study examined the effects of rutin hydrate on cisplatin-associated changes in adult Wistar rat hippocampus, focusing on body and brain weight, hippocampal histology, oxidative stress markers and selected metabolic enzyme activities. The pre-treatment regimen was interpreted using the time-matched cisplatin-only group; the post-cisplatin regimen was treated as a descriptive comparison because its sacrifice time was not matched to the cisplatin-only group.

Experimental

Animals and experimental design

Thirty adult male Wistar rats weighing 150g–220g were obtained from a registered animal facility and acclimatized for two weeks. Animals were housed under standard laboratory conditions with free access to standard chow and clean drinking water at approximately 22 ± 2 °C under a 12-h light/dark cycle. Thirty animals were allocated at study start, with six animals (n=6) assigned to each of the five treatment groups. Although adult male wistar rats were used for the study; sex effects were not investigated, so the findings should not be assumed to generalize to female animals.

The study began with six animals (n=6) per group (30 animals total), No formal a priori power calculation was performed; the group size was determined according to the available experimental resources, tissue requirements and the study protocol. Animals were selected according to the predefined study criteria and acclimatized for two weeks before treatment. Body weight was monitored during acclimatization, and animals meeting the study eligibility criteria at the time of allocation were assigned to the experimental groups.

Six animals (n=6) were initially assigned to each experimental group. For biochemical analyses, three animals (n=3) per group were selected for hippocampal tissue analysis, while two animals (n=2) per group were initially designated for histological processing. Where a selected histological specimen was unsuitable for processing or assessment, the remaining animal from the same experimental group was used as a replacement.

Table 1. Experimental groups, doses, routes and treatment schedules.

Group	Treatment	Dose/route	Schedule
I	Vehicle control	0.5% CMC, oral	Daily vehicle
II	Cisplatin	2.5 mg/kg, intraperitoneal	Cisplatin day 15; sacrificed 24 h later on day 16
III	Cisplatin + rutin hydrate	Cisplatin 2.5 mg/kg IP; rutin 100 mg/kg oral	Cisplatin day 1; rutin days 2–15; sacrificed day 16 (15 days after cisplatin)
IV	Rutin hydrate + cisplatin	Rutin 100 mg/kg oral; cisplatin 2.5 mg/kg IP	Rutin days 1–14; cisplatin day 15; sacrificed day 16 (24h after cisplatin)
V	Rutin hydrate	100 mg/kg, oral	Days 1–14; sacrificed day 15

Group II and Group IV were both evaluated 24 h after cisplatin exposure, allowing a time-matched comparison of cisplatin alone with the pre-treatment regimen. Group III was evaluated 15 days after cisplatin and therefore is not a time-matched test of post-treatment protection.

Rutin hydrate was prepared daily as a suspension in 0.5% carboxymethyl cellulose (CMC). The suspension concentration was 20 mg/mL, corresponding to an intended dose of 100 mg/kg body weight. 20mg/mL concentration was considered due to the smaller stomach volume of the study animal and ad libitum feeding and drinking. For each animal, the administration volume was calculated as: volume (mL) = [body weight (kg) × 100 mg/kg] / 20 mg/mL. The control group received the vehicle. (Mostafa D.G. et al.)

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Ethical approval and sample processing

Ethical approval was granted by the Departmental Committee on Animal Research, Department of Anatomy, Lead City University, Ibadan, Nigeria on 24/08/2026, with approval reference number LCU/FBMHS/ANA/REC/2026/050. Procedures followed institutional requirements and the National Institutes of Health Guide for the Care and Use of Laboratory Animals, with measures taken to minimize discomfort, stress and pain.

Animals were observed throughout the experimental period for general health and treatment-related changes. Body weight and visible clinical signs were monitored during the study, with particular attention to changes in activity, feeding, grooming and general condition. Any adverse events, unexpected morbidity or mortality were recorded. Animals were monitored in accordance with the welfare requirements specified in the approved experimental protocol.

At the end of each experimental period, animals were euthanized under moderate anesthesia (0.1ml of 50mg/ml Ketamine intraperitoneal 'IP') followed by cervical dislocation. Brain samples were removed, weighed and recorded, and relative brain weight was calculated as [brain weight/final body weight × 100]. Brain specimens of three (n=3) animals from each group were used for biomarker analysis while brain samples from two (n=2) animals were allocated for histological processing, with the one last brain specimen from each group serving as substitute for erred samples during histological processing procedure.

Histological analysis

Brain tissues were fixed in 10% formol-calcium for about 48 h, processed using standard paraffin-embedding procedures and sectioned at 5µm. Haematoxylin and eosin staining was used to assess general hippocampal cytoarchitecture and neuronal morphology, while Cresyl Violet staining was used to assess Nissl substance and neuronal integrity. The dentate gyrus, CA1 and CA3 regions were examined microscopically.

Biochemical analysis and Statistics

Hippocampal tissue was assessed for SOD activity, MDA/lipid peroxidation, SDH activity and LDH activity.

SOD: SOD activity was determined using a Bio-diagnostic kit (Cat. SD 25 21), based on inhibition of nitroblue tetrazolium reduction, with absorbance measured at "560 nm for 5 min at 25 °C". SOD was expressed as "U/mg protein" after normalization to tissue protein concentration. ([Nature][2])

MDA: MDA activity was determined using a Bio-diagnostic kit (Cat. MD 2529), based on the reaction of MDA with thiobarbituric acid to produce a pink-coloured complex measured at "534 nm". ([Nature][2])

SDH: SDH activity was determined using the "Abcam colorimetric kit (Cat. ab228560)", which is based on conversion of succinate to fumarate with transfer of electrons to an artificial electron acceptor. ([Nature][2])

LDH: LDH activity was determined using the "Elabscience colorimetric kit (Cat. E-BC-K046-M)", based on conversion of lactate to pyruvate, followed by formation of a colored pyruvate–dinitrophenylhydrazone product. The resulting color intensity is related to LDH activity. ([Nature][2])

Brain-tissue preparation: A published rat-brain protocol homogenized tissue in "1 mL of 0.1 M phosphate buffer (pH 7.4)", centrifuged the homogenate at "10,000 rpm for 15 min", and stored the resulting supernatant at "−20 °C" before biochemical analysis. ([Nature][2])

Protein normalization: Protein concentration was determined using the "Lowry method", and biochemical measurements were corrected according to the corresponding total protein concentration. ([Nature][2])

Results were expressed as mean ± standard error of the mean (SEM). One-way ANOVA was done and a Tukey Post hoc analysis was done to compare result between groups.

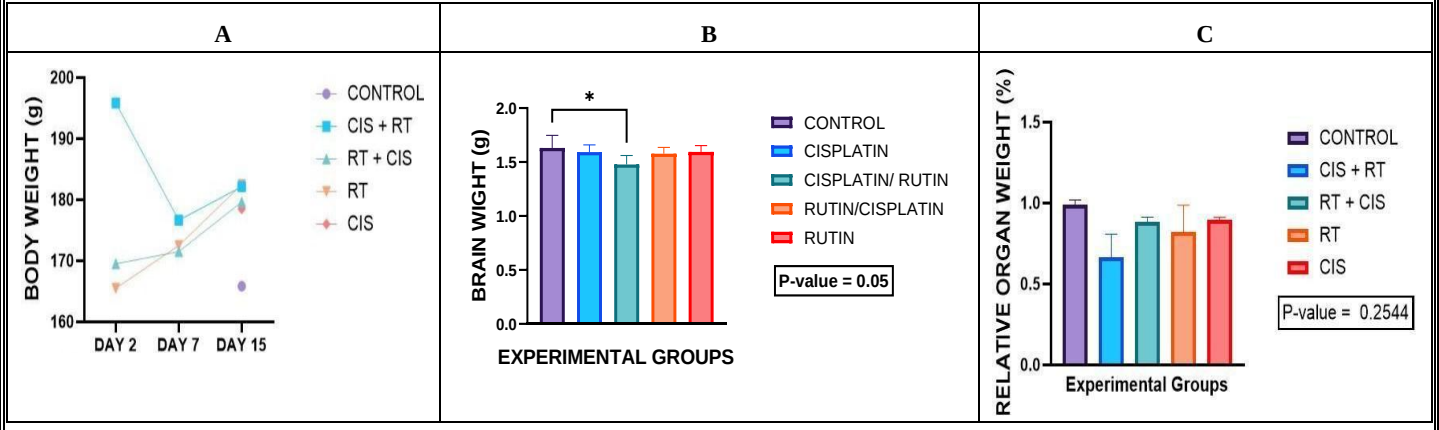
Results

Body, brain and relative brain weights

Body weight was recorded on days 2, 7 and 15. The day-2 measurement should not be described as a common baseline because Group III had already received cisplatin on day 1, and other treatment groups had also begun their assigned regimens. Group III showed a marked reduction in body weight from day 2 to day 7, followed by an increase by day 15.

Absolute brain weight differed significantly among groups ($p = 0.0011$). Mean values were 1.632 g in controls, 1.478 g in the cisplatin + rutin group, 1.577 g in the rutin + cisplatin group, 1.782 g in the rutin-only group and 1.592 g in the cisplatin-only group.

Figure 1. Body weight trajectory, absolute brain weight and relative brain weight across experimental groups. (A) Body weight on days 2, 7 and 15; (B) absolute brain weight; (C) relative brain weight.



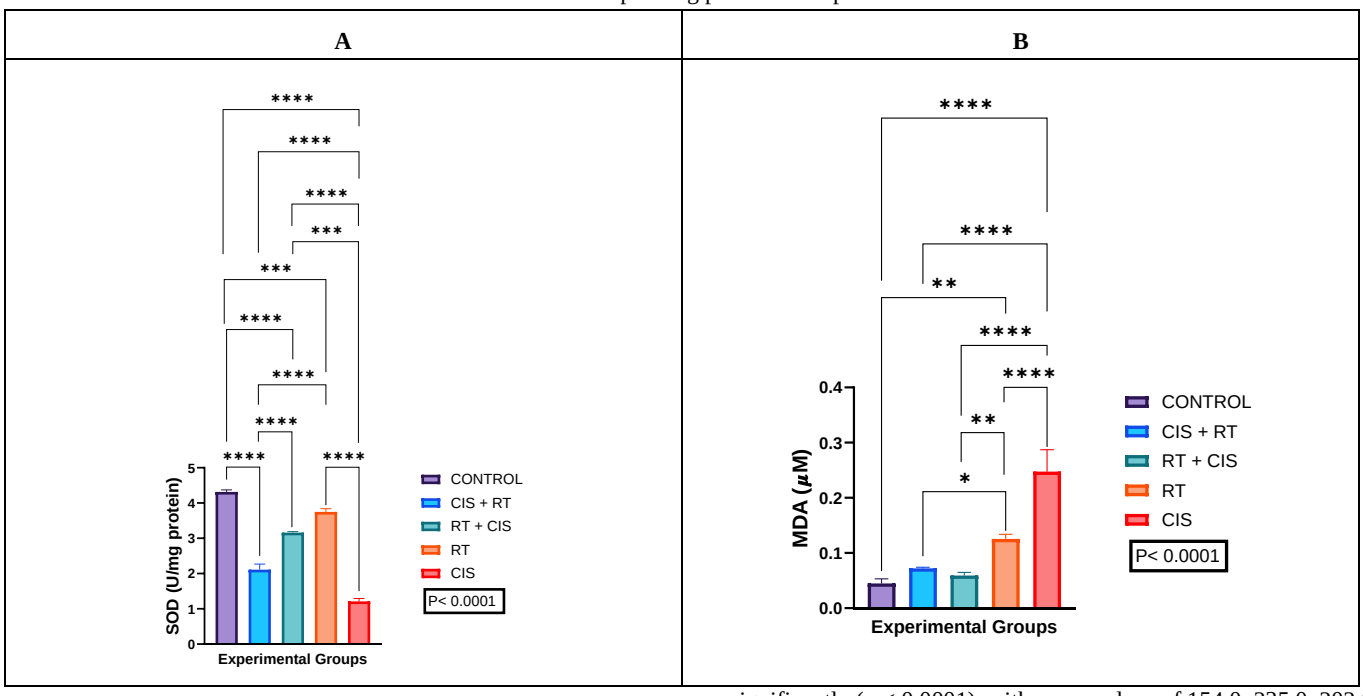
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Oxidative stress and metabolic enzyme activities

SOD activity differed significantly among groups ($p < 0.0001$). Mean SOD activity was 4.314 U/mg protein in controls, 3.158 in cisplatin + rutin, 3.749 in rutin + cisplatin, 1.209 in rutin alone and 2.109 in cisplatin alone.

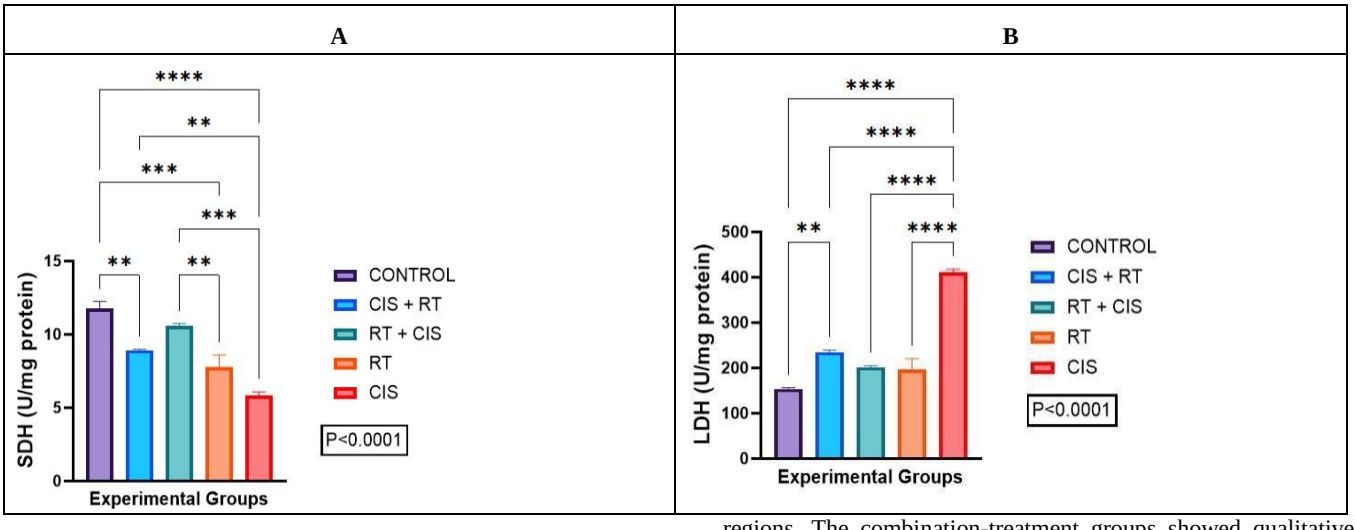
MDA concentration differed significantly among groups ($p < 0.0001$). Mean values were 0.04492 µM in controls, 0.07220 µM in cisplatin + rutin, 0.05909 µM in rutin + cisplatin, 0.2476 µM in rutin alone and 0.1254 µM in cisplatin alone.

Figure 2. Oxidative stress markers across experimental groups. (A) Superoxide dismutase (SOD) activity; (B) malondialdehyde (MDA) concentration. Values are presented as mean ± SEM. Statistical symbols: *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001; brackets indicate the corresponding pairwise comparisons.



SDH activity differed significantly among groups ($p < 0.0001$). Mean values were 11.79 U/mg protein in controls, 8.930 in cisplatin + rutin, 10.56 in rutin + cisplatin, 7.767 in rutin alone and 5.823 in cisplatin alone. LDH enzyme activity also differed significantly ($p < 0.0001$), with mean values of 154.0, 235.0, 202.0, 196.4 and 411.0 U/mg

Figure 3. Mitochondrial and metabolic enzyme activities across experimental groups. (A) Succinate dehydrogenase (SDH) activity; (B) lactate dehydrogenase (LDH) activity. Values are presented as mean ± SEM. Statistical symbols: *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001; brackets indicate the corresponding pairwise comparisons.



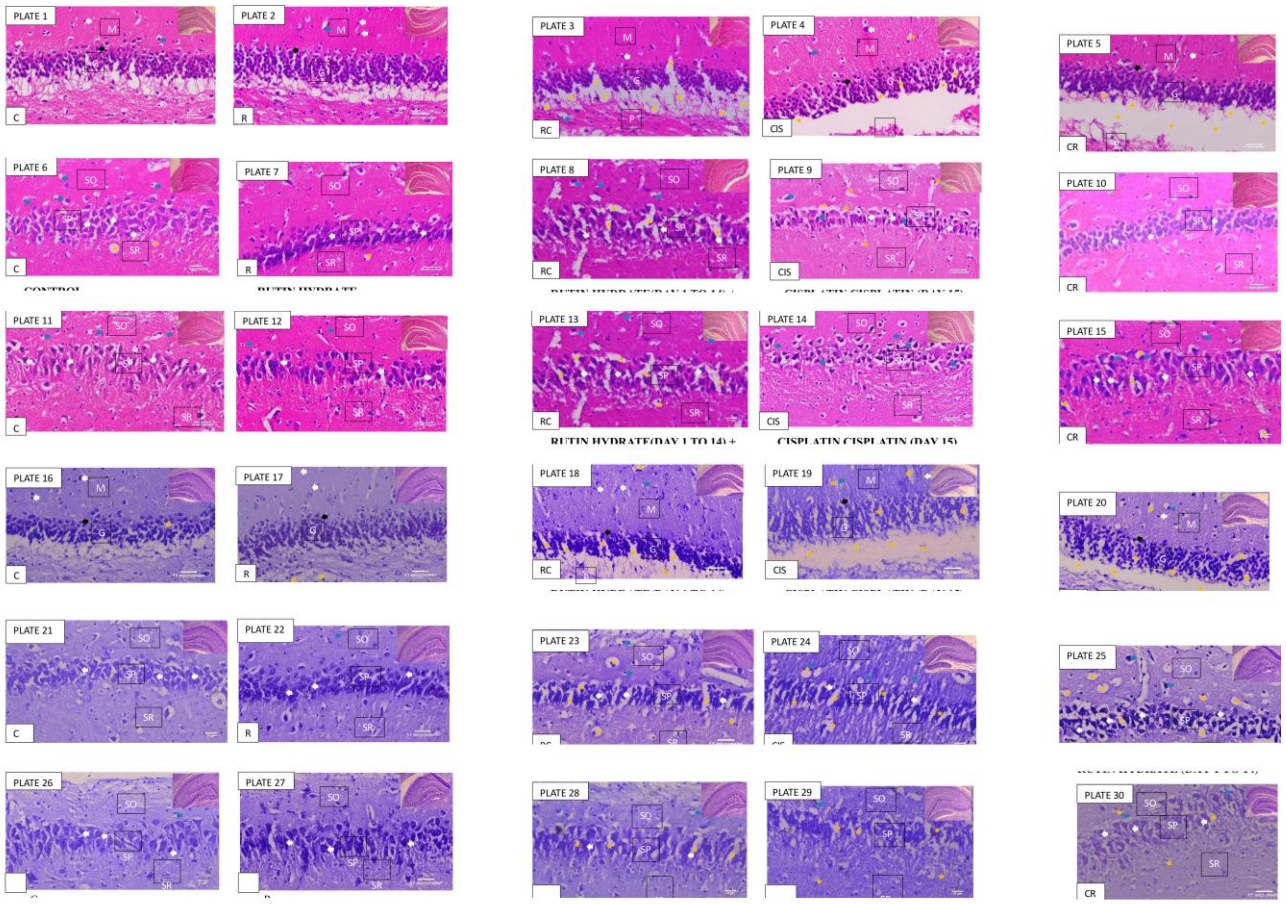
Hippocampal histology

H&E sections from control animals showed preserved hippocampal architecture. Cisplatin exposure was associated with pyknotic neurons, vacuolation, irregular cellular spacing and altered neuronal morphology in the dentate gyrus, CA1 and CA3 regions. The combination-treatment groups showed qualitatively fewer apparent abnormalities than the cisplatin-only group.

Cresyl Violet staining similarly showed relatively preserved neuronal morphology and Nissl substance in the combination groups compared with cisplatin exposure.

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Figure 4. Representative hippocampal photomicrographs from the experimental groups. H&E staining is shown for the dentate gyrus, CA1 and CA3 regions, followed by Cresyl Violet staining of the same regions. Scale bars are shown on the photomicrographs. The plate codes in the original montage are mapped as follows: C = control; R = rutin hydrate; RC = rutin hydrate + cisplatin; CIS = cisplatin; CR = cisplatin + rutin hydrate. Micrographs were taken at X40 and X400 magnification.



Discussion

The present findings indicate that cisplatin exposure was associated with biochemical and structural alterations in the hippocampus. The cisplatin-only group showed lower SOD and SDH activity and higher MDA and LDH activity than the control group. The reduction in SOD is consistent with impaired antioxidant defence, while the higher MDA concentration is compatible with increased lipid peroxidation. These observations fit the broader evidence that oxidative stress contributes to cisplatin-related neurotoxicity.

The SDH results indicate altered mitochondrial enzyme activity. Cisplatin alone produced the lowest SDH activity, whereas both combination regimens showed higher values. In the time-matched pre-treatment comparison, the rutin-plus-cisplatin group also had lower MDA and LDH activity and higher SOD and SDH activity than cisplatin alone. These findings are consistent with attenuation of several biochemical alterations when rutin was given before cisplatin. They do not, however, establish that the post-cisplatin regimen was protective, because Group III was assessed 15 days after cisplatin whereas Group II was assessed 24 h after dosing.

The histological observations provide structural context for the biochemical results. Pyknotic neurons, vacuolation and altered cellular organization were most apparent after cisplatin exposure, while the combination groups appeared comparatively preserved.

The rutin-only group requires particular attention. It had the lowest SOD activity and the highest MDA concentration in the experiment, and its SDH activity was also below the control value. Although the histological sections were described as largely preserved, the biochemical pattern does not support an uncomplicated beneficial effect of rutin at 100 mg/kg in this experiment.

The present findings differ from the earlier rat study by Almutairi et al., which used 30 mg/kg rutin and 5 mg/kg cisplatin and evaluated gene-expression outcomes in brain tissue. The present experiment used 100 mg/kg rutin hydrate and 2.5 mg/kg cisplatin and focused on hippocampal biochemical and histological outcomes. These differences in dose, treatment schedule and endpoints limit direct comparison between the studies.

The findings can also be considered alongside reports describing rutin-related modulation of glutamate uptake and neuroinflammatory signalling.[8,9] However, the present study did not directly measure glutamate transport, inflammatory cytokines or apoptosis. These mechanisms should therefore be regarded as possible explanations supported by the wider literature rather than as mechanisms demonstrated by this experiment.

Conclusion

Within the time-matched pre-treatment comparison, rutin hydrate administration was associated with higher SOD and SDH activity and lower MDA and LDH activity than cisplatin alone, alongside qualitatively better hippocampal architecture. The experiment does not provide a time-matched assessment of post-cisplatin protection,

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and the rutin-only group showed an adverse oxidative-stress profile that requires verification. The findings therefore support further investigation of rutin hydrate as a potential modulator of cisplatin-associated hippocampal alterations, rather than establishing a general neuroprotective effect across both treatment schedules.

Acknowledgements

The author acknowledges the Department of Human Anatomy, Lead City University, Ibadan, and all individuals who contributed to the conduct of the research. The study was self-funded by the researcher.

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DOI: 10.5281/zenodo.24126589