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Landolphia owariensis Reduces Alcohol-Induced Neuroinflammation, Oxidative Stress, and Modulates GFAP and NF Expressions in the Prefrontal Cortex of Rats Exposed to Binge Alcohol

Charles A. Oyinbo[1], Tamuno-Olobo Johnbull[1], Abraham S. Ebor[2], Atoni A. Dogood[1], Daminola A. U. Francis[1]

[1]Department of Human Anatomy, [2]Departments of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Nigeria

ABSTRACT

Alcohol use disorder (AUD), a neurodegenerative-driven mental health condition, lacks satisfactory treatment, prompting the exploration of polypharmacological approaches. Medicinal plants offer a natural solution for polypharmacology, as they contain multiple pharmacologically active compounds, thus eliminating the complexities and risks of combining multiple synthetic pharmaceutical agents. This study investigated the neuroprotective potential of Landolphia owariensis (LO), a medicinal plant, on alcohol-induced neurodegeneration. Thirty-two male rats were randomly allocated into three groups (n = 10 per group) and subjected to a four-day binge alcohol regimen. The control rats received daily oral doses of 5 g/kg of a nutritional shake diet (NSD), Vitamilk®. A set of rats was given 5 g/kg of 25% ethanol diluted NSD 50% v/v. The last cohort of rats received an oral dosage of 100 mg of LO and 5 g/kg of 25% ethanol in diluted (50% v/v) NSD. Rats were sacrificed at the end of the 4th day; brain samples were isolated and subjected to histological, immunohistochemical, and biochemical analysis. Results revealed that alcohol intake increased lipid peroxidation and neurodegeneration in the prefrontal cortex (PFC), with elevated glial fibrillary acidic protein (GFAP) and neurofilament (NF) expression. In contrast, LO administration reduced lipid peroxidation and neurodegeneration, downregulating GFAP and NF protein expression. These findings demonstrate the neuroprotective effects of LO and suggest its potential development as a supplementary agent for AUD management. The mechanism underlying this neuroprotection may partly involve the downregulation of GFAP and NF protein expression, highlighting that LO has the potential to modulate neuroinflammatory responses.

Keywords

Alcohol use disorder, Neuroinflammation, Medicinal plant, Nutraceuticals, Landolphia owariensis

Correspondence: Charles A. Oyinbo, PhD. Department of Human Anatomy, Faculty of Basic Medical Sciences, Niger Delta University, P.M.B. 071 Yenagoa, Nigeria. E-mail: charles.oyinbo@ndu.edu.ng; Phone Number:+234-8134752922; ORCID: 0000-0001-7911-1392 Tamuno-Olobo Johnbull – johnbulltammy@ndu.edu.ng, 0000-0001-6127-0301; Abraham S. Ebor - eboh.abraham@ndu.edu.ng, 0009-0005-0613-0388 Atoni A. Dogood - atoniatoni@ndu.edu.ng, 0000-0002-1352-5055, Daminola A. U. Francis - obomuriah@gmail.com, 0009-0000-4275-305X

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INTRODUCTION

The accessibility of alcohol has increased due to the proliferation of diverse alcoholic cocktails presented in convenient and secure packaging. Recently, Nigerian authorities expressed concern over a potential rise in alcohol-related crime and juvenile delinquency, similar to trends observed in India (Singh and Bhadra, 2022; NAFDAC, 2024). Reports suggested that alcoholic beverages are readily available in convenient sizes and ready-to-drink packaging in stores adjacent to and within university campuses (Odeigah and Patton, 2024). A concerning trend has surfaced, with recent research revealing a notable increase in alcohol consumption among undergraduates (Ay *et al*., 2025). Indeed, the majority of university students are 18 or older and assume legal responsibility; however, behavioural or binge alcohol consumption has significant consequences. Binge drinking is linked to impaired judgement, neglect of

duties, and cognitive deficits (MacKillop *et al*., 2022). Additionally, it leads to alcohol use disorders (AUDs), which are associated with neurodegeneration in various brain regions (MacKillop *et al*., 2022). The prefrontal cortex (PFC) is our main focus of investigation because of its susceptibility to alcohol-induced neurodegeneration (Fowler *et al*., 2014). This brain region is responsible for executive function, decision-making, and emotional regulation, all of which are compromised in individuals with alcohol consumption disorder (Araujo *et al*., 2021). Excessive alcohol use inflicts considerable harm on the PFC due to neuroinflammation and oxidative stress, resulting in neurodegeneration (Tsermpini *et al*., 2022; Ajayi *et al*., 2023). Excessive alcohol intake, such as binge drinking, activates glial cells, causing the production of pro-inflammatory cytokines and increased expression of glial fibrillary acidic protein (Yang and Zhou, 2019; Guerin *et al*., 2023). Furthermore, alcohol metabolism produces reactive oxygen species, leading to oxidative stress and lipid peroxidation, which contribute to the onset of alcohol use disorder.

The treatment of AUD remains a momentous challenge because no satisfactory pharmacological intervention is available (Mason, 2022). The multifaceted factors that drive the development of an AUD are responsible for this impediment (Mason, 2022; Lohoff, 2022). Consequently, there is a growing interest in exploring alternative therapeutic opportunities, with botanical remedies offering promising potential (Sahoo, 2018).

Landolphia owariensis P. Beauv, is a tropical African plant with promises; it possesses anti-inflammatory, antioxidant, and analgesic properties (Baumgärtel and Lautenschläger, 2023; Oladeji *et al*., 2024) and also certain neuroprotective properties (Oyinbo *et al*., 2016). It is a plant species found in the rainforests of West and Central Africa. It belongs to the Apocynaceae family. This climber, characterised by its long, slender stems, navigates through the dense forest canopy, utilising available supports to ascend. The leaves of *L. owariensis* (LO) are elliptical or ovate, featuring a pointed tip and smooth margin. The plant's floral arrangement consists of small, greenish-yellow flowers clustered together in a manner indicative of its Apocynaceae lineage (Okonkwo *et al*., 2016). Documented ethnomedicinal use of LO includes treatment of fever, rheumatism, and digestive issues (Baumgärtel and Lautenschläger, 2023; Oladeji *et al*., 2024). The latex of the plant is used as an anthelmintic (Baumgärtel and Lautenschläger, 2023), and the sap expressed from the leaves is employed to alleviate symptoms of giddiness and epilepsy (Oladeji *et al*., 2024). People consume a decoction of the roots as a purgative and to treat urethral discharge. Furthermore, this decoction is used in steam baths to alleviate feverish aches. The leaves, when boiled, are applied topically to treat sprains (Atawodi and Alafiatayo, 2007; Oladeji *et al*., 2024).

The medicinal properties of LO can be attributed to its diverse phytochemical constituents, which include alkaloids, glycosides, and phenolic acids (Baumgärtel and Lautenschläger, 2023; Oladeji *et al*., 2024). The pharmacological properties of LO include antibacterial, antioxidant, anti-inflammatory, analgesic, antiulcer, and anti-secretory actions in the stomach (Ezike *et al*., 2026). However, we had described a neuroprotective function in alcohol-induced cerebellar assault (Oyinbo *et al*., 2016). Research suggests that LO may possess significant therapeutic potential, warranting further investigation into its pharmacological activities and their potential applications. The primary endeavour of this study is to evaluate the neuroprotective effect of LO using a behavioural binge-alcohol rat model of alcohol-induced neurodegeneration, with a specific focus on assessing its potential to mitigate neuroinflammation and oxidative stress.

MATERIALS AND METHODS

Plant Collection and Identification

LO (woody climber) was collected from its natural habitat in Amassoma, Nigeria. On-site identification of the plant was conducted by a botanist affiliated with the Department of Pharmacognosy and Herbal Medicine, Niger Delta University, Nigeria. The sample was later authenticated in the departmental herbarium and deposited with voucher specimen number NDUP/2024/027. Plant materials were identified and vouchered according to established protocols (Adewunmi and Sofowora, 1980).

Extraction of LO

Extracts of LO were obtained by a previously described common protocol (Syahputra *et al*., 2022). Briefly, the bark of LO was cleaned with water to remove dirt and debris. It was then oven-dried at 45°C and ground into a coarse particle. Two hundred and fifty grams of the coarse particles were macerated in ethanol (70%) for 48 h at an ambient temperature of 24-32°C. The extract was concentrated by vacuum filtration at 35°C, followed by drying in a desiccator containing silica gel. The per cent yield was 7.7% (19.3 g). The ethanol used was of analytical grade (AnalaR, UK).

Dose Determination

The dose of LO was chosen based on its knowledge of its LD_{50}, which was previously determined to be over 3,000 mg/kg in rats (Oyinbo *et al*., 2016). Several studies also reported a similar range of over 3,000 to 5,000 mg/kg (Okonkwo *et al*., 2016; Ismael *et al*., 2020). The long-term goal of this study was to explore the potential of LO as a botanical dietary supplement. Consequently, we selected a 100 mg/kg dose for this evaluation.

Rationale for Vitamilk® in the 4-day Binge Alcohol Model

There are documented studies on the use of Ensure® original vanilla nutritional shake as the sole source of nutrition during the binge alcohol model of neurodegeneration (Obernier *et al*., 2002; Marshall *et al*., 2013; Tajuddin *et al*., 2014). In our laboratory, we have employed both Vanilla Ensure® and Vitamilk® as nutritional shakes in this binge rodent model (Charles *et al*., 2016; Oyinbo *et al*., 2016; Robert *et al*., 2017; Oyinbo *et al*., 2018; Robert and Oyinbo, 2018). Both nutritional shakes exhibit comparable calorie contents and essential vitamins, with vanilla Ensure® (237 mL) providing 220 calories, 6 g of fat, 32 g of carbohydrates, and 9 g of protein, whereas Vitamilk® (300 mL) offers 240 calories, 10 g of fat, 29 g of carbohydrates, and

9 g of protein (Abbott Nutrition, 2024; Nutritionix, 2024). For this study, Vitamilk® was selected as the nutritional shake during the 4-day binge treatment due to its availability and comparable calorie content to vanilla Ensure®. The similar nutritional profiles of both brands make Vitamilk® shake a suitable alternative that can also sustain rats in the 4-day binge alcohol model.

Animal Groupings

Thirty male Sprague Dawley (SD) rats (220-250 g) underwent a four-day binge alcohol rodent model of AUD as previously described (Obernier *et al*., 2002; Crews and Nixon, 2009; West *et al*., 2021). Briefly, the rat was randomized into three groups: control, alcohol-exposed, and alcohol-exposed plus LO treatment. The control SD rats were administered 5 g/kg of a nutritional shake diet (NSD, Vitamilk®). The rats in the alcohol-exposed and alcohol-exposed plus LO treatment groups received 5 g/kg of ethanol orally (25% w/v in diluted NSD at 50% v/v). In addition, the latter group received LO (100 mg/kg) orally in distilled water as a supplement once daily. These treatments, except LO supplementation, were carried out every eight hours for four days. Water was freely accessible for rats, but they were denied their normal rat ration for the four days of diet administration (Obernier *et al*., 2002; Marshall *et al*., 2013; West *et al*., 2021). The alcohol-exposed rats without LO supplementation (AER) and the LO-supplemented (LOS) rats were observed for signs of alcohol intoxication (Knapp and Crews, 1999). All administration was oral via an orogastric tube.

Brain Isolation

At the end of the fourth day, rats were terminally anaesthetised with ketamine (75 mg/kg) and diazepam (2.5 mg/kg) (ip). Intracardiac perfusion (n = 5 per group) was done routinely with phosphate buffer formalin. These subsets of the brain were carefully dissected out and fixed in the same fixative for 48 h in preparation for histological study. The rest of the brains (n = 5 per group) were used for biochemical study.

Histological Study

Brain samples underwent routine paraffin section processes. Sections of 5 µm were obtained for a haematoxylin and eosin (H&E) examination. Sectioning for immunohistochemical labelling was done at 3 µm. The tissue process for H&E was done in line with well-established protocols (Cardiff *et al*., 2014).

Immunohistochemical Study

Neuroinflammation was examined by two central nervous systems (CNS) microfilament labellings. These were the IHC labelings of neurofilament (NF) and glial fibrillary acidic protein (GFAP) in paraffin-embedded brain tissue sections for the study of neuroinflammation. The avidin-biotin immunoperoxidase technique (Hsu *et al.*, 1981; Chauhan *et al*., 2024) was employed to label NF and GFAP antigens in paraffin-embedded tissue sections. Sections (3 µm) were deparaffinised and rehydrated routinely. Sections were treated in 0.01 M citrate buffer (pH 6.0) for 15 min. Subsequently, endogenous peroxidase activity was inhibited with 3% hydrogen peroxide for 30 min. Sections were then incubated for 1 h in 1% bovine serum albumin (BSA) in TRIS-buffered saline (TBS) to block non-specific binding. Sections were then incubated with mouse monoclonal anti-NF or anti-GFAP antibodies (1:100 dilution) in TBS containing 1% BSA for 12 h at 4°C. Consequently, sections were exposed to biotinylated secondary antibody (anti-mouse IgG) in TBS containing 1% BSA for 1 h at ambient temperature. The avidin-biotin complex (ABC) was formed by incubating sections with the ABC kit reagent for 1 h at ambient temperature. The antigen-antibody complexes were visualised in section using 3,3'-diaminobenzidine tetrahydrochloride chromogen for 15 min at ambient temperature and counterstained with haematoxylin for 5 min, dehydrated, cleared, and mounted in distyrene-plasticizer-xylene. All antibodies and kits used were products of Novocastra, Leica Biosystems Newcastle, UK.

IHC Image Analysis

To elucidate protein expression levels in tissue samples, we employed ImageJ software to quantify diaminobenzidine (DAB) staining intensity in immunohistochemical (IHC) images. The analytical approach involved colour deconvolution using the colour deconvolution plugin, effectively separating DAB staining from haematoxylin counterstain. We then isolated the DAB channel, applying thresholding to segment staining and optimising threshold levels for precise segmentation. Following segmentation, we analysed and integrated mean grey values and proportions of GFAP- and NF-positive cells, providing a comprehensive assessment of protein expression (Cizkova *et al*., 2021).

Lipid Peroxidation Determination

The method of Halliwell and Gutteridge (Gutteridge and Wilkins, 1982) was adopted, and a 10 % homogenate of PFC was prepared in phosphate-buffered saline, pH 7.4. Thereafter, 1 mL of it was added to a tube containing 1,500 µL of 10 % trichloroacetic acid and was incubated at ambient temperature for 10 min. Thereafter, all tubes were centrifuged at 4,000 x g for 10 min, and the supernatant was taken and mixed with 1,500 µL of 0.67% thiobarbituric acid dissolved in 50% acetic acid. The mixture was boiled for 30 min. The developed pink colour due to the reaction between malondialdehyde and thiobarbituric acid complex was measured at 535 nm. Values were calculated and reported as mmol/g wet tissue.

Statistical Analysis

Intergroup differences were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (GraphPad Prism 9, San Diego, USA). Data are presented as mean ± standard error of the mean (SEM). Statistical significance was defined as $p < 0.05$.

RESULTS

Lipid Peroxidation Results

Lipid peroxidation levels served as a biomarker to assess the extent of cellular damage. In this particular instance, lipid peroxidation levels sum up the net oxidative damage in the PFC. A high level of lipid peroxidation is an indica-

tion of a high level of oxidative damage and, hence, a high degree of oxidative stress (Jena *et al*., 2023). The effect of LO on the level of lipid peroxidation in the PFC of binge alcohol-exposed rats was evaluated. The mean level of lipid peroxidation in various groups (Fig. 1) was compared in a one-way ANOVA analysis. The results indicate that binge alcohol intake significantly increases the levels of lipid peroxidation in the two groups exposed to alcohol compared with the control, $p < 0.001$ and $p < 0.01$, respectively (Fig. 1). There was a significant difference between the LO-supplemented rats and non-supplemented rats, $p < 0.01$. This finding suggests that LO minimises oxidative damage caused by the intake of alcohol in rats' PFC.

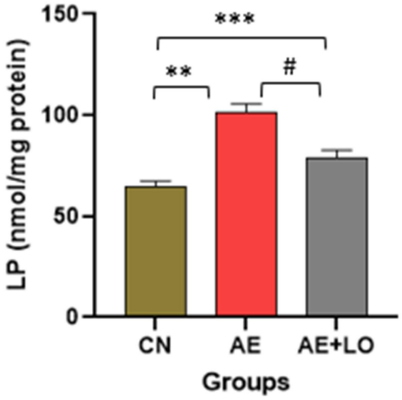

Fig. 1: Effects of LO supplementation on lipid peroxidation (LP) levels in the PFC of binge alcohol-exposed rats. LO significantly reduced the level of lipid peroxidation in alcohol-exposed rats. # $p < 0.01$; $p < 0.01$, * $P < 0.001$. CN, control; AE, alcohol-exposed; AE+LO, alcohol-exposed plus *L. owariensis* supplementation.

Histology of the PFC of Alcohol-Exposed Rats

Histopathological examination of the PFC revealed marked intergroup differences in neuronal morphology. The sections from control rats exhibited normal PFC architecture, which comprised polygonal neurons with dense, well-defined nuclei (Fig. 2A). Sections from rats exposed only to alcohol showed that alcohol induced significant neuropathological alterations, including a high proportion of neurons with rounded cell bodies, neuropil vacuolation, and the appearance of degenerating neurons (Fig. 2B).

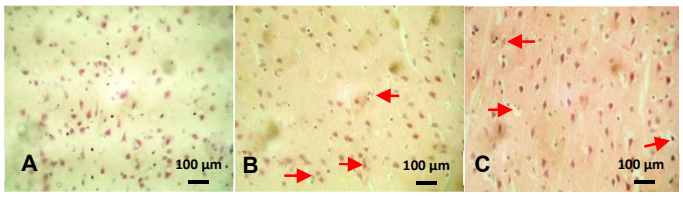

Fig. 2: Effects of LO on alcohol-induced cytoarchitectural alteration. A). PFC of control rats showing no histopathology, and neurons are mostly polygonal-shaped cyton with dense nuclei. B). PFC of the alcohol-treated group showing a higher number of neurons with round cytons, degenerating neurons (red arrow) and slight neuropil vacuolation. C). PFC of the alcohol-exposed plus LO-supplemented group showing a higher number of neurons with round cytons and polygonal neurons, a non-vacuolated neuropil. LO - *L. owariensis*. H&E (hematoxylin and eosin) stain. Scale bar: 100 µm

The LO supplementation group showed an ameliorative effect on the alcohol-induced changes. Rats that received concurrent LO and alcohol demonstrated a significantly improved histological outcome. Specifically, LO supplementation resulted in a greater proportion of polygonal neurons, similar to the control group, and no clear neuropil vacuolation (Fig. 2C). Even though degenerating neurons were still present, the overall morphology of the neurons revealed a significant protective action of LO on alcohol-induced damage in the PFC.

GFAP Immunohistochemistry in the PFC of Alcohol-Exposed Rats

LO attenuated alcohol-induced cerebral neurodegeneration by downregulating the expression of GFAP proteins (Fig. 3). GFAP-immunoreactivity (GFAP-IR) was overexpressed in the alcohol treated rats compared to the control rats (Fig. 5). GFAP-IR in the LO-fed rats was less intense than that of the alcohol group. However, they were more intense compared with the control rats (Fig. 5). Astrocytes in the PFC of rats exposed to alcohol with or without LO supplementation (Fig. 3B and C, respectively) were all in the activated state, but the packing density of the astrocytes appeared loose in LO supplement rats compared to the alcohol-exposed rats without supplementation. In the control rats (Fig. 3A), we observed that the astrocytes' processes generally exhibited non-overlapping territories, and numerous astrocytes showed no detectable GFAP expression. In LOS rats, astrocytes generally displayed moderate astrogliosis with clear GFAP expression and exhibited hypertrophy, but with each astrocyte's domains relatively preserved, without implicit interlacing of astrocyte processes (Fig. 3B). In contrast, the alcohol group had a profuse astrogliosis, accompanied by GFAP overexpression, astrocyte hypertrophy, and a substantial overlap of astrocyte processes that disrupted distinct astrocyte domains (Fig. 3C).

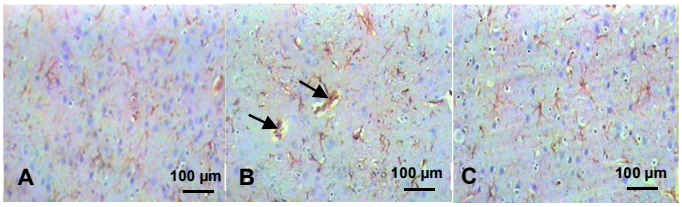

Fig. 3: Effect of LO on alcohol-induced GFAP activation. The GFAP was expressed in the PFC of the control (A), alcohol-exposed (B), and *LO* supplementation (C) groups. Astrocytes were predominantly resting in control (A) as against an activated state (i.e., GFAP overexpression) in the alcoholic rats (B). Astrocytes in LOS rats (Fig. 3C) were activated (positive GFAP-IR), but note the low packing density and less intensely labelled astrocytes compared to non-supplemented alcohol-exposed rats (Fig. 3B). Furthermore, note that in the control rats (Fig. 3A), the territories of astrocyte processes do not overlap, and most astrocytes do not exhibit appreciable levels of GFAP. In AER, note the marked astrogliosis and GFAP-IR (black arrows) and extensive intersection of astrocyte processes leading to the interruption of specific astrocyte areas. The LOS rats showed moderate astrogliosis with most astrocytes showing positive GFAP-IR and hypertrophy but with maintenance of distinct astrocyte territories and lacking conspicuous intersection of astrocyte processes. LO - *L. owariensis*. GFAP labelling. Scale bar: 100 µm

NF immunohistochemistry in the PFC of alcohol-exposed rats

LO attenuated alcohol-induced cerebral neurodegeneration by reducing the expression of NF proteins (Fig. 4 and 5). Neurons were generally NF-positive. We observed no detectable difference in the expression of NF protein in the molecular layers of the PFC of all groups. However, differences in NF-IR became clear in the outer granular and outer molecular layers. NF-IR was mild in the control rats. This was overexpressed in the alcohol-exposed rats. The NF-IR intensity in the LO groups was less intense compared with the alcohol group.

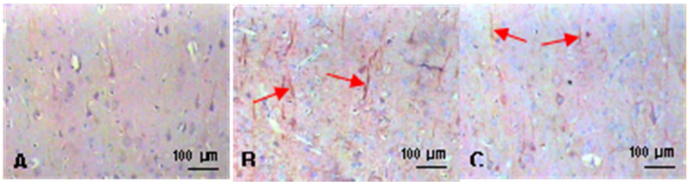

Fig. 4: Effect of LO on alcohol-induced NF activation. Representative images show NF protein expression in the PFC of (A) control, (B) alcohol-exposed, and (C) *L. owariensis*-treated rats. Note the increased NF protein expression in axons (red arrow) of the outer granular and outer pyramidal layers in alcohol-exposed rats, which is reduced following *L. owariensis* treatment. LO - *L. owariensis*. NF labelling. Scale bar: 100 µm

GFAP and NF Immunointensity and Positive Cell Proportion

GFAP-Positive Cells

Quantitative analysis of GFAP-positive cells revealed significant differences in mean stain intensity, expressed in grey values, across the experimental groups. The control group exhibited a mean grey value of 235 ± 11, whereas a substantial reduction was observed in the alcohol-treated group, with a mean value of 141 ± 6. Notably, animals co-treated with LO demonstrated a partially restored staining intensity of 213 ± 13, suggesting a neuroprotective effect exerted by the extract. In addition to labelling intensity, GFAP-positive cell population density was assessed. The control group displayed a density of 247 ± 17 cells per 10^6 µm², which increased significantly to 317 ± 14 cells per 10^6 µm² in the alcohol-treated group. Co-treatment with LO effectively normalised this increase, resulting in a density of 249 ± 16 cells per 10^6 µm², closely resembling control levels.

NF-Positive Cells

A similar pattern was observed in NF-positive cells. The control group exhibited a mean grey value of 240 ± 5.1, which declined markedly to 155 ± 12 following alcohol administration. Co-treatment with LO preserved NF labelling, yielding a mean grey value of 235 ± 10, nearly equivalent to that of the control. Assessment of NF-positive cell population density revealed values of 193 ± 14, 396 ± 14, and 248 ± 27 cells per 10^6 µm² in the control, alcohol-treated, and co-treated groups, respectively. These findings indicate that alcohol induces both a reduction in neuronal structural integrity and an increase in reactive cellular responses—effects that are notably mitigated by LO co-treatment (Fig. 5).

Our one-way ANOVA results demonstrated that alcohol administration led to a significant reduction in the grey values of GFAP and NF labelling when compared to the control group ($P < 0.001$ for both markers). In the context of immunohistochemistry measured via greyscale analysis, lower grey values correspond to more intense (darker) staining, which in turn reflects increased expression of the respective proteins. So, these results show that alcohol exposure greatly increased the levels of both GFAP and NF proteins, probably as a reaction to brain damage and the activation of astrocytes.

Importantly, co-administration of LO alongside alcohol mitigated this effect. In the co-treated group, the labelling intensity of both GFAP and NF returned to levels statistically similar to those of the control group ($P > 0.05$), suggesting that LO may exert a protective or regulatory influence, possibly by attenuating alcohol-induced neuroinflammation and neural damage. In addition to labelling intensity, significant differences were also observed in the cell population densities of GFAP-positive and NF-positive cells across the groups ($P < 0.05$ and 0.01, respectively). Specifically, alcohol treatment increased the number of immunopositive cells, which may reflect a reactive gliosis and stress-induced neuronal changes. However, these expressions were also suppressed in the LO-treated group, indicating that the extract not only modulated protein expression but also helped restore cellular homeostasis.

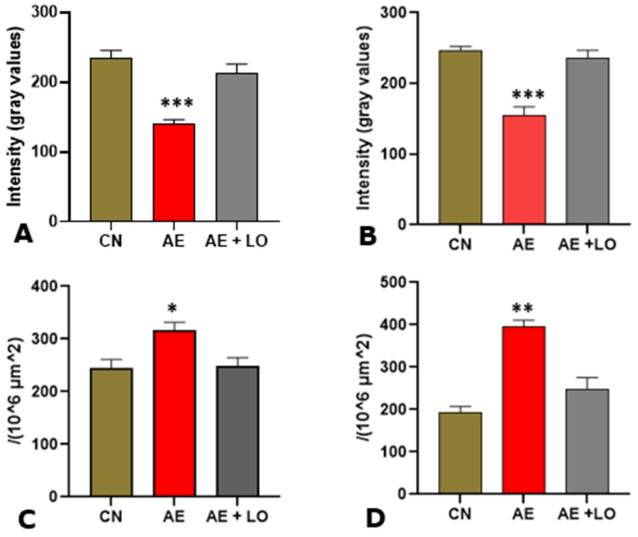

Fig. 5: Numerical expression of GFAP and NF positive cell. A). GFAP-IR. B). NF-IR. C). GFAP+ve cell. D). NF+ve cells. Binge-alcohol exposure significantly increased GFAP and NF immunoreactivity ($p < 0.001$), indicating astrocyte and neuronal stress. However, co-treatment with LO mitigated these markers to near-control levels, suggesting a neuroprotective effect. Furthermore, alcohol increased GFAP-positive cells ($p < 0.05$), indicative of reactive gliosis, whereas *L. owariensis* co-treatment mitigated this effect. Notably, *L. owariensis* reduced the proportion of NF-positive neurons in alcohol-exposed rats ($p < 0.01$). These findings highlight *L. owariensis*'s potential as a therapeutic agent against alcohol-induced neural damage.

DISCUSSION

This study examined the influence of LO administration on alcohol-induced degeneration in the PFC of rats. Results indicate that LO attenuates alcohol-induced cerebral neurodegeneration. Previous studies have indicated that alterations in cortical cytoarchitecture and neuronal loss in the cerebral cortex are associated with declined cerebral functions (Yang *et al*., 2024). There is a dynamic relationship between morphological change and functional decline. The present study showed cytoarchitectural alterations in the PFC of alcoholic rats that did not receive LO supplementation. These alterations were particularly distinct in the molecular, outer granular, and outer pyramidal layers, where most neurons showed a rounded profile as against the characteristic polygonal profile, which is consistent with normal neurons (Lemon *et al*., 2021). In addition, the neuropil of alcohol-exposed rats without LO supplementation exhibited mild vacuolations, a classical indicator of cellular stress (Obrador *et al*., 2021; Furukawa *et al*., 2023). Though these findings are significant on their own, since they constitute evidence that the administration of LO attenuates the severity of neurodegeneration seen in AUD-like exposure, moreover, the report suggests that the effects of LO against alcohol-induced neurodegeneration are broad and protective to other brain regions, such as the cerebellum (Oyinbo *et al*., 2016).

We investigated LO on GFAP expression in alcohol-induced cerebral neurodegeneration. Numerous CNS disorders are linked to aberrant GFAP expression (Bennett and Viaene, 2021; Verkhratsky *et al*., 2023). Our result indicates LO could attenuate alcohol-induced cerebral neurodegeneration by suppressing the expression of GFAP proteins in PFC. expression/upregulation of GFAP is associated with many neurodegenerative conditions (Verkhratsky *et al*., 2023). Glial scarring, a consequence in several neurodegenerative conditions, is initiated partially by excessive astrocyte reactivity (GFAP overexpression) (Escartin *et al*., 2019). Hence, suppressing over-activation of astrocytes (overexpression of GFAP), as demonstrated in this present study, may be a critical step in reducing the severity of neurodegeneration seen in chronic alcoholics. On the other hand, GFAP knockout mice experience various degenerative processes, such as aberrant myelination, deterioration of white matter structure, and disruption of the blood–brain barrier (De-Luca *et al*., 2022; Yue and Hoi, 2023). Therefore, tight control of GFAP is necessary for many critical roles in the CNS. However, the factors determining whether GFAP upregulation (overexpression) or downregulation would be beneficial or detrimental in a particular neurodegenerative disease are unknown. Nevertheless, it is known from years of observation that certain neurodegenerative diseases express a downregulation of GFAP; these include Down's syndrome, schizophrenia, bipolar disorder, depression, and Wernicke's encephalopathy (Fan and Huo, 2021). On the contrary, GFAP is upregulated in dementia and Parkinson's disease (Fan and Huo, 2021; Gradisnik and Velnar, 2023). Studies utilising knockout and transgenic mouse models, in which specific astrocyte proteins were either knocked out or overexpressed, have elucidated the crucial roles of astrocytes in both neuroprotection and neurodegeneration, especially in response to cerebral insults (Ding *et al*., 2021; Fan and Huo, 2021). However, the present study reveals that alcohol administration alone resulted in pronounced GFAP expression and increased neurodegeneration in rats, whereas co-administration with LO led to a notable reduction in GFAP expression (downregulation) and attenuated neurodegeneration.

Our findings further indicate that LO mitigates alcohol-induced cerebral neurodegeneration by suppressing NF protein expression in the context of alcohol toxicity. Notably, aberrant neurofilament modifications, including oxidation and phosphorylation, have been implicated in the pathogenesis of various neurodegenerative diseases (Yuan and Nixon, 2021).

The immunohistochemistry (IHC) studies demonstrate that binge alcohol exposure significantly alters glial fibrillary acidic protein (GFAP) and neurofilament (NF) expression in the rat PFC, indicative of cellular injury or stress. Astrocytic activation and NF upregulation, hallmarks of neuronal injury or stress, were observed (Yuan and Nixon, 2021; Andersen *et al*., 2023). Notably, treatment with LO potentially offers neuroprotection by regulating GFAP and NF protein expression in binge alcohol-exposed rats. The neuroprotective effects of LO may be attributed to its antioxidant and anti-inflammatory properties, as well as its ability to modulate neurotransmitters and regulate neurotrophic factors, thereby maintaining neuronal integrity. Astrocytes play a crucial role in regulating neurotransmitter systems, and disruptions in these interactions have been implicated in various neurological disorders (Andersen *et al*., 2023).

To adhere to the 3Rs principles, the LO-only' group was excluded due to the large disparity between the selected dose (100 mg/kg) and the LD50 value (>3000 mg/kg), which may have limited our observations and potentially masked underlying mechanisms, representing a potential study limitation. However, our results suggest that GFAP and NF overexpression may exacerbate cortical neurone susceptibility to alcohol-induced damage. LO administration reduced GFAP and NF expression in this binge drinking rat model, indicating potential neuroprotection. These findings highlight a link between astrocyte function and neuronal integrity in alcohol-related neuropathology.

Conclusion

Our findings indicate that LO exhibits neuroprotective properties in the rat PFC under conditions of binge alcohol exposure, potentially mediated via modulation of GFAP and NF expression. Specifically, LO administration resulted in significant downregulation of GFAP and NF expression, concomitant with a reduction in lipid peroxidation and attenuation of neurodegenerative pathology. These results suggest that LO may possess therapeutic potential as an adjunctive treatment for alcohol-induced neurodegenerative disorders, warranting further investigation to elucidate its molecular mechanisms and clinical applications.

DECLARATION

Acknowledgements
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Conflict of Interest
None declared.

Ethical Approval
All procedures employed in this study were approved by the FBMS research ethics committee (FMBS RE147/24) of the Niger Delta University.

Consent to Participate and Publish Data
Not applicable.

Authors' Contribution
CAO: conception, design, experimentation, data analysis, and writing. JTO: design, experimentation, data analysis, and writing. ASE: design, validation, data analysis, and writing. ADA: design, experimentation, data analysis, and writing. DUF: design, experimentation, data analysis, and writing.

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