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Carica Papaya Leaf Crude-extract Ameliorates Lipopolysaccharide-induced Neurotoxicity in Mice Cerebellum by Downregulating GFAP and IBA-1 Expression

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ABSTRACT

This study assessed the ameliorative role of *Carica papaya* leaf crude extract (CPLCE) in lipopolysaccharide (LPS) model of neurotoxicity in mice. Thirty-two adult male mice (25 - 30g) were randomly assigned to three groups (A & C; n=8, B; n= 16 i.e. B_1 and B_2). B_1 was treated with LPS once/daily for seven days. B_2 was initially treated with LPS once/day for 7 days, followed by treatment with CPLCE once daily for the last seven days. Groups A and C received distilled water and CPLCE respectively for the duration of the experiment. During the treatment, physical clinical signs were monitored for in the mice and a neurobehavioral test (wire hanging test) was carried out to ascertain muscle strength. The mice's brains were harvested and their cerebellum excised. Immunostaining for GFAP and Iba1 was performed using standard procedures as well standard H& E technique. Results from this study suggest significant cerebellar degeneration in group B_1 treated with LPS only when compared to groups A & C. There was a marked recovery of the degenerating cerebellar cytoarchitecture in group B (B_2) when treated with CP for the next 7days when compared to those treated with LPS only. This was further confirmed by reduced expression of cerebellar GFAP and Iba-1 in this group. The absence of cerebellar degeneration and normal expression of GFAP and Iba-1 was observed in group C treated with CP only for 14 days. In conclusion, CP could prove to be promising for possible neuroinflammatory diseases treatment.

Keywords: Neuroglia, Immunohistochemistry, Acute neurotoxicity, Cerebellum, Neurodegenerative changes.

INTRODUCTION

The cerebellum which is located inferior to the cerebrum, functions in the coordination of muscle involved in maintaining posture, and balance. Although the location of this structure inferiorly depicts its protection right from early development, however, it also reveals why serious damage to it impairs its functions resulting in ataxia [1]. These progressive neurodegenerative disorders are characterized by atrophy or death of the cerebellar cells resulting in motor dysfunction, in-balance, and limb and gait ataxia [2]. Such illnesses that involved cell death of certain parts of the brain are referred to as neurodegenerative diseases. Although, the pathophysiology of such diseases is not clearly defined, however, an important body of evidence points toward the role of various inflammatory processes [3,4]. The microglial cell being the main representative of the immune system in the central nervous system (CNS) can sense foreign or harmful pathogens and trigger its activation and the generation of neuroinflammatory processes and release of cytokines and interleukins [5]. Research has further proven that lipopolysaccharide (LPS), a classic inflammatory agent and the main component of the cell wall of Gram-negative bacteria can induce neuroinflammatory responses with the release of chemokines, cytokines, and reactive oxygen species (ROS) in mice [6]; such cascade of biological responses is key to the understanding of the pathophysiology of neurodegenerative diseases (NDs). When LPS is administered to experimental animals, it can induce cognitive impairment and elucidates different behaviors such as anorexia, weight loss, exploratory behavior, increased anxiety, somnolence, decreased locomotion, and general depression [7]. Most of these clinical signs are very akin to those observed in humans with a neurodegenerative disease, hence its implication in animal research involving neuroinflammation is of huge benefit.

Although, several studies involve the development of drugs or technology for the cure or treatment of NDs, unfortunately up till now, few effective management procedures are currently available for the amelioration of these neurological-related diseases [8]. This has led to the emergence of alternative means of management including the use of *Carica papaya* and even other herbs from the tropics as a herbal remedy. The presence of a strong chemical defense system in tropical plants like *Carica papaya* (CP), comprising secondary metabolite compounds, has drawn many researchers interested in the bioactive phytochemicals of this plant and others alike [9]. The interest in the usage of CP as an anti-neuroinflammatory agent in the index study stems from recent research on a mouse model of allergic airway inflammation which revealed that CP leaf extract significantly reduced the infiltration of inflammatory cells in both blood and bronchoalveolar lavage fluid samples. This was possible by a downregulation of the inflammatory genes: IL-4, IL-5, eotaxin, TNF-α, nuclear factor-κB (NF-κB), and inducible nitric oxide synthase (iNOS) [10]. Further, an ethanol acetate fraction of CP leaf water extract was shown to inhibit the release of TNF-α (60.2% inhibition) by human monocytic cells derived from acute monocytic leukemia (THP-1) following Porphyromonas gingivalis lipopolysaccharide activation. The activity of the extract was significant when compared to dexamethasone which is a major bioactive component in Gencix, a toothpaste recommended for the protection of gums and prevention of gingivitis. Hence, the anti-inflammatory effect was associated with the flavanol content of the extract [11]. In another study, CP leaf ethanol extract was found to significantly decrease carrageenan-induced paw edema and cotton granuloma tissue formation. It further reduced edema in a formaldehyde-induced arthritis model [12]. Therefore, with these interesting remarks about its potency in the reduction of inflammatory processes, its possible use as an anti-neuroinflammatory agent is of huge

importance to patients with brain diseases in low- and middle-income countries with huge financial burdens from other diseases which are endemic to them. This study is therefore aimed at determining and establishing the novelty of an ameliorating efficacy of aqueous crude extract of *Carica papaya* leaf in LPS-induced neurotoxicity in male mice.

MATERIALS AND METHODS

Ethical Consideration: Ethical clearance was requested and obtained from the University of Medical Sciences Research and Ethics Committee on Animal Use and Care, and all experiments on mice were carried out in accordance with the "Guidelines on Ethical Treatment of Experimental Animals" described by the European Union directive (Directive 2010/63/EU).

Drug and Dose Constitution: 5g of Lipopolysaccharide (LPS) was obtained from Boster Biological Technology Co., LTD, 3942 Valley Ave Pleasanton, CA 94566, USA and was used as a mycotoxin for neurotoxicity induction. The stock of LPS was reconstituted in distilled water to a concentration of 5 mg/mL. The LPS stock was then diluted with sterile phosphate-buffered saline (PBS) to a working concentration of 0.2 ug/ul and administered intraperitoneally at a dose of 10 ul/g as described by Radulovic et al.[13].

Plant Material and Extraction: *Carica papaya* leaf (Paw paw leaf) was sourced from the Herbal Garden of the University of Medical Sciences, Ondo, Nigeria. The harvested leaves of *C. papaya* were washed and air dried under shade after which they were ground to powdered form using an Athena Reciprocating Laboratory electric blender produced by Athena Tech, Mumbai. 520 g of the powdered sample was macerated in 2 L of methanol. The macerated solution was filtered and evaporated in a carefully regulated water bath (maintained at 50 °C) which gave a brownish-green semi-solid extract with a yield of 30.2 g. The extract was stored in a refrigerator at 4°C and prepared for oral administration. Using distilled water as a vehicle, extract was administered orally at a dose of 200 mg/kg using a cannular. The dose employed for this study was sublethal as described by [14].

Experimental Animals and Experimental Design: Thirty-two (32) adult mice (BALB/c) with a weight of 25-30g were procured from the Animal House of the University of Medical Sciences, Ondo, Ondo State, Nigeria and randomly assigned to three (3) groups A, B and C. n= 8 for groups A & C, while group B, n= 16 mice, which was further divided into two subgroups: B_1& B_2 (n=8). Group A (Control) received water and vital feed for 14 days. Group B received LPS daily for the 1st 7 days (as B_1) and then CP daily for the next 7 days (as B_2). Group C received CP only daily for 14 days, please see Table 1. After each daily administration, physical observation was done and recorded to identify possible clinical signs as a result of the treatment. Preceding the experiment, the mice were allowed to be acclimatized for 14 days and were cared for with best practices, as described in the Ethical Guidelines for the Use of Animals in Research by the National Committee for Research Ethics in Science and Technology (NENT).

Table 1: Treatment Protocol

Groups	Sub-groups	Administration	Duration
Group A-control (n=8)		Received 100ml/kg/day of water as placebo	14 days
	B1- neurotoxicity mod	Received 10 ul/g of LPS only	7 days as standard toxicity treatment
Group B- (n= 16)- B_1 &B_2	B2- Anti-toxicity mode	Received 10 ul/g of LPS only for initial 7days, and 200mg/kg CPLCE or an additional 7 days	14 days total treatment
Group C- CPLCE control		Received 200mg/kg of CPLCE only	14 days

Behavioral Test: After treatment for 3 days, neurobehavioural assessment; wire hang test was carried out to evaluate the mice's motor activity. This test is based on the latency of a mouse to fall off a wire upon exhaustion. During the test, a time of 180 seconds was set as the optimal time for a mouse to stay on the wire. This task was used to check for muscle strength and prehensile reflex (capacity of the mouse to hold a tightly stretched horizontal wire with its forepaws and to remain suspended on the wire). A training session was initially carried out on the second day of the experiment, while the test begun on the third day of the experiment. During the test, the suspension time (time to drop from the wire) was recorded, with which the neuromuscular abnormalities of motor strength was estimated. The test was repeated on day 5, 7, 10, 12 and 14. The method was modified from the standard operating procedure as described by Maaike [15]. In the setup, a wire grid was used for the experiment. A video camera connected to the computer was suspended from the ceiling directly above the cage on top of the platform supporting the wire grid. Barriers were placed on each side of the wire grid, to prevent the mice from moving towards the other side of the grid or possibly falling off, and then the trial sessions began and recorded by the connected computer from a separate room in the lab.

Animal Sacrifice and Sample Collection: At the end of the experiment, following 24 hours of the last administration, the body weight of the mice were measured using a weighing scale. The mice were sacrificed by anesthetizing with diazepam and amphetamine intraperitoneally. The skulls were dissected using forceps to expose the brain of the mice and the whole brain harvested and fixed in 10% neutral buffered formalin. The cerebellum was carefully located and excised for histological and immunohistochemical studies.

Histological Studies: The Hematoxylin and Eosin (H and E) method was carried out to display the general histoarchitecture of the cerebellum. The fixed tissues were subsequently processed using a standard manual tissue processing technique. The pr ocessed tissue were examined under a light microscope for histological changes and subsequently captured with a Brunel Light Microscope, 20 megapixels (Brunel SP35 Digital Trinocular, United Kingdom).

Immunohistochemical Studies: Ionized calcium-binding adaptor molecule-1 (Iba-1) was used to evaluate the expression of microglia in neuroinflammation and it is known to be upregulated during activation of microglia, while Glial fibrillary acidic protein (GFAP) [16], and was used to demonstrate the expression of Microglia in neuroinflammation. The method used for Iba-1 and GFAP in this study was modified from the protocol described by Ijomone et al. 16. The paraffin-embedded sections were deparaffinized in xylene and taken to water through descending grades of alcohol. Then, antigen retrieval was performed before the tissue slides were washed in phosphate-buffered saline (PBS) for about 2 minutes. Endogenous peroxidase blocking was carried out using 0.3% hydrogen peroxide in PBS for 10 minutes and slides washed with PBS. The sections were incubated in primary antibody (anti-iba-1) at 1:1250 and GFAP at 1:7500 2 hours at room temperature then washed again in PBS for 5 minutes respectively. The resultant sections were incubated in 1 mm PRESS (peroxidase) Polymer Anti- Rabbit IgG Reagent (HRP) or 30 min, and then washed twice, 5 min at a time. The color was developed on the sections using 3, 3′-diaminobenzidine Peroxidase (HRP) Substrate Kit (Vector ®) Burlingame, CA. The sections were then rinsed in Scott tap water and counter-stained in hematoxylin after which dehydration using graded alcohols was carried out, clearing using xylene, mounted and cover slipped using Distyrene Plasticizer Xylene (DPX). Quality control assessment was done on the tissue slides and they were finally stored at room temperature before photomicrography.

Photomicrography and Image Analysis: The processed immunohistochemistry tissues were viewed under a digital light microscope and digital photomicrographs were taken by an attached camera at x400 and x100 magnifications, using OMAX software. The number of GFAP and IBA-1 reactive cells were counted using Image J software and, for digital analysis of photomicrographs using the immunoreaction plugin as described by Erukainure et al. [17].

Data Analysis: Data generated from the study were presented in a bar charts, as mean SEM. Test of significance was done using One-way Analysis of Variance with Tukey's post hoc test, set at 95% (0.05) confidence limit. Statistics was done with the aid of the GraphPad Prism (v 8) software (GraphPad Inc, USA).

RESULTS|

Physical Observation: The mice in the control group (group A) were normal and showed no adverse clinical physical changes. Mice maintained normal appetite as observed at the beginning of the experiment and across the treatment period. However, group B1 (LPS treated group) upon LPS administration, the mice progressively began to show signs of lethargy with loss of appetite, a reduction in physical activities. While, group B2 (LPS+ CPLCE group) showed improved appetite and general improvement in motor activities. In group C (CPLCE only), there was a notable increase in activity and appetite.

Morphological Studies: From the bar charts in Fig 1, it was observed that group A (control group) had a steady normal increased mean body weight gain which is expected (Fig 1a. However, group B_1 (LPS treated group) showed a considerable decrease in body weight, while groups B_2 (LPS+CPLCE) and C (CPLCE) showed a marked increase in weight (Fig 1 b and c respectively).

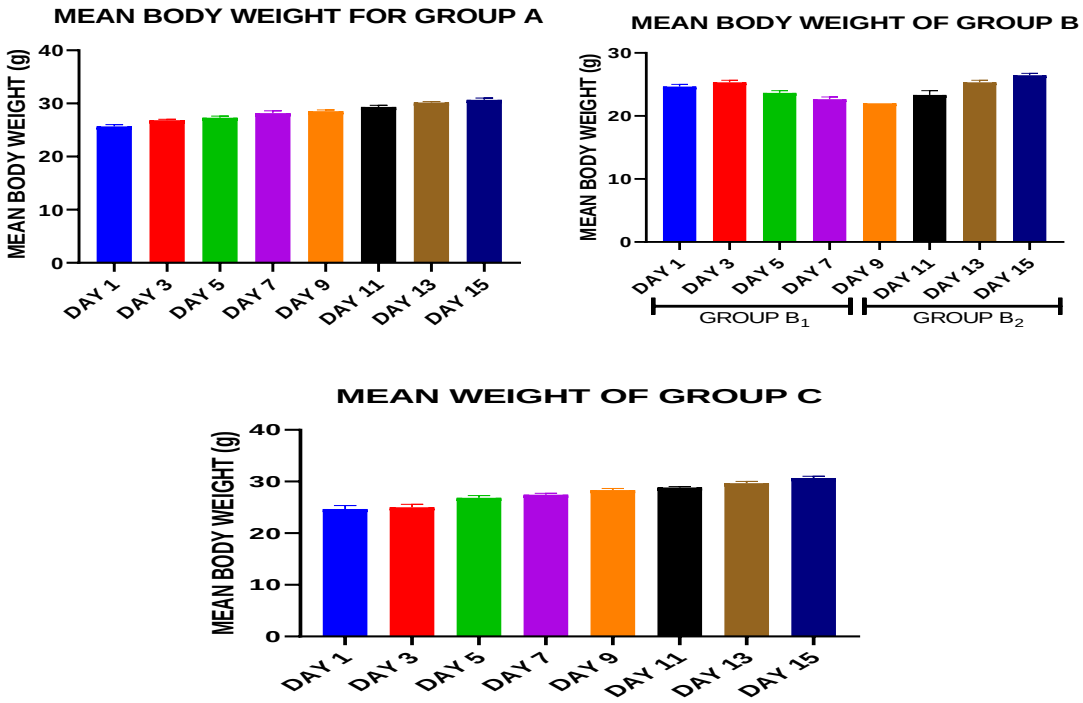

Figure 1 (a-c): Bar chart showing mice`s weight response to the experimental protocol.

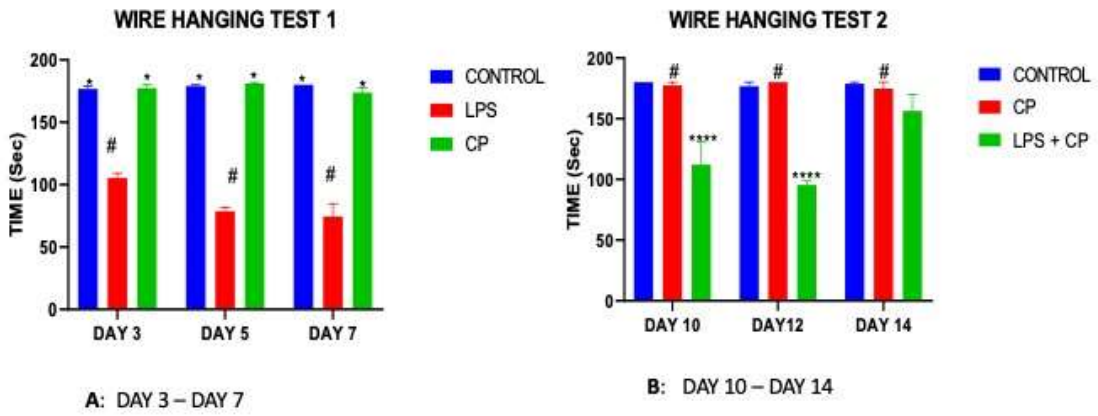

Figure 2: Bar chart showing mean falling time

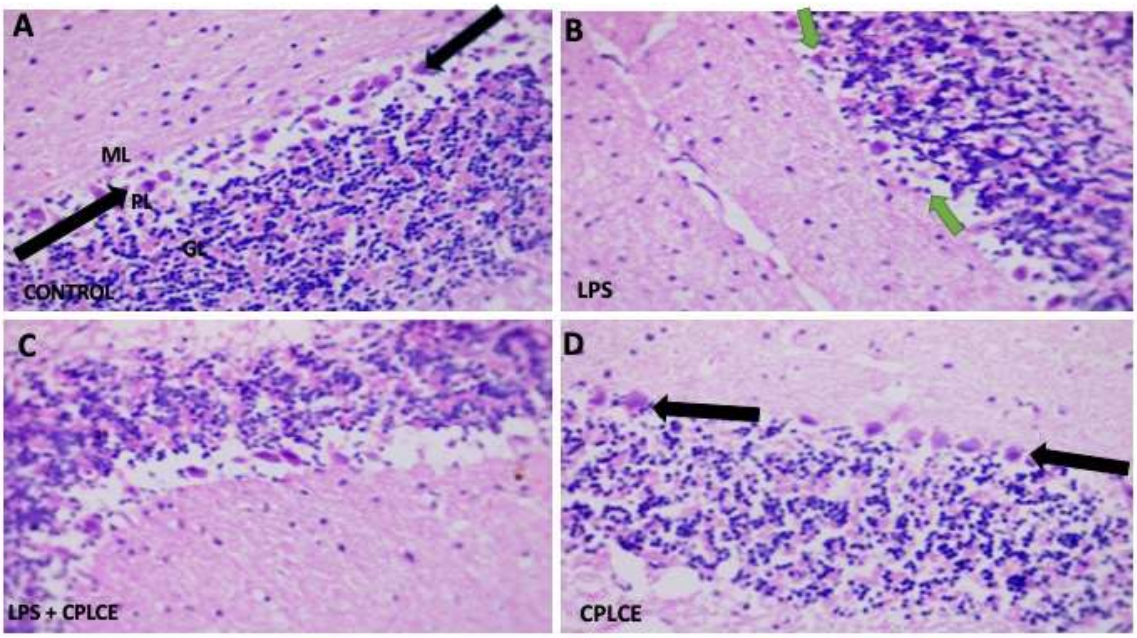

Figure 3: (A- D) Photomicrographs of histologic changes in the cerebellar cortex of con and treatment groups (H and E, x400). ML-molecular layer; PL-Purkinje layer; GL-granu layer; black arrows - intact Purkinje neurons; Green arrows - degenerating Purkinje neurons

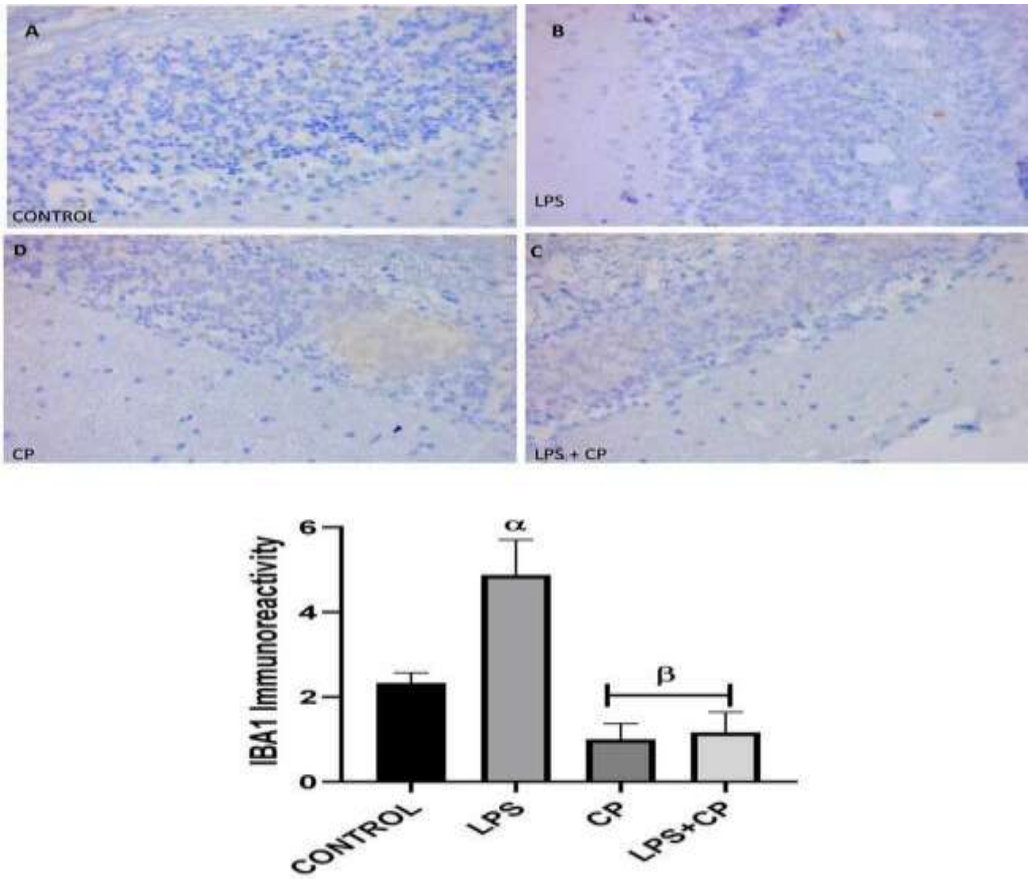

Figure 4 Plate A - D: Photomicrographs and bar chart of the cerebellum for control and treatment group at x400. The bar graph depicts the number of Iba-1 immunoreactive cells in control and treated groups. Bars are mean ± SEM. n=8/group. α denotes significant increase ($p < 0.05$) compared to control group. β denotes a significant decrease ($p < 0.05$) compared to LPS only treated group using One-way ANOVA followed by the Tukey pos hoc test.

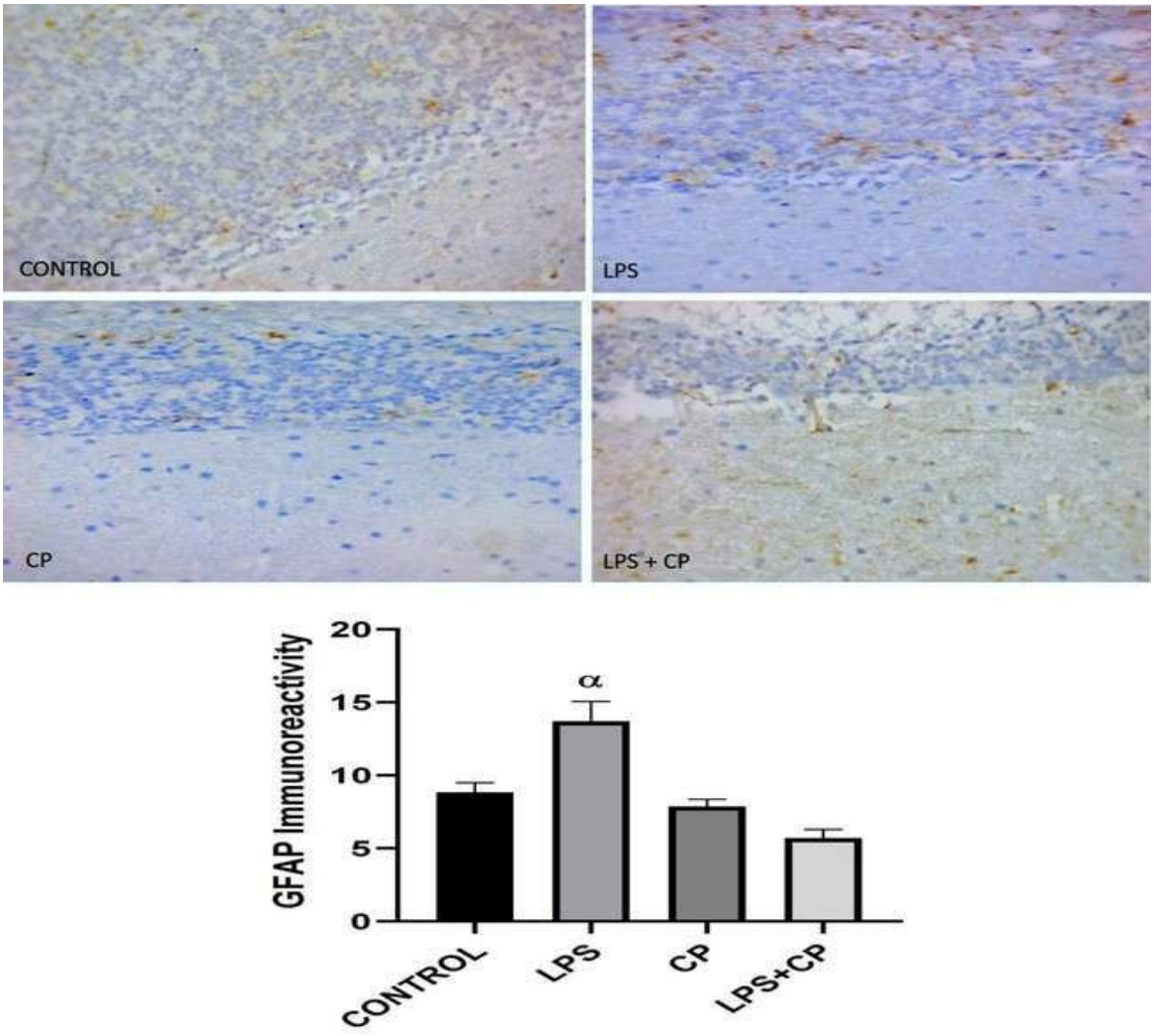

Figure 5: Plate A- D; Photomicrographs of the cerebellum for control and treatment group. Iba1, x400**.** The bar graph depicts the number of GFAP immunoreactive cells in control and treated groups. Bars are mean ± SEM. n=8/group. α denotes a significant increase ($p < 0.05$) compared to the control group using one-way ANOVA followed by the Tukey pos hoc test.

Wire Hanging Test (muscular strength): The charts in Fig 2 A and B) show that there was no significant decrease in time before falling from the wire for the control group from days 3 to day 14, suggesting normal muscular strength. For the LPS only group (group B_1), there was a significant reduction ($p< 0.05$) in muscular strength from days 3 to day 7 compared with the control group. For LPS + CPLCE (group B_2), there was a significant increase ($p < 0.05$) in muscular strength compared to the LPS group, while for the CPLCE group, there was no significant difference ($p > 0.05$) in muscular strength from days 3 to day 14 signifying normal muscular strength (chart C).

This showed that the trend of the muscular strength of the CPLCE group is similar to that of the control group. In addition, we can infer that treatment with CPLCE after LPS treatment completion, had a significant effect on the muscular strength as shown on days [10, 12, 14].

Histological Assessments: Normally, the histology of cerebellar cortex features three distinct layers: the granule layer with small tightly packed rounded granular neurons, the Purkinje cell layer consisting of a single layer of large Purkinje neurons with a large round nucleus, and prominent nucleoli, and the molecular layer with sparse cells. Control Fig 3 (Plate A) and CPLCE (Plate D show mostly this intact cerebellar histology. LPS group (Plate B) shows obvious degenerative features in Purkinje neurons characterized by cellular shrinking and Perineuronal vacuolation. Moreover, there are fewer granular neurons in the granular layer compared to Control and CP groups. In contrast, the LPS+CP group (Plate C) shows preserved cortical cerebellar histology with fewer Purkinje neurons showing degenerative features. However, there are still few granular neurons compared to Control and CP groups.

Immunohistochemical Assessments (Expression of Iba-1 and GFAP) - Iba-1 expression: The immunohistochemical localization of IBA-1 in Fig 4 shows distinct IBA-1 expression in cortical cerebellar neurons. LPS only groups show elevated IBA-1 immunoreactive neurons than control, CPLCE-only, and LPS+CPLCE groups Fig 4 (Plate A – D). Further quantification shows that there is a significant increase (p □ 0.05) of Iba-1 expression in LPS treated group [4.88 ± 0.83] compared to control group [2.33 ± 0.24], CPLCE group [1.00 ± 0.39] and LPS+CPLCE group [1.17 ± 0.48]. There is no significant decrease in Iba-1 expression between control group [2.33 ± 0.24], CPLCE group [1.00 ± 0.39] and LPS+CPLCE group [1.17 ± 0.48].

GFAP expression: The immunohistochemical localization of GFAP in Fig 5 shows distinct GFAP expression in cortical cerebellar neurons. LPS treated group show elevated GFAP immunoreactive neurons compared to the control, CP only, and LPS+CPLCE groups Fig 5 (Plate A- D). Additionally, quantification of GFAP expression shows that there is a significant increase (p □ 0.05) in GFAP immunoreaction in LPS treated group [13.71 ± 1.34] compared to the control group [8.83 ± 0.65], CPLCE group [7.88 ± 0.48], and LPS+CPLCE group [8.83 ± 0.65]. There is no significant decrease in GFAP immunoreactivity between the control group [8.83 ± 0.65], the CPLCE group [7.88 ± 0.48], and the LPS+CPLCE group [8.83 ± 0.65].

DISCUSSION

The physical observation carried out throughout the duration of the experiment, affirms the role of LPS as a neurotoxicant, because the mice treated with LPS had clinical signs such as lethargy, reduced physical (motor) activity and anorexia. These signs have earlier been reported by the study on LPS by Zhao et al. [7].

Surprisingly, CPLCE markedly improved on these symptoms in the mice. Additionally, the changes in mean body weight observed in our study reflects the anorexia experienced by the LPS treated mice. Moreover, weight measurement have been identified to serve as a general guide for health status in research animals [18], therefore, the present findings in this study suggest that although LPS might have resulted in a slight decrease in body weight probably due to anorexia, conversely, CP leaf crude extract was beneficial and did not interfere negatively with the growth of the mice and improved the weight of those pre-treated with LPS. The increase in weight gain perhaps could be due to an increased appetite across the mice treated with the extract. Besides, CPLCE has earlier been identified by Nivaasini,[19] to improve loss of appetite in patients with low appetite, presumably due to its rich fiber content. Consequently, more research is needed to confirm the actual biological component in CP that serves as an appetite booster.

Furthermore, the wire hanging test was adopted to measure motor activity in the mice following LPS induction. This test has been widely used to assess the motor activity of mice [20]. The result obtained from our experiment revealed a sharp decline in motor activities by a marked decline in muscle grip-strength after LPS administration in mice. This decline in muscle strength depicts a poor motor coordination and muscle tone, which is attributable to cerebellar impairment. This our finding corroborates that of Dadsetan et al. who observed a significant loss in motor function after LPS-induced cerebellar damage [21]. However, it was unexpected to observe that with the treatment of CP leaf crude extract at 200 mg/kg, there was an improved motor functions in the LPS-induced mice as well as those treated with CP crude leaf extract only. This amelioration of LPS-induced muscle strength weakness in mice was reflected by an increased hanging time in the mice treated with CPLCE. Note that the duration of time for a mouse to hold onto the hanging wire is used as an indirect method to determine a measure of grip, muscle strength, and coordinating activities of the cerebellum [22], hence we can infer that CP leaf extract may have a beneficial role in the general improvement of motor coordination in mice cerebellum.

Additionally, light microscopic examination of H & E stained cerebellar sections of the LPS-induced group (10 µl/g) revealed marked degenerative changes in the Purkinje and granular layers of the cerebellar cortex. The Purkinje cells had small dark stained nuclei with eosinophilic cytoplasm and some of the cells were completely lost leaving empty spaces. The granule cell layer was thin and had many degenerating granule cells. Foregoing studies reported degenerative changes in the cerebellum following LPS induction [23, 24]. Further to these, the findings from this study likewise revealed the presence of congested blood vessels with inflammatory cellular infiltration. Conversely, with the treatment of the mice with 200 mg/kg CP, there was improved cerebellar cyto- and histoarchitectures with fewer Purkinje neurons showing degenerative features and an observed well-reduced blood vessels congestion due to the probable anti-inflammatory properties of the crude extract. This anti-inflammatory property of CPLCE has been described by several researchers [12, 25]. CP, a tropical plant is used in folklore medicine for the management of several disease conditions including those associated with inflammation. This is probable because of its strong chemical defense system and secondary metabolite compounds [9]. Based on this, we hypothesize that treatment with CP leaf crude extract could help reduce neurodegenerative changes associated with LPS exposure in mice's cerebellum.

Furthermore, in this study, marked over-expression of cerebellar Iba-1 was

established following LPS-induced neurotoxicity and was marked by an increased number of brown-stained Iba-1-expressing microglia cells (Iba-1 positive cells). This protein reactivity is recognized to be induced by cytokine and interferon actions and fully expressed during inflammatory response [26]. As a marker for microglia, Iba-1 expression is upregulated upon brain damage or during CNS degeneration, and more highly expressed in the aged brain [27]. Moreover, microglia are CNS-resident defense cells that police the brain and spinal cord for pathogens and play a critical role in CNS homeostasis and plasticity 28. Nonetheless, these glial cells are vulnerable to over-activation in response to aberrant stimulation, such as from an endotoxin (LPS) as well as injuries 29. Earlier studies have demonstrated that microglia are responsible for the onset and progression of neuroinflammatory-mediated neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD) [30, 31]. As a consequence, the inhibition of microglial and astrocytes hyperactivation could help attenuate neuroinflammation and neuronal damage [32]. In our result, we showed a lower number of Iba-1 positive cells with the treatment of mice with CP leaf crude extract. The marked reduction in Iba-1 immuno-stained cells reflects a decrease in microglia activities and correspondingly reduced inflammatory response, as demonstrated less secretion of cytokines and subsequent neurotrophic factors. This finding is in agreement with those from Owoyele et al. and Lee et al. on CP [12, 25].

Besides, lipopolysaccharide (LPS) is a neurotoxic substance that has been widely used experimentally to induce neuroinflammatory responses in the central nervous system [33]. These responses are majorly mediated by glial cells, such as microglia and astrocytes 34. Unsurprisingly, following LPS exposure in several animal models, microglia and astrocytes have been overactivated leading to the over-reactivity of these cells [35]. Glial fibrillary acidic protein (GFAP) and ionized calcium-binding adaptor molecule-1 (Iba-1) are proteins involved in the mediation of neuroinflammation and have been observed to be well expressed in both astrocytes and microglia [27, 36]. Their selective expression makes them important as markers for identifying specific glial cell groups. Therefore, this study considers the advantage of specific expression of GFAP and Iba-1 to evaluate the ameliorative effect of *Carica papaya* leaf extract (CP) following LPS-induced neuroinflammation in mice's cerebellum. The cerebellum, a part of the central nervous system is known to play a vital role in maintaining balance and motor coordination 37. However, a previous study has shown that systemic administration of LPS triggers neurotoxicity and neuroinflammation in the cerebellum, which induces motor deficits in mice 38. Hence, in the current study, GFAP and Iba-1 were immunoexpressed and quantified to assess the extent of LPS-induced neuroinflammatory changes and the anti-inflammatory role of CP leaf crude extract on the cerebellum. The present study reports marked overexpression of cerebellar GFAP following LPS-induced neurotoxicity, characterized by an increased number of brown-stained GFAP-expressing astrocytic cells. This increase reflects the extent of activation of astrocytes in the mice cerebellum due to LPS neurotoxicity. As an endotoxin, it initiates and promotes the release of inflammatory cytokines and other neurotrophic factors such as GFAP as observed in this study. However, the LPS-induced neurotoxicity which resulted in increased GFAP immunoreactivity was greatly mitigated by CP leaf crude extract. Our data clearly showed a marked reduction in the overexpression of GFAP with treatment with the extract. Currently, to the best of the author's knowledge at the point of the research work, there was no other study reporting the anti-neuroinflammatory effect of CP leaf crude extract on LPS-induced neurotoxicity via downregulation of cerebellar GFAP in mice's brain. The

downregulation of GFAP by CP reflects reduced astrocyte reactivity. It is important to note here that neuroinflammation is a fundamental inflammatory response that is generated to protect the central nervous system. Nonetheless, uncontrolled neuroinflammation is potentially harmful and can result in neuronal dysfunction and death [39]. Therefore, GFAP is a key component of the astrocytes cytoskeleton that functions in maintaining cell integrity and resilience. Classically, it is upregulated upon brain damage or during CNS degeneration and is highly expressed in the aged brain [40]. Astrocytes respond to various brain injuries likely to result in neurodegeneration by hyper-proliferating (astrogliosis)[41] and have been implicated in the pathogenesis of ND like AD, PD, and HIV-associated dementia [42].

CONCLUSION

This study provides behavioral, histological, and immunohistochemical pieces of evidence that CP leaf crude extract ameliorated LPS-induced neurodegeneration in the mice cerebellum via downregulation of GFAP and IBA-1 expressing glial cells. Also, CP improved motor activity in LPS-induced mice and may have promising therapeutic potential against neuroinflammation-induced neurodegeneration.

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Conflict of interest

The authors disclose no conflict of interest.

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Submission declaration and verification

This manuscript has not been submitted to any journal.

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