JOPAT Vol 23(2} 1516– 1527, July – December, 2024 Edition.
ISSN2636 – 5448 https://dx.doi.org/10.4314/jopat.v23i2.9

NEUROPROTECTIVE AND ANTI-OXIDANT PROPERTIES OF WALTHERIA AMERICANA LEAF IN MALE WISTAR RATS

Owemidu, Idowu Olumorin[1] [2], Samuel Adetunji Onasanwo [1], Abayomi M. Ajayi[3] and Oyetola T. Oyebanjo[1,4]

[1] Neurosciences and Oral Physiology Unit, Department of Physiology, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Nigeria, samphil2002@yahoo.com
[2] Department of Physiology, Faculty of Basic Medical Sciences, Kogi State University, Anyigba, Nigeria, owemidu.io@ksu.edu.ng
[3] Departments of Pharmacology & Therapeutics, University of Ibadan, Ibadan, Nigeria; yomexj@yahoo.com
[4]Department of Physiology, Babcock University, Ilishan-Remo, Ogun State; oyebanjoo@babcock.edu.ng

ABSTRACT

Neurodegenerative disorders are linked with neuroinflammation in specific areas of the brain. *Waltheria americana* is used in folkloric medicine for the treatment of pain and inflammation. Most drugs used in managing neuroinflammation are expensive and associated with adverse effects thereby necessitating the need for safe, potent and affordable agents. This study, therefore, evaluated the neuroprotective and anti-oxidant properties of methanol extract of *Waltheria americana* leaf (MEWA) in laboratory rodents.

Leaves of *W. americana* were obtained at the College of Agriculture, Kabba, Kogi State, and authenticated at the Herbarium, Forestry Research Institute of Nigeria, Ibadan (FHI:111064). The leaves were extracted by maceration in methanol and concentrated. Neuroinflammation was induced by intra-peritoneal injection of Lipopolysaccharide (2 mg/kg) in 15 rats and were grouped (n=5) as follows: Group1 (MEWA 200 mg/kg), Group 2 (Quercetin 50 mg/kg), Group 3 received vehicle only (control 10 mL/kg) while another group (not induced) received vehicle only. The treatment was done for 30 days. Memory function was assessed using Y Maze Test (YMT). At termination, Striatum, Prefrontal cortex (PFC) and Hippocampus were sectioned. Glutathione, malondialdehyde and acetylcholinesterase were evaluated by spectrophotometry. Nissl stains were used for neuronal morphology. Data were analysed using ANOVA at $\alpha_{0.05}$.

The MEWA (200 mg/kg) significantly increased percentage alternation. The MEWA (200 mg/kg) significantly increased glutathione level (µmol/g tissue) in PFC (67.38±7.11) and Hippocampus (105.40±4.80) compared to control PFC (39.75±8.30), Hippocampus (55.54±2.0) and significantly decreased malondialdehyde level (ηmol/g tissue), acetylcholinesterase activity (µmol/min/g tissue), in Striatum (52.69±7.95, 7.96±0.31), PFC (57.94±3.81, 23.81±0.37), Hippocampus (111.0±12.90, 68.01±0.73), compared to control Striatum (83.51±3.85, 13.21±0.78), PFC (146.30±7.71, 32.27±1.49) and Hippocampus (151.40±8.80, 73.85±1.40), respectively. The MEWA 200 mg/kg preserved neuronal morphology.

Waltheria americana leaf extract ameliorated neurodegeneration in striatum, prefrontal cortex and hippocampus by reducing levels of free radicals, acetylcholinesterase and preventing neuronal damage in laboratory rodents.

Keywords: *Waltheria americana*, Neuroinflammation, oxidative stress, acetylcholinesterase

*** Correspondence:** owemidu.io@ksu.edu.ng

INTRODUCTION

Neuroinflammation is a phenomenon linked to the initiation of many neurodegenerative diseases, and it plays a role in the pathogenesis and development of these neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease [1]**.** The diseases or neurological disorders have become a global health problem affecting millions of people according to the World Health Organization (WHO) 2007. Drugs such as NSAIDs and cholinesterase inhibitors that are used to improve cognitive deficits in neurological conditions do present adverse effects and are not always effective in managing these diseases especially at their advanced stages [2]. Thus, modern studies are now directed towards the search for alternative therapy especially those targeted at preventing or reducing the over-activation of glial cells in the brain [3].

Traditional medicine and plants are being used to prevent and treat a variety of health issues in rural populations around the world, improving their quality of life. Because of the high cost and limited availability of modern drugs in rural areas of tropical Africa, a significant proportion of the population relies on traditional herbal medicines [3,4]. In recent years, natural products have sparked a lot of interest because of their possible pharmacological uses. Therefore, research into phytomedicine, with the aim of standardizing extracts of plant species that may possess physiological effects as alternatives to common synthetic drugs are ongoing [5,6]

Waltheria americana L., also known as velvet leaf, marshmallow, monkey bush, boater bush etc, is a member of the Sterculiaceae family. It can be found all over the tropics and warm subtropics. *Waltheria americana* is used in herbal medicine to treat both mild (e.g., sore throat, cough) and complex illnesses (e.g., inflammation, asthma) [7,8]. *Waltheria americana* L. is a plant that has sparked medical attention due to its use in conventional medicine to treat disorders involving the central nervous system, such as pain, neuralgia [9,10], headaches, seizures, and sleep issues [11, 12]. Reports on its analgesic and anti-inflammatory activities have been reported to validate the folkloric claim on its ability to manage pain [13]. This study was therefore conducted to investigate the neuroprotective and anti-oxidant effect of *W. americana* Linn in lipopolysaccharide-induced neuroinflammatory model in rats.

MATERIALS AND METHODS

Plant Material

Waltheria americana leaves were obtained around the College of Agriculture, Kabba, Kogi state, and authenticated by Mr S. A. Odewo and Mr K. A. Adeniji of the Herbarium of Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria where the plant voucher specimen was kept with a voucher number FHI 111064.

Preparation of plant extract

The leaves were spread in a room in order to make the leaves dry at room temperature. The blender was used to get a fine powdered form of the leaves. Four hundred gram (400g) of powdered form was soaked in a solvent (methanol) for 72 hours. The extract was sieved and subjected to filtration using a filter paper. An oven set at 40 ºC was used to concentrate the filtrate. The extract weight was found to be 9.6 g. The percentage yield of the extract was calculated to be 2.4 percent using the formular:
% yield = extract weight Initial weight× 100
A regulated refrigerator of 4ºC was used to store the dried extract until use.

Animals

Male Wistar rats weighing between 120-150g were used for this study. They were acclimatized for 2 weeks at the Central Animal House, College of Medicine, University of Ibadan. They were kept under standard laboratory conditions, and fed on rodent cubes (Ladokun Feeds, Ibadan, Nigeria). All experimental procedures on rodents

were conducted in accordance with established protocols under the guidelines of the Principle of Laboratory Animal Care (National Institute of Health Publication No. 85-23) and ethical guidelines for investigation of experimental pain in conscious animals by Zimmerman.

Experimental Design

Twenty (20) rats were divided into four groups (n=5). Quercetin 50 mg/kg was used as a reference antioxidants and neuroprotective agent.

Group A: Normal Control (not induced)
Group B: LPS + Vehicle (10 ml/kg orally) served as the control
Group C: LPS + MEWA (200 mg/kg orally)
Group D: LPS + Quercetin (50 mg/kg orally)

Induction of Neuroinflammation by Lipopolysaccharides (LPS)

Neuroinflammation was induced by single intraperitoneal (i.p) injection of 2 mg/kg *Escherichia coli* bacterial lipopolysaccharide (LPS) (serotype 055: B5, Sigma, St Louis, MO, USA) dissolved in normal saline. Animals were treated on the day of induction of neuroinflammation and this was done continuously on a daily basis for 30 days. Rats were sacrificed 30 days after LPS injection. After sacrifice, three (3) regions of the brain (Striatum, Prefrontal cortex, and Hippocampus) were removed.

Behavioral Assessment
Y Maze Test
The effect of MEWA on LPS-induced memory impairment was assessed using the Y maze test as described [14]. The randomly grouped rats (n=5) were treated with MEWA for 30 days. On the last day, each animal was placed individually at the center of the Y-maze for memory function assessment. For a period of 5 minutes each animal placed at the centre of the maze was allowed to move freely. The sequence by which the animals enter the maze was recorded manually (i.e., ABCBAC). The apparatus was cleaned with 10% ethanol after each test to prevent odor bias. An actual alternation was determined from successive entries of the three arms on overlapping triplets set in which three different arms are entered (i.e. BAC, CAB, ABC, and not CAC or BAB). Percentage alternation was given as (actual alternations/maximal alternations) X 100, where maximal alternation is the total number of entry minus two.

Determination of Acetylcholinesterase (AChE) Activity in Rat Brain
Acetylcholinesterase activity was assessed using the method of Ellman [15]. The absorbance was measured with a spectrophotometer at a wavelength of 412 nm, and the change in absorbance was reported every two minutes for 10 minutes. The rate of AChE activity was calculated as mol/min/g tissue by measuring the increase in color created by thiocholine when it reacts with 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB).

Determination of Reduced Glutathione (GSH) Concentration

Reduced glutathione (GSH) was evaluated according to studies by Moron *et al* [16]. An equal volume (0.4 ml) of brain homogenate and 20% TCA (0.4 ml) were combined and centrifuged for 20 minutes at 10,000rpm at 4 °C in a cold centrifuge. The supernatant (0.25 mL) was mixed with 2 mL DTNB, and the final volume was increased to 3 mL using phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412 nm using a spectrophotometer and concentration of GSH was expressed as (µmol/g tissue).

Estimation of Brain Level of Malondialdehyde (MDA)

Malondialdehyde (MDA) level was estimated according to the method of Adam-Vizi and Seregi, [17]. An aliquot of 0.4ml of the sample was mixed with 1.6 ml of Tris–KCl buffer to which 0.5ml of 30% TCA was added. Then, 0.5ml of 0.75% TBA was added and placed in a water bath for 45min at 80 °C. This was then cooled in ice and centrifuged at 3000rpm for 15min. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532nm using a

spectrophotometer. The MDA concentration was calculated using a molar extinction coefficient of 1.56 × 105 M−1 cm−1 and values were expressed as µmol of MDA.

Histological analysis using Nissl Staining

The brain tissue for histological evaluation was isolated after intra-cardiac perfusion with phosphate buffer formalin. The brain tissue was fixed in 10% formalin and cresyl violet staining was carried out using the method described by [18] to stain the Nissl substances within the neurons. The brain samples were sectioned into prefrontal cortex, striatum and hippocampus and viewed under a light microscope.

Statistical analysis

Data were presented as mean ± Standard Error of Mean (SEM) using graph pad prism version 5. Comparisons between groups were made using the one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test, 95% confidence level, and at $p < 0.05$ was considered statistically significant.

RESULTS

MEWA increased number of arm entry in the Y -maze test in rats.

Figure 1 shows the effect of MEWA on number of arm entries in Y maze test during LPS induced neuro-inflammation in rats. There was a significant ($p < 0.05$) increase in the number of arm entry in the MEWA 200 mg/kg, Quercetin 50 mg/kg and Normal control groups when compared with LPS + Veh group.

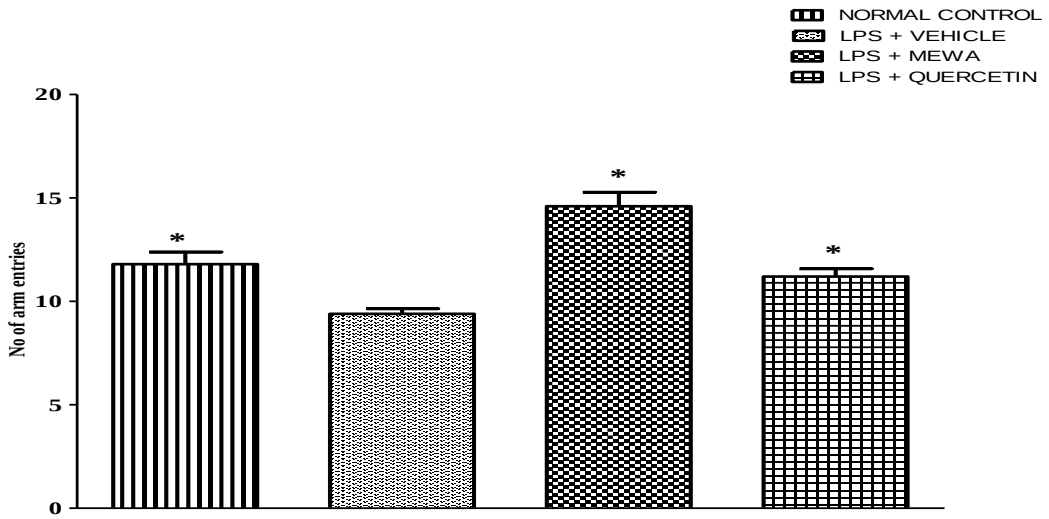

Figure 1: Effects of MEWA on number of arm entries in Y maze test in rats after LPS-induced neuroinflammation. Values are expressed as Mean ± S.E.M of five rats, .*p<0.05 compared to LPS + Veh group (One way ANOVA followed by Newman-keuls) *post hoc* test.

MEWA increased percentage alternation in the Y- Maze Test

Figure 2 shows the effect of MEWA on percentage alternation in Y maze during LPS-induced neuro-inflammation. There was a significant ($p<0.05$) increase in percentage alternation in the 200 mg/kg of MEWA, Normal Control and Quercetin 50 mg/kg when compared to LPS + Veh group. The percentage alternation was comparable in the MEWA 200 mg/kg group and Normal Control group and both groups have higher percentage alternations than the Quercetin 50 mg/kg group.

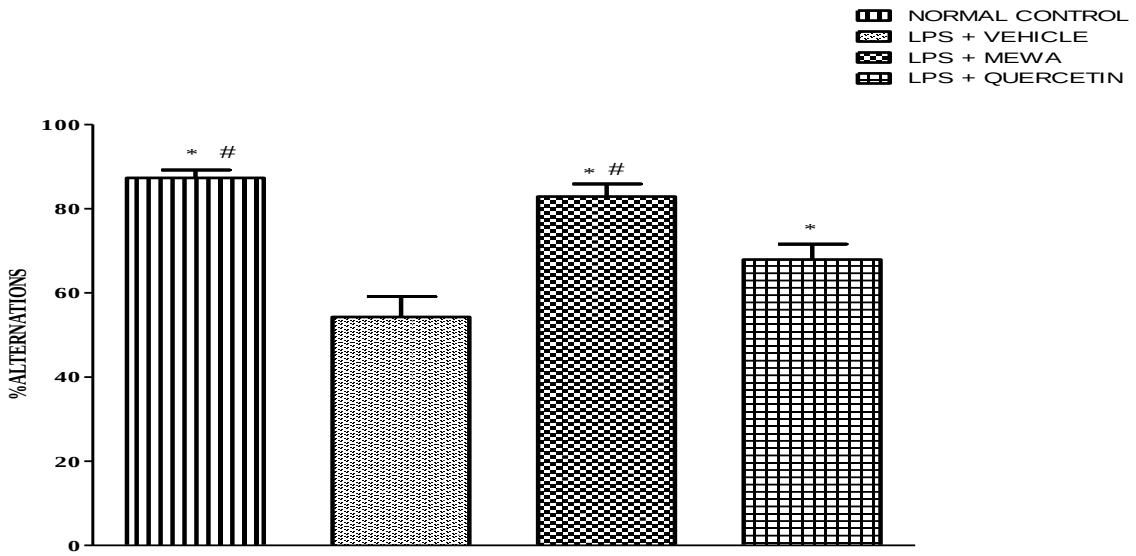

Figure 2: Effects of MEWA on Percentage alternations in Y maze test in rats after LPS-induced neuroinflammation. Values are expressed as Mean± S.E.M of five rats. *p<0.05 and #p<0.05 compared to LPS + Vehicle and LPS + Quercetin group respectively.

MEWA increased reduced glutathione in striatum, prefrontal cortex and hippocampus.

Figure 3 shows the effects of MEWA on the level of GSH in rats' striatum, prefrontal cortex and hippocampus after LPS-induced neuroinflammation. In the striatum (STR), the level of GSH showed no significant ($p<0.05$) increase in MEWA 200 mg/kg group when compared to the LPS + Veh group. Whereas, in the prefrontal cortex, (PFC) there is a significant difference in Quercetin 50 mg/kg and MEWA 200 mg/kg groups when compared to the LPS + Veh group. Also, in the hippocampus (HPC) there were significant increases in GSH levels in Quercetin 50 mg/kg and MEWA 200 mg/kg compared to the LPS + Veh group.

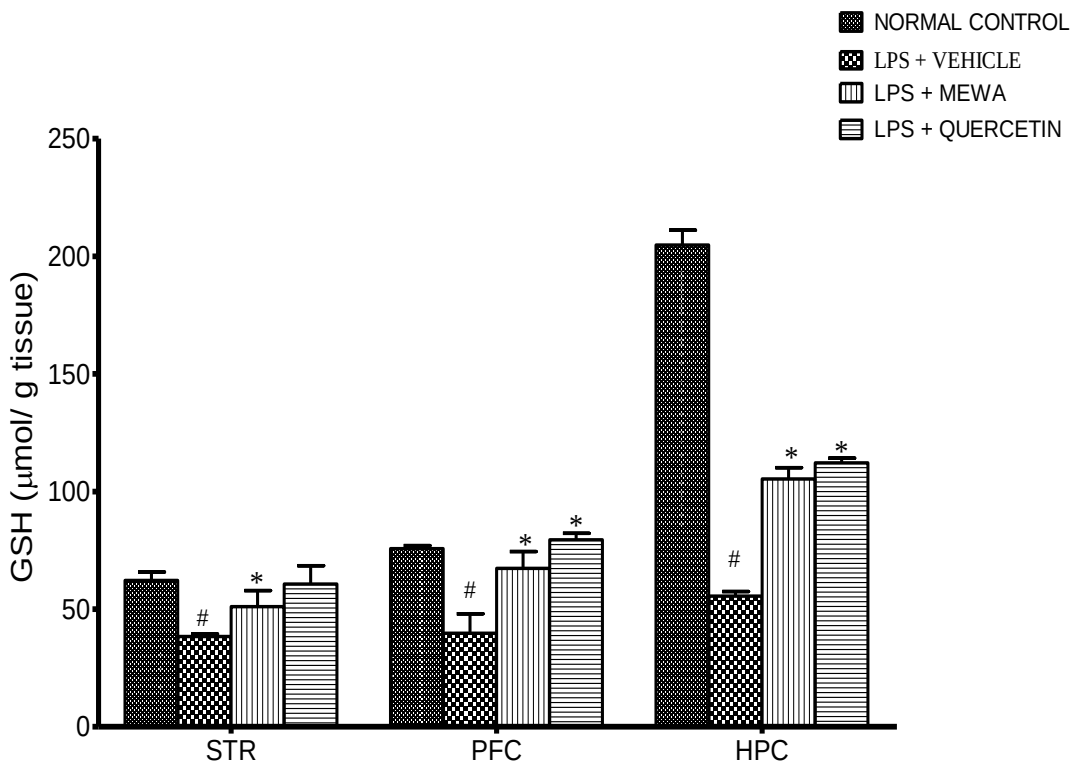

Figure 3: Effects of MEWA on the level of GSH in rats striatum (STR), prefrontal cortex (PFC) and hippocampus (HPC) after LPS-induced neuroinflammation. *p<0.05 and #p<0.05 compared to LPS + Vehicle and Normal control respectively.

MEWA reduced malondialdehyde levels striatum, prefrontal cortex and hippocampus.

Figure 4 shows the effects of MEWA on the level of MDA in rats' striatum, prefrontal cortex and hippocampus after LPS-induced neuroinflammation. In the Striatum (STR), the level of MDA showed a significant reduction in Quercetin 50 mg/kg and MEWA 200 mg/kg when compared to the LPS+ Veh group. Moreso, in the Prefrontal Cortex, (PFC) there was a significant difference in Quercetin 50mg/kg and MEWA 200 mg/kg when compared to the LPS + Veh group in the MDA level. Also, in the hippocampus (HPC) the level of MDA showed a significant reduction in Quercetin 50 mg/kg and MEWA 200 mg/kg compared to the LPS + Veh group.

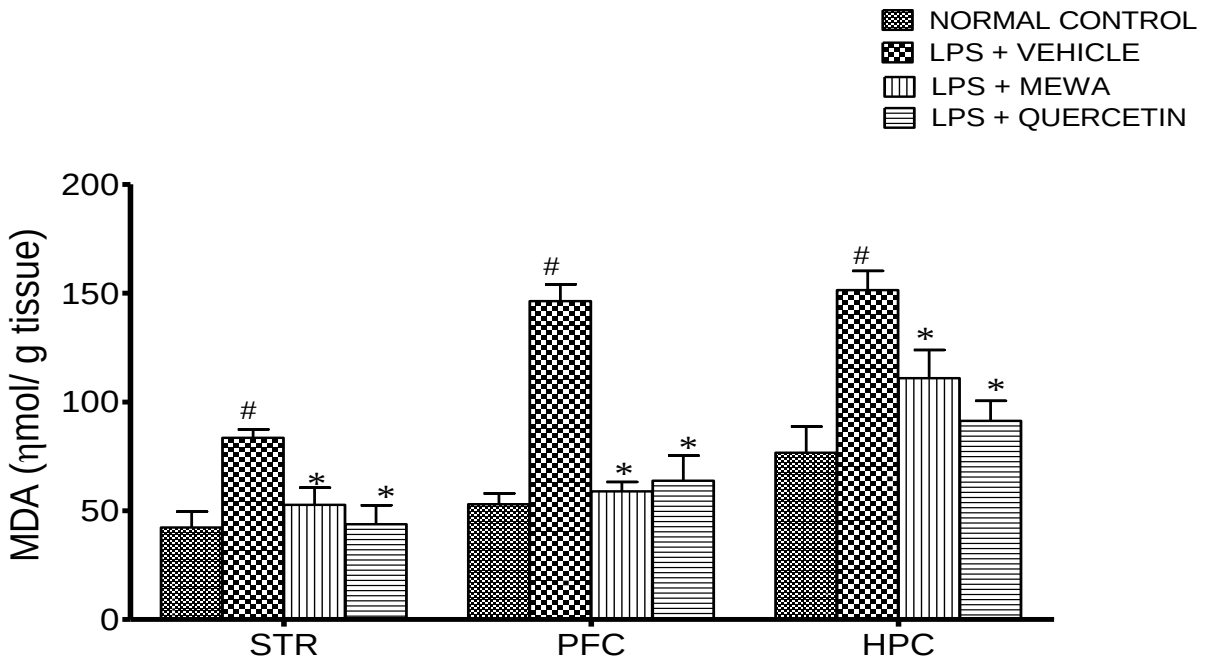

Figure 4: Effects of MEWA on the level of MDA in Rats Striatum (STR), Prefrontal cortex (PFC) and Hippocampus (HPC) after LPS-induced neuroinflammation. *p<0.05 and #p<0.05 compared to LPS + Vehicle and Normal control respectively.

MEWA reduced acetylcholinesterase levels in the striatum, prefrontal cortex and hippocampus

Figure 6 shows the effect of MEWA on the level of (AChE) in rats' striatum, prefrontal cortex and hippocampus after LPS-induced neuroinflammation. In the (STR), there was a significant reduction in the level of (AChE) in Quercetin 50 mg/kg and MEWA 200 mg/kg groups when compared to the LPS + Veh group. Also in the (PFC) a significant reduction was seen in the (AChE) level of Quercetin 50 mg/kg and MEWA 200 mg/kg when compared to the LPS + Veh group. Similarly, in the (HPC) a significant reduction was seen in the (AChE) level of Quercetin 50 mg/kg and MEWA 200 mg/kg when compared to the LPS + Veh group.

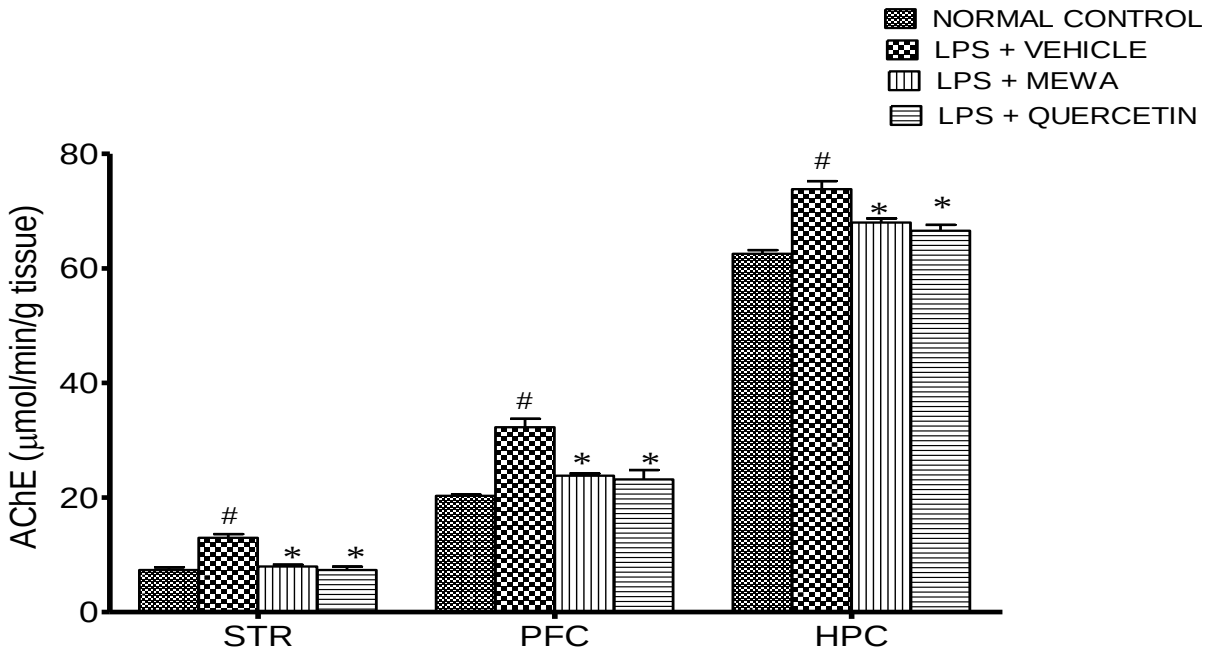

Figure 6: Effects of MEWA on the level of Acetylcholinesterase in rats Striatum (STR), Prefrontal cortex (PFC) and Hippocampus (HPC) after LPS-induced neuroinflammation. *p<0.05 and #p<0.05 compared to LPS + Vehicle and Normal control respectively.

Histological evaluation of the effect of MEWA on the prefrontal cortex, striatum and Hippocampus of Wistar rats after LPS- induced neuroinflammation

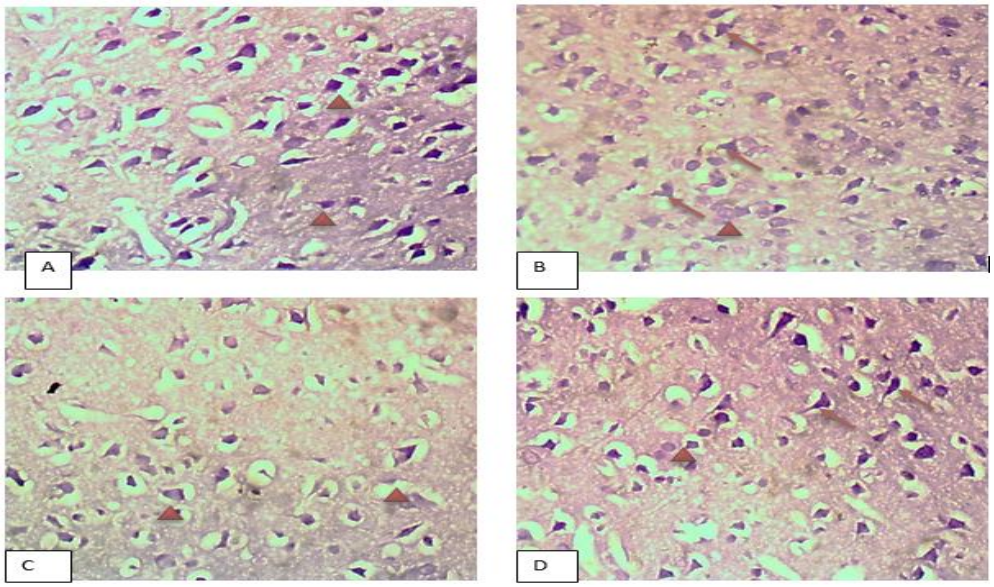

Figure 7: Representative sections of the prefrontal cortex of experimental rats stained with cresyl violet. Neurons of the LPS-treated group displayed some necrotic neurons with few normal neurons (red arrow) while MEWA and Quercetin-treated groups displayed normal neurons with a few necrotic neurons (red arrows). A= Control B= LPS + Vehicle C= LPS + MEWA (200 mg/kg), D= LPS + Quercetin (50 mg/kg). LPS= Lipopolysaccharide.

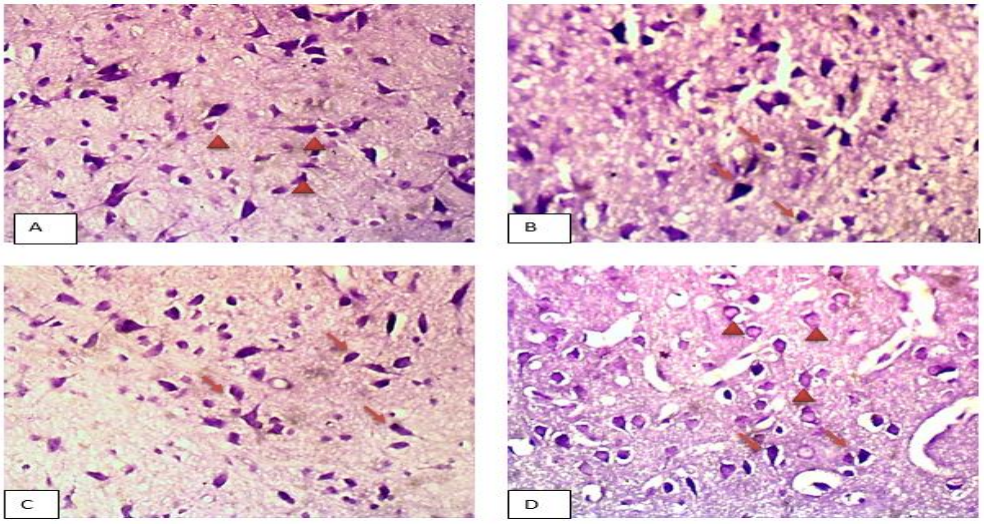

Figure 8: Representative sections of the striatum of experimental rats stained with cresyl violet. Neurons of the LPS-treated group displayed few normal neurons while MEWA and Quercetin-treated groups showed normal Nissl staining distribution among their neurons. A= Control B= LPS + Vehicle C= LPS + MEWA (200 mg/kg), D= LPS + Quercetin (50 mg/kg). LPS = Lipopolysaccharide.

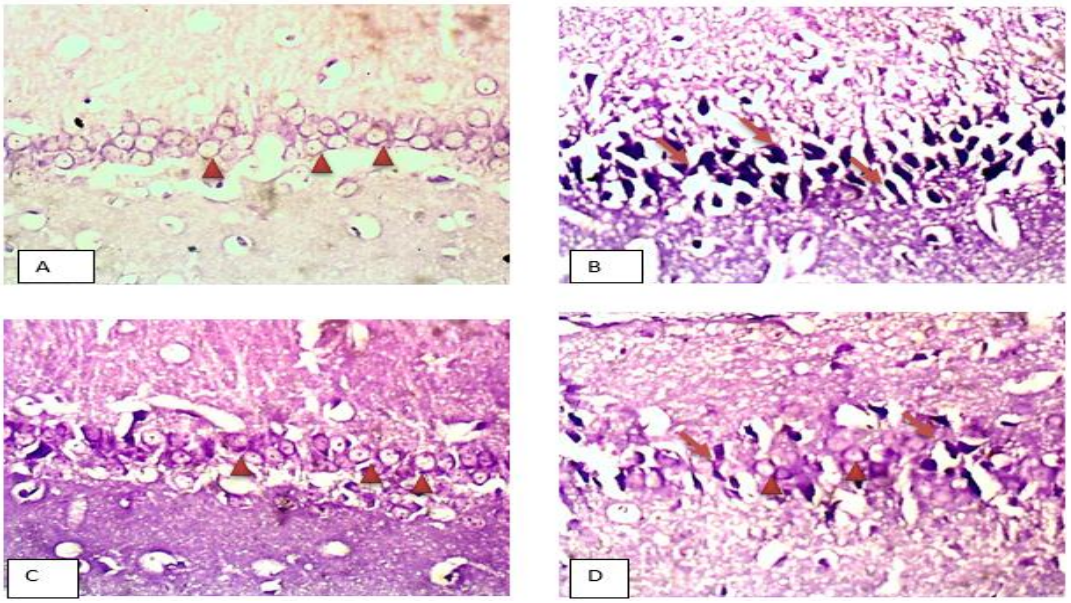

Figure 9: Representative sections of the hippocampus of experimental rats stained with cresyl violet. Neurons of the LPS-treated group displayed preponderance of highly pyknotic (dying) neurons (red arrow) while MEWA and Quercetin-treated groups displayed normal pyramidal neurons with a few pyknotic neurons (red arrows). A= Control B= LPS + Vehicle C= LPS + MEWA (200 mg/kg), D= LPS + Quercetin (50 mg/kg). LPS= Lipopolysaccharide.

DISCUSSION

In the present study, investigation of the neuroprotective and anti-oxidant properties of *Waltheria americana* was investigated using the LPS- induced model of neuroinflammation in rodents. This model has been previously used as an accepted model [19,20,21]

To examine the effect of MEWA on spatial memory, the Y Maze test was employed. This test is based on the mechanism of rodents to explore new environment and verify its spatial memory capacity. Hallucinogenic drugs impair this learning in Y maze [22]. In this study LPS impaired the spatial memory, this impairment was comparable with the work of Kim *et al*, 2016 [23] that used scopolamine to induce amnesia. Spatial memory loss was reversed by the administration of MEWA b**y** increasing both number of entries and percentage alternations. This shows the ability of the extract as a potential target for improving spatial working memory as seen in Alzheimer's disease patients. Glutathione is a known antioxidant responsible for mopping up free radicals, peroxides and heavy metals that might be injurious to cells [24]. Low levels of this enzyme are seen in conditions such as inflammatory diseases. From this study, induction of neuroinflammation via LPS caused a decrease in glutathione which was restored by MEWA in the striatum, hippocampus and prefrontal cortex of the brain. The ability of MEWA to mop up these free radicals indicates that it could be used as an intervention in the management of neurodegenerative diseases. Malondialdehyde, an index of lipid peroxidation, has been reported to be initiated by the production of reactive oxygen species (ROS) and can cause neuronal death in the brain especially cholinergic neurons [25]. From this study, MDA levels were reduced by MEWA in all the brain regions investigated, which reveals that MEWA could be a target to managing neurodegenerative diseases linked with oxidative stress. Our findings from this study on oxidative stress reduction by the extract during neuroinflamation is similar to the work of Etienne *et.al*, [26]. Acetylcholinesterase (AChE) is an enzyme found in cholinergic neurons and it is primarily involved with the breakdown of acetylcholine into acetate and choline [25]. Acetylcholine is primarily involved with the transmission of neuronal signals for to enhance learning and memory functions [26]. From the results, AChE levels were attenuated across all the regions signifying the ability of MEWA to enhance learning and memory functions in the brain.

Nissl staining revealed preponderance of highly pyknotic and necrotic neurons in the hippocampus and striatum of LPS-treated rats. This effect was observed to be reduced by MEWA suggesting the ability of MEWA to prevent neuronal damage in the cortex, striatum and hippocampus that cause cognitive deficits in various neurodegenerative diseases.

CONCLUSION

The extract of the leaf of *Waltheria Americana* ameliorated neuroinflammation in the striatum, prefrontal cortex and hippocampus by reducing levels of free radicals, acetylcholinesterase and preventing neuronal damage in laboratory rodents.

REFERENCES

[1] Guzman-Martinez L, Maccioni RB, Andrade V, Navarrete LP, Pastor MG, Ramos-Escobar N. Neuroinflammation as a Common Feature of Neurodegenerative Disorders. Front Pharmacol. 2019 Sep 12;10:1008. doi: 10.3389/fphar.2019.01008.

[2] McKee, A. C., Carreras, I., Hossain, L., Ryu, H., Klein, W. L., Oddo, S., et al. (2008). Ibuprofen reduces Abeta, hyperphosphorylated tau and memory deficits in Alzheimer mice. Brain Res. 1207, 225–236. doi: 10.1016/j.brainres.2008.01.095.

[3] Mundo J., Castillo-España P., Gutiérrez M.C., Abarca C., León-Rivera I., Arellano-García J., Perea-Arango I (2015). Methanolic extracts from roots and cell suspension cultures of Waltheria americana Linn induce GABA release in cerebral slices of mouse brain, *Afr. J. Pharm. Pharmacol*. 9: 139–144.

[4] Oladele A.T., Alade G.O., Omobuwajo O.R (2011). Medicinal plants conservation and cultivation by traditional medicine practitioners (TMPs) in Aiyedaade Local Government Area of Osun State, Nigeria. Agric Biol J N Am; 2(3):476–87.

[5] León-Rivera I, Herrera-Ruiz M, Estrada-Soto S, Gutiérrez MdelC, Martínez-Duncker I, Navarrete-Vázquez G, Rios MY, Aguilar B, Castillo-España P, Aguirre-Moreno A (2011). Sedative, vasorelaxant, and cytotoxic effects of convolvulin from Ipomoea tyrianthina. J. Ethnopharmacol. 135:434–439.

[6] Huerta-Reyes M, Herrera-Ruiz M, González-Cortazar M, Zamilpa A, León E, Reyes-Chilpa R, Aguilar-Rojas A, Tortoriello J (2013). Neuropharmacological in vivo effects and phytochemical profile of the extract from the aerial parts of Heteropterys brachiata (L.) DC. (Malpighiaceae). J. Ethnopharmacol. 146:311–317.Burkill HM. The useful plants of West Tropical Africa. Royal Botanic Gardens,Kew, London, United Kingdom 2000;686

[7] Burkill HM. The useful plants of West Tropical Africa. Royal Botanic Gardens,Kew, London, United Kingdom 2000;686.

[8] Zongo F., Ribuot C., Boumendjel A., Guissou I (2013). Botany traditional uses, phytochemistry and pharmacology of Waltheria indica L. (syn. Waltheria americana): a review, J. Ethnopharmacol. 148: 14–26.

[9] Saunders J.G (2007). Sterculiaceae of Paraguay. II. Waltheria. Bonplandia 16:143-180.

[10] Borokini TI, Omotayo, FO (2012). Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria. J. Med. Plant. Res. 6:1106–1118.

[11] Leonard D.B (1998). Medicine at your feet: healing plants of the Hawaiian kingdom. Waltheria indica ('Uhaloa). Roast Duck Producktions, US.

[12] Hamidu LJ, Ayo JO, Adelaiyre AB, Abubakar MS (2008). Sedative and anti-convulsant effects of ethyl acetate fraction of Waltheria indica in mice. J. Pharm. Toxicol. 3:261-266.

[13] Idowu Olumorin Owemidu, Mujeedat Adebukola Olubori, Oluwaseun Samuel Faborode, Oluwatobi Stephen Oloyede , Samuel Adetunji Onasanwo (2018). Anti-nociceptive and anti-inflammatory activities of the methanol extract of Waltheria americana Linn. leaf in experimental animals. *Journal of Intercultural Ethnopharmacology*. 9 (2): 47-54.

[14] Hughes RN. The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. *Neuroscience & Biobehavioral Reviews*. 2004;28(5):497–505.

[15] Ellman G. L., Courtney K. D., Andres V. Jr., and Featherstone R. M. (1961), A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88Ð95.

[16] Moron et al., 1979 M.S. Moron, J.W. Depierre, B. Hamervi ck Levels of GR and GST activity in lung and liverBiochem. Biophys. Acta, 582 (1979), pp. 67-78

[17] Adam-vizi V., Seregi M., (1982): Receptor dependent stimulatory effect of noradrenaline on Na+/K+ ATPase in rat brain homogenate: Role of lipid peroxidation. Biochem.Pharmacol., 31: 2231-2236.

[18] Oduola T, Bello I, Idowu T, Avwioro G, Adeosun G, Olatubosun L. Histopathological changes in Wistar albino rats exposed to aqueous extract of unripe Carica papaya. N Am J Med Sci. 2010.

[19] Zhao J., Bi W., Xiao S., Lan X., Cheng X., Zhang J., Lu D., Wei W., Wang Y., Li H., et al. Neuroinflammation Induced by Lipopolysaccharide Causes Cognitive Impairment in Mice. *Sci. Rep.* 2019;9:5790.

[20] Alzahrani N.A., Bahaidrah K.A., Mansouri R.A., Alsufiani H.M., Alghamdi B.S. Investigation of the Optimal Dose for Experimental Lipopolysaccharide-Induced

Recognition Memory Impairment: Behavioral and Histological Studies. *J. Integr. Neurosci.* 2022;21:049.

[21] Yang L., Zhou R., Tong Y., Chen P., Shen Y., Miao S., Liu X. Neuroprotection by Dihydrotestosterone in LPS-Induced Neuroinflammation. *Neurobiol. Dis.* 2020;140:104814. doi: 10.1016/j.nbd.2020.104814.

[22] Tursun Alkam, Toshitaka Nabeshima, Chapter 4 - Modeling the Positive Symptoms of Schizophrenia,Editor(s): Mikhail V. Pletnikov, John L. Waddington, Handbook of Behavioral Neuroscience,Elsevier,Volume 23,2016,Pages 39-54.

[23] Jong-Bo Kim, Spandana Rajendra Kopalli and Sushruta Koppula (2016) Indigofera tinctoria Linn (Fabaceae) attenuates cognitive and behavioral deficits in scopolamine-induced amnesic miceTrop J Pharm Res, 15(4): 778

[24] Abd-el-fattah M.A., Abdelakader N.F., Zaki H.F (2014). Pyrrolidine dithiocarbamate protects against scopolamine-induced cognitive impairment in rats. *European Journal of Pharmacology* 723: 330-338.

[25[Tabet N, Mantle D, Orrell M (2000): Freeradicals as mediators of toxicity in Alzheimer's disease: a review and hypothesis. *Adverse Drug Reactions and Toxicological Reviews*. 19(2): 127-152.

[26] E Djeuzong, AK Kandeda, S Djiogue, L Stéphanie, D Nguedia, F Ngueguim, et al. Antiamnesic and neuroprotective effects of an aqueous extract of Ziziphus jujuba Mill. (Rhamnaceae) on Scopolamine-Induced Cognitive Impairments in RatsEvid. Based Complement. Alternat. Med., 2021 (2021, August 11), Article 5577163, 10.1155/2021/5577163

[27] Munoz-Torrero D (2008): Acetylcholinesterase inhibitors as Disease-Modifying Therapies for Alzheimer's disease. *Current Medicinal Chemistry*. 15(24): 2433-2455.

[28] Leonardo Guzman-Martinez, Ricardo B. Maccioni1, Víctor Andrade, Leonardo Patricio Navarrete, María Gabriela Pastor, and Nicolas Ramos-Escobar (2019). Neuroinflammation as a Common Feature of Neurodegenerative Disorders. *Frontiers in pharmacology*. 10: 1008, 1-17.