

Effect of Ethanol Extract of *Annona muricata* on Lipopolysaccharide Induced Neuroinflammation in Wistar Rats

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Abstract

Introduction: *Annona muricata* is a lowland tropical fruit bearing tree of the Annonaceae family which can be found in the rainforest of Africa, South America, and Southeast Asia. It is commonly known as soursop, graviola, guanabana, or brazilian pawpaw. It has a large, glossy, dark green leaves with edible, green heart-shaped fruit. The leathery skin of the fruit is covered with soft, curved spines each of which may contain 55-170 black seeds distributed in a creamy white flesh with a characteristic aroma and flavour. **Objectives:** The study aims to investigate the effect of ethanol extract of *Annona muricata* on Lipopolysaccharide induced Neuroinflammation in Wistar rats. **Method:** Prostaglandin E1 & E2 markers were used for biochemical assays. The Neurobehavioral assessment of memory and locomotion coordination was assessed using the Y maze and Open field and the data was presented as Mean \pm Standard Error of Mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by a post hoc Newman-Keuls multiple comparison test. All statistical analysis were done by using Prism software, version 5 (GraphPad software Inc., San Diego, CA, USA). **Results:** The effects of ethanol extract of *Annona muricata* (AME) on PGE1 on lipopolysaccharide induced neuroinflammation in wistar rats shows the control, LPS, AME + LPS and Donepezil + LPS. From the result, LPS + Extract (AME) showed significant effects when compared to LPS which showed no significant effect when compared to control. The effects of ethanol extract of *Annona muricata* (AME) on % Alternation on lipopolysaccharide induced Neuroinflammation in wistar rats shows the control, LPS, AME + LPS and Donepezil + LPS. From the result LPS + Extract (AME) showed significant effects when compared to LPS. LPS showed no significant effect when compared to control. The effects of ethanol extract of *Annona muricata* (AME) on No of entry on lipopolysaccharide induced neuroinflammation in wistar rats shows the control, LPS, AME + LPS and Donepezil + LPS. From the result LPS + Extract (AME) showed no significant effects when compared to LPS which showed significant effect when compared to control. **Conclusions:** This study concludes that *Annona muricata*, at 200mg/kg possess excellent anti-inflammatory qualities and it could be further explored as a potential choice for the treatment of neurodegenerative diseases.

Keywords: *Annona muricata*, neuroinflammation, neurodegenerative diseases, Lipopolysaccharides.

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INTRODUCTION

Neuroinflammation is described as an inflammatory response within the brain or spinal cord. This inflammation is mediated by the production of cytokines, chemokines, reactive oxygen species, and secondary messengers produced by resident CNS glia (microglia and astrocytes), endothelial cells and peripherally derived immune cells (DiSabato *et al.*, 2016), which plays an important defensive role against

various pathogens (Tohidpour *et al.*, 2017). There are immune, physiological, biochemical and psychological consequences of these neuroinflammatory responses (DiSabato *et al.*, 2016). Although acute neuroinflammation plays a protective role, chronic neuroinflammation is frequently considered detrimental and damaging to nervous tissue (Pintado *et al.*, 2012).

Neuroinflammation induces and accelerates pathogenesis of Parkinson's disease (PD), Alzheimer's

disease (AD) and Multiple sclerosis (MS), while on the other side, it favors the recovery of the injured neurons (Tohidpour *et al.*, 2017). Recent evidence supports that neuroinflammatory processes involving immune cells, glial cells and neuronal cells are very crucial and fundamental to understanding the origin or pathogenesis of a disease, such as a progressive loss of dopaminergic neurons in PD (Chung *et al.*, 2010; More *et al.*, 2013; Kong *et al.*, 2017; Visan, 2017).

Neuroinflammation develops chronic in chronic neurological disorders, resulting in neuronal cell death. Several studies have suggested that neuroinflammation is a principal pathology in neurodegenerative and other CNS disease. Herbal medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous system of medicine (Alschuler *et al.*, 1997). Their first documented use in Indian, Chinese, Egyptian, Greek, Roman, and Syrian writings dates back about 5000 years. Herbal remedies/traditional medicines have thus been derived from ancient civilizations' rich traditions and scientific history (Kamboj, 2000). Herbs and plants can be processed and consumed in a variety of ways, including whole herbs, teas, syrups, essential oils, ointments, salves, rubs, capsules, and tablets containing a ground or powdered form of a raw herb or a dried extract.

Different plants has been reputed to have relevance and has been used to manage diseases e.g. *Annona muricata*, Arnica montana, Aloe vera, Capsicum frutescens, etc. *Annona muricata* is a lowland tropical fruit bearing tree of the Annonaceae family which can be found in the rainforests of Africa, South America, and Southeast Asia. It is commonly known as soursop, graviola, guanabana, or brazilian pawpaw. It has a large, glossy, dark green leaves with edible, green heart-shaped fruit (Moghadamtousi *et al.*, 2015). The leathery skin of the fruit is covered with soft, curved spines each of which

may contain 55-170 black seeds distributed in a creamy white flesh with a characteristic aroma and flavour (Coria-Tellez *et al.*, 2016).

MATERIALS AND METHODS

ANIMAL USE AND CARE

24 female Wistar rats weighing between 70g-140g were obtained from the animal holding unit, Babcock University, Ilishan-Remo and kept in a well-ventilated Wistar rats cage for two weeks to acclimatize. They were given feed and water and their beddings were changed every three days.

PLANT MATERIAL

The graviola leaves (*Annona muricata*) were gotten from the market at IlishanRemo, Ogun state. They were cut into smaller pieces and air dried at 40 degree Celsius and then homogenized into powder form. The ratio of ethanol used in soaking the powdered form of *Annona muricata* was 1:8 (1mg of annona powder was dissolved in 8ml of ethanol as the standard measurement). The powdered leaves of *Annona muricata* were then macerated with ethanol for a week, sieved, and decanted. Thereafter, the filtrate was concentrated using a rotary evaporator to obtain the ethanol extract. The extract was then lyophilized using a freeze dryer to further reduce the moisture content.

PREPARATION OF STOCK SOLUTION FOR LIPOPOLYSACCHARIDE (LPS)

Lipopoly saccharide (B5:111) was obtained from Sigma Aldrich, Belgium. 6 mg of lipopolysaccharide was dispensed into a glass bottle, 17.46 ml of normal saline was dissolved in 0.54 mg/ml of LPS. The solution was shaken and fresh solution was prepared on a daily basis. Lipopolysaccharide (LPS) was administered intraperitoneally using an insulin syringe to induce neuroinflammation at a dose of 250mcg/kg.

EXPERIMENTAL DESIGN

The rats were divided into 4 groups of 6 rats each as follows:

GROUPS	NUMBER OF ANIMALS	ADMINISTRATION	DURATIONS
1	6	Distilled water (10mL/Kg p.o)	14 days
2	6	LPS (250mcg i.p)	14 days
3	6	AME (200mg/kg p.o) + LPS(200mcg i.p)	14 days (7days each)
4	6	Donepezil (1mg/kg p.o) + LPS (250mcg/kg i.p)	14 days (7days each)

NEUROBEHAVIOURAL STUDIES

Neurobehavioral assessment of memory was done using the Y maze.

Y MAZE

The device is a three-arm maze formed like a capital 'Y,' with each arm spaced at a 120-degree angle. Arm lengths range from 30 to 50 cm, and mazes are

typically symmetrical, though asymmetrical mazes are frequently employed. The route widths are about 10 cm. The maze is typically utilized as an enclosed maze with roughly 30 cm high walls and elevated to a height of 50 cm from the floor. The two-goal arms may include a well with a food reward, or they could have an exit tube that permits the animal to escape the maze (Deacon 2013).

Rats were carried to the test room in their home cages and were handled by the base of their tails at all times. Each rats were placed into the center of the apparatus and allowed to explore the apparatus for 5 minutes. Each arm of the y-maze was labelled A, B, and C. After the 5 minute test, rats were returned in their home cages and the ymaze was cleaned with 70% ethyl alcohol and permitted to dry between tests. Observation was done using a timer.

The behaviors scored with the y-maze apparatus are:

- Number of entry: Frequency with which each rats entered each arm of maze
- Alternation: Frequency with which each rats enters a new arm of the maze rather than returning to one previously visited.

BIOCHEMICAL ASSAY

The brain of the rats were excised, weighed using digital weighing balance and placed in ice in order to protect the biochemical components. The whole brain tissues were macerated to form the tissue homogenate and the homogenate was centrifuged to form the supernatant solution. The supernatant solution was used for biochemical assay of ELISA test.

ELISA TEST

ELISA tests for Prostaglandins E1 & E2 were carried out according to manufacturer's instructions.

STATISTICAL ANALYSIS

All the data are presented as Mean \pm Standard Error of Mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by a post hoc Newman-Keuls multiple comparison test. All statistical analysis were done by using Prism software, version 5 (GraphPad software Inc., San Diego, CA, USA). The statistical differences at level $p < 0.05$ were considered significant.

RESULTS

LIPOPOLYSACCHARIDE INDUCED NEUROINFLAMMATION IN WISTAR RATS

- Effects of ethanol extract of *Annona muricata* in Prostaglandin E1 and PGE2 on lipopolysaccharide induced neuroinflammation in wistar rats

The effects of ethanol extract of *Annona muricata* (AME) on PGE1 on lipopolysaccharide induced neuroinflammation in wistar rats are shown in Figures 4.1 and 4.2. The results shows the control, LPS, AME + LPS and Donepezil + LPS. From the result, LPS + Extract (AME) showed significant effects when compared to LPS.

LPS showed no significant effect when compared to control.

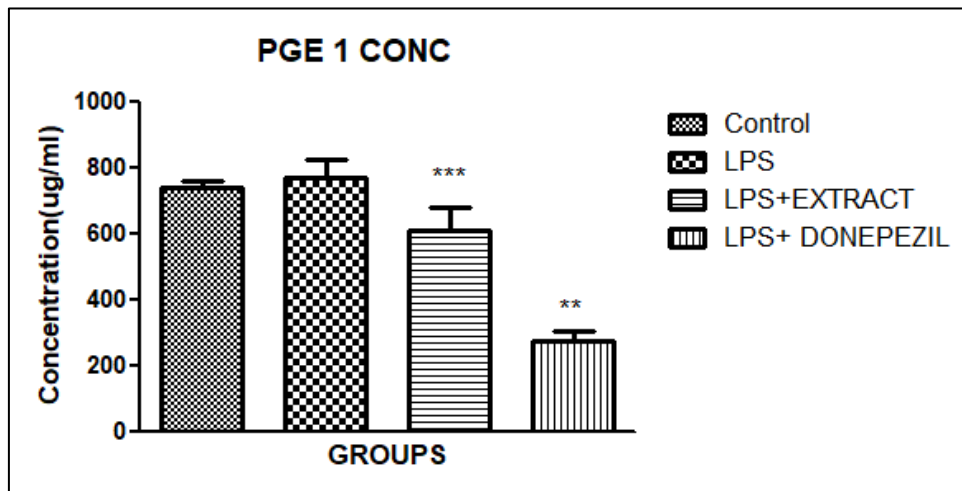


Figure 4.1: Effects of ethanol extract of *Annona muricata* in PGE1 on lipopolysaccharide induced neuroinflammation in wistar rats. Data are expressed as Mean \pm SEM (n=6). Comparisons were made using one-way ANOVA followed by post-hoc Newman-Keuls test. The symbol denotes significant levels: * $P < 0.05$ compared with the LPS group**

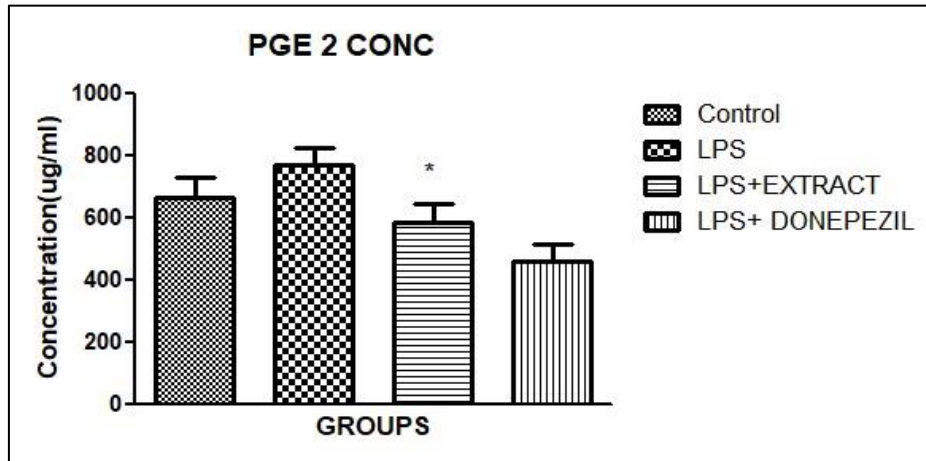


Figure 4.2: Effects of ethanol extract of *Annona muricata* in PGE2 on lipopolysaccharide induced neuroinflammation in wistar rats. Data are expressed as Mean ± SEM (n=6). Comparisons were made using one-way ANOVA followed by post-hoc Newman-Keuls test. The symbol denotes significant levels: *p<0.05. compared with the LPS group

EFFECTS OF ETHANOL EXTRACT OF *ANNONA MURICATA* IN Y MAZE ON LIPOPOLYSACCHARIDE INDUCED NEUROINFLAMMATION IN WISTAR RATS

- **Effect of ethanol extract of *Annona muricata* on spontaneous Alternation following LPS- induced Neuroinflammation in Wistar Rats**

The effects of ethanol extract of *Annona muricata* (AME) on % Alternation on lipopolysaccharide induced Neuroinflammation in wistar rats are shown in Figures 4.3. The results shows the control, LPS, AME + LPS and Donepezil + LPS. From the result LPS + Extract (AME) showed significant effects when compared to LPS. LPS showed no significant effect when compared to control.

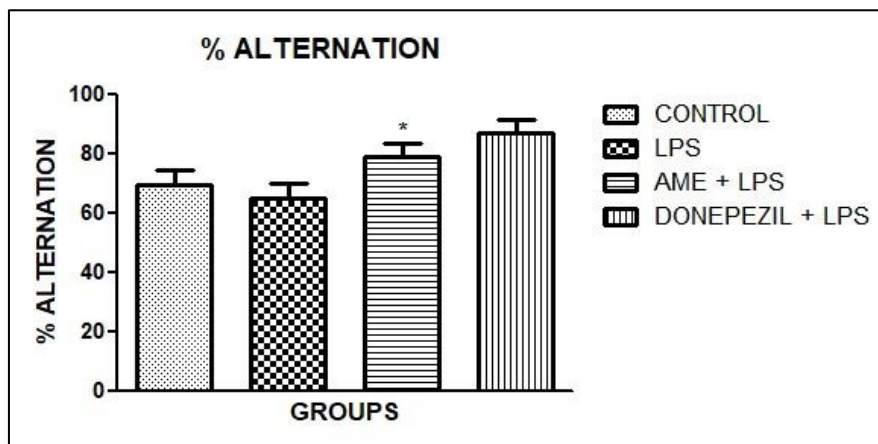


Figure 4.3: Effects of ethanol extract of *Annona muricata* on %Alternation on lipopolysaccharide induced neuroinflammation in wistar rats. Data are expressed as Mean ± SEM (n=6). Comparisons were made using one-way ANOVA followed by post-hoc Newman-Keuls test. The symbol denotes significant levels: *p<0.05 compared with the LPS group

- **Effect of Ethanol extract of *Annona muricata* on No of Entry following LPS- induced Neuroinflammation in Rats Wistar**

The effects of ethanol extract of *Annona muricata* (AME) on % Alternation on lipopolysaccharide induced neuroinflammation in wistar rats are shown in

Figures 4.4. The results shows the control, LPS, AME + LPS and Donepezil + LPS. From the resul, t LPS + Extract (AME) showed no significant effects when compared to LPS.

LPS showed significant effect when compared to control.

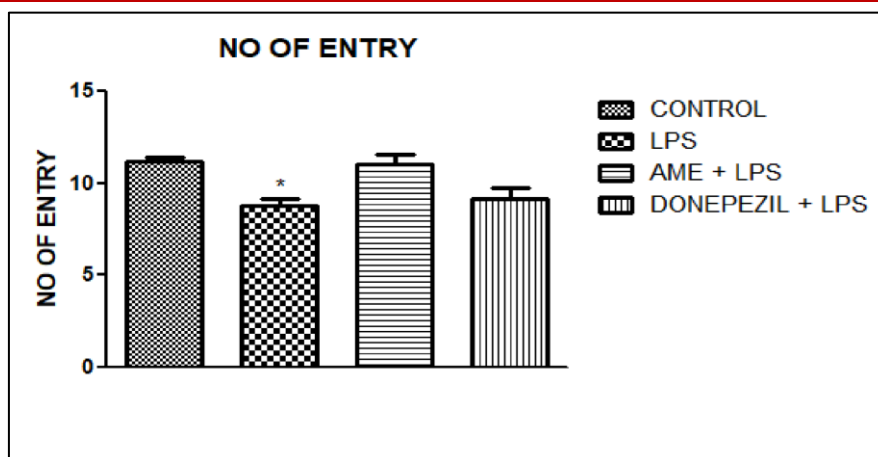


Figure 4.4: Effects of ethanol extract of *Annona muricata* in No of entry induced neuroinflammation in wistar rats. Data are expressed as Mean ± SEM (n=6). Comparisons were made using one-way ANOVA followed by post-hoc NewmanKeuls test. The symbol denotes significant levels: * $p < 0.05$ compared with the LPS group

DISCUSSION

The present day study elucidated the effect of ethanol extract of *Annona muricata* leaf extract on Neuroinflammation models in laboratory animals. Neuroinflammation was induced using the Lipopolysaccharide while the choice of laboratory animals used for the study is female Wistar rats. The choice of this plant source could be traced to its vast use in folklore medicine to treat many common diseases by folklore medicine practitioners.

From the result, group two animals showed marked increase in prostaglandin E₁ and E₂ levels when compared to that of the control. However, the prostaglandin E₁ and E₂ levels in both group three and four were significantly reduced. The marked increase in group two animals could be due to inflammatory effect of lipopolysaccharide. Lipopolysaccharide (LPS) is the major component of Gram-negative bacteria cell walls and can cause an acute inflammatory response by triggering the release of a vast number of inflammatory cytokines in various cell types. LPS is widely recognized as a potent activator of monocytes/macrophages. However, according to Yuan *et al.*, (2009), inflammation is characterized by increase prostaglandin E₂ levels and this could be attributed to the cyclooxygenase conversion of arachidonic acid which occur in response to localized injury and infection. High levels of prostaglandins are produced in response to injury or infection and cause inflammation, which is associated with the symptoms of redness, swelling, pain and fever. This is an important part of the body's normal healing process. Administration of ethanol extract of *Annona muricata* however significantly reduced the prostaglandin level in group three relative to group four and these changes could be due to the inhibitory activities of ethanol extract of *Annona muricata* on both cyclooxygenases one and two thereby preventing the release of prostaglandin. The inhibition of cyclooxygenases within the neuronal cells

by *Annona muricata* has been previously reported by Ishmael *et al.*, (2014).

Neurobehavioural studies in laboratory rodents has been reputed as a sure way to assess the effectiveness and side effects of many administered substances targeted at different structures of the brain and as a matter of fact, this behavioural assessment strategy helps in assessing changes in behavioural pattern of an animal following the administration of a known substance (McKinney, 2009). Spontaneous alternation is a behavioral test for measuring the willingness of rodents to explore new environments. Rodents typically prefer to investigate a new arm of the maze rather than returning to one that was previously visited. Many parts of the brain--including the hippocampus, septum, basal forebrain, and prefrontal cortex--are involved in this task (McKinney, 2009). The result of this study showed that group two animals showed significant decline in spontaneous alternation in the three arms of the Y maze apparatus when compared to that of control group. This result is in concordance with that of Zhao *et al.*, (2016) who reported that Peripheral administration of LPS activates the immune system and neurotransmitter in the hippocampus as well as the other brain regions leading to decreased synaptic transmission, memory, and locomotion. However, ethanol extract or *Annona muricata* significantly improved spontaneous alternation in group three animals. This could be due to the presence of natural agents like flavonoid which possess excellent membrane stabilizing properties and which also has the potential to improve healing process of Neurilemma following Neuroinflammation. The result also tallies with the findings of Yang (2015) who attributed the anti-inflammatory potentials of *Annona muricata* leaf extract to presence of flavonoids and alkaloids which are present in abundance in the extract and which possess excellent membrane stabilizing and wound healing qualities.

CONCLUSIONS

This study concludes that *Annona muricata*, at 200mg/kg possess excellent antiinflammatory qualities and it could be further explored as a potential choice for the treatment of neurodegenerative diseases.

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