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Full-Length Research Article

Anti-Inflammatory Potentials of *Anacardium occidentale* L. Nut Methanol Extract in Wistar Rats

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Summary: The Anacardium occidentale nut is increasingly gaining attention due to its economic, nutritional value and biological properties. This study is aimed at evaluating the anti-inflammatory potential of Anacardium occidentale nut Methanol extract (AOME) in carrageenan-induced paw oedema and the carrageenan-induced air pouch models of inflammation. AOME (200 mg/kg) significantly ($p<0.05$) inhibited carrageenan-induced increase in paw volume and increase in paw size, respectively, at 5 hours post-carrageenan injection. In the carrageenan induced air pouch inflammation model, 200 mg/kg AOME significantly ($P<0.05$) reduced exudate volume, Total leucocyte count, Neutrophil cell count, Monocyte count and Lymphocyte count. 200 mg/kg AOME also reduced significantly MDA, Nitrite, TNF-α, IL-6, and significantly increased GSH and catalase levels. Anacardium occidentale nuts showed anti-inflammatory effects through reduction in lipid peroxidation and TNF-α and IL-6 activity, alongside amelioration of oxidative stress.

Keywords*:* Inflammation, *Anacardium occidentale* Nut, Carrageenan, Antioxidant, Oedema model, Air pouch model

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INTRODUCTION

Inflammation is the defensive response of the body to injury, either through physical damage, immunological agents, chemical agents, or actions of pathogens. An inflammatory response in the body is generally due to two purposes: tissue homeostasis and defence of tissues against pathogens (Lo *et al*., 1999; Dzoyem *et al*., 2017). Inflammation has historically been hailed to be solely a beneficial response to injurious stimuli. Still, it has been implicated over the years in the pathogenesis of several disease states, either as the cause or an aggravator of these conditions. Typical examples of the diseases include cancer, cardiovascular diseases, respiratory diseases like chronic bronchitis, emphysema, asthma, metabolic conditions like diabetes and obesity, classic inflammatory conditions like rheumatoid arthritis and inflammatory bowel diseases, and also neurodegenerative diseases like Alzheimer's, Parkinson's, and depression (Haslett, 1992). Inflammatory conditions are managed primarily through lifestyle modifications, such as dietary changes, exercise, and stress management, as well as, when necessary, medication, including nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and, in some cases, targeted therapies specific to the cause and severity of inflammation (Chen *et al*., 2017). Use of NSAIDs and other drugs has not been particularly successful, as they have been associated with drug-related toxicities, iatrogenic reactions and other adverse effects (Dyozem *et al*., 2017). Therefore, the investigation into natural alternatives, particularly plant-derived medicines, to synthetic anti-inflammatory agents has gained ground over time.

Nuts are one of the most nutritionally concentrated foods known, as they contain about 55-70% fat, 10-30% protein, alongside a good proportion of carbohydrates (Blomhoff *et al*., 2006). They also contain several bioactive and health-promoting components, including numerous types of antioxidants with varying properties (Alasalvar and Bolling, 2015). The cashew nut is composed of about 40 – 57% oil and about 21% protein (Kluczkovski and Martins, 2015). Cashew nut is one of the most popular tree nuts worldwide, and its annual production is higher than that of any other tree nut, with about 3.5 million tons produced annually (Kluczkovski and Martins, 2015).

Edible phytochemicals have been shown to provide a multitargeted therapy alternative to contemporary anti-inflammatory drugs. The leaf and stembark of *Anacardium occidentale* are used in folkloric medicine to treat inflammation-associated diseases. Extracts from different parts of *Anacardium occidentale* have been found to possess antimicrobial activity (the leaves and stem), and anti-inflammatory property (leaves, stem and bark) (Onasanwo *et al*., 2012; Dias-Souza *et al*.,2013; Olajide *et al*., 2013). Roasted cashew nuts were found to reverse hypertension related to liver failure in male rats (Akintunde *et al*., 2023). Cashew nuts have also been shown to improve diabetic (type-2) condition, oxidative stress and inflammation in arthritis and colitis (Damavandi *et al*., 2019; Fusco *et al*., 2020; Siracusa *et al*., 2020). Methanolic extract of the cashew nut has also been shown to

possess anti-diabetic, anti-hyperlipidemic, and anti-oxidative effects (Ajao *et al.,* 2021). Consuming nuts was found to lower blood lipid levels, albeit mainly in Individuals with low body mass (Sabate, 2010). Nuts consumption has also been shown to reduce cardiovascular and diabetes risk. However, this effect is independent of body mass and other factors, likely due to a reduction in inflammatory markers (Jiang *et al*., 2005). Thus, concerns still remain regarding the increase in weight due to the high fat and energy-dense content of cashew nuts and other nuts. This study is then aimed at evaluating the anti-inflammatory potential of *Anacardium occidentale* nut Methanol extract (AOME), which is devoid of the oily components of the nut.

MATERIALS AND METHODS

Drugs and Reagents: λ-carrageenan, Ellman's reagent, Thiobarbituric acid, Trichloroacetic acid were products from Sigma-Aldrich (USA). Sodium chloride, Sodium monobasic phosphate, sodium dibasic phosphate (BDH, England). Tumor necrosis factor-alpha (TNF-α) and Interleukin-6 were ELISA kits from Biolegend, USA.

Preparation of *Anacardium occidentale* Nut Methanol Extract: Cashew nuts were purchased from local suppliers in Ogbomosho, Oyo State, Nigeria. The nuts were identified by Voucher Number: FHI: 111039. The kernels were separated from the shells manually using a knife to cut the shells transversely, and the kernels were quickly removed from the shells to minimise contact with the cashew nutshell liquor. The Kernels were then oven-dried at 40°C for 6 days, and the dried kernels were blended into a powdery form. The nut powder was then macerated in n-hexane for 72 hours, 3 times to completely defat it, before further maceration in Methanol for 72 hours. The filtrate was concentrated in a vacuum rotary evaporator at 40 °C. The percentage yield was determined to be 2%. The resulting paste extract denoted as *Anacardium occidentale* nut methanol extract (AOME) was stored at 4 °C in a refrigerator and daily prepared in distilled water for pharmacological assays.

Experimental Animals: Male Wistar rats weighing 150-200g were obtained from the laboratory animal facility in the University of Ibadan. The animals were housed in cages maintained under standard environmental conditions of light and humidity and fed with pelletized feed (Vital Feeds Ltd, Jos) They had an unlimited supply of food and water. Ethical approval (UI-ACUREC/18/0077) was given by the Animal Care and Use Research Ethics Committee (ACUREC) of the University of Ibadan. All procedures related to animals were carried out in compliance with ACUREC's guidelines.

Qualitative Phytochemical Screening: Conventional standard protocols described by Harborne, (1998), Odebiyi and Sofowora (1978) for detecting the presence of different phytochemical constituents in plant extracts were employed. Secondary metabolites tested for include alkaloids, anthraquinones, tannins, saponins, flavonoids, steroids, cardiac glycoside, terpenoids, and phenols. The presence of the compounds tested was rated as positive (+) or negative (-).

Acute Oral Toxicity Test: An acute toxicity test of the methanol extract of *Anacardium occidentale* L. nuts was performed in accordance with the OECD 423 criteria, (Schlede, 2002). A dose of the extract at a concentration of 2000 mg/kg body weight to a group of three female rats that had fasted for 24 hours beforehand, while the control received distilled water (10 mL/kg). The rats were under continuous observation for signs of toxicity during the initial four hours and for a duration of 14 days post-administration.

Assessment of Anti-Oedema Activity of *Anacardium occidentale* Nut Methanol Extract (AOME) in Carrageenan-Induced Paw Oedema in Rats: Twenty-five male Wistar rats were randomly divided into five groups. The animals were pre-treated with AOME and the selected standard drug (Indomethacin) one hour before the induction of oedema, following an overnight fast. The administered doses include 50mg/kg, 100mg/kg, and 200mg/kg for AOME, and 5mg/kg for Indomethacin. Oedema was induced in the right hind paw by injection of 0.1 ml of 1% carrageenan suspension in 0.85% NaCl solution in the sub-plantar tissue of the paw. The paw volume was measured immediately before oedema induction and hourly for 5 hours after induction using the Ugo Basile Plethysmometer. This was done according to the method described by Winter *et al*. (1962). The percentage inhibition of the development of oedema was determined with;

$$\%\text{inhibition} = \frac{\text{AUC of control} - \text{AUC of treatment}}{\text{AUC of control}} * 100$$

AUC – Area under the curve

Assessment of the Effect of *Anacardium occidentale* Nut Methanol Extract (AOME) on Carrageenan-Induced Air Pouch Model of Inflammation: Air pouch was induced in all the groups as described by Sedgwick *et al*. (1985) but with adjustments made by Ajayi *et al*. (2017). A total of thirty male Wistar rats were divided into six groups of five. These groups were given: 50 mg/kg AOME, 100 mg/kg AOME, 200 mg/kg AOME, 5 mg/kg indomethacin, Carrageenan-only group, and normal saline control group. Following the protocol laid out by Sedgwick *et al*. (1985), air pouches were formed in all of the groups. The rats were anesthetised, and air pouches were created by subcutaneously injecting 20 ml of sterile air into the intrascapular region of their backs on the first day of the experiment. To maintain the pouch's patency, it was inflated with an additional 10 ml of sterile air on the fourth day. The oral treatment of the AOME and indomethacin groups began on the 4th day and continued until the 6th day. Inflammation was induced on the 6th day by administering 2 ml of 2% carrageenan in the pouch. Twenty-four hours later, the pouch was cleansed by infusion of 2 ml of normal saline. The pouch was subsequently incised, allowing for the aspiration and measurement of the exudates.

Exudate Volume, Total Leukocyte and Differential Cell Count: The cavity was flushed with 2 ml of phosphate buffer saline after it was opened, and the exudates were extracted and placed in sterile, ice-cold tubes. The quantity of exudates collected was measured using a syringe. Total leukocyte count was done by staining with Turk's solution and counted with the Neubauer chamber hemocytometer under a light microscope. Differential white blood cell count was done using May Grunwarld-Giemsa stain on cytopsin slide smears.

Estimation of Protein concentration: The biuret method as described by Gornall et al., (1949), was used to measure the quantity of proteins in the exudates. The supernatant (50 µl) was mixed with distilled water (1.950 ml), and Biuret reagent (3ml) was further added to the mixture. The mixture was incubated at room temperature for half an hour. The absorbance was measured at 540 nm, and the concentration was ascertained from the standard curve.

Assessment of Lipid Peroxidation by Measuring Malondialdehyde: Thiobarbituric acid reactive substances (TBARS) was the indicator used to measure level of lipid peroxidation. TBARS measurement carried out following the method of Nagababu et al. (2010) as adjusted by Ajayi et al. (2017). Supernatant from the exudates (0.1 mL) was mixed in Tris-KCl buffer (0.15 M) twenty times. 0.75% Thiobarbituric acid was mixed with thirty percent of Trichloroacetic acid (TCA) (0.5 mL), then the mixture was left to incubate in a water bath at 80 °C for 45 minutes. The mixture was then ice-cooled for 10 minutes and centrifuged for 5 minutes at 4000 revolutions per minute. Using a spectrophotometer, the amount of light absorbed by the supernatant was measured at 532 nm. The outcome was determined by utilising an absorption index for malondialdehyde (molar extinction coefficient 1.56 × 10^5/M/cm) unit of measurement for thiobarbituric acid in the exudates is nmol/ml.

Estimation of Nitrite Concentration: The assay for nitrite was conducted as described by Palmer et al. (1988). Griess reagent was used to measure the amount of nitrite present in cell-free exudates. The Griess reagent used was freshly prepared by mixing a 1:1 ratio of 0.1% N-1-naphthyl ethylenediamine dihydrochloride and 1%

sulfanilamide in 5% phosphoric acid. Exudate samples were treated with Griess reagent and analysed with a spectrophotometer at 540 nm. The amount of nitrite in the exudates, measured in µmol/ml, was determined using a standard curve plotted with sodium nitrite concentrations ranging from 0 to 100 µM. The amount of nitrite in exudates was measured in micromoles per millilitre.

Estimating the Activity of Reduced Glutathione Enzyme: The assay for glutathione (GSH) as non-enzymic antioxidant was conducted following the method described by Yan et al. (1997) which is a modification of the methodology by Beutler et al. (1963). The amount of reduced glutathione, present in the exudates was measured using Ellman's reagent. 1 mL of 20% TCA was used to dilute the supernatant (0.1 mL) from the exudates ten times. Centrifugation of the solution was done for 10 minutes at 10,000 rpm in a cold centrifuge at 4 °C. 51-Dithios-nitrobenzoic acid (2mL, 0.0006 M) and sodium phosphate buffer (0.75 mL, 0.1 M, pH 7. mL, 0.1 M, pH 7.4) were mixed with the supernatant (0.25 mL). Using a spectrophotometer at 412 nm, the mixture's absorbance was measured. Standard glutathione (0–200 µM) was used to plot a standard curve, which was then used to estimate the concentration of glutathione in the exudates (µmol/mL of exudates).

Estimation of the Activity of Catalase Enzyme: The methodology stated by Goth (1991) was used to assess Catalase activity. A 96-well microtiter plate was used to prepare and incubate a 50 µL mixture for 5 minutes at a temperature of 37°C. The mixture contained H_2O_2 (50 mM) and ionic liquids at phosphate buffer at pH 7.4. Subsequently, catalase solution (50 µL; 50 µg/mL in 0.2 M, pH 7.4 phosphate buffer) was added to the mixture, and then incubated at 37°C for an additional 5 minutes. A microplate reader was used to record the absorbance at 405 nm after 100 µL of 64.8 mM ammonium molybdate was added to stop the enzymatic process.

Assay for Tumour Necrosis Factor alpha (TNF-α) and Interleukin-6 (IL-6) by ELISA: The amount of TNF-α and IL-6 in the exudates from the air pouches was measured using the enzyme-linked immunoassay method. This was done using standard ELISA kit; ELISA MAX™ Deluxe kit (BioLegend, USA) following the guidelines provided by the manufacturer.

Statistical Analysis

Data obtained were presented as mean ± SEM. The data were analysed using one-way ANOVA, followed by the Tukey's post hoc multiple-comparison test. Significant difference was taken at $p < 0.05$

RESULTS

Acute Toxicity test: There was no mortality after administration of a dose of 2000 mg/kg MAON or within fourteen days of observation.

Change in paw volume and paw size in carrageenan-induced oedema inflammation: Injection of carrageenan into the paw of rats resulted in time dependent increase in volume and size of the paw. The 50 mg/kg, 100 mg/kg, and 200mg/kg doses of AOME significantly inhibited the carrageenan-induced increase in volume and size in a dose dependent manner. This is illustrated in Figures 4.1, 4.2, 4.3, and 4.4 below. 50 mg/kg, 100 mg/kg and 200 mg/kg of AOME inhibited the increase in paw volume by 33.8%, 49.6% and 52.4% respectively, and the paw size was inhibited by 35.1%, 64.4%, and 46.7% respectively. Similarly, 5 mg/kg Indomethacin inhibited increase in paw volume and paw size by 70.7% and 80.1% respectively. This is shown in Figure 1.

Table 1:
Phytochemical Components of Methanol Extract of *Anacardium occidentale* L. Nut

Phytochemicals	Relative Abundance
Tannins	++
Saponins	++
Flavonoids	++
Anthraquinone	-
Cardiac Glycoside	-
Terpenoids	+
Steroids	+
Alkaloids	++
Phenols	++

++ = Abundantly present; + = Moderately present; - = Absent

Effect of AOME on Cellular migration and exudate formation in carrageenan-induced air pouch: Carrageenan injection significantly increased volume of exudates (3.9±0.1291) produced, total leucocytes (44.31±3.322), and specifically neutrophils (41.12±3.50), monocytes (0.6983±0.1489), and lymphocytes (13.91±0.6888). Treatment with AOME significantly reduced the exudate volume (100 mg/kg – 3.0±0.1225, 200 mg/kg – 3.150±0.1190) and, Total leucocytes (50 mg/kg – 27.75±3.085, 100 mg/kg – 16.68±0.9311, 200 mg/kg – 1.779), neutrophils (50 mg/kg – 15.94±1.843, 100 mg/kg – 10.96±0.6761, 200 mg/kg – 21.99±3.3), monocytes (50 mg/kg – 0.2783±0.03124, 100mg/kg – 0.2±0.03114), and lymphocytes (50 mg/kg – 10.83±0.8459, 100 mg/kg – 5.710±0.1847, 200 mg/kg – 8.902±0.4008). This is shown in Figure 2.

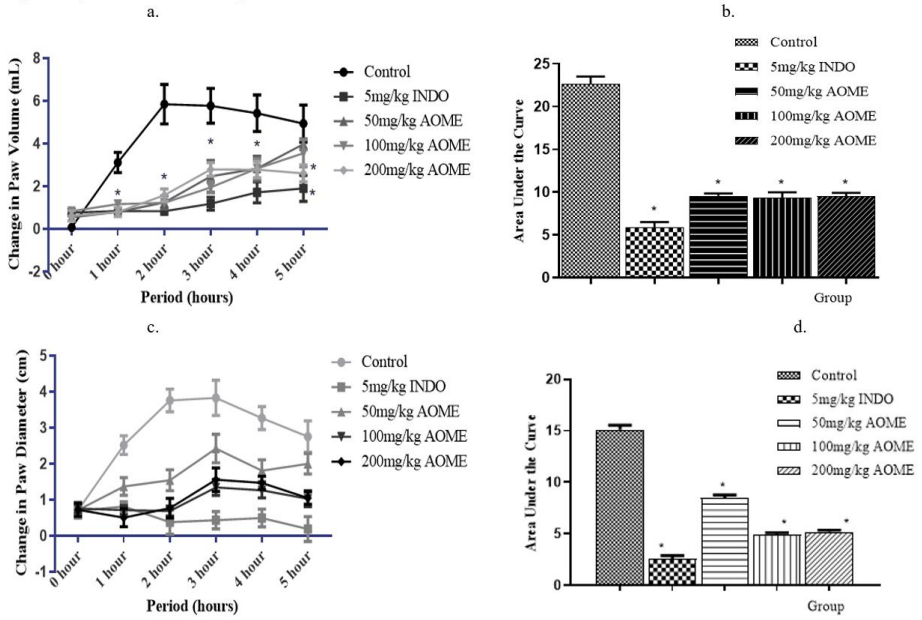

Figure 1:
The Effect of AOME administration on paw volume across 5 hours after induction of inflammation. a. Change in paw volume, b. Area under the curve (AUC) of change in paw volume, c. Change in paw size, d. Area under the curve (AUC) of change in paw size. The area under the curve for increase in rat paw oedema in 4 h. Values are Mean ± SEM (n = 5). Significant difference denoted by *p < 0.05 compared to control by one-way ANOVA followed by Tukey's post hoc test

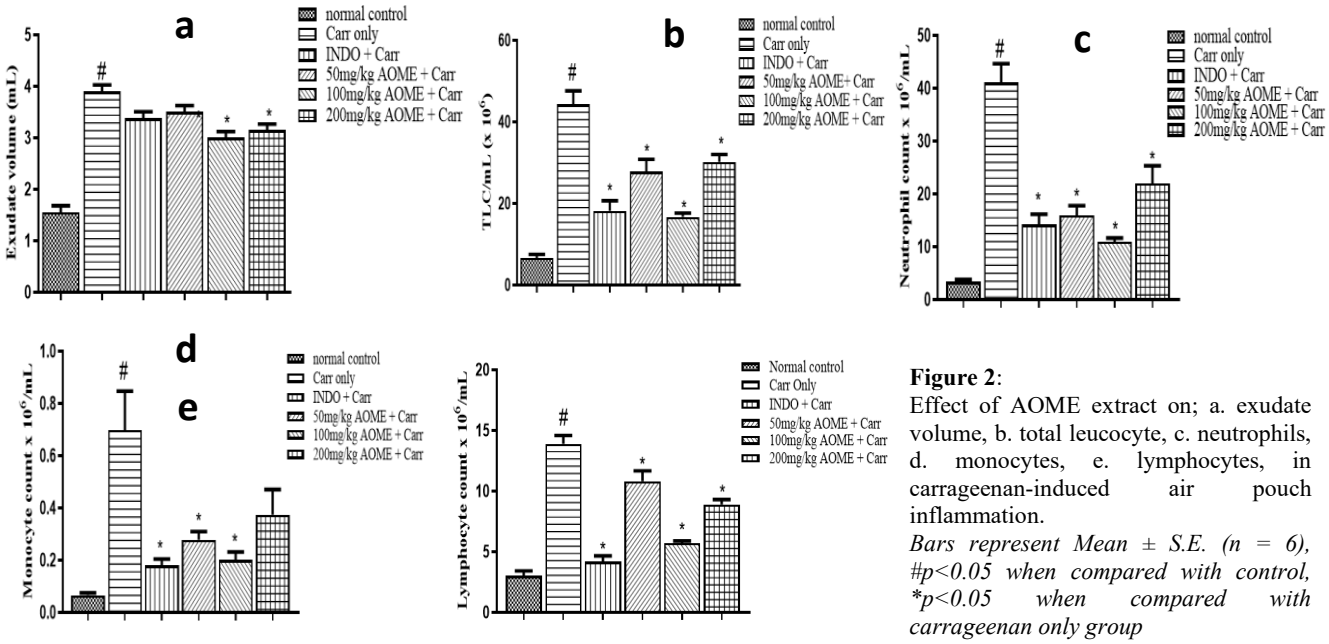

Figure 2:
Effect of AOME extract on; a. exudate volume, b. total leucocyte, c. neutrophils, d. monocytes, e. lymphocytes, in carrageenan-induced air pouch inflammation.
*Bars represent Mean ± S.E. (n = 6), #p<0.05 when compared with control, *p<0.05 when compared with carrageenan only group*

Effect of AOME on Lipid peroxidation and Nitrite in carrageenan-induced air pouch: Injection of carrageenan into six-day-old pouches significantly increased lipid peroxidation when compared to the control, with malondialdehyde as a metric. Malondialdehyde levels were significantly reduced by AOME (100mg/kg group - 24.54±1.251; 200mg/kg group – 21.88±1.734) in a dose-dependent manner when compared to the carrageenan only group (36.42± 0.8848). Nitrite levels were also significantly increased when compared to controls by carrageenan administration (101.7±4.448), and this was significantly inhibited by pretreatment with AOME (50 mg/kg – 72.84±3.068, 100 mg/kg – 74.88±4.058, and 200 mg/kg – 66.18±2.581). This is shown in Figure 3

Effect of AOME extract on Antioxidant enzymes in carrageenan air pouch: Injection of carrageenan into 6-day-old pouch significantly decreased glutathione (GSH) and catalase levels when compared to controls. Pretreatment with AOME (50 mg/kg – 8.571±0.5578, 100mg/kg – 9.007±0.4167, and 200mg/kg – 8.108±0.3663) significantly increased GSH when compared to the carrageenan only group (5.074±0.4168). Catalase (50 mg/kg – 22.60±0.9641, 100 mg/kg – 22.78±0.6785, 200mg/kg – 22.73±0.8716) levels was also significantly increased when compared to the carrageenan only group. This is shown in Figure 4.

Effect of AOME extract on proinflammatory cytokines in carrageenan-induced air pouch: Injection of carrageenan into 6-day-old pouches significantly increased the presence of TNF (231.6±10.24) and IL-6 (94.54±2.145) in the pouches. Pretreatment with AOME significantly reduced TNF levels (50 mg/kg – 145.0±4.009, 100 mg/kg – 61.79±8.311, 29.55±13.98)in a dose dependent manner across three doses, while only 200 mg/kg produced a significant reduction in IL-6 (200 mg/kg – 69.46±1.056) concentration when compared to carrageenan group. This is shown in Figure 5.

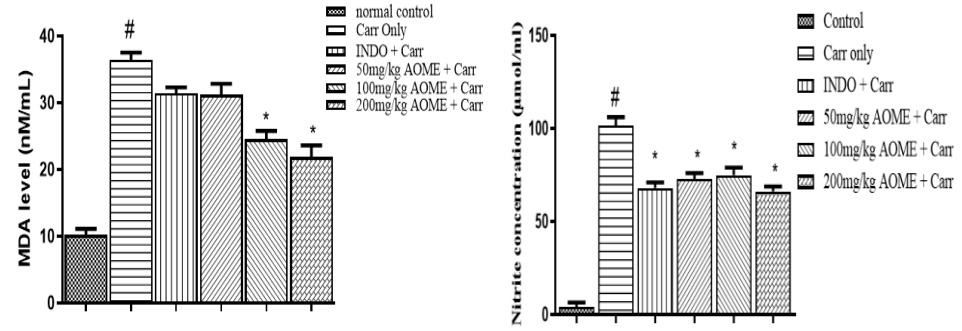

Figure 3:
Effect of AOME administration on a. malondialdehyde (MDA) and b. Nitrite concentration in carrageen-induced air pouch. Bars represents Mean ± S.E. (n = 6), #p<0.05 when compared with control, *p<0.05 when compared with carrageenan only group.

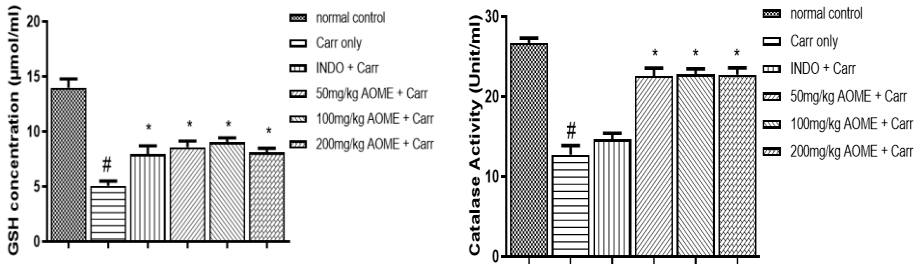

Figure 4:
Effect of AOME on antioxidant enzymes; a. reduced Glutathione, b. Catalase, in carrageenan-induced air pouch. Bars represent Mean ± S.E. (n = 6), #p<0.05 when compared with control, *p<0.05 when compared with carrageenan only group

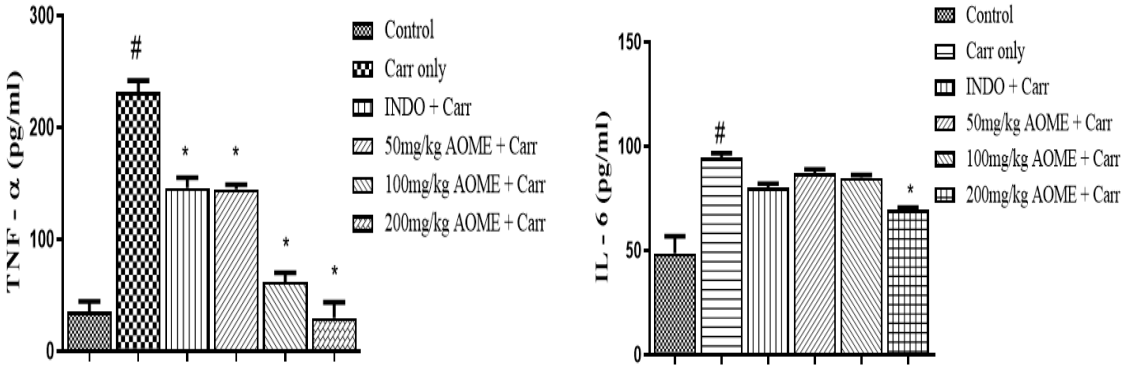

Figure 5:
Effect of AOME on a. Tumour Necrosis Factor (TNF-α); b. IL-6, concentration in carrageenan-induced air pouch inflammation. Bars represents Mean ± S.E. (n = 6), #p<0.05 when compared with control, *p<0.05 when compared with carrageenan only group

DISCUSSION

Inflammation, especially in the acute phase, involves adjustments in the blood supply to the site of injury, leading to accumulation of fluid and migration of cells to the site of injury under the influence of inflammatory mediators (Higgins and Lees, 1984; Medzhitov, 2008). Polymorphonuclear leucocytes, like neutrophils, are attracted to the point of inflammation following dilation of blood vessels due to the influence of inflammatory mediators, like cytokines, on the target tissues. These phagocytic cells identify, attack, ingest, and dispose of invading pathogens and other particulate matter, aimed at resolving inflammation (Sugishita *et al*., 1981).

In this study we investigated the anti-inflammatory property of *Anacardium occidentale* nuts methanol extract. The anti-inflammatory effect was evaluated in carrageenan-induced rat paw oedema, and carrageenan-induced air pouch models of inflammation.

The rat paw oedema study showed that *Anacardium occidentale* extract prevented the inflammatory and oxidative stress response induced by carrageenan in rats. Carrageenan-induced rat paw oedema is a widely used test to determine anti-inflammatory activity. It constitutes a routine animal model for evaluating pain at the site of inflammation without any injury or damage to the inflamed paw (Sugishita *et al*., 1981; Henriques *et al*., 1987; Jain *et al*., 2001; Sini *et al*., 2010). The development of oedema in the rat hind paw following the injection of carrageenan is essentially a biphasic, age-weight dependent event in which various mediators operate in sequence to produce the inflammatory response. The first phase of the oedema formed is uninhibited by NSAIDs, and the mediators involved include histamine, serotonin, and bradykinin. The second phase is associated with the elevated production of prostaglandins and, in recent times, inducible cyclooxygenase (COX-2) (Nantel *et al*., 1999). Despite the phases in the inflammatory response, oedema formation due to carrageenan is known to peak approximately 3 hours post-induction. Oral treatment of *Anacardium occidentale* (50, 100, and 200 mg/kg) caused significant inhibition of oedema formation at the third hour. While only *Anacardium occidentale* nut at 200 mg/kg significantly reduced oedema formation at the fifth hour. This is consistent with findings by Siracusa, *et al*., (2020) who also found *Anacardium occidentale* nut to reduce significantly paw oedema formation induced by carrageenan. Indomethacin (5 mg/kg) showed more potency in anti-oedema activity than the AOME.

The air pouch model of inflammation was further used to evaluate the anti-inflammatory mechanism of AOME. The air pouch model produces a lining that is morphologically and functionally like the synovium. But unlike the synovium large quantity of inflammatory exudates can be collected for biochemical analysis and the histological and angiogenesis analysis of the air pouch lining can also be performed. This model produces only a local inflammatory response without any systemic changes (Romano *et al*., 1997).

In this study, the injection of carrageenan into a 6-day-old air pouch significantly increased exudate volume, indicating vascular leakage and increased vascular permeability to leukocytes and plasma. Vascular permeability is a primary response in acute inflammation caused by histamine, serotonin, and prostaglandins. Inflammatory sensors on resident tissue macrophages and mast cells induce production of cytokines like TNF-α and IL-6, as well as chemokines, prostaglandins, histamine and serotonin, which act on target tissue and the vasculature to induce vasodilation, extravasation of neutrophils and leakage of plasma into affected tissue (Medzhitov, 2010).

This study showed that TNF-α and IL-6 significantly increased with the injection of carrageenan into 6-day-old air pouches compared to controls. This is consistent with several studies which have demonstrated TNF and IL-6 to increase in the presence of carrageenan. TNF-α is known to be involved in the migration of leucocytes in the early phase of carrageenan inflammation, with the presence of activated leucocytes leading to further production of TNF-α in the late phase (Romano *et al*., 1997). TNF-α levels were suppressed significantly by pre-treatment of AOME extract (50 mg/kg, 100 mg/kg, and 200 mg/kg).

Injection of carrageenan into the air pouch significantly increased leucocytes and differentially increased neutrophils, monocytes, and lymphocytes when compared to the control in this study. Several studies have shown neutrophils, monocytes and lymphocytes increase with injection of carrageenan into air pouch (Ajayi, *et al*., 2017; Onasanwo, *et al*., 2017; Oyeleke, *et al*., 2018; Ajayi, *et al*., 2020). Neutrophils are the first inflammatory cells that are recruited to the inflammation site (Abdulkhaleq *et al*., 2018). Neutrophils function mainly by attacking microorganisms and foreign invaders as the body's primary line of defence. They also regulate the release of chemoattracting factors like azurodicin, which is involved in the recruitment of other immune cells, especially monocytes (Kumar *et al*., 2018). Neutrophils have also been shown to actively synthesise and secrete cytokines, chemokines, leukotrienes and prostaglandins, and accumulation of neutrophils in large numbers in inflammatory conditions and within inflamed tissues may contribute significantly to local production of mediators (Wright, *et al*., 2010).

Activated neutrophils, as seen at inflammation sites, generate rapid reactive oxygen species (ROS) via the action of NADPH oxidase, and the resultant oxidative stress has been implicated in diseased conditions like rheumatic arthritis and other life-threatening disease states (Wright *et al*., 2010). Pathways involved in carrageenan induced inflammatory cascade has been shown to include ROS-mediated response and a TLR4 mediated pathway leading to the activation of nuclear factor kappa B (NFκB) (Bhattacharyya *et al*., 2008). In this study, generation of ROS and thus oxidative stress was shown in the significant increase in lipid peroxidation marker, malondialdehyde (MDA) levels, and significant reduction in Glutathione (GSH) levels, and catalase concentration in carrageenan only group when compared to controls. This agrees with previous studies (Alabi, *et al*., 2017; Onasanwo *et al*., 2017; Ajayi *et al*., 2020), who have showed

changes in oxidative stress products in the presence of carrageenan. The antioxidant potential of AOME extract was shown in that the extract significantly decreased MDA levels, significantly increased GSH and catalase concentrations in the presence of carrageenan and in a dose dependent manner. This might be due to the richness of the extract in flavonoids, saponins, and phenols.

Beyond the observed inhibitory effects on oedema formation, leucocyte migration, cytokine production, and oxidative stress, the anti-inflammatory activity of *Anacardium occidentale* methanol extract (AOME) may also involve modulation of key intracellular signalling pathways that coordinate acute inflammatory responses. Carrageenan-induced inflammation is known to activate receptor-mediated pathways, notably toll-like receptor 4 (TLR4), resulting in downstream activation of nuclear factor kappa B (NF-κB), a transcription factor central to the induction of inflammatory genes including TNF-α, IL-6, COX-2, and inducible nitric oxide synthase (iNOS) (Bhattacharyya et al., 2008). Increases in TNF-α and IL-6 observed in the carrageenan groups of this study are consistent with this pathway. The ability of AOME to significantly reduce TNF-α levels suggests that the extract may attenuate the activation or nuclear translocation of NF-κB, thereby limiting the transcriptional drive that sustains and amplifies the inflammatory cascade. This interpretation aligns with the phytochemical profile of *Anacardium occidentale*, as flavonoids and phenolic compounds have previously been demonstrated to interfere with NF-κB signalling through inhibition of IκB phosphorylation and prevention of NF-κB nuclear entry.

Additionally, the reduction of oedema in the second phase of carrageenan inflammation observed in this study suggests a possible modulatory effect on cyclooxygenase pathways. The late phase of carrageenan-induced oedema is characterised by elevated prostaglandin synthesis, particularly via COX-2 induction (Nantel et al., 1999). Although AOME was less potent than indomethacin, the significant inhibition at the third and fifth hours, particularly at the 200 mg/kg dose, may indicate partial suppression of COX-2 activity or expression. Phytochemicals present in AOME—especially phenolic metabolites—are known in other botanical preparations to inhibit COX-derived prostanoids, thereby contributing to decreased vasodilation, plasma extravasation, and nociceptive sensitisation typical of the late phase of carrageenan inflammation.

The extract's pronounced effect on oxidative stress markers further suggests involvement of redox-sensitive pathways that intersect with inflammatory signalling. Excessive ROS generation by activated neutrophils not only contributes to lipid peroxidation but also acts as a secondary messenger for activation of NF-κB and other pro-inflammatory transcription factors. The ability of AOME to significantly decrease MDA levels and restore endogenous antioxidant systems such as GSH and catalase indicates that the extract may enhance cellular antioxidant defences or directly scavenge reactive species. This antioxidant activity could attenuate ROS-driven amplification of inflammatory pathways, thereby reducing tissue damage and limiting the recruitment and activation of additional immune cells. The enrichment of the extract in flavonoids, saponins, and phenols, compounds widely recognised for their free-radical scavenging and enzyme-stabilising activities, likely contributes to this protective effect.

Taken together, the findings of this study suggest that AOME exerts anti-inflammatory effects through multiple converging mechanisms: suppression of early inflammatory mediators such as TNF-α; attenuation of COX-2-associated late-phase oedema; and mitigation of oxidative stress that otherwise perpetuates inflammatory signalling. These combined activities underscore the therapeutic potential of *Anacardium occidentale* nuts in modulating both the cellular and biochemical components of acute inflammation.

In conclusion, our results demonstrated the potential of the methanol extract of cashew nuts (AOME) in ameliorating inflammatory response through the modulation of the release of proinflammatory cytokines, cytokines mediated immune cells proliferation, migration and associated oxidative stress in carrageenan models. And the anti-inflammatory effect may be through inhibition of proinflammatory cytokines and oxidative stress. Further studies to investigate the possible mechanisms by which AOME exerts its anti-inflmmamatoory effect, and also investigate the anti-inflammatory effect of AOME in neuroinflammation is to be considered.

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