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Evaluation of Carrageenan Induced DNA Damages, Inflammation and the Effects of Treatment with Ethanol Seed Extract of *Persea americana* (Avocado) on Enzymatic and Non-Enzymatic Antioxidant Profiles in Wistar Rats

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Abstract:

- ***Background:***

Inflammatory processes are closely associated with disturbances in cellular redox homeostasis, particularly through excessive generation of reactive oxygen species. When ROS production exceeds the capacity of endogenous antioxidant systems, oxidative stress may develop and contribute to the progression of both acute and chronic inflammatory conditions. *Persea americana* (avocado) seeds contain various bioactive phytochemicals, including phenolic compounds and flavonoids, which have been associated with antioxidant and anti-inflammatory properties. However, the capacity of avocado seed extract to modulate enzymatic and non-enzymatic antioxidant defenses during carrageenan-induced inflammation remains insufficiently characterized.

- ***Aim:***

This study investigated the effects of carrageenan induced DNA Damages, inflammation on glutathione peroxidase (GPx), catalase (CAT), and reduced glutathione (GSH) levels in rats. It further examined whether treatment with an ethanol extract of *Persea americana* seeds could attenuate alterations in these antioxidant parameters and restore redox balance, using a standard anti-inflammatory agent as a reference for comparison.

- ***Methods:***

Seventy-five male wistar rats weighing between 100g -120g were used for the study. This Seventy-five male wistar rats were acclimatized for two weeks. Then after the rats were divided into five groups of 15 male wistar rats each. The groups are: Normal control as group 1; Negative control group as group 2; 3000mg/kg ethanol seed extract of *persea americana* group as group 3. 1500mg/kg ethanol seed extract of *persea americana* group as group 4; 20mg/kg Ibuprofen group as group 5. Administration of the extract and ibuprofen drug was once a day and in the morning. It was administered orally using oral gavage. All the groups expect group 1 were induced of inflammation. The right leg paw sizes were measured to know the baseline value of the paw size using Electronic Vernier Caliper. Then they were injected into their right leg paw 0.2ml of 1% carrageenan solution. After three hours their various paw sizes were measured again to confirm that the paws were

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swollen (inflamed) using Electronic Vernier caliper (Paw-size oedema). Afterwards six wistar rats from each group were sacrificed for blood sample collection to assay some biochemical parameters in order to know their baseline values before commencing their treatment (baseline or 0th day). The biochemical assay are: GPx, Catalase, and GSH. Afterwards the remaining nine rats were returned back to their respective cages for treatment and continual feeding. On 14th day following night fasting, with light chloroform anesthesia from the same wistar rats another blood sample were collected from six rats out of the nine rats in all the groups through ocular puncture for the same biochemical assay. The rats were returned back to their respective group and cage for continuation of treatment, they were feed normal rat pellets and water. Finally, on the 28th day, following night fasting, six rats were sacrificed for the last sample collection for the same biochemical assay. The vehicle for the extract and ibuprofen drug was water.

- ***Results:***

The negative control group serum concentration of GPx, Catalase, and GSH decreased significantly ($p<0.05$), at baseline, 14th and 28th day. They also decreased significantly between baseline and 14th, 28th day, 14th day and 28th ($p<0.05$). The 20mg/kg ibuprofen treated group serum concentration of GPx, Catalase, and GSH increased significantly ($p<0.05$), at baseline, 14th and 28th day. They also increased significantly between baseline and 14th, 28th day, 14th day and 28th ($p<0.05$). The 3000mg/kg ESEPA group,1500mg/kg ESEPA group and normal control group serum concentration of GPx, Catalase, and GSH increased significantly ($p<0.05$), at baseline, 14th and 28th day. They also increased significantly between baseline and 14th, 28th day, 14th day and 28th ($p<0.05$). This significant increase was in relative to the standard reference anti-inflammatory drug (ibuprofen) used for this study.

- ***Conclusion:***

Ethanol seed extract of *Persea americana* ameliorated carrageenan induced suppression of enzymatic and non-enzymatic antioxidant defenses in rats in a dose- and time-dependent manner, supporting its potential as an adjunct or alternative anti-inflammatory and antioxidant agent worthy of further pharmacological and toxicological evaluation.

Keywords: *Persea americana; Carrageenan; Oxidative Stress; Catalase; Glutathione Peroxidase; Reduced Glutathione; DNA Damages; Inflammation.*

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I. INTRODUCTION

Inflammation is a protective physiological response to tissue injury, infection, or irritation, but when deregulated or prolonged it becomes a driver of tissue damage and chronic disease. A central feature of the inflammatory cascade is the generation of reactive oxygen species (ROS) by activated phagocytes and other resident cells; these species serve important signaling and antimicrobial functions but, when produced in excess, overwhelm the cell's redox buffering capacity and cause oxidative injury to lipids, proteins and DNA(Ngo *et al.*, 2022). The balance between reactive oxygen species generation and antioxidant defense is regulated in large part by the transcription factor- nuclear factor erythroid 2- related factor 2(Nrf2), which governs the expression of a broad panel of cytoprotective and antioxidant enzymes, and disruption of this axis has been linked to the persistence of inflammatory injury across multiple disease states (Ngo *et al.*, 2022; Saha *et al.*, 2024).

Among the first-line enzymatic defenses against oxidative injury are catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione. Catalase decomposes hydrogen peroxide to water and oxygen, GPx reduces peroxides using reduced glutathione as a cofactor, GSH directly reacts with reactive oxygen species (ROS) and other oxidants, helping to prevent oxidative damage to cellular components. Its cysteine sulfhydryl (–SH) group is primarily responsible for this reducing activity and GSH serves as a substrate for glutathione peroxidase (GPx). GPx uses GSH to reduce hydrogen peroxide and lipid hydroperoxides to less harmful products. During this process, GSH is oxidized to glutathione disulfide (GSSG). Because these enzymes are consumed and their synthesis suppressed during sustained oxidative and inflammatory challenge, their activities in blood or tissue are widely used as biomarkers of the severity of oxidative stress and of the efficacy of candidate anti-inflammatory and antioxidant interventions (Ngo *et al.*, 2022; Saha *et al*., 2024).

The carrageenan-induced paw oedema model remains one of the most extensively used experimental paradigms for evaluating anti-inflammatory activity because it reproduces the neutrophil-dependent, biphasic oedematous response characteristic of acute inflammation, together with a measurable fall in endogenous antioxidant enzyme activity and a rise in lipid peroxidation products at the site of injury(Semis *et al.*, 2021). Recent studies using this model have repeatedly demonstrated that carrageenan challenge depresses CAT, GPx and superoxide dismutase activities while elevating malondialdehyde, and that plant-derived polyphenolic extracts can restore this balance, often to an extent comparable with standard reference drugs (Semis *et al*., 2021).

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Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen remain the mainstay of pharmacological management of inflammatory pain, but their long-term use is constrained by well-documented gastrointestinal toxicity, including mucosal erosion, ulceration and bleeding, arising both from cyclo-oxygenase inhibition and from direct mucosal effects (Tawfik *et al*., 2026). Recent pharmaco-epidemiological analyses continue to confirm a measurable, dose-related risk of gastrointestinal bleeding even with ibuprofen, the NSAID generally considered to carry the lowest relative risk within the class (Tawfik *et al*., 2026). This safety profile has sustained interest in plant-derived alternatives or adjuncts that combine anti-inflammatory activity with antioxidant protection and a more favourable tolerability profile.

Persea americana Mill. (avocado), a widely cultivated tropical fruit tree, has attracted growing scientific attention for the phytochemical richness of its seed, which contains substantial quantities of flavonoids (including catechin and epicatechin), procyanidins, and phenolic acids(Semis *et al*.,2021). Ethanol and aqueous seed extracts have demonstrated antioxidant, anti-inflammatory, antidiabetic, hepatoprotective and anti-apoptotic properties in several rodent models. In a chemically induced hepatocarcinogenesis model, hydroethanolic avocado fruit and seed extracts prevented the elevation of lipid peroxidation and ameliorated carcinogen-induced reductions in glutathione peroxidase, glutathione-S-transferase and superoxide dismutase activities (Ahmed *et al*., 2022). Similarly, an aqueous extract of *Persea americana* seeds attenuated oxidative stress and inflammatory markers, including interleukin-6, tumour necrosis factor-alpha and nuclear factor kappa B, while improving insulin signaling in alloxan-induced diabetic rats (Ojo *et al*., 2022). These findings suggest that avocado seed extract may exert its protective effects, at least in part, through preservation or restoration of enzymatic antioxidant defenses, a mechanism that has not previously been characterized in a carrageenan-induced inflammation model with sequential (baseline, day 14, day 28) assessment.

The aim of the present study was therefore to evaluate carrageenan-induced changes in GPx, CAT and GSH activities in rats, and to assess the ameliorative effects of treatment with ethanol seed extract of *Persea americana* (ESEPA), relative to the standard reference anti-inflammatory drug ibuprofen.

II. MATERIALS AND METHODS

➢ *Study Area*

This work was carried out in Human Biochemistry department, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, located in Okofia Nnewi, Anambra State. The biochemical assay was done in Onamec Medical and Research Laboratory, Nnewi, Anambra State.

➢ *Procurement of Animals and Plant Materials*

Animals for the study were obtained from the Animal Facility Unit of College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. *Persea americana* (avocado) fruit was obtained from Eke Amaobi local market, in Otolo Nnewi, in Nnewi North Local Government Area, Anambra State, Nigeria. The seed was separated from the pulp, and was authenticated by a taxonomist from Botany Department, Nnamdi Azikiwe University, Awka, with the Herbarium number, NAUH-123^{A}.

➢ *Procurement of Chemicals and Standard Drugs*

All chemicals that were used for this study were of analytical grade. A standard anti- inflammatory drug Ebu-200 was purchased from a pharmaceutical shop in Nnewi.

➢ *Preparation of Plant Material, Chemicals and Standard Drug*

The *Persea americana* seed extracts was prepared according to the method described by Sundarganapath (2013). I removed the seeds from the pulp and cut the seed into smaller sizes and air dried them at room temperature for about 16weeks to avoid the escape of volatile components by oven drying. I air dried them to a constant weight. The dried seed was milled into fine powder using the Christy-Norris hammer grinding mill. The ethanol seed extract was obtained by soaking 200g of powdered dried seed into a beaker containing 1000ml of absolute ethanol for 48hours. Afterward the beaker containing absolute ethanol solvent was filtered using Muslin cloth and concentrated using Rotary evaporator (model:TT22, USA) at 64.7^{0}C to obtain extracts of *Persea americana* dried seed preparation. The extract was in a gel-like form and was stored in container inside the refrigerator for further usage. I obtained the extract administered to the experimental male wistar rats by dissolving 2000mg of the extract in 20ml of distilled water. The stock solution is 100mg/ml. It was administered according to the weight of the wistar rats in each group.

$$\text{Volume of extract (ml)} = \frac{\text{weight of rats (kg)} \times \text{Dosage (mg/kg)}}{\text{Stock solution (100mg/ml)}}$$

The carrageenan solution used for the induction of inflammation in the male wistar rats was prepared by dissolving 1g of carrageenan in 100ml of distilled water (1% carrageenan), (Sini *et al*., 2010).

The ibuprofen drug was prepared by dissolving 1000mg of ibuprofen in 50ml of distilled water to obtain dosage of 20mg of ibuprofen. This method was described by Apioku *et al*., (2014). It was administered according to the weight of the wistar rats in each group using the following:

$$\text{Volume of extract (ml)} = \frac{\text{weight of rats (kg)} \times \text{Dosage (mg/kg)}}{\text{Stock solution (20mg/ml)}}$$

➢ *Experimental Design*

Seventy-five male wistar rats weighing between 100g - 120g were used for the study. The Seventy-five male wistar rats were acclimatized for two weeks. The rats were divided into 5groups of 15rats each and were sub divided into two groups for biochemical studies on (baseline) 0th, 14th day and 28th day. The groups are: Normal control as group 1; Negative

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control group as group 2; 3000mg/kg ethanol seed extract of *persea americana* group as group 3. 1500mg/kg ethanol seed extract of *persea americana* group as group 4; 20mg/kg Ibuprofen group as group 5. Administration of the extract and ibuprofen drug was once a day and in the morning. It was administered orally using oral gavage. All the groups expect group 1 were induced of inflammation. The right leg paw sizes were measured to know the baseline value of the paw size using electronic Vernier caliper. Then they were injected into their right leg paw 0.2ml of 1% carrageenan solution. After three hours their various paw sizes were measured again to confirm that the paws were swollen (inflamed) using electronic Vernier caliper (Paw-size oedema). Afterwards six wistar rats from each group were sacrificed for blood sample collection to assay some biochemical parameters in order to know their baseline values before commencing their treatment (baseline or 0^{th} day). The biochemical assay are: GP_X, CATALASE and GSH. Afterwards the remaining nine rats were returned back to their respective cages for treatment and continual feeding. On 14^{th} day following night fasting and light chloroform anesthesia, from the same wistar rats another blood sample were collected from six rats out of the nine rats in all the groups through ocular puncture for the same biochemical assay. The rats were returned back to their respective group and cage for continuation of treatment, they were feed normal rat pellets and water. Finally, on the 28^{th} day following night fasting, six rats were sacrificed for the last sample collection for the same biochemical assay. The vehicle for the extract and ibuprofen drug was water.

➢ *Grouping of Animals*

- Group 1: Normal control- No carrageenan. They were feed with standard rat pellet diet and tap water. Using oral gavage once daily for twenty-eight days.
- Group 2: Negative control – induced with 0.2ml of 1% carrageenan and no treatment. They were feed with standard pellet diet and tap water for twenty-eight days.
- Group 3: Group 3: Induced with 0.2ml of 1% carrageenan and treated with (high dose) 3000mg/kg ethanol extracts of *Persea americana* seed p.o. using oral gavage once daily for twenty-eight days. They were fed with standard rat pellet diet and tap water.
- Group 4: Induced with 0.2ml of 1% carrageenan and treated with (low dose) 1500mg/kg ethanol extracts of *Persea americana* seed p.o.using oral gavage once daily for twenty-eight days. They were feed with standard rat pellet diet and tap water.
- Group 5: Positive control- Induced with 0.2ml of 1% carrageenan and treated with 20mg/kg ibuprofen standard drug p.o.using oral gavage once daily for twenty-eight days. They were feed with standard rat pellet diet and tap water.

➢ *Analytical Methods*

- *Estimation of Glutathione Peroxidase [GPx]*

The activity of glutathione peroxidase was determined by the method of Rotruck *et al*., (1973).

GPx in the presence of H_2O_2 oxidizes reduced glutathione (GSH) to for H_2O. The amount of GSH consumed is directly proportional to the activity of GPx and it is expressed as U/ml (µmol of GSH consumed/minute). The GSH remaining after the reaction is allowed to react with 5'-5' dithiobis-2-nitrobenzoic acid (DTNB) to form a yellow complex that absorbs maximally at 412 nm.

$$2GSH + H_2O_2 \xrightarrow{GP_X} GSSG + 2H_2O$$

- *Procedure*

The reaction mixture contained 0.4ml of phosphate buffer (pH 7.0), 0.1ml sodium azide and 0.2ml of the plasma or standard or blank, 0.2ml of glutathione and 0.1ml of H_2O_2. The resulting solution will be thoroughly mixed and incubated at 37°C for 10 minute. The reaction will be stopped by the addition of 0.4ml of 10% trichloroacetic acid (TCA). The tubes will be centrifuged at 4000 rpm for 5minutes. Thereafter, 0.5ml of the supernatant will be added into a cleaned test tube followed by the addition of 2ml of phosphate buffer (pH 7.0) and 0.5ml of 40 mM DTNB. The solution will be thoroughly mixed and the resulting yellow colour will be read at 412nm. The blank will be treated the same way except that it will contain 0.2ml of distilled water instead of sample 20mg/100ml of GSH. Standard (0.651µmol/ml) will be also used. The activity of glutathione peroxidase will be expressed as U/mL of plasma (µmoles of GSH utilized/ minute).

- *Calculation:*

Actual Test OD = OD Blank – OD Test

Actual Std OD = OD Std – OD Blank.

$$\text{GPx activity (U/mL)} = \frac{\text{Actual OD Test}}{\text{Actual OD}_{std}} \times \text{conc. of STD}(0.651\mu\text{mol/ml})$$

- *Estimation of Catalase*

The activity of catalase was determined by the method of Hadwan *et al*., 20216.

✓ *Principle:*

Catalase catalyzes the following reaction:

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Catalase activity was assessed by incubating the enzyme sample in substrate hydrogen peroxide in sodium–potassium phosphate buffer, (pH7.4) at 37 °C for three minutes. The reaction was stopped with ammonium molybdate. Absorbance of the yellow complex of molybdate and hydrogen peroxide is measured at 374nm against the blank.

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- *Procedure:*

Reagent	Test (S) (uL)	Test-BK (M) (uL)	Std (S°) (uL)	Blank (uL)
Sample	25	25	-	-
Water	-	250	25	275
H_2O_2	250	-	250	-
Mix, and incubate for 3 minutes at 37 °C.				
Ammonium molybdate	1000	1000	1000	1000
Read at 374 nm against reagent blank				

- *Calculation:*

Catalase activity of test in kU/L = 2.303/t x (log S°/S-M) x Vt/Vs = (log S°/S-M) X 53.7

Where: t = time, S° = absorbance of standard tube, S = absorbance of test tube, M = absorbance of control-test (correction factor), Vt = total volume of reagents in test tube and Vs = volume of serum.

- *Estimation of Reduced Glutathione (GSH)*

The activity of reduced glutathione was determined by the method of Mozer, 1983.

- *Principle:*

GST utilizes 1-chloro-2, 4-dinitrobenzene (CDNB) which is suitable for the broadest range of GST isoenzymes. Upon conjugation of the thiol group of the glutathione to the CDNB substrate, there is an increase in the absorbance at 340nm. DTNB reacts with the free thiol group on GSH to yield a yellow chromophore (TNB), which is quantified colorimetrically using a spectrophotometer or microplate reader at 412nm.

$$GSH + CDNB \xrightarrow{GST} GS\text{-}DNB\ Conjugate + HCL$$

- *Procedures*

I added 600ul of reaction buffer, 40ul of 0. 4% DTNB and 10ul of the sample supernatant into a cuvette, I incubated for 5mins at room temperature. The free thiol group on GSH reacts with DTNB to yield a yellow colored product called TNB(5-thio-2-nitrobenzoic acid).Then I absorbance at 412nm, with the blank.

- *Calculations*

$$GSH = \frac{\text{Abs of test}}{\text{Abs of STD}} X \text{ Std GSH}$$

Into a clean cuvette I added 980µL of PBS; then I added 10µl of CDNB, I mixed properly by pipetting up and down using 1ml pipette. Then I placed the cuvette in the spectrophotometer set at 340nm and the blank the spectrophotometer. Then I pipetted 10µl of the GST enzyme sample. Then I read every 30seconds over a period of 5minutes after a lag time of 1minute.Then I transfer 1ml of the substrate solution to a cuvette and read the blank absorbance at 340nm. I also added 2ul of GST control sample, provided with the kit, directly to the cuvette containing up to 1ml substrate solution, then I mixed by covering the cuvette with a parafilm and inverting several times. Then I recorded the absorbance readings according to the kinetics program.

- *Calculations:*

$$\text{Change in absorbance/ minute in} = \frac{\text{Abs(final read)- A(initial read)}}{\text{Reaction time(min)}}$$

Subtract the change in absorbance /min of the blank from the change in absorbance/min of the sample, the reaction product GS-DNB conjugate. Absorbs at 340nm. The rate of increase in the absorption is directly proportional to the GST activity in the sample.

➢ *Blood Sample Collection and Biochemical Assays*

Blood samples were collected from six rats in each group at three time points: Day 0 (baseline, immediately following oedema confirmation), but for day 14th and day 28th following night fasting samples were obtained through ocular puncture under light anaesthesia from the same wistar rats. Serum was separated by centrifugation and stored at -20°C pending analysis. Biochemical parameters assayed included $GP_{X,}$ Cat and GSH. Standard colorimetric and enzymatic methods were used for all assays.

➢ *Statistical Analysis*

Data are expressed as mean ± standard deviation (SD) for n=6. Within-group comparisons across time points were performed using paired t-tests. A p-value of less than 0.05 was considered statistically significant. All analyses were conducted using SPSS version 26.0.

III. RESULTS

➢ *Effect of ESEPA on Glutathione Peroxidase (GPx) Activity*

Table 1 shows GPx activity (IU/L) across the three time points. In the normal control group, GPx activity increased significantly from baseline (0.88 ± 0.12) to day 28 (0.95 ± 0.76; p = 0.036). There was also significant increased serum GPX concentration between baseline and 14th, 28th day, and between 14th and 28th day (p‹0.05). In the negative (carrageenan-only) control group, GPx activity decreased progressively and significantly from 0.74 ± 0.12 at baseline to 0.64 ± 0.08 by day 28 (p = 0.030), consistent with

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carrageenan-induced suppression of this antioxidant enzyme. The ibuprofen treated group also showed a significant increase, from 0.71 ± 0.09 at baseline to 0.87±0.11 at 28th day (p = 0.000). There was also significant increased serum GPx concentration between baseline and 14th, 28th day, and between 14th and 28th day (p‹0.05). Both ESEPA treated groups showed a reversal of this trend: the 3000 mg/kg group increased from 0.74 ± 0.19 at baseline to 0.93 ± 0.17 at day 28 (p = 0.000), while the 1500 mg/kg group increased from 0.73 ± 0.16 at baseline to 0.88±0.15 at 28th day (p = 0.010). There was also significant increased serum GPx concentration between baseline and 14th, 28th day, and between 14th and 28th day (p‹0.05).This is in relative to the standard reference anti-inflammatory drug used (ibuprofen). This significant increase was dose and time dependent.

Table 1 Glutathione Peroxidase Levels (IU/L) in Baseline, 14th Day and 28th Day Across the Various Groups of Carrageenan Induced Inflamed (Paw Oedema) Male Wistar Rats and Treated with Ethanol Seed Extract of *Persea americana* or Ibuprofen (mean±SD).

Groups	Baseline(A)	14th day(B)	28th day(C)	A vs B(p)	A vs C(p)	B vs C(p)
Normal control(group 1)	0.88±0.12	0.94±0.08	0.95±0.76	0.039	0.036	0.041
Negative control (group 2)	0.74±0.12	0.67±0.08	0.44±0.08	0.003	0.030	0.042
3000mg/kg ESEPA(group 3)	0.74±0.19	0.85±0.13	0.93±0.17	0.008	0.000	0.020
1500mg/kg ESEPA(group 4)	0.73±0.16	0.83±0.15	0.88±0.11	0.010	0.010	0.071
20mg/kg Ibuprofen(group 5)	0.71±0.09	0.81±0.18	0.87±0.11	0.002	0.000	0.044

Values represent the mean ± SD for N= 6. P values of (p‹0.05) is considered significantly different. Legend: ESEPA – Ethanol seed extract of *Persea americana*.

➢ *Effect of ESEPA on Catalase (CAT) Activity*

Table 2 presents catalase activity (IU/L). Catalase activity in the negative control group decrease significantly from 24.13 ± 7.24 at baseline to 18.40 ± 3.57 by day 28 (p = 0.003), reflecting the expected carrageenan-induced depletion of this enzyme. There was also significant decreased serum Cat concentration between baseline and 14th, 28th day, and between 14th and 28th day (p‹0.05).

In contrast, the normal control group showed a significant increase (25.19 ± 6.15 at baseline to 38.61 ± 4.15IU/L at 28th day; p = 0.038). There was also significant increased serum Cat concentration between baseline and 14th, 28th day, and between 14th and 28th day (p‹0.05).

The ibuprofen group increased significantly from 24.25 ± 8.92 at baseline to 36.48±4.90 at 28th day (p = 0.038). There was significant increased serum Cat concentration between baseline and 14th, 28th day, and between 14th and 28th day (p‹0.05). Both ESEPA doses produced marked, statistically significant increase in catalase activity: the 3000 mg/kg group rose from 24.30 ± 9.88 at baseline to 41.10±6.07 at 28th day (p = 0.003), and the 1500 mg/kg group rose from 24.05 ± 8.14 at baseline to 37.19 ± 10.68 at 28 day (p = 0.011). There was also significant increased serum Cat concentration between baseline and 14th, 28th day, and between 14th and 28th day (p‹0.05).This is in relative to the standard reference anti-inflammatory drug used for this study (ibuprofen).

Table 2 Catalase Levels (IU/L) in Baseline, 14th Day and 28th Day Across the Various Groups of Carrageenan Induced Inflamed (Paw Oedema) male Wistar Rats and Treated with Ethanol Seed Extract of *Persea americana* or Ibuprofen (mean±SD).

Groups	Baseline(A)	14th day(B)	28th day (C)	A vsB(p)	A vs C(p)	B vs C(p)
Normal control(group 1)	25.19±6.15	36.68±4.13	42.61±4.15	0.038	0.020	0.016
Negative control(group 2)	24.13±7.24	21.05±4.85	18.40±3.57	0.016	0.003	0.002
3000mg/kg ESEPA(group 3)	24.30±9.88	38.65±7.29	41.10±6.07	0.016	0.003	0.002
1500mg/kg ESEPA(group 4)	24.05±8.14	32.57±5.53	37.19±10.68	0.015	0.011	0.001
20mg/kg Ibuprofen(group 5)	24.25±8.92	31.37±8.72	36.48±4.90	0.037	0.016	0.038

Values represent the mean ± SD for N= 6. P values of (p‹0.05) is considered significantly different. Legend: ESEPA – Ethanol seed extract of *Persea americana*.

➢ *Effect of ESEPA on Reduced Glutathione Activity*

Table 3 shows GSH activity (mg/dL). GSH activity decreased significantly in the negative control group, from 31.32 ± 3.77 at baseline to 25.83 ± 4.78 at day 28 (p = 0.003), while it increased significantly in the normal control group from 39.49 ± 10.22 at baseline to 42.22 ± 10.26 at 28day (p = 0.022). There was also increased GSH serum concentration between baseline and 14th, 28 day and between 14th day and 28th day (p‹0.05). The ibuprofen-treated group increased from 34.32 ± 3.99 at baseline to 38.59±3.03 at 28th day (p = 0.001).Treatment with 3000mg/kg ESEPA increased GSH activity from 34.22 ± 3.51 at baseline to 42.83 ± 5.97 at 28th day (p = 0.002), a value that exceeded that of the normal control group at the same time point. There was also increased GSH serum concentration between baseline and 14th, 28 day and between 14th day and 28th day (p‹0.05). The 1500 mg/kg ESEPA group also showed a significant, dose-related increase, from 24.05 ± 8.14 at baseline to 40.06±2.64 at 28th day (p = 0.000). There was also increased GSH serum concentration between baseline and 14th, 28 day and between 14th day and 28th day (p‹0.05). This is in relative to the standard reference anti-inflammatory drug used for this study (ibuprofen). Overall, GSH activity was restored most effectively in the high-dose (3000 mg/kg) ESEPA group, followed low-dose (1500 mg/kg) ESEPA and ibuprofen treated group.

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Table 3 GSH Levels (mg/dl) in Baseline, 14th Day and 28th Day Across the Various Groups of Carrageenan Induced Inflamed (Paw Oedema) Male Wistar Rats and Treated with Ethanol Seed Extract of *Persea americana* or Ibuprofen (mean±SD).

Groups	Baseline(A)	14th day(B)	28th day (C)	A vs B	A vs C	B vs C
Normal control(group 1)	39.49±10.22	41.39±10.18	42.22±10.26	0.034	0.022	0.342
Negative control(group 2	31.32±3.77	27.58±3.87	25.83±4.78	0.003	0.003	0.028
3000mg/kg ESEPA(group 3)	34.22±3.51	38.39±2.13	42.83±5.97	0.003	0.002	0.004
1500mg/kg ESEPA(group 4)	24.05±8.14	38.09±3.03	40.06±2.64	0.010	0.000	0.010
20mg/kg Ibuprofen(group 5)	34.32±3.99	31.37±8.72	38.59±3.03	0.005	0.001	0.044

Values represent the mean ± SD for N= 6. P values of ($p<0.05$) is considered significantly different. Legend: ESEPA – Ethanol seed extract of *Persea americana*.

IV. DISCUSSION

Enzymatic antioxidants such as Glutathione peroxidase, catalase rapidly neutralize harmful free radicals by converting toxic reactive oxygen species into harmless water and oxygen, protecting cells from damage, reducing chronic disease risks, and maintaining cellular health. The present findings confirm that carrageenan-induced inflammation is associated with a significant, time-dependent decline in the activities of the enzymatic antioxidants GPx, CAT and GSH, consistent with the well-established pattern of ROS-mediated depletion of endogenous antioxidant defenses observed in carrageenan paw oedema and related inflammatory models (Semis *et al*.,2021). This decline reflects both the direct consumption of these enzymes and their cofactors during the neutralization of carrageenan-induced ROS, and the suppressive effect of sustained oxidative and inflammatory signaling on the transcriptional programmes, including the Nrf2–antioxidant response element axis, that normally sustain antioxidant enzyme synthesis (Ngo *et al*., 2021; Saha *et al.*, 2024).

Treatment with ethanol seed extract of *Persea americana* produced a clear, dose-related recovery of all three enzymes over the 28-day observation period, with the higher dose (3000 mg/kg) generally producing the greatest restorative effect, in several instances numerically exceeding the effect of the standard reference drug, ibuprofen. This pattern is consistent with previous reports describing the antioxidant and anti-inflammatory properties of avocado fruit and seed extracts. In a chemically induced hepatocarcinogenesis model, hydroethanolic avocado extracts similarly ameliorated carcinogen-induced reductions in glutathione peroxidase, glutathione-S-transferase and superoxide dismutase activities while preventing the elevation of lipid peroxidation (Ahmed *et al*., 2025). Likewise, an aqueous seed extract of *Persea americana* improved antioxidant capacity and suppressed pro-inflammatory cytokine expression, including interleukin-6, tumour necrosis factor-alpha and nuclear factor kappa B, in alloxan-induced diabetic rats, alongside restoration of insulin signaling through the PI3K/AKT pathway (Ojo *et al*., 2024). Together with the present results, these observations support the interpretation that the phenolic and flavonoid constituents of *Persea americana* seed, which include catechin, epicatechin and related procyanidins, act at least in part by preserving or restoring the activity of first-line enzymatic antioxidant defenses, either through direct free-radical scavenging that reduces the oxidative burden on these enzymes, or through upstream modulation of redox-sensitive transcriptional pathways(Ngo *et al*.,2022; Saha *et al*., 2024).

The comparability, and in some measures superiority, of the 3000 mg/kg ESEPA dose relative to ibuprofen is of particular interest given the well-documented gastrointestinal liability of NSAIDs. Even ibuprofen, generally regarded as carrying the lowest relative risk of gastrointestinal bleeding among non-selective NSAIDs, has been shown in recent pharmaco-epidemiological analyses to carry a significant, dose-dependent risk of gastrointestinal bleeding relative to non-use, a risk that increases substantially with other agents in the class (Tawfik *et al*., 2026). An orally administered plant extract capable of restoring antioxidant enzyme activity to an extent comparable with ibuprofen, without the accompanying cyclo-oxygenase-mediated mucosal injury, would therefore represent an attractive candidate for further development as an anti-inflammatory adjunct, particularly for populations at elevated risk of NSAID-related gastrointestinal complications.

V. CONCLUSION

Carrageenan-induced inflammation in rats was associated with significant, time-dependent suppression of the enzymatic antioxidants glutathione peroxidase, catalase and reduced glutathione. Treatment with ethanol seed extract of *Persea americana* ameliorated this suppression in a dose- and time-dependent manner, with effects at the higher dose (3000 mg/kg) that were broadly comparable to those of the standard anti-inflammatory drug ibuprofen. These findings support the antioxidant and anti-inflammatory potential of avocado seed extract and provide a rationale for further pharmacological, phytochemical and toxicological investigation of *Persea americana* as a candidate adjunct or alternative to conventional anti-inflammatory therapy.

- *Conflict of Interest*

The authors declare no conflict of interest.

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