n-Hexane extract of Anacardium occidentale ameliorates spatial memory impairment, anxiety, and depressive-like behavior through the suppression of gut-brain inflammation and cholinergic disruption in acetic acid-induced experimental colitis

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n-Hexane extract of *Anacardium occidentale* ameliorates spatial memory impairment, anxiety, and depressive-like behavior through the suppression of gut-brain inflammation and cholinergic disruption in acetic acid-induced experimental colitis.

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Abstract

Inflammatory bowel disease has been implicated in mood disorders and neurodegeneration through systemic inflammation and the tryptophan-kyneurin pathway, and cashew kernel has been reported to possess anti-inflammatory and antioxidant potential in models of ulcerative colitis. However, little is known about the potential of *n*-Hexane extract of *Anacardium occidentale* (HEAO) in ameliorating ulcerative colitis and associated neuroinflammation, which this study was designed to evaluate.

Thirty female Wistar rats assigned into six groups (n=5) were used for this study. Group I (10 mL/kg vehicle), group II received acetic acid (A.A) only (0.2ml of 3% AA i.r). Groups III-IV received A.A + (50, 100, 200 mg/kg p. o) of HEAO, while group VI received A.A + prednisolone 2 mg/kg p.o. The animals received treatments after intrarectal administration of acetic acid for 14 days. Followed by an assessment of anxiety, depression, and cognitive deficit, the brain and colon were harvested for biochemical and histological assessments. All data are expressed ± standard error of mean (SEM), and statistical analysis was conducted using ANOVA ($P<0.05$).

HEAO treatment significantly increased the time spent in the open arm, reduced immobility time, and increased alternation preference in the EPM, FST, and Y-maze tests, respectively. HEAO also significantly improved redox status balance, suppressed proinflammatory cytokine levels, and modulated acetylcholinesterase activities. This was supported by the preservation of neuronal and enterocyte integrity in the brain and colon.

n-Hexane extract of *Anacardium occidentale* (HEAO) exerted its anxiolytic, antidepressant, and nootropic effects by suppressing oxido-inflammatory damage and modulation of cholinergic transmission in the colon and the brain.

Keywords: Neuroinflammation, Colitis, Alzheimer's disease, Inflammatory bowel disease

Introduction

Inflammatory bowel disease (IBD) is a chronic progressive immunological disorder affecting the gastrointestinal tract characterized by ulcerations of the gastrointestinal tract. The disease is characterized by hypersensitivity of the immune system to the contents of the gastrointestinal tract. The degree of the gastrointestinal tract affected depends on the type of IBD, which is either Crohn's disease or ulcerative colitis (Dahlhamer *et al.*, 2016).

IBD affects about 7 million people globally, mainly in the United States of America, Canada, and Australia. However, the disease is currently on the rise, and there is an increase in its emergence in areas where it was not known before. IBD is of unknown etiology; however, genetics, gut dysbiosis, gut immune system dysfunction, and environmental stressors have been implicated in the pathogenesis of the disease (Kaplan *et al.*, 2019).

Affected individuals experienced abdominal discomfort, diarrhea, rectal bleeding, anemia, and weight loss. In addition, IBD has been implicated in the pathogenesis of some neurological diseases, especially mood disorders (Depression and Anxiety), and neuroinflammation-implicated neurodegenerative diseases (Alzheimer's and Parkinson's disease), as part of the gut-brain crosstalk, also known as the gut-brain axis (Masanetz *et al.*, 2022).

Nitrated alpha-synuclein results from nitrosative stress in the gastrointestinal system, which is then transported from the enteric nervous system to the central nervous system as Lewy neurites via the sympathetic and parasympathetic innervation of the enteric nervous system. The nitrated-synuclein impairs mitochondrial activity in the dopaminergic neurons of the substantia nigra, leading to neuronal damage and oxidative stress damage of the dopaminergic neurons (Klingelhoefer & Reichmann, 2015).

In Alzheimer's disease and depression, gut microbiome metabolites, bacteria lipopolysaccharide, short-chain fatty acid (SCFA), and tryptophan metabolites transported to the brain through the nervous system and systemic circulation due to the increased intestinal permeability which is found in IBD have been implicated in impaired amyloid clearance in the brain and neurotoxic products like quinolinic acid leading to neurodegeneration in Alzheimer's disease and serotonin depletion implicated in depressive behavior (Hestad *et al.*, 2022; Zajac *et al.*, 2022).

In addition, proinflammatory cytokines from the gut enter the brain through the circumventricular organ, triggering a cascade of neuroinflammation-mediated neurodegeneration in the brain (Kempuraj *et al.*, 2017).

Therefore, both oxidative stress and inflammation in the gut in inflammatory bowel disease could mediate neurodegeneration in the brain through the gut-brain crosstalk.

Currently, the management of IBD involves the use of non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying drugs like sulfasalazine. While the drugs have been beneficial, they usually present serious side effects, as a serious limitation that prevents their long-term use for a lifelong disease like IBD. In addition, there is little interest in the potential of the drugs in preventing or ameliorating oxidative stress and neuroinflammation-mediated neurodegeneration and associated neurological symptoms in IBD (Newman & Muscat, 2023).

Therefore, there is interest in developing novel therapies that can address both gastrointestinal and neurological disorders in inflammatory bowel disease through the modulation of the oxido-inflammatory response implicated in both conditions. Of growing interest in the management of IBD and associated neuroinflammation is the use of natural products and nutraceuticals owing to their antioxidants, anti-inflammatory potential, relative tolerance and global reliance in herbal extracts for primary especially in low-income economies with growing interest in developing countries. The natural product of interest in this study is the oil extract from cashew kernel (Larussa *et al.*, 2017).

Cashew (Anacardium occidentale L) is a tropical tree plant cultivated extensively in North America and Africa, respectively. The fleshy apple of the cashew plant is consumed for its pleasant taste, and the nut kernel is consumed as a snack (Oliveira *et al.*, 2020). Anacardium occidentale has a long history of ethnomedicinal usage across different cultures, and various parts of the plant, including the leaves, bark, fruit, and Anacardium nut shell oil, have found industrial usage. Anacardium occidentale leaves and shell oil have been used traditionally in the management of inflammatory diseases like arthritis and edema. Its tissue regeneration has also been reported for its wound healing potential, and its potential in managing intestinal inflammation, stomach ulcers, and diarrhea has also been reported (Vilar *et al.*, 2016).

All these ethnomedicinal potentials have been attributed to the presence of bioactive compounds such as anacardic acids, cardanol, and polyphenols. Making Anacardium occidentale nutraceutical products a good source of antioxidants and anti-inflammatory agents in managing inflammation implicated in brain and gut disorders (Baptista *et al.*, 2018; Y.-Y. Chen *et al.*, 2023).

Evidence from previous studies has shown that consumption of *Anacardium occidentale* nut ameliorated experimental colitis through the modulation of nuclear factor kappa B (NFkB) pathways, and the nut is capable of suppressing tissue inflammation and oxidative stress in hyperhomocysteinemic rats (Siracusa *et al.*, 2020). Therefore, this study aimed to investigate the potential of Anacardium occidentale nut oil extract to ameliorate gut and brain inflammation and behavioral deficits in acetic acid-induced colitis, through oxido-inflammatory response amelioration.

Materials and Methods

Drugs, reagents, and chemicals

The glacial acetic acid utilized in this investigation was bought from Sigma-Aldrich, based in St. Louis, MO, USA. Prednisolone was procured from A. Martins Pharmacy in Ibadan, Nigeria. Additionally, Tween-20, Phosphate Buffered Saline, Phosphate Buffered Formalin, Thiobarbituric acid (TBA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), and ammonium molybdate were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The ELISA kit for tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) was sourced from Biolegend, based in the U.S.A. All substances utilized in this investigation were of analytical grade and high purity.

Plant Material

The *Anacardium occidentale* nuts were obtained from the plot of the Cocoa Research Institute of Nigeria, Ibadan. The kernels were isolated using a simple cutter. This was used to slit each

nut open, and a knife was employed to remove the kernel immediately from the shell to minimize contamination with the cashew nut shell liquid (CNSL). The kernels were subjected to roasting at 70°C for 30 minutes, which ensures the removal of the kernels. The kernels were then blended using an automated blender to obtain a powdered sample (Yahaya, 2012).

CASHEW KERNEL OIL EXTRACTION

The Anacardium occidentale nuts were deshelled manually and oven-dried at 40°C. The nut oil was then extracted with n-hexane under agitation, following the procedures described by Onasanwo *et al.,* (2012), and the extract was collected. The oil obtained was kept in the refrigerator at 4°C until needed (Onasanwo *et al.*, 2013).

PROXIMATE ANALYSIS

After bringing the samples to a uniform size, they were analyzed for moisture content, crude protein, crude fat, ash content, carbohydrates, and energy value according to (Aremu *et al.*, 2006).

MOISTURE DETERMINATION

Moisture was determined by the loss in weight that occurs when a sample is dried to a constant weight in an oven. About 2g of a feed sample was weighed into a silica dish previously dried and weighed. The sample was then dried in an oven at 65 °C for 36 hours, cooled in a desiccator, and weighed. The drying and weighing continued until a constant weight was achieved. Since the water content of feed varied very widely, ingredients and feed were usually compared for their nutrient content on a moisture-free or dry matter (DM) basis.

CRUDE PROTEIN

Crude protein was determined by measuring the nitrogen content of the feed and multiplying it by a factor of 6.25. This factor was based on the fact that most protein contains 16% nitrogen. Crude protein was determined by the Kjeldahl method, namely, digestion, distillation, and titration.

CRUDE FAT

The ether extract of a feed represents the fat and oil in the feed. The Soxhlet apparatus was the equipment used for the determination of ether extract. It consists of 3 major components: an extractor that comprises the thimble, which holds the sample; a condenser for cooling and condensing the ether vapor; and a 250ml flask.

ASH CONTENT

Ash was the inorganic residue obtained by burning off the organic matter of feedstuff at 400-600 °C in a muffle furnace for 4 hours. 2g of the sample was weighed into a pre-heated crucible. The crucible was placed into a muffle furnace at 400-600 °C for 4 hours or until whitish-grey ash was obtained. The crucible was then placed in the desiccator and weighed

CRUDE FIBRE

The organic residue left after the sequential extraction of the feed with ether was used to determine the crude fiber.

EXPERIMENTAL ANIMALS

Thirty (30) female Wistar rats (160-180g) obtained from the Central Animal House of the College of Medicine, University of Ibadan, Nigeria were used in this study. The animals were housed 7 in clear plastic cages with wood shavings, at 28 ±2 ℃, 50 ± 5% humidity, and had access to a standard pellet chow and tap water ad libitum throughout the study. Animals were acclimated for at least 2 weeks to habituate before the experiment were conducted. The animals were handled in accordance with the criteria outlined in the principles of Laboratory Animal Care (NIH US publication No. 85-23, revised 1996) (PHS, 1996).

EXPERIMENTAL DESIGN

In this study, female Wistar rats weighing 120-150g were randomly divided into 6 groups of 5 animals per group. Group one received oral (p.o) and intrarectal (i.r.) normal saline only. Group two received acetic acid (A.A) only intrarectally (0.2ml of 3% A.A i.r.), Group three to five received acetic acid (0.2ml of 3% i.r.) followed by varying concentrations of HEAO (25mg/kg, 50mg/kg, and 100mg/kg), group six received acetic acid (0.2ml of 3% i.r.) followed by the standard drug prednisolone (2 mg/kg). The prednisolone dose was according to (Araújo *et al.*, 2016). After acetic acid administration, the rats were held horizontally for 2 min to prevent acetic acid leakage. Control animals underwent the same procedure using an equal volume of normal saline instead of an acetic acid solution. Prednisolone and cashew kernel oil were administered the day after acetic acid injection for 14 days, as represented in Figure 1.

NEUROBEHAVIORAL ASSESSMENT OF THE ANXIOLYTIC, ANTIDEPRESSANT, NOOTROPIC EFFECT OF CASHEW KERNEL OIL

Elevated plus maze for anxiety assessment

The elevated plus maze (EPM) is a behavioral test conducted to assess anxiety-like behaviors in rodents. It consists of two open arms (50 cm*10cm) and two covered arms (50cm*40cm*10cm). The arms were extended from a central platform (10cm*10 cm), and the maze was kept elevated to a height of 50cm from the floor. All the experimental Wistar rats were transferred to the testing room 30 minutes before the beginning of the experiment. The subject rat was placed at the edge of the open arm with its head directed away from the apparatus. The time spent in the open arms and closed arms was recorded, and these measurements serve as an index of anxiety-like behavior. The duration for this test was 5 minutes. The subject rat was then gently removed from the apparatus and returned to its home cage. The fecal pellets were removed, and all spots of urination were wiped up. The floor and walls of the apparatus were wiped with cotton wool dabbed in 95% ethanol (Komada *et al*., 2008).

Forced swimming test for the assessment of depression

The Forced Swimming Test (FST) is a behavioral test conducted to assess depressive-like behaviors in rodents. A transparent cylindrical glass container (50cm*20cm) was used for the test. The FST was conducted for 2 days, i.e., 15th to 16th day of the protocol schedule. The rats were acclimated to the testing environment by transporting the animals in their home cages 30 minutes before behavioral testing. The cylindrical glass containers were filled with tap water at 23 ± 1 °C, and the water depth was adjusted according to the rat's size, so that it didn't touch the bottom of the container with its hind legs. The rats were placed in the water-filled cylinder container for 6 minutes (1 minute for the pretest stage and 5 minutes for the test stage). The rats were removed from the container after the time had elapsed and placed in a transient drying

cage with the heat lamp above it and the heat pad under it. The water was changed after every session to avoid any influence on the next rat (Yankelevitch-Yahav *et al*., 2015).

Neurobehavioral assessment of special cognition using the Y-maze test

Three wooden branches that were connected in the middle to form a Y-shaped figure. The mouse could see distal spatial landmarks because of the arms' 9 cm-thick walls. There was no difference between the insides of the arms. There were no intra-maze hints. This crucial ethological evaluation relies on the mice's intrinsic desire to explore novel environments in the absence of either negative or positive reinforcers and mild levels of stress. With modifications to adapt the system to rats, the Y-maze drawing and process were based on a previously published technique. The dimensions (120°; 23.5 x 4 x 5.25 inches) are uniformly spaced. In a Y-maze, the effectiveness of spontaneous alternation was evaluated. The rats were placed in one of the branch chambers and allowed free access to the area for five minutes. The sequence of the arm entrance was manually captured. The "Y-maze" between testing was cleaned with 70% ethyl alcohol. "Alternation" was defined as selecting each of the three arms in succession. By dividing the total number of arm entrance choices by the number of possibilities, the spontaneous alternation % was calculated.

$$\%\ \text{spontaneous alternations} = \frac{\text{number of alternations}}{\text{total arm entries} - 2} * 100$$ (Kraeuter *et al.*, 2019).

BIOCHEMICAL ANALYSIS

Following treatment and neurobehavioral assessment, the representative animals from each group were euthanized using cervical dislocation, their brains were harvested and processed for biochemical assessment by homogenizing and centrifuging the brain tissues at 4^{o}c. The supernatant of the homogenates was collected and processed for colorimetric and enzyme-linked immunosorbent assay methods of analysis.

EVALUATION OF REDOX STATUS IN THE BRAIN AND THE COLON IN ACETIC ACID-INDUCED GUT-BRAIN INFLAMMATION.

Determination of malonaldehyde (MDA)

Malondialdehyde (MDA) forms a 1:2 adduct with thiobarbituric acid (TBA), which was measured by spectrophotometry. The thiobarbituric acid test for malondialdehyde, as described by Garcia et al. (2005), was used to assess lipid peroxidation. The deproteinization of brain synaptosomal preparation used 30% trichloroacetic acid (TCA), 5N HCI, and 2% (w/v) thiobarbituric acid in 0.5 M NaOH. The reaction mixture was brought to a temperature of 90°C in a water bath for 15 minutes, allowed to cool to room temperature, and then centrifuged at 12000 g for 10 minutes. Using a Bio-Tek Instruments, Inc. ELISA plate reader, the pink chromogen was measured at 532 nm. In terms of nM/mg of protein basis, the data were expressed (Garcia *et al.*, 2005).

Nitrite level estimation in the brain tissue

Griess reagent (0.1 percent N-(1-Naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5 percent phosphoric acid) was prepared by mixing 0.01 grams of NEDA, 0.1 grams of sulfanilamide, and 250 µL of phosphoric acid to make a volume of 10.0 milliliters. After being incubated at 3700C for 10 min, an equal volume of Griess reagent and brain

synaptosomes was treated with the regulators 1mM NADPH, Nitrate reductase 1.0 U/ml, and 1 M Zinc acetate. The absorbance was then measured spectrophotometrically at 542 nm wavelengths in the ELISA plate reader (Bio-Tek Instruments, Inc.). It was produced as a protein of ug/mg (Green *et al.*, 1982).

Evaluation of reduced glutathione level in the brain and colon

GSH (glutathione), a naturally occurring antioxidant in the body, was quantified by its reaction with 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) at 412 nm wavelength (Ellman, 1959). The processed sample of brain tissue was subjected to an equivalent volume of 5% TCA treatment. The mixture was centrifuged for 0 minutes at 3000 rpm. Supernatant was introduced together with 0.1 ml of phosphate buffer (pH 8.4), 0.05 ml of DTNB, and 0.05 ml of double-distilled water (DH20). After that, absorbance was determined spectrophotometrically at 412 nm in a Biotek ELISA reader within 15 minutes. GSH was expressed as an ug/mg (Rahman *et al.*, 2006).

Evaluation of superoxide dismutase activity in the brain and colon

Superoxide dismutase activity was quantified in collected tissue samples using a colorimetric assay kit. Before analysis, samples will be prepared by homogenization in an ice-cold buffer suitable for enzyme extraction, followed by centrifugation at 10,000 x g for 15 minutes at 4 ^{o}C to obtain the supernatant. For the SOD assay, samples were incubated with a substrate solution containing xanthine and xanthine oxidase, which generates superoxide radicals. These radicals react with a detection chromogen, producing a colored product whose absorbance can be measured spectrophotometrically. SOD present in the samples will scavenge superoxide radicals, thereby inhibiting the chromogen's color development. The rate of inhibition was measured at a specific wavelength, 450 nm. One unit of SOD activity was defined as the amount of enzyme that inhibits the rate of superoxide-driven chromogen reduction by 50%. Results were normalized to the protein content and expressed as units per milligram of protein (U/mg protein) (Weydert & Cullen, 2010).

Determination of Catalase Activity

Catalase activity will be assessed in prepared tissue homogenates of brain and colon tissue using a spectrophotometric method based on the decomposition of hydrogen peroxide H_2O_2. Samples were prepared by homogenizing them in a cold potassium phosphate buffer, pH 7.0, to preserve enzyme activity, followed by centrifugation at a specific speed (e.g., 10,000 x g for 15 minutes at 4 ^{o}C to isolate the supernatant containing soluble proteins. The assay principle involves monitoring the decrease in H_2O_2concentration over time as it is broken down by catalase. The reaction is typically initiated by adding a known concentration of H_2O_2 to a reaction mixture containing the sample. The decomposition of H_2O_2 will be measured directly by observing the decrease in absorbance at 240 nm at 30-second intervals for a total of 2-3 minutes using a spectrophotometer. One unit of catalase activity is commonly defined as the amount of enzyme that catalyzes the decomposition of 1 µmol of H_2O_2 per minute. The results were normalized to the protein content of the sample and expressed as units per milligram of protein (U/mg protein) or similar appropriate units (Góth, 1991).

Glutathione S-transferase (GST) assays

To determine glutathione transferase activity, microtiter plate assays were conducted using 1-chloro-2,4-dinitrobenzene (CDNB; Aldrich, Milwaukee, WI). The pre-reaction mixture (100 µL) consisted of 8 mM glutathione (Sigma, St. Louis, MO), 10 µL enzyme homogenate, and 0.1 M phosphate buffer (pH 8.0). The reaction was initiated by the addition of 200 µL CDNB

in 0.1 M phosphate buffer (pH 8.0) containing 15% glycerol, yielding a final CDNB concentration of 0.5 mM. A Bio-Tek plate reader (model ELx808IU) equipped with a 340 nm filter was used to monitor the enzyme activity at 25 ºC for 10 min, with absorbance readings taken every 15 s (Ottea et al., 2000). Inhibitors of glutathione transferase activity included EA (final concentration 0.19 mM), sulfobromophthalein sodium hydrate (Aldrich, Milwaukee, WI; final concentration 0.01 mM), and DM (final concentration 0.712 mM) (Prapanthadara et al., 2000). The inhibitors were added to the enzyme homogenate and incubated at 37 ºC for 10 min before substrate addition (Moatamedi Pour *et al.*, 2014).

EVALUATION OF GUT-BRAIN INFLAMMATION IN THE BRAIN AND COLON TISSUES OF ACETIC ACID-INDUCED COLITIS

The colon and brain tissues harvested from the Wistar rats were homogenized in ice-cold Tris buffer (pH 7.2, 4 °C) containing 50mM Tris, ImM EDTA, OmM MgCl2, and 5% (w/v) protease inhibitor cocktail (Sigma). After homogenization, samples were sonicated for 10s using an ultrasonic processor (Heat Systems Ultrasonic Inc.) at a setting of 20 duty cycles and then centrifuged at 10,000 RPM for 20 min at 4°C in 3K300 centrifuges. Supernatants were collected and cytokines were estimated using ELISA kits (RandD Systems), and readings were taken in an ELISA plate reader (Bio-Tek Instruments, Inc). Cytokine levels were expressed as a picogram of cytokines/microgram protein (Sturm *et al*., 2010).

EVALUATION OF THE BRAIN CHOLINERGIC SYSTEM DISRUPTION

Acetylcholinesterase activity was evaluated to determine the level of the brain cholinergic system disruption using the method described by Ellman. This assay was carried out using acetylthiocholine iodide (a synthetic substrate for ACHE) and DTNB. The DTNB reacts with thiocholine produced from the hydrolysis of acetylthiocholine iodide by Acetylcholine in tissue samples. The absorption intensity of the DTNB adduct is used to measure the amount of thiocholine formed, which is proportional to the Acetylcholine activity.

Procedure

The brain samples (0.03M sodium phosphate buffer, pH 7.4), a 10% (w/v) were prepared by using an Ultra-Turrax T25 (USA) homogenizer at a speed of 10,000 rpm. The brain homogenate was mixed with an equal volume of 1% Triton X-100 (1% w/v in 0.03M sodium phosphate buffer, pH 7.2) and centrifuged at 10,000 revolutions per minute (RPM) at 4°C in a Beckman Ultracentrifuge (LE 80, USA), using a fixed-angle rotor (80ti) for 60 min. Supernatant was collected and used for acetyl cholinesterase activity. An acetylcholinesterase kinetic study was done by Ellman's method (1961) at 412 nm wavelength by using a double-beam spectrophotometer. Processed brain samples were mixed with 0.1mM sodium phosphate buffer (pH 8.0), and after 5 min incubation, acetylthiocholine iodide (154.38 mM) and DTNB (10 mM) were added just before reading. The specific activity of acetyl cholinesterase is expressed in umoles/min/mg of protein. The study of Acetylcholine activity is based on measuring the change in the rate of hydrolysis of a thiocholine ester (acetylthiocholine iodide, the substrate), which exchanges with DTNB to produce a yellow product, 5-mercapto-2-nitrobenzoate. The specific activity of ACHE was calculated by following the formula;

$$Acetylcholinesterase\ activity = \frac{(\Delta E\ x\ V)\ x\ 1000}{1.36\ x\ 10^4\ X\ v}$$

Where:

ΔE = Extinction/Absorbance change/min
1000 = Conversion factor for μmoles
V = Volume of the total reaction mixture
1.36 x 10000 = Extinction Coefficient
v = volume of the enzyme used
The specific activity of Acetylcholine is expressed in moles/min/mg of protein (Ellman *et al.*, 1961)

Hematoxylin and Eosin study of the colon and the hippocampus

Brain and colon samples were preserved using 10% formalin. Samples were transferred through a series of progressively more concentrated ethanol baths, up to 100% ethanol, to remove traces of water. This dehydration was followed by a clearing agent, xylene, which removes the alcohol and is miscible with the wax. Finally, melted paraffin wax is added to replace the xylene and infiltrate the tissue. Once infiltrated in paraffin, tissues were oriented in molds that were filled with wax; once positioned, the wax was cooled, solidifying the block and tissue. A knife mounted in a microtome is used to cut tissue sections 10 micrometers thick, which are mounted on a glass microscope slide. Staining with hematoxylin and eosin (H&E) was employed to give contrasts to the tissues and highlight particular features of interest. The features of the samples were observed using a light microscope (de Haan *et al.*, 2021).

Statistical analysis

All data are expressed ± Standard Error of Mean (SEM), and statistical analysis to estimate differences within and between the groups was analyzed using one-way analysis of variance (one-way ANOVA) (unless otherwise stated) and followed by Tukey post-hoc test. All statistical analysis was carried out using GraphPad Prism version 5.0, with statistical significance expressed at the level $P < 0.05$.

Results

Evaluation of cashew kernel oil nutritive potential and Total Phenol assessment

The n-Hexane extract of *Anacardium occidentale* nut used for this study was subjected to proximate analysis, qualitative and quantitative assessment of its phenolic contents to confirm the presence of nutrient macromolecules, phenols, and quantification of the phenolic content in the oil sample. The results obtained are presented in Tables 1-3 and Figure 2.

Evaluation of the effect of HEAO on anxiety-like behaviors

The anxiolytic potential of *HEAO* on acetic acid-induced colitis implicated anxiety-like behavior was evaluated using the elevated plus maze, and data obtained were analyzed using one-way ANOVA for the time spent by the mice on both the open and closed arms. As shown in Figure 3A, there was significant ($p<0.05$) anxiety-like behavior in the acetic acid-only group as evidenced by decreased time spent in the open arms and increased time spent in the closed arm, which indicates anxiety-like behavior ($F(5, 6) = 0.003054$; $P<0.0001$). However, the administration of *HEAO* significantly ($p<0.05$) increased the amount of time spent in the open arm, indicating reduced anxiety. This shows the potential of *HEAO* in reducing the manifestation of anxiety-like behavior in mice.

Effects of HEAO on Depressive-like Behaviors Using the Forced Swimming Test

The potential of *Anacardium occidentale* on depressive-like behavior was evaluated using the forced swimming test, and the data obtained were analyzed using one-way ANOVA. As shown in Figure 3B, ANOVA revealed a significant ($p<0.05$) effect of treatment on immobility time ($F(5, 24) = 10.84$; $P<0.0001$). The acetic acid group (57.20 ± 9.73) demonstrated a significant ($p<0.05$) increase in immobility time when compared to the control group (20.40 ± 5.68). Treatment with 25 mg/kg *HEAO* ($55.20 + 11.54$) and 50 mg/kg *HEAO* (33.60 ± 14.15) significantly ($p<0.05$) reduced immobility time compared to the acetic acid group, indicating a decrease in depressive-like behaviors. Additionally, the 100 mg/kg *HEAO* (53.20 ± 5.02) and the prednisolone group (39.40 ± 10.24) also showed reductions in immobility time, suggesting that these treatments may mitigate depressive symptoms in this model.

Effects of HEAO on Exploratory preference using the Y Maze test in spatial memory assessment.

The nootropic potential of *Anacardium occidentale* on spatial learning in the Y-maze test, as shown in Figure 3C, was evaluated. The results for the Y-maze exploratory preference (PEP) percentage ($F(5,18) = 6.209$, $p = 0.0010$) indicate significant differences among the treatment groups. The acetic acid-only group exhibited a marked decrease in PEP (28.86 ± 6.07) compared to the control group (40.24 ± 6.43), suggesting impaired cognitive function due to neuroinflammation. However, treatment with 50 mg/kg (47.25 ± 7.76) and 100 mg/kg (43.32 ± 9.15) of HEAO, as well as prednisolone (48.75 ± 8.29), significantly improved PEP, indicating a restoration of cognitive function and exploration behavior. This suggests that the n-hexane extract of *Anacardium occidentale* has potential neuroprotective effects in ameliorating cognitive deficits induced by acetic acid.

Effect of HEAO on MDA, nitrite, glutathione, catalase, GST and SOD levels of the brain and colon of Acetic acid-induced experimental colitis

In the colon, MDA levels exhibited a more pronounced response to the treatments, as shown in Figure 4A. The acetic acid-only group recorded MDA levels of 1.96 ± 0.08, significantly higher than the control group (1.63 ± 0.07), with a strong statistical significance ($F(5, 18) = 13.38$, $p < 0.0001$). Treatment with 25 mg/kg of HEAO reduced MDA levels to 1.60 ± 0.18, and further reduction was noted with 50 mg/kg (1.65 ± 0.11) and 100 mg/kg (1.19 ± 0.04) of HEAO. The prednisolone treatment also showed a decrease in MDA levels to 1.73 ± 0.23. These results indicate that HEAO effectively mitigates lipid peroxidation in the colon, highlighting its potential therapeutic benefits in reducing oxidative stress associated with acetic acid-induced colitis.

As shown in Figure 4B, the levels of malondialdehyde (MDA) in the brain were significantly altered by the treatments. The acetic acid-only group exhibited a significant increase in MDA levels (3.11 ± 0.56) compared to the control group (1.73 ± 0.27), with a statistically significant difference ($F(5, 18) = 8.232$, $p = 0.0003$). Treatment with 25 mg/kg of HEAO resulted in a reduction of MDA levels to 1.99 ± 0.39, while further reductions were observed with 50 mg/kg (1.76 ± 0.27) and 100 mg/kg (1.68 ± 0.36) of HEAO. The comparative drug, prednisolone, also reduced MDA levels to 1.94 ± 0.31. These suggest that HEAO has a protective effect against lipid peroxidation in the brain induced by acetic acid-induced colitis.

In Figure 4C, the results obtained for brain nitrite levels ($F(5,18) = 19.31$, $p < 0.0001$) indicated a significant increase in nitrite levels in the acetic acid-treated group (9.86 ± 0.12) compared to the control group (7.69 ± 0.34). Treatment with HEAO at 25 mg/kg (9.95 ± 0.99) and 50 mg/kg (9.59 ± 0.12) did not significantly reduce the elevated nitrite levels compared to the acetic group. However, the 100 mg/kg HEAO group (7.89 ± 0.27) and the prednisolone-treated group (8.78 ± 0.20) exhibited a significant decrease in nitrite levels, suggesting a potential ameliorative effect of these treatments on nitrite production in the brain.

The levels of glutathione (GSH) were significantly altered in both the brain and colon of the experimental groups. For the colon in Figure 5A, GSH levels were similarly affected, with the acetic acid-only group showing a significant reduction (29.53 ± 0.99) compared to the control (47.75 ± 5.39), supported by a one-way ANOVA ($F(5,18) = 37.26$, $p < 0.0001$). Interestingly, pretreatment with 50 mg/kg of HEAO resulted in a notable improvement in GSH levels (43.86 ± 2.94), while the 100 mg/kg dose significantly elevated GSH levels to 57.29 ± 5.96, surpassing the control values. Prednisolone treatment also provided a protective effect, with GSH levels recorded at 39.30 ± 3.70. These indicate that the HEAO (HEAO) has a significant impact on restoring GSH levels in both the brain and colon, suggesting its potential as a therapeutic agent in ameliorating oxidative stress associated with experimental colitis.

In the brain in Figure 5B, GSH levels were significantly decreased in the acetic acid-only treated group (17.99 ± 1.77) compared to the control group (25.00 ± 1.25), as indicated by a one-way ANOVA ($F(5,18) = 14.77$, $p = 0.0003$). Notably, pretreatment with 25 mg/kg of HEAO increased GSH levels (21.83 ± 0.80), while higher doses of 50 mg/kg (21.23 ± 0.79) and 100 mg/kg (21.65 ± 1.43) also demonstrated a trend toward restoring GSH levels, although not reaching control levels. Prednisolone treatment (20.95 ± 0.32) showed a comparable protective effect against the decline in GSH.

In the colon, as shown in Figure 5C, Catalase activity was significantly decreased in the acetic acid group (7.79 ± 0.36) compared to the control (11.15 ± 0.61) ($F(5,18) = 23.92$, $p < 0.0001$). Notably, the 50 mg/kg (12.58 ± 0.13) and 100 mg/kg HEAO groups (12.69 ± 0.38) showed significant increases in Catalase activity, indicating a protective effect against oxidative stress. The Prednisolone-treated group (10.27 ± 1.37) also showed improvement but did not reach control levels.

In the brain in Figure 5D, Catalase activity was significantly reduced in the acetic acid-treated group (18.70 ± 1.56) compared to the control group (29.35 ± 1.07) ($F(5,18) = 27.72$, $p < 0.0001$). Treatment with 25 mg/kg HEAO (21.41 ± 0.94) and 50 mg/kg HEAO (23.28 ± 0.82) showed a partial recovery in Catalase activity, while the 100 mg/kg HEAO group (27.84 ± 3.11) approached control levels. The acetic group treated with Prednisolone (20.49 ± 0.85) also exhibited a significant increase compared to the acetic group alone, though it remained lower than the control.

SOD activity was assessed in the brain and colon tissues, revealing significant differences among treatment groups. In the colon Figure 5E, SOD activity was markedly reduced in the acetic-only group (0.15 ± 0.19) compared to the control (0.72 ± 0.20), with $F(5, 18) = 6.166$ and $p = 0.0017$ indicating a strong statistical significance. Pretreatment with 25 mg/kg (0.25 ± 0.14) and 50 mg/kg (0.38 ± 0.19) HEAO showed slight improvements; however, the 100 mg/kg group (0.68 ± 0.15) exhibited a significant increase in SOD activity, closely approaching control levels. The prednisolone group also showed a modest recovery (0.63 ± 0.25).

In the brain, SOD activity was significantly reduced in the acetic acid-only group (0.89 ± 0.03) compared to the control group (1.105 ± 0.098), with an overall analysis showing ($F(5, 18) = 3.927$, $p = 0.0139$) as seen in Figure 5F. Treatment with 25 mg/kg (1.00 ± 0.11), 50 mg/kg (0.96 ± 0.06), and 100 mg/kg (1.07 ± 0.05) HEAO did not restore SOD levels to control values, but the 100 mg/kg group showed a notable improvement compared to the acetic-only group. The prednisolone group (0.97 ± 0.08) also indicated some recovery in SOD activity.

For the colon, GST activity was also analyzed ($F(5,18) = 3.536$, $p = 0.0211$). The control group displayed GST levels of 0.10 ± 0.02 in Figure 5G, while the acetic group showed a decrease (0.07 ± 0.01). Treatment with 25 mg/kg (0.09 ± 0.01), 50 mg/kg (0.10 ± 0.02), and 100 mg/kg (0.10 ± 0.01) HEAO, along with prednisolone (0.09 ± 0.01), did not significantly differ from the control but demonstrated an improvement compared to the acetic group.

In the brain, Figure 5H, GST activity was assessed ($F(5,18) = 3.730$, $p = 0.0171$). The acetic acid group exhibited similar GST levels (0.23 ± 0.02) compared to the control group (0.25 ± 0.23). However, administration of 25 mg/kg (0.25 ± 0.02), 50 mg/kg (0.26 ± 0.02), and 100 mg/kg (0.27 ± 0.02) HEAO, as well as prednisolone (0.27 ± 0.01), resulted in a significant increase in GST activity relative to the acetic group.

Effects of n-Hexane Extract of Anacardium occidentale on the inflammatory status and acetylcholinesterase activity of the brain and colon of Acetic acid-induced experimental colitis

For the Colon TNF-alpha results as seen in Figure 6A, a significant difference was observed ($F(5, 18) = 17.87$, $p < 0.0001$). The acetic acid group (76.74 ± 11.72) demonstrated a marked increase in TNF-alpha levels compared to the control (11.86 ± 1.23). Treatment with 25 mg/kg HEAO (52.88 ± 12.41) and 50 mg/kg (51.70 ± 13.24) resulted in significant reductions in TNF-

alpha levels, while the 100 mg/kg group (37.89 ± 10.83) and Prednisolone (57.07 ± 6.83) also showed notable decreases, indicating the potential anti-inflammatory effects of the treatments.

In Figure 6B, the results obtained for Brain TNF-alpha showed a significant increase ($F(5, 18) = 12.36$, $p < 0.0001$) in the level of TNF-alpha in the acetic acid group (95.11 ± 8.86) compared to the control group (37.16 ± 2.85). Treatment with 25 mg/kg (45.36 ± 1.39) and 50 mg/kg (46.35 ± 5.47) HEAO significantly reduced TNF-alpha levels, bringing them closer to control values. The 100 mg/kg HEAO group (45.48 ± 11.06) also showed a significant decrease, while the Prednisolone group (61.26 ± 24.96) exhibited a moderate reduction compared to the acetic group only.

For the Colon IL-1β results in Figure 6C, a significant difference was observed ($F(5, 18) = 17.87$, $p < 0.0001$). The acetic acid group (76.74 ± 11.72) demonstrated a marked increase in IL-1β levels compared to the control (11.86 ± 1.23). Treatment with 25 mg/kg HEAO (52.88 ± 12.41) and 50 mg/kg (51.70 ± 13.24) resulted in significant reductions in IL-1β levels, while the 100 mg/kg group (37.89 ± 10.83) and Prednisolone (57.07 ± 6.83) also showed notable decreases, indicating the potential anti-inflammatory effects of the treatments.

In Figure 6D, the results obtained for Brain IL-1β showed a significant increase ($F(5, 18) = 12.36$, $p < 0.0001$) in the level of IL-1β in the acetic acid group (95.11 ± 8.86) compared to the control group (37.16 ± 2.85). Treatment with 25 mg/kg (45.36 ± 1.39) and 50 mg/kg (46.35 ± 5.47) HEAO significantly reduced IL-1β levels, bringing them closer to control values. The 100 mg/kg HEAO group (45.48 ± 11.06) also showed a significant decrease, while the Prednisolone group (61.26 ± 24.96) exhibited a moderate reduction compared to the acetic group only.

In addition, acetylcholinesterase (AChE) activity was assessed in this study, and the results obtained ($F(5, 18) = 10.35$, $p < 0.0001$), as shown in Figure 7, demonstrated that the acetic acid group (0.38 ± 0.02) significantly increased AChE activity compared to the control group (0.28 ± 0.03). Treatment with 25 mg/kg (0.26 ± 0.05), 50 mg/kg (0.27 ± 0.01), and 100 mg/kg (0.26 ± 0.04) of HEAO, as well as prednisolone (0.26 ± 0.01), effectively restored AChE activity to levels similar to that of the control group, indicating a protective effect against the increase in AChE activity induced by acetic acid.

As shown in Figure 8A, hematoxylin and eosin histological staining of the colon showed atrophy of the villi and hyperplasia of the colonic crypt, which was not observed in the groups treated with HEAO and prednisolone. In Figure 8B, hippocampal CA3 was histologically stained with hematoxylin and eosin. However, there were no observable lesions across all treatment groups, including the acetic-acid group.

Discussion

In this study, we investigated the potential of HEAO as a supplemental nutraceutical oil in the management of colitis and associated cognitive and mood disorders through the use of acetic acid in modulating experimental colitis. Our findings showed that HEAO demonstrated nootropic, anxiolytic, and anti-depressant potential by modulating the oxido-inflammatory and cholinergic system response in the gut and brain to intrarectal acetic acid administration.

In this study, we showed that intrarectal administration of colitis leads to impaired spatial learning and memory through the impairment in the sequence of alternation in the Y-maze apparatus test. This is a test used to assess the spatial cognition in rodents by measuring the spontaneous behavior of rodents to remember and follow a sequential pattern of entry into the arms of the Y-maze apparatus. This evidence supports previous studies that showed that acetic acid-induced colitis resulted in impaired spatial learning and memory in rodents (de Santana Souza *et al.*, 2017; Omayone *et al.*, 2023). HEAO, however, improved the rodents' performance in the Y-maze test task through the percentage alternation ratio, showing that treatments of the rodents with HEAO improved their sequence of entry into the arms of the apparatus.

Anxiogenic behavior, well-being, and depressive-like behavior were also assessed in this study using an elevated plus maze test, open field test, and forced swimming test. Individuals with either ulcerative colitis or Crohn's disease have been reported to have higher rates of anxiety and depression when compared to the general population, according to the Crohn's and Colitis Foundation, 2024.

Utilizing rodents' natural aversion to open spaces, the elevated plus has two arms: the open and the closed arm. An anxious rodent will prefer the closed arm to the open, while the open arm exploration is used to evaluate the anxiolytic potential of pharmacological agents. Acetic acid-induced colitis has been shown to increase the time spent in the closed arm, demonstrating the anxiogenic effect of acetic acid intrarectal administration and mimicking the anxiogenic disorder associated with colitis (Noubissi *et al.*, 2022).

Our results showed that colitis increased the time spent in the closed arm of the elevated plus maze and decreased the time spent in the open arm. In contrast, HEAO showed a dose-dependent response in reducing the time spent in the closed arm and gradually increasing the time spent in the open arm. This result corroborates other studies on cashew products, such as anacardic acid and cashew nut consumption, having anxiolytic potential (de Castro Querino Dias *et al.*, 2023; Gomes Júnior *et al.*, 2018). Our results showed that the extracted oil exerts a similar effect on anxiety as a central nervous system disorder.

The antidepressant potential of HEAO was assessed in this study using the forced swimming test (FST). FST is a test of despair used to screen pharmacological agents with antidepressant potential, where the animals are exposed to unavoidable situational despair, and the latency to give up on the test is recorded as immobility time due to hopelessness, with longer immobility time used as a marker of depressive-like behavior and experimental acetic acid-induced colitis has been shown to increase immobility time on the forced swimming test (Minaiyan *et al.*, 2015). HEAO at 50 mg/kg exerted an antidepressant-like effect by reducing the immobility time of the rodents on the forced swimming tests, which is similar to the results obtained for the hydroethanolic extract of cashew nut and anacardic acid, respectively (Désiré *et al.*, 2023; Júnior *et al.*, 2019).

Evidence of general well-being was assessed in this study using spontaneous self-grooming behavior in an open field test, which has been reported as a measure of hygiene lacking in rodents and humans with mood disorders (Scarola *et al.*, 2019). Previous studies have shown that acetic acid-induced colitis reduced spontaneous grooming behavior time (Noubissi *et al.*, 2022). However, HEAO increased the spontaneous self-grooming behavior time; our results are in contrast to the work of Dias et al, 20323 (de Castro Querino Dias *et al.*, 2023), which reported a reduction in grooming behavior of rodents that consumed cashew nuts. It is worth noting that our study is on the extracted kernel oil.
We investigated the potential of HEAO to ameliorate the neuropsychiatric behavior impairment that resulted from acetic acid-induced experimental colitis. We assayed oxido-inflammatory biomarkers in the colon and brain, which is also evidence of the gut-brain oxido-inflammatory crosstalk (Craig *et al.*, 2022).

The redox status of the colon and brain was evaluated through the assessment of prooxidants and antioxidant markers. The marker of membrane lipid peroxidation, malondialdehyde (MDA), was quantified in the colon and the brain. Acetic acid increased MDA levels in the colon and the brain, respectively. This agrees with previous studies that showed acetic acid-induced colitis increased MDA levels in the brain and colon, respectively (El-Akabawy & El-Sherif, 2019; Noubissi *et al.*, 2022; Omayone *et al.*, 2023). Brain nitrites were evaluated, which are a product of the highly reactive peroxynitrite from reactive nitrogen species, which was increased by the induced colitis. this observation was reversed in a dose-dependent manner in the colon and brain by HEAO. Protection of the endogenous enzymatic and non-enzymatic antioxidants was evaluated for glutathione (GSH), Glutathione S-transferase (GST), Catalase (CAT), and superoxide dismutase (SOD) in the colon and brain following colitis induction. We observed that acetic acid-induced colitis leads to an imbalance in the redox status through an increase in the prooxidants mentioned above and a decrease in antioxidants GSH, GST, CAT, and SOD levels, which was also reported in previous studies with acetic acid-induced experimental colitis (El-Akabawy & El-Sherif, 2019; Shahid *et al.*, 2022). HEAO intervention improved and protected the levels of antioxidants in the colon and brain, respectively. These results agree with earlier reports supporting the potential of raw cashew consumption and its ethanolic extracts to improve redox status in chronic stress, hyperhomocysteinemia, and hyperlipidemic rat models (D'Amico *et al.*, 2022; de Castro Querino Dias *et al.*, 2023).

The inflammatory components of the colitis-induced gut-brain neuroinflammation crosstalk were evaluated through the evaluation of tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1 β), which are major proinflammatory cytokines known to be involved in the neuropsychiatric behavior, such as anxiety, depression, and neuroinflammation-mediated neurodegeneration associated with human and experimental colitis through tryptophan-kynurenine metabolism by the proinflammatory cytokines activation of indoleamine 2,3-dioxygenase (IDO) (L.-M. Chen *et al.*, 2021).

Nutraceuticals have been shown to modulate this pathway through the suppression of proinflammatory cytokines, demonstrating the ameliorative potentials of nutraceuticals in gut-mediated neuropsychiatric conditions and neurodegenerative disorders (Lin *et al.*, 2022). In this study, HEAO suppressed the level of proinflammatory cytokines, tumor necrosis factor-alpha (TNF-α), and interleukin-1beta (IL-1 β) in the colon and brain, respectively. Our observation is in tandem with other reports of roasted cashew nuts and cashew nut shell liquid having anti-inflammatory potentials in different central and peripheral neuroinflammation models (Akomolafe & Asowata-Ayodele, 2022; Freschi *et al.*, 2019; Medeiros-Linard *et al.*, 2018).

The cholinergic system is the major neurotransmission system involved in cognitive processes in the brain, and the hydrolysis of acetylcholine by the enzyme acetylcholinesterase has been a therapeutic target with drugs such as donepezil in the maintenance of cholinergic dysfunction in conditions like Alzheimer's disease (Bohnen *et al.*, 2022).
In this regard and with respect to our results in the Y-maze test, acetylcholinesterase activity was evaluated to determine the potential of HEAO in modulating acetic acid-induced increase in acetylcholinesterase activity (Elbaz *et al.*, 2023). HEAO suppressed acetylcholinesterase's activity, which may be responsible for its previously observed nootropic effects in the spatial memory assessment.
The results observed in this study may result from the presence of phenols, alkaloids, and other bioactive components present in the HEAO, known for their antioxidant, anti-inflammatory, and anti-cholinesterase potentials (Shahidi & Tan, 2008).

The histological assessments affirmed the effectiveness of acetic acid in modeling experimental colitis as reported in previous studies characterized by villous atrophy and hyperplasia of colonic crypt, which are features associated with mucosal irritation, compromised epithelial turnover, and inflammation. These pathological changes were ameliorated by HEAO and prednisolone in this study. The impact of colitis on the hippocampus showed no significant neurodegenerative effects. These suggest that the duration of treatment with acetic acid may not offer neurodegenerative effects, but neurochemical alterations that are yet to manifest in neurodegenerative effects. Immunohistochemical study of regulatory proteins may offer significant insight into potential neuroinflammatory cascade effects, including microgliosis and astrogliosis.

Conclusion

Hexane oil extract of cashew kernel ameliorates experimental colitis and improves associated neurological outcomes through the modulation of oxido-inflammatory response and acetylcholinesterase activity. Demonstrating its potential usefulness as a nutraceutical in the management of ulcerative colitis and associated neuropsychiatric behaviors, as represented in Figure 9.

Declarations

Ethical approval

This research was done in line with the principles of Laboratory Animal Care (NIH US publication No. 85-23, revised 1996) (PHS, 1996). Ethical approval was granted by the University of Ibadan Animal Care and Research and Ethics Committee (06/06/2023) with approval number UI-ACUREC/22/098.

Consent for publication

The authors consent to the publication of this manuscript

Availability of data and materials

Data will be provided on request to the corresponding author.

Competing interests

The authors declare that there are no competing interests.

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Author(s) contribution

IAA (0000-0003-0872-1286) and SAO (0000-0003-0493-7995) designed the experiment, oil extraction was done by GS. RA, AL & SA conducted the animal experimentation and Phytochemical screening. The data analysis of the manuscript was done by IAA & DA. IAA, DA & SAO prepared the manuscript. Funding was provided by R.A, S.A., IAA & SAO. All authors contributed to the research and preparation of the manuscript.

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List of Abbreviations

HEAO	*n*-Hexane Extract of *Anacardium occidentale*
A.A.	Acetic acid
EPM	Elevated plus maze
FST	Forced Swimming Test

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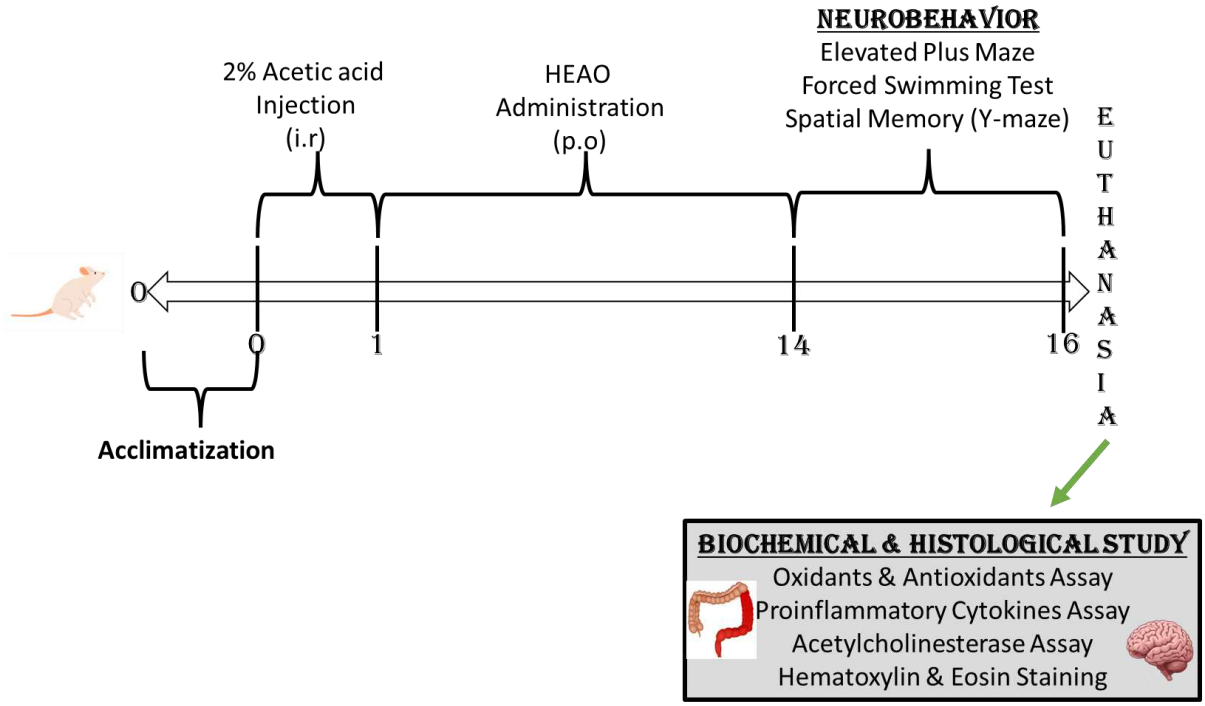


Figure 1: Schematic representation of experimental protocol

Table 1: Proximate analysis of nutritional macromolecules in the oil sample

% weight/weight of macromolecules in 5g of Oil Sample

Sample name	% ash content (%w/w)	% crude fibre (%w/w)	% fat content (%w/w)	% moisture content (%w/w)	% crude protein (%w/w)	% carbohydrate (%w/w)
Oil sample	0	0	45.08	0.17	0.7	8.98

Table 2: Qualitative Phytochemical screening of oil sample

Parameters	**Oil sample**
Saponins	+ve
Tannis	+ve
Flavonoids	-ve
Cardiac glycosides	+ve
Anthraquinones	++ve
Steroids	-ve
Terpenoids	+ve
Alkaloids	+ve
Phenol	+ve

INTERPRETATIONS

+ve: present

++ve: abundant

-ve: absent

Table 3: Total Phenol concentration of the oil sample (ug) at gallic acid equivalent

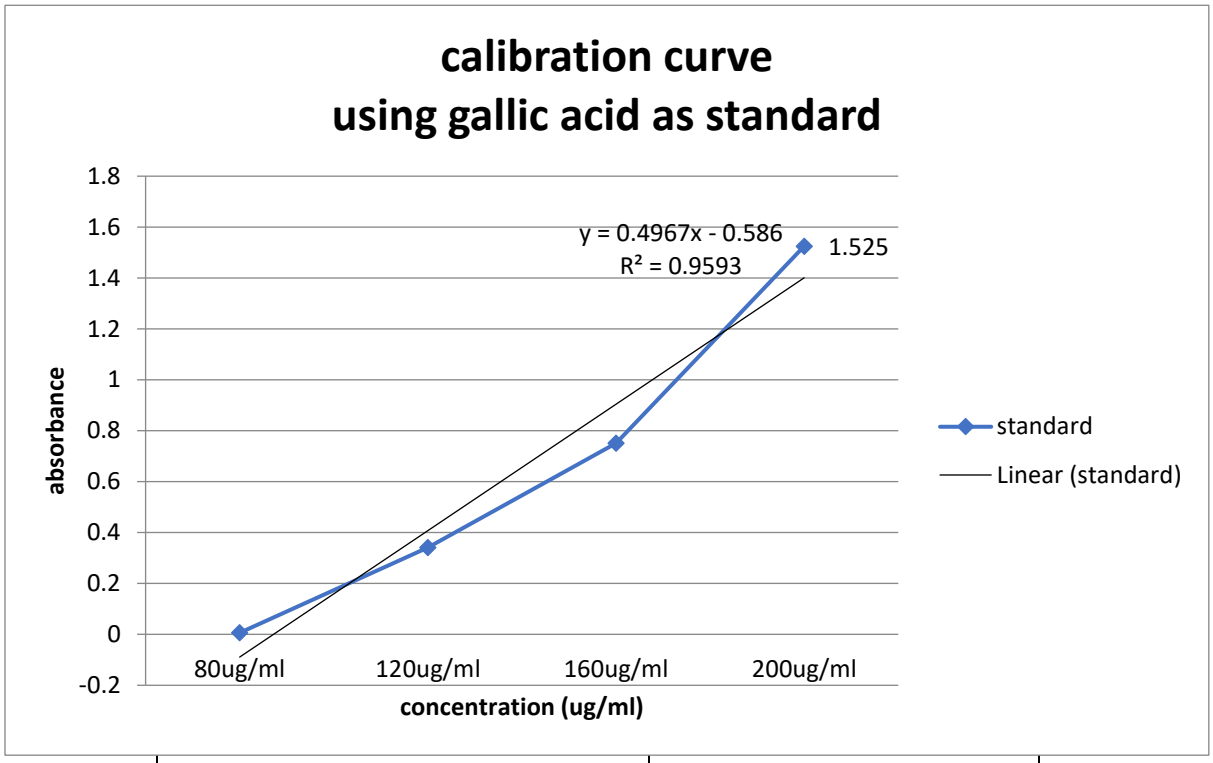


Figure 2:

200ug/ml	1.38
400ug/ml	1.42
600ug/ml	1.46
800ug/ml	1.48
1000ug/ml	1.49

Quantitative estimation of phenol concentration in the Cashew Kernel Oil

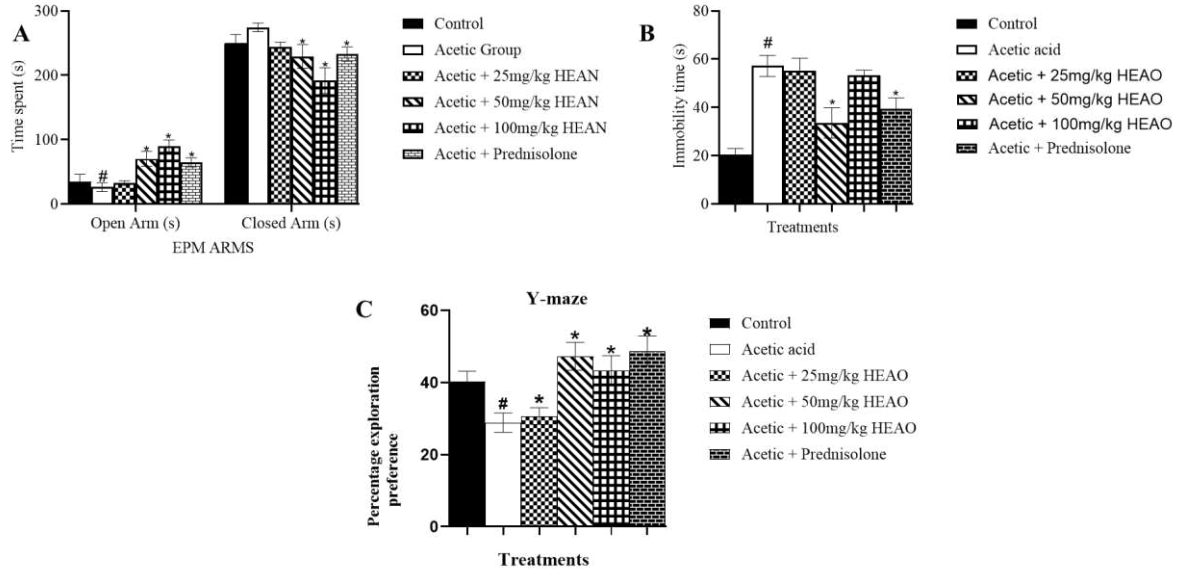


Figure 3A-C. Effect of cashew kernel oil on anxiety, depressive-like behavior, and spatial memory in acetic acid-induced colitis rats. Values represent mean ± SEM (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. $p < 0.05$ was considered significant.

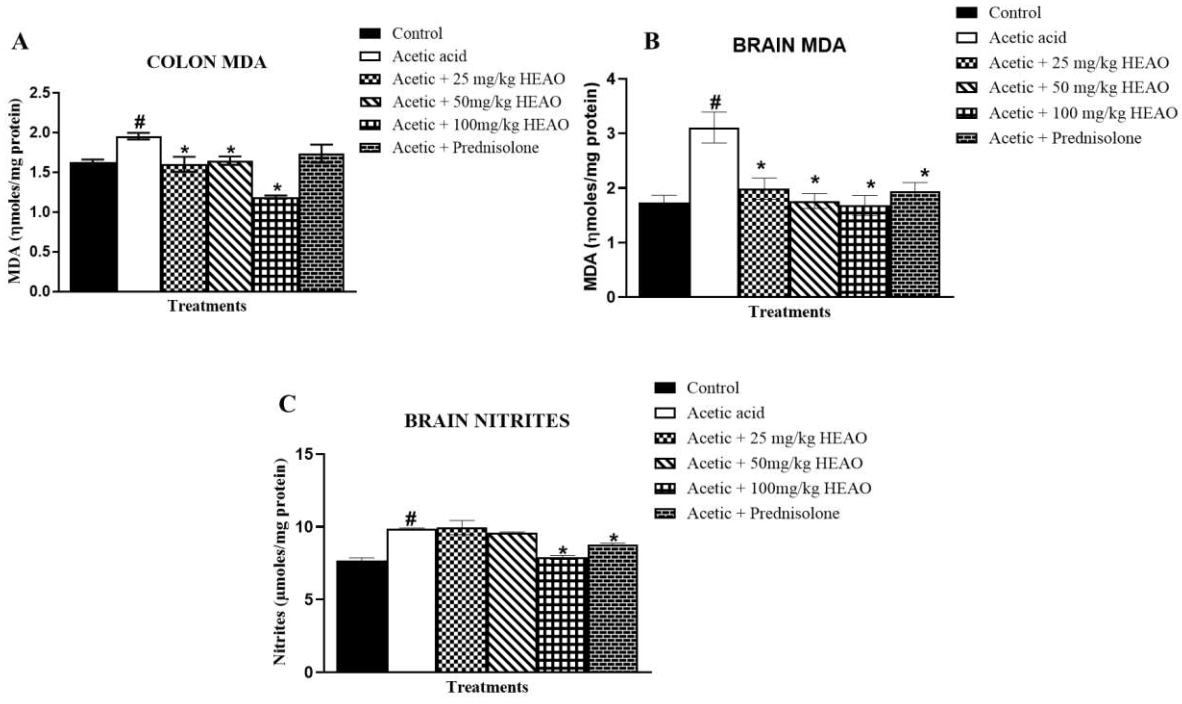


Figure 4A-C. Brain and colon oxidative stress status of rats treated with cashew kernel oil following acetic acid-induced colitis. Values are expressed as mean ± SEM (n = 5). One-way ANOVA followed by Tukey's post hoc test; $p < 0.05$ indicates statistical significance.

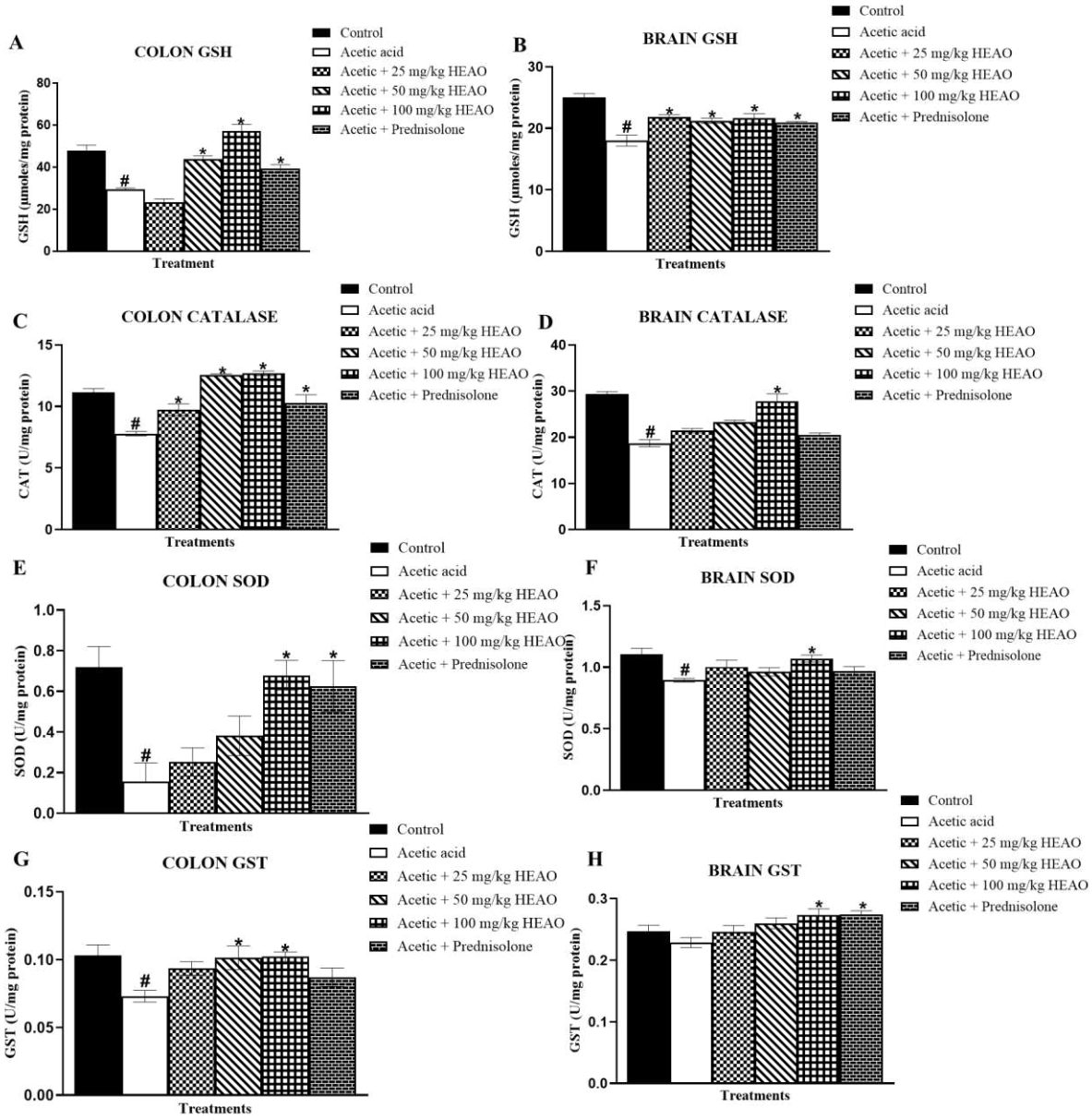


Figure 5A-H. Antioxidant defense status in the brain and colon of acetic acid-induced colitis rats treated with cashew kernel oil. Values are mean ± SEM (n = 5). One-way ANOVA followed by Tukey's post hoc test. $p < 0.05$ indicates significance.

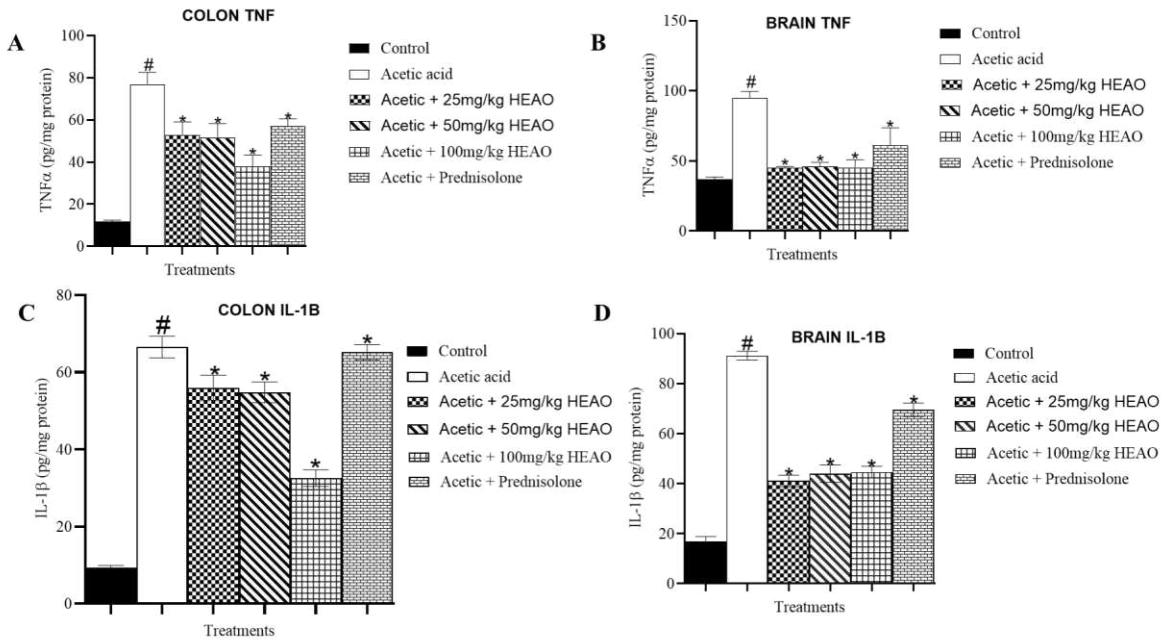


Figure 6A-D. Proinflammatory cytokine levels in the brain and colon tissues of acetic acid-induced colitis rats treated with cashew kernel oil. Data are mean ± SEM (n = 5). One-way ANOVA followed by Tukey's post hoc test. $p < 0.05$ was considered significant.

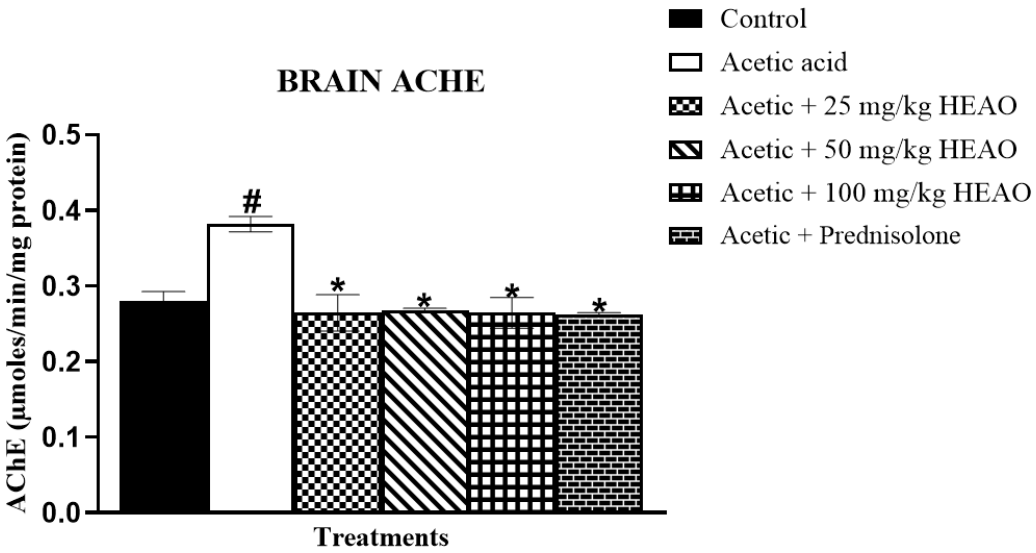
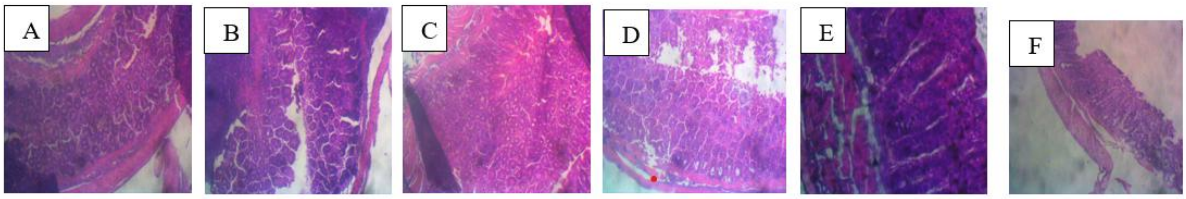


Figure 7. Acetylcholinesterase (AChE) activity in brain homogenates of acetic acid-induced colitis rats treated with cashew kernel oil. Values are expressed as mean ± SEM (n = 5). One-way ANOVA with Tukey's post hoc test; $p < 0.05$ indicates significance.

HISTOLOGICAL EVALUATION

N-HEXANE EXTRACT OF *Anacardium occidentale* NUT ameliorated atrophy of villi and cryptal hyperplasia in acetic acid-induced colitis

Magnification: (x100)



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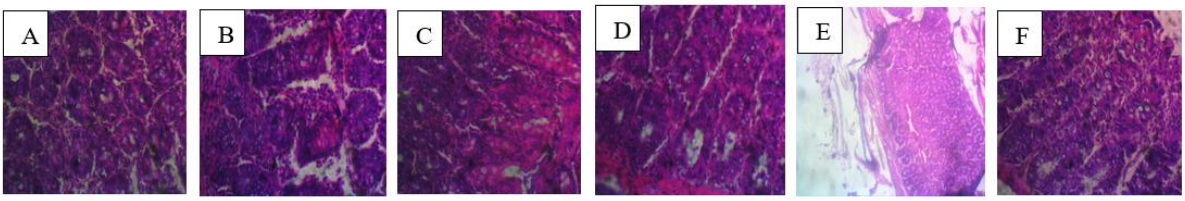
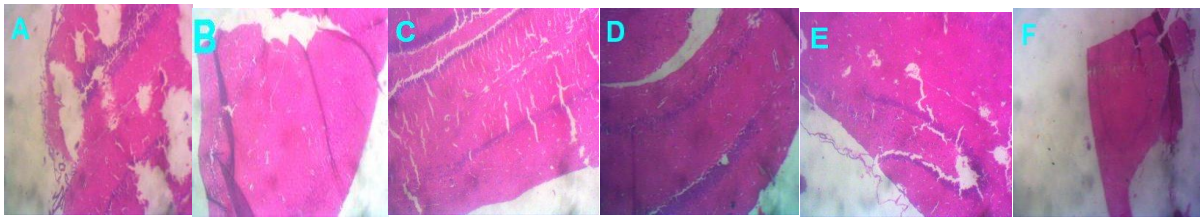


Figure 8A: Representative micrograph of H&E-stained colonic tissues on the effect of *n*-hexane extract of *Anacardium occidentale* (HEAO) in Wistar rats of experimental colitis. A = Control – there is no observable lesion; B = Acetic only – there is atrophy of villi and cryptal cell hyperplasia; C = Acetic + 25 mg/kg HEAO – there is no observable lesion; D = Acetic + 50 mg/kg HEAO – there is no observable lesion; E= Acetic + 100 mg/kg HEAO – there is no observable lesion; F= Acetic + Prednisolone – there is no observable lesion.

Acetic Acid-induced colitis has no degenerative impact on the hippocampus in N-HEXANE EXTRACT OF *Anacardium occidentale* NUT Wistar rats of experimental colitis

x100



x400

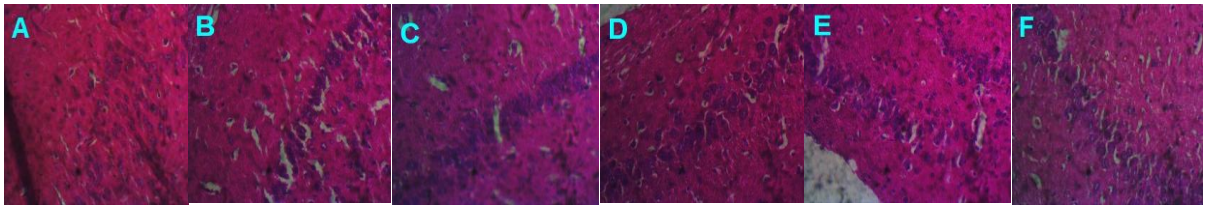


Figure 8B: Representative micrograph of H & E-stained hippocampus on the effect of *n*-hexane extract of *Anacardium occidentale* (HEAO) in Wistar rats of experimental colitis.
A= Control – there is no observable lesion; B= Acetic only – there is no observable lesion; C= Acetic + 25 mg/kg HEAO – there is no observable lesion; D= Acetic + 50 mg/kg HEAO – there is no observable lesion; E= Acetic + 100 mg/kg HEAO – there is no observable lesion; F= Acetic + Prednisolone – there is no observable lesion.

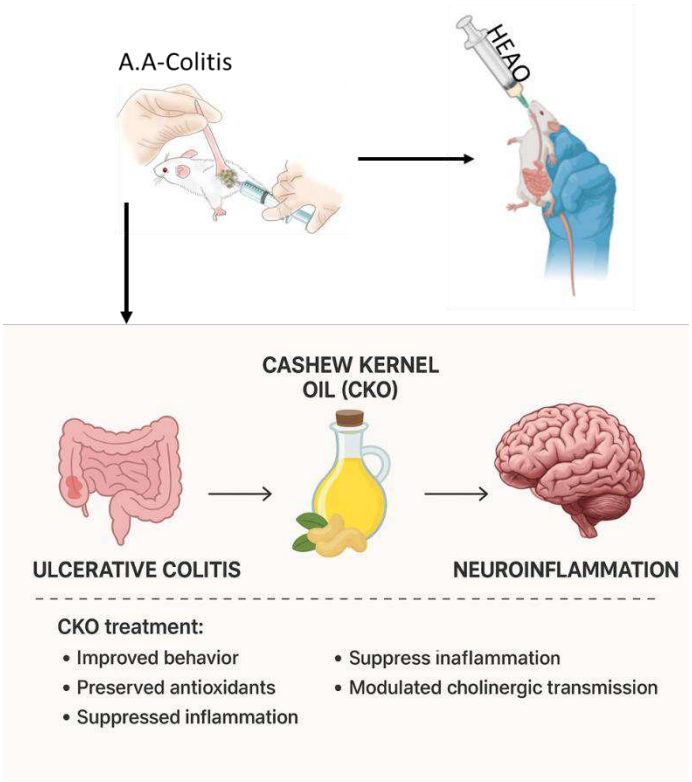


Figure 9: Summary of findings