Original Research Article

NEUROPROTECTIVE EFFECTS OF *HEINSIA CRINITA* AND COCONUT WATER IN A SCOPOLAMINE-INDUCED ALZHEIMER'S MODEL IN ALBINO RATS: BIOCHEMICAL CHANGES, CELLULAR HEALTH, AND NEURONAL ACTIVITY

ABSTRACT

Aim: This study aimed to investigate the potential therapeutic effects of *Heinsia Crinita* and Tender Coconut water (TCW) on Alzheimer's disease, focusing on their phytochemical composition, antioxidant activity, and anti-inflammatory effects. The study aimed to identify potential therapeutic agents for Alzheimer's disease and contribute to the development of more effective and natural treatments for this devastating disorder.

Study design: The study used a randomized controlled trial design, with six groups: negative control (Group A), positive control (Group B), Donepezil-treated group (Group C), TCW-treated group (Group D), *Heinsia Crinita*-treated group (Group E) and TCW + *Heinsia Crinita*-treated group (Group F).

Place and Duration of Study: The study was carried out in the Department of Anatomical Sciences, University of Calabar, between February 2022 and January 2024.

Method: The rats were induced with Alzheimer's disease-like cognitive dysfunction using scopolamine; 1.0 mg/kg, intraperitoneally (i.p.) and then treated with the respective interventions; TCW (2ml/kgBW) and *Heinsia Crinita* (500mg/kgBW). Histological analysis of the hippocampal tissue was performed using Hematoxylin and Eosin stain and Glial Fibrillary Acid Protein (GFAP) stain. Biochemical assays were conducted to determine Malondialdehyde (MDA) levels and glutathione peroxidase (GPx) activity.

Results: The results of the study showed that *Heinsia Crinita* may provide greater health benefits than Tender Coconut water due to its higher concentrations of bioactive compounds like alkaloids, flavonoids, and saponins, enhancing its medicinal properties, particularly its antioxidant and anti-inflammatory effects. When combined with Tender Coconut water, *Heinsia crinita* (Group F) shows improved efficacy in reducing inflammation, cellular damage, and oxidative stress compared to Tender Coconut water alone. This combination is also as effective as Donepezil (Group C) in alleviating immunochemical alterations in a scopolamine-induced Alzheimer's model in Wistar rats, marked by reduced astrocyte activation, lower malondialdehyde (MDA) levels, and increased glutathione peroxidase (GPx) activity $p < 0.05$.

Conclusion: This study suggest that the combination of *Heinsia Crinita* and *Tender Coconut* water may serve as a promising therapeutic approach for addressing neurodegenerative conditions like Alzheimer's disease. The enhanced medicinal properties of *Heinsia Crinita*, particularly its ability to reduce oxidative stress and inflammation, coupled with the nutritional benefits of tender coconut water, could provide a synergistic effect that supports neuronal health and mitigates disease progression. This finding highlights the potential for natural products to be integrated into traditional medicine and modern therapeutic strategies.

Keywords: *Synergistic Effects, Heinsia Crinita, Tender Coconut water, Combination therapy, Antioxidant activity, Anti-inflammatory effects, Oxidative stress, Lipid damage.*

1.1 INTRODUCTION

Alzheimer's disease is a devastating neurological disorder that affects millions of people worldwide, causing significant cognitive decline, memory loss, and emotional distress (1, 1). Despite its prevalence, there is currently no cure for Alzheimer's disease, and available treatments primarily focus on managing symptoms and slowing down disease progression (3, 3). The development of effective treatments for Alzheimer's disease is urgently needed to improve the quality of life of affected individuals and their families. In recent years, there has been growing interest in the potential therapeutic effects of natural compounds and herbal remedies for Alzheimer's disease (5, 5). Coconut water, a popular beverage made from the clear liquid inside young coconuts, has been touted for its potential health benefits, including anti-inflammatory, antioxidant, and anti-diabetic effects (6, 7). *Heinsia crinita*, a lesser-known plant species native to tropical regions, has also been reported to have potential medicinal properties, including anti-inflammatory and antioxidant effects (9, 9, 11, 11).

However, despite its potential benefits, the therapeutic effects of coconut water and *Heinsia crinita* on Alzheimer's disease have not been extensively studied. This study aimed to investigate the potential therapeutic effects of these two natural compounds on Alzheimer's disease, with a focus on their phytochemical composition, antioxidant activity, and anti-inflammatory effects. By comparing the two plants and exploring their mechanisms of action, this study hopes to identify potential therapeutic agents for Alzheimer's disease and contribute to the development of more effective and natural treatments for this devastating disorder.

According to the World Health Organization (WHO), Alzheimer's disease is the most common cause of dementia, affecting over 50 million people globally, with numbers expected to triple by 2050 (12). The economic burden of Alzheimer's disease is also staggering, with estimates suggesting that it costs over $1 trillion annually in the United States alone (14).

The exact causes of Alzheimer's disease are still unknown, but it is believed to be related to a combination of genetic, environmental, and lifestyle factors (15). The disease is characterized by the

accumulation of beta-amyloid plaques and neurofibrillary tangles in the brain, leading to neuronal death and cognitive decline (16). Current treatments for Alzheimer's disease primarily focus on managing symptoms and slowing down disease progression, but they have limited effectiveness and are often associated with significant side effects (17).

In recent years, there has been growing interest in the potential therapeutic effects of natural compounds and herbal remedies for Alzheimer's disease. This interest is driven by several factors, including the desire for more effective and safer treatments, as well as the need to reduce the economic burden of the disease (18). Coconut water, a popular beverage made from the clear liquid inside young coconuts, has been touted for its potential health benefits, including anti-inflammatory, antioxidant, and anti-diabetic effects (6).

However, despite its popularity, coconut water has not been extensively studied for its potential therapeutic effects on Alzheimer's disease. *Heinsia crinita*, a lesser-known plant species native to tropical regions, has also been reported to have potential medicinal properties, including anti-inflammatory and antioxidant effects (9). However, its potential therapeutic effects on Alzheimer's disease are unknown.

The study of *Heinsia crinita* and coconut water offers several advantages. Firstly, both plants are relatively understudied compared to other medicinal plants, making them attractive targets for further research (19). Secondly, they have been reported to have potential therapeutic effects that may be relevant to Alzheimer's disease, such as anti-inflammatory and antioxidant effects (9, 9, 11). Finally, their phytochemical composition is relatively complex and may offer synergistic effects that could be beneficial for treating Alzheimer's disease (20).

Overall, this study aimd to investigate the potential therapeutic effects of *Heinsia crinita* and coconut water on Alzheimer's disease, with a focus on their phytochemical composition, antioxidant activity, and anti-inflammatory effects. By comparing the two plants and exploring their mechanisms of action, this study hopes to identify potential therapeutic agents for Alzheimer's disease and contribute to the development of more effective and natural treatments for this devastating disorder.

Plate 1 & 2: Tender Coconut (A) and *Heinsia Crinita* (B)

2.0 MATERIAL AND METHODS

2.1 Materials

Adult Male Wister Rats, scopolamine hydrobromide, Donepezil, Tender coconut water (TCW), Heinsia crinita, Animal feed, Whatman No.1 filter paper, Rotary evaporator, Cheesecloth, Chloroform, Formal saline, PBS (Phosphate-Buffered Saline), BSA (Bovine Serum Albumin), Primary antibodies (GFAP), Secondary antibodies (anti-mouse immunoglobulins), Dextran polymer (Dako, Denmark), Glutathione peroxidase assay kit, TBA-reactive material (Malondialdehyde assay kit)

2.1.1 Phytochemical Screening

Qualitatice phytochemical screening and proximate analysis were carried out to identify the various phytochemicals present in these samples, as well as their quantities and proportions while quantitative phytochemical screening allowed us to detect and quantify a wide range of phytochemicals, including alkaloids, glycosides, phenolics, flavonoids, and other bioactive compounds.

2.1.1.1 Qualitative Phytochemical Screening.

The presence of alkaloids was detected by boiling the sample with 2% aqueous HCl and then treating the filtrate with Dragendorff's reagent, Meyer's reagent, Wagner's reagent, and picric acid (1%). The results

showed that the reagents produced a red precipitation or turbidity, indicating the presence of alkaloids (21).

To detect flavonoids, the sample was heated with ethyl acetate in boiling water, filtered, and then treated with dilute ammonia solution (1%) and aluminum chloride solution (1%). The Ammonium test and Aluminum chloride test showed the presence of flavonoids by producing a yellow coloration (22).

The saponin test was performed by shaking the sample extract with distilled water, allowing for frothing to take place, and then warming the mixture. The persistence of frothing on warming was taken as preliminary evidence for the presence of saponins (23).

For tannin detection, the sample extract was steamed with 45% ethanol, cooled, filtered, and then treated with lead sub-acetate solution and ferric chloride solution. The Lead sub-acetate test and Ferric chloride test showed the presence of tannins by producing a cream gelatinous precipitate and a transient greenish to black color (24).

To detect and quantify phenol in the sample, a procedure was followed. The sample was mixed with ether to form a slurry, agitated for 15 minutes, and then developed with 5 ml of amyl alcohol. The concentration of phenol was determined using a UV-Vis spectrophotometer at a wavelength of 505 nm (22).

2.1.1.2 Quantitative Phytochemical Screening.

According to the method described by Harborne (21), the following parameters were determined: alkaloids, flavonoids, saponins, tanins and phenols.

2.1.1.3 Proximate Analysis

The moisture content of the samples was determined by drying them at 105°C, following the method described by Alzeer**,** (25). The protein content was determined using the micro-Kjeldahl method, as outlined in AOAC (26). The crude lipids were extracted from the samples using a Soxhlet apparatus and petroleum ether.

The ash content of the samples was determined gravimetrically, based on the methods outlined in AOAC (26). Additionally, the total carbohydrate content of the samples was calculated using the difference

method, where the values of moisture, crude protein, ash, and crude fat (ether extract) were summed and then subtracted from 100, as described by Ijarotimi et al. (27).

In summary, the proximate analysis of the samples involved drying them to determine moisture content, using a micro-Kjeldahl method to determine protein content, extracting crude lipids using a Soxhlet apparatus and petroleum ether, determining ash content gravimetrically, and calculating total carbohydrate content using a difference method.

2.1.2 Animal Breeding and Husbandry

The rats were sourced from the Department of Physiology, University of Calabar and were housed in the animal room of the Department of Human Anatomy for a 2-week acclimatization period. To maintain standard conditions, the room was maintained at a temperature range of 27°C to 30°C, with a 12-hour natural light cycle and 12-hour dark period. The animals were fed a standardized diet of rat chow manufactured by Agro Feed Mill Nigeria Ltd, obtained from No. 58 Mount Zion Road, Calabar, and had access to drinking water *ad libitum*. Following the acclimatization period, the animals were randomly assigned to six groups of five rats each, comprising two control groups and five experimental groups.

2.1.3 Harvest and authentication of Tender Coconut water (TCW)

Young coconut fruits were harvested from a coconut tree grown in a farm located at Nyangasang, within the Calabar Municipal Local Government Area of Cross River State. The fruits were subsequently identified and authenticated by the Botany Department at the university, with a voucher number assigned: BOT/NUT/UC/23C. The outer husk was removed, and the tender coconut water was extracted through a small hole in the hard shell and transferred to a sterile beaker for use. Fresh coconut water was utilized daily, ensuring optimal quality and minimizing the risk of contamination.

2.1.4 Preparation of *Heinsia crinita* extract

Fresh, mature green leaves of *Heinsia crinita* were obtained from Watt market, a local market in Cross River State, and were subsequently identified and authenticated by the Botany Department at the university, with a voucher number assigned: BOT/HERB/UC/45A. The leaves were cleaned and air-dried in the laboratory, after which they were blended into a powder using an electric blender. A 100g sample of

the powder was weighed and soaked in 80% ethanol, followed by agitation using an electric blender and storage at a cool temperature (0-80°C) for 48 hours. The mixture was then filtered twice, first using a cheesecloth and then a Whatman No.1 filter paper, to obtain a homologous filtrate. The filtrate was then concentrated in a vacuum at low temperature (40-45°C) using a rotary evaporator to obtain a crude paste, which was stored in a sterile container and kept in a cool, dry place until it was used for daily administration.

2.2 Experimental Design

The study used a randomized controlled trial design, with six groups:

Group A: Negative control group (n = 5)

Group B: Positive control group (n = 5)

Group C: Donepzil - treated group (n = 5)

Group D: Tender coconut water (TCW) treated group 2 (n = 5)

Group E: *Heinsia crinita* - treated group (n = 5)

Group F: TCW + *Heinsia crinita* - treated group (n = 5)

2.3 Methods

2.3.1 Induction of Alzheimer's disease model

Scopolamine was administered to rats at a dose of 1.0 mg/kg, intraperitoneally (i.p.), to induce Alzheimer-like cognitive dysfunction for a period of 7 days. Following this 7-day period, scopolamine was given to the rats every three days.

2.3.2 Randomization and Grouping

The rats were randomly assigned to one of the six groups described above which includes;

Group A: Negative Control - This group receive no treatment and serves as a baseline for comparison and helps to establish a "normal" level of outcome measures.

Group B: Positive Control - This group serves as a baseline for comparison and helps to establish Alzheimer's disease model.

Group C: Donepzil - treated group: This group received Donepezil (5mg/kgBW).

Group D: Tender coconut water (TCW) treated group: This group received Tender Coconut Water (2ml/kgBW).

Group E: Heinsia crinita - treated group: This group received *Heinsia crinita* (500mg/kgBW).

Group F: TCW + Heinsia crinita - treated group: This group received TCW + *Heinsia crinita* (2ml + 500mg/kgBW).

2.3.3 Treatment Administration

The treatments were administered orally once daily for 28days.

2.3.4 Termination of Experiment

After administration, the rats were euthenized by chloroform through inhalation. The rats were then dissected to harvest the brain with the full skull, which was immediately fixed in 10% formal saline. Blood samples were collected using 5ml syringes with 21G needles via cardiac puncture into pre-labelled plain vials and allowed to settle and coagulate. The brain tissue samples were used for histological assessment, while the blood samples were used for biochemical assessment.

2.3.5 Histological Tissue Processing

2.3.5.1 Histological Tissue Processing for Hematoxylin and Eosin Stain.

The full skull was cracked to expose the brain, which was then grossed to present the hippocampus. The brain tissue samples were dehydrated through an ascending series of ethanol concentrations (50%, 70%, 80%, 95%, and 100%) and then cleared in xylene. The samples were infiltrated with a molten paraffin wax, embedded in it, and then mounted in a rotator microtome to cut them into thin sections (5-8 µm) using a rotating blade. The sections were floated in a warm water bath and then picked up on albumized slides using a pair of forceps. The paraffin slides were dewaxed using xylene and then rehydrated through a series of decreasing ethanol concentrations (95%, 80%, 70%, and 50%). The slides were then stained with haematoxylin for 15 minutes, differentiated in acid alcohol for 1 minute, and blued with Eosin for 1 minute. After staining, the slides were rinsed under tap water, cleared in xylene, and allowed to dry

before being coverslipped with DPX. The thorough processing and staining protocol resulted in specific staining of the cytoplasm and nucleus of the cells, enabling researchers to analyze and interpret the hippocampal structure and potential abnormalities. The staining pattern visualized cellular morphology, including cell shape, size, and organization, as well as the identification of specific cellular components such as nucleoli and cytoplasmic inclusions. This allowed for a detailed examination of the hippocampal tissue and the detection of any potential abnormalities or pathologies that may be present.

2.3.5.2 Histological Tissue Processing for Glial Fibrillary Acid Protein (GFAP) Staining

The glass slides containing tissue samples of the hippocampus were also processed for Glial Fibrillary Acid Protein (GFAP) staining. The slides were deparaffinized and dehydrated before being treated with 0.05 percent hydrogen peroxide in absolute alcohol to block endogenous peroxidase activity for 30 minutes. A 5-minute wash in phosphate-buffered saline (PBS) at a pH of 7.4 followed this treatment. The slide was then incubated in a 0.01 M citrate buffer (pH 6) in a microwave for 5 minutes to unmask antigenic sites. To prevent nonspecific background staining, the slide was incubated in 1 percent Bovine Serum Albumin (BSA) dissolved in PBS for 30 minutes at 37°C. Primary antibodies, including GFAP at a dilution of 1:100, were applied to the slide and incubated for 90 minutes at room temperature. After rinsing with PBS, the slide was incubated with anti-mouse immunoglobulins (secondary antibody) conjugated to a peroxidase-labeled dextran polymer (Dako, Denmark) for 60 minutes. To detect the reaction, the slide was incubated in 3,3'-diaminobenzidine for 15 minutes, followed by counterstaining with Mayer's hematoxylin. Finally, the slide was dehydrated, cleared, and mounted by Di-N-Butyle Phthalate in Xylene (DPX). The resulting stained slides showed GFAP-positive cells appearing brown and nuclei appearing blue, allowing for analysis and interpretation of the results.

2.3.6 Biochemical Assay

The MDA Assay was performed to determine MDA levels while glutathione peroxidase assay was performed to determine serum glutathione levels

2.3.6.1 MDA (Malondialdehyde) Assay

Serum from the blood samples were added to clean centrifuge tubes, followed by the addition of 10% Trichloroacetic acid, 0.05M H_2SO_4, and 0.67% thiobarbituric acid (TBA). The mixture was incubated for

10 minutes before being heated in a boiling water bath for 2 minutes, followed by the addition of butanol. The resulting TBA-reactive material was extracted and its absorbance was measured at a wavelength of 532nm. A standard curve was prepared by extrapolating the values of the TBA-reactive material, based on the reaction between MDA and TBA, which forms a red compound with a maximum absorption peak at 532nm.

2.3.6.2 Glutathione Peroxidase Assay

Reaction mix was added to sample, positive control, and reagent control wells, which were then mixed and incubated at room temperature for 15 minutes to deplete all GSSG in the samples. The reaction was started by adding cumene hydroxide solution to each well, followed by mixing. The output (A1) was measured on a microplate reader at OD340nm at time point T1, and the mixture was then incubated at 25°C for 5 minutes, protected from light. The output (A2) was then measured on a microplate reader at OD340nm at time point T2. The decrease in absorbance at OD340nm between time points T1 and T2 was used to calculate the glutathione peroxidase activity in the samples, based on the reaction between glutathione peroxidase enzyme and cumene hydroxide, which results in the reduction of glutathione disulfide (GSSG) to glutathione (GSH).

2.4 Statistical Analysis

Descriptive statistics were calculated to summarize the characteristics of the MDA values in each treatment group. The mean values of MDA levels and GPx activity across the 6 treatment groups were then compared using a one-way ANOVA, which identified significant differences between the groups. In response, Tukey's HSD test was performed to actively determine which specific treatment groups have significantly different mean values of MDA levels and GPx activity, thereby providing more nuanced insights into the differences between the groups.

3.0 RESULTS

3.1 Phytochemicals Analysis

3.1.1 Qualitative Phytochemical Analysis

The results of the phytochemical analysis reveals significant differences in the phytochemical profiles of Heinsia Crinita and coconut water. The result show that Heinsia Crinita may have more more potent medicinal properties, more effective antioxidant and anti-inflammatory properties compared with tender coconut water with the presence of flavonoids, alkaloids, and saponins.

Table 1: Phytochemical profiles of Heinsia Crinita and Tender coconut water.

	Alkaloids	Flavonoids	Saponnins	Tannins	Phenols
Coconut water	++	-	++	++	+
Heinsia crinite	+++	++	+++	++	+

The values are expressed as;

+	Present in low concentration
++	Present in moderate concentration
+++	Present in high concentration
_	Absent

3.1.2 Quantitative Phytochemical Analysis

The result of quantitative phytochemical analysis reveal significant differences in the phytochemical concentration profile of Tender Coconut water and Hensia Cristina. The results show that Heinsia Crinita May have higher potential medicinal properties, potential health benefits, potential uses in traditional medicine and potential applications in pharmaceuticals and cosmetics compared to tender coconut water with higher concentrations of alkaloids, saponins, and tannins.

Table 2: Phytochemical Concentration Profile of Heinsia Crinita and Tender Coconut water

	Alkaloids	Flavonoids	Saponnins	Tannins	Phenols
Tender coconut water (mg/g)	0.48	-	0.17	0.29	0.04
Heinsia crinita (mg/g)	3.71	0.91	2.29	1.36	0.30

**The values are expressed as milligrams/g (mg/g). The "Difference" column shows the absolute difference between the values for coconut water and Heinsia crinita. There is higher concentrations of other phytochemicals in Heinsia Crinita*

3.1.3 Proximate analysis of Tender Coconut water and Hensia Cristina

The result of the Proximate analysis suggests that Heinsia Crinita may have higher Potential health benefits compared with Tender Coconut water with higher water content, fiber content, and protein content whereas coconut water has a higher carbohydrate content. This suggests that Heinsia Crinita and coconut water have distinct nutritional profiles, with Heinsia Crinita being a more nutrient-rich and hydrating option

Table 3: Proximate Data of Tender Coconut water and Heinsia crinita

	Moisture content	Ash	Fat	Fibre	Protein content	Carbohydrates
Tender coconut water *(%)*	-	0.43	0.15	-	0.06	99.39
Heinsia crinita (%)	55.39	3.22	2.13	6.84	3.78	24.41

**The values are expressed as percentages of the total weight of the sample. The "Difference" column shows the absolute difference between the values for coconut water and Heinsia crinita. Heinsia Crinita have a higher water content, fiber content, and protein content, whereas coconut water has a higher carbohydrate content.*

3.2 Histopathological Analysis

3.2.1 Histological Evaluation of Hippocampal Tissues with Hematoxylin and Eosin staining

Positive control group (Group B) show that scopolamine induced histomorphological alternations of scopolamine induced Alzheimer's diseases in Albino Wister rats with with reduction in pyknosis and karyorrhexis, Cytoplasmic vacuolation and gliosis which inflammation, cellular damage or stress and neuronal cell death.

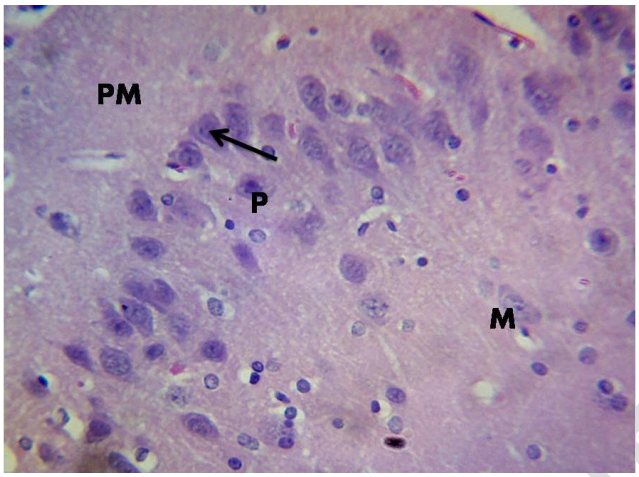
Negative control group (Group A) show not show the histomorphological alterations associated with Alzheimer's diseases in Albino Wister rats.

Treatment with Heinsia crinita + tender coconut water (Group F) show that it may not be as effectient in reducing inflammation, cellular damage or stress and neuronal cell death compared with the treatment with Donepzil alone with reduction.

Treatment with Heinsia crinita (Group E) show that it may be more efficient in reducing inflammation, cellular damage or stress and neuronal cell death compared with the treatment with tender coconut water (Group D) with reduction in reduction in pyknosis and karyorrhexis, Cytoplasmic vacuolation and gliosis.

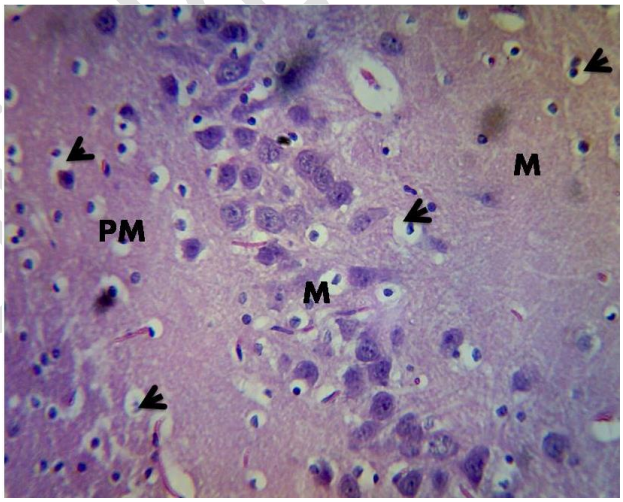
Treatment with Heinsia crinita + tender coconut water (Group F) show that it may be more efficient in reducing inflammation, cellular damage or stress and neuronal cell death compared with the treatment with tender coconut water alone with reduction in pyknosis and karyorrhexis, Cytoplasmic vacuolation and gliosis.

Figure 1: Photomicrographs of the hippocampal tissue section stained with hematoxylin and eosin



Panel A

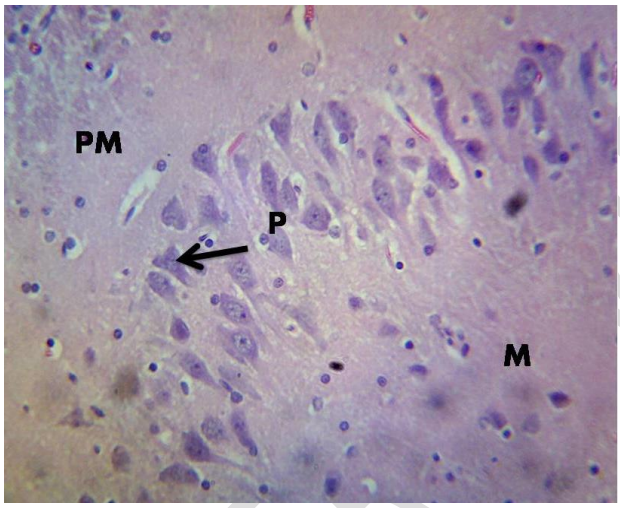
Panel A: Hippocampal tissue section tissue section of Group A (Negative control group) shows the typical three cell layers, including the Molecular layer (M), Pyramidal cell layer (P), and Granule cell layer (PM). The pyramidal neurons appeared normal having characteristic appearance, with large, lightly staining nuclei and lightly eosinophilic cytoplasm. They exhibit a lack of severe nuclear changes, such as pyknosis or karyorrhexis, and do not show evidence of significant cytoplasmic vacuolation or deposition of abnormal substances



Panel B

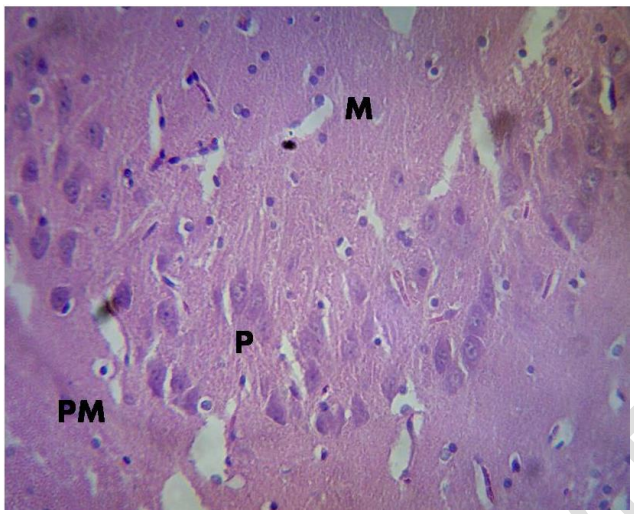
Panel B: Hippocampal tissue section tissue section of Group B (Positive control group) shows the three cell layers including the Molecular layer (M) Pyramidal cell layer (P), Granule cell layer (PM). The Pyramdal neurons show severe pyknosis and karyorrhex with darkly staining, shrunken nuclei, loss of

nuclear details and increased eosinophilia in the pyramidal layer (P). Moderate Cytoplasmic vacuolation with Pink or empty spaces within the cytoplasm, cytoplasmic clear cells and mildly eosinophilic with deposits. Severe gliosis With fibrous tissue formation and scaring



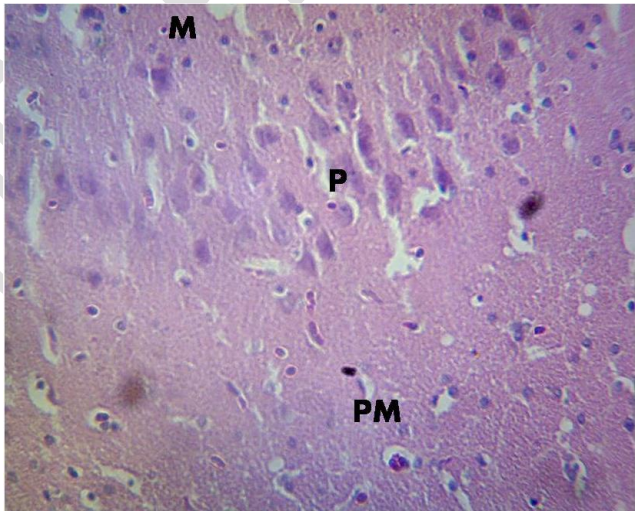
Panel C

Panel C: Hippocampal tissue section tissue section of Group C (Donepzil treated group) shows the three cell layers including the Molecular layer (M) Pyramidal cell layer (P), Granule cell layer (PM). The Pyramdal neurons show mild pyknosis and karyorrhex with darkly staining, shrunken nuclei in the pyramidal layer (P). Mild cytoplasmic vacuolation with Pink or empty spaces within the cytoplasm. Mild gliosis With vacoulations



Panel D

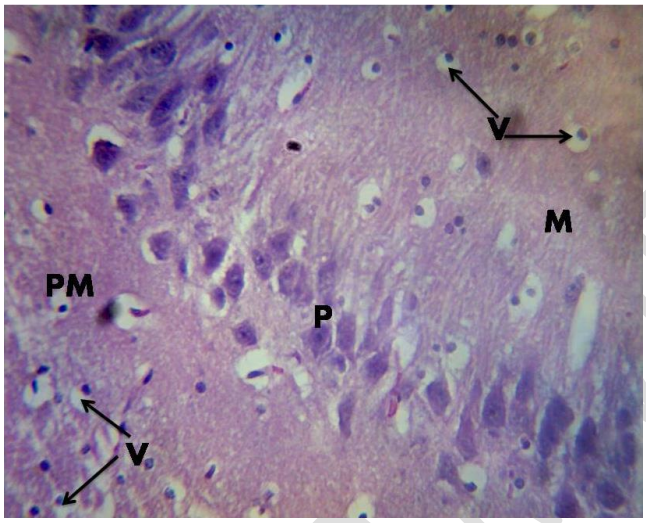
Panel D: Hippocampal tissue section Group D (Tender coconut water (TCW) - treated group, shows the three cell layers including the Molecular layer (M) Pyramidal cell layer (P), Granule cell layer (PM). The Pyramdal neurons show moderate pyknosis and karyorrhex with darkly staining, shrunken nuclei, loss of nuclear details and increased eosinophilia in the pyramidal layer (P). Moderate Cytoplasmic vacuolation with Pink or empty spaces within the cytoplasm, cytoplasmic clear cells and mildly eosinophilic with deposits. Mild gliosis With proliferation and vacoulations.



Panel E

Panel E: The hippocampal tissue section of Group E (Heinsia crinita-treated group) shows the three cell layers including the Molecular layer (M) Pyramidal cell layer (P), Granule cell layer (PM). The Pyramdal

neurons show mild pyknosis and karyorrhex with darkly staining, shrunken nuclei in the pyramidal layer (P). Mild Cytoplasmic vacuolation with Pink or empty spaces within the cytoplasm. Mild gliosis With fibrous tissue formation and scaring



Panel F

Panel F: The hippocampal tissue section of Group F (Heinsia crinita + tender coconut water - treated group) shows the three cell layers including the Molecular layer (M) Pyramidal cell layer (P), Granule cell layer (PM). The Pyramdal neurons show mild pyknosis and karyorrhex with darkly staining, shrunken nuclei in the pyramidal layer (P). Mild Cytoplasmic vacuolation with Pink or empty spaces within the cytoplasm. Moderate gliosis With fibrous tissue formation and scaring

Hematoxylin and Eosin staining x40, Molecular layer = M, Pyramidal cell layer = P, Granule cell layer = PM.

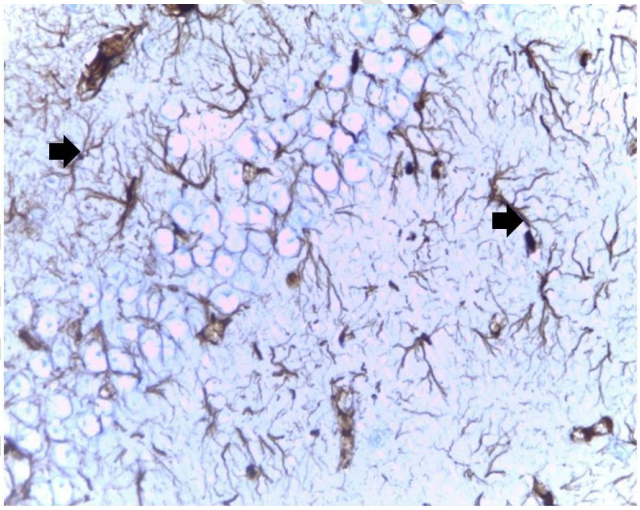
3.2.2 GFAP Staining of Hippocampal Tissues

The analysis of the hippocampal tissue sections reveal the effectiveness in reversing the injury among the experimental groups. The effectiveness in reversing the injury among the experimental groups can be ranked as follows:

Positive control group (Group B) show that Scopolamine induction show immunochemical alterations associated with Alzheimer's disease with severe astrogliosis. This is expected, as astrogliosis is a

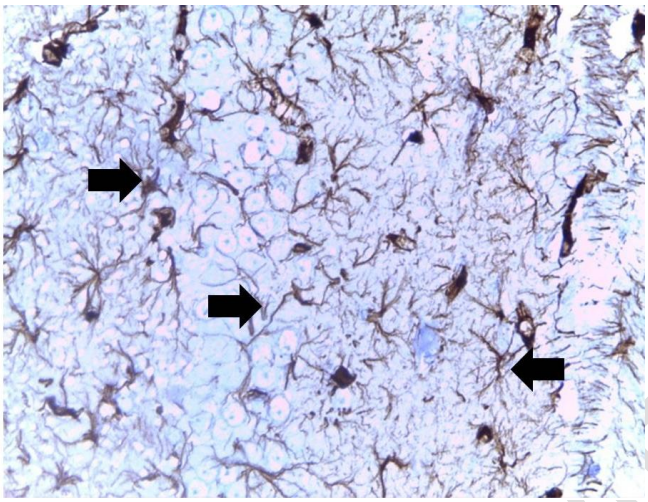
common response to injury or damage. Negative control group (Group A) show no sign of neuropathological features associated with scopolamine induced Alzheimer's disease with resting astrocytes. This is expected, as it is the control group. Treatment with Heinsia crinita (Group E) may be more effective in ameliorating the immunochemical alterations of scopolamine induced Alzheimer's disease model in Wister rats compared to tender coconut water treatment with reduced severity of astrocyte activation. Treatment with Heinsia crinita + tender coconut water (Group F) show that it may be more efficient in ameliorating the immunochemical alterations of scopolamine induced Alzheimer's disease model in Wister rats compared to tender coconut water treatment alone with reduced severity of astrocyte activation. Treatment with Heinsia crinita + tender coconut water (Group F) show that it may be as efficient as Donepzil treatment (Group C) in ameliorating the immunochemical alterations of scopolamine induced Alzheimer's disease model in Wister rats with reduced severity of astrocyte activation similar to Donepzil treatment.

Figure 2: Analysis of the hippocampal tissue sections



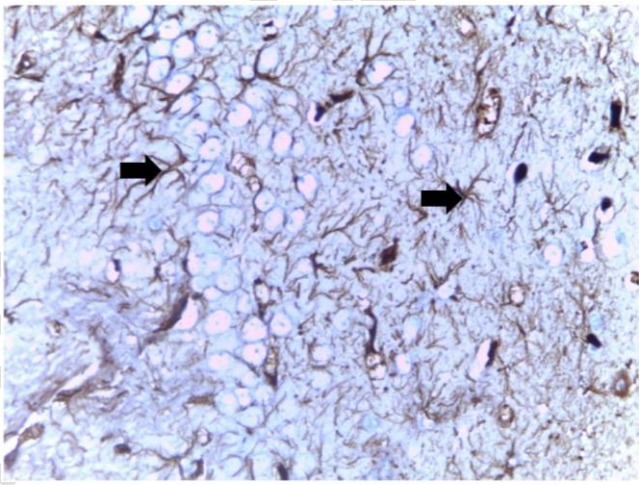
Panel A

Panel A: Hippocampal tissue section of Group A (Negative control group) shows resting astrocytes characterized by low level of astrocyte activation, normal expression of glial fibrillary acidic protein (GFAP) and normal morphology and size.



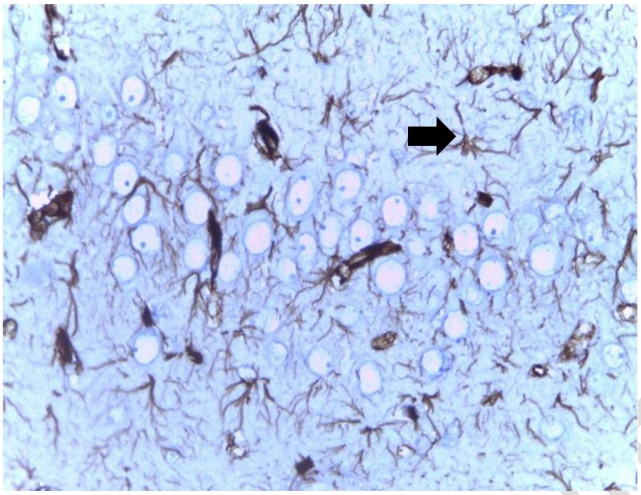
Panel B

Panel B: Hippocampal tissue section of Group A (Positive control group) shows astrogliosis characterized by chronic overexpression of GFAP and changes in astrocyte morphology, such as hypertrophy and hyperplasia.



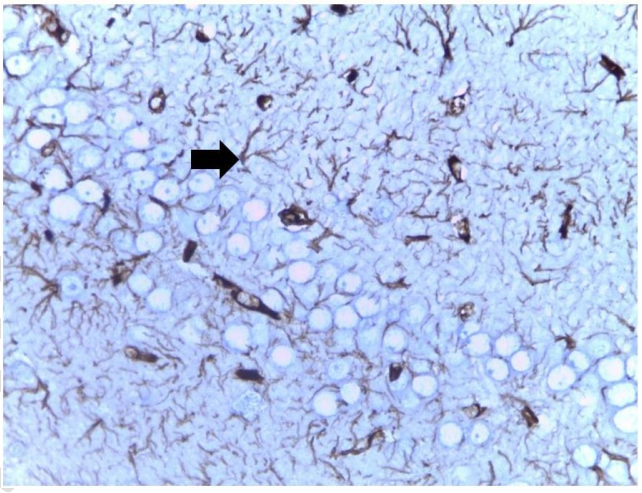
Panel C

Panel C: Hippocampal tissue section of Group C (Donepzil treated group) shows mild astrocyte activation characterized by increased expression of GFAP and Changes in astrocyte morphology, such as increased process length and branching.



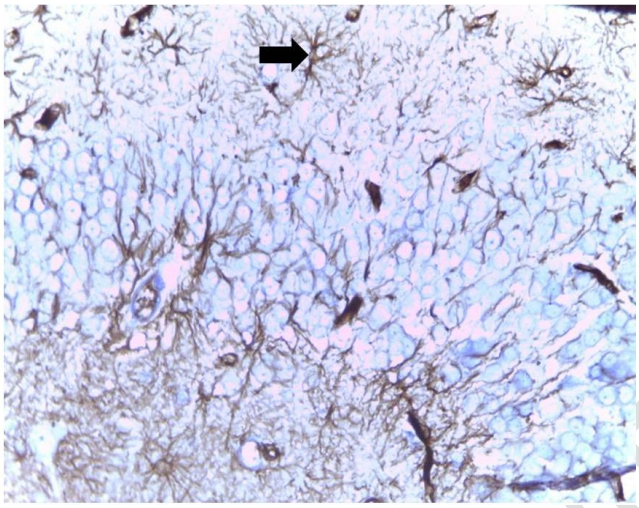
Panel D

Panel D: Hippocampal tissue section Group D (Tender coconut water (TCW) - treated group) moderate astrocyte activation characterized by overexpression of GFAP and significant changes in astrocyte morphology, such as swelling and fragmentation.



Panel E

Panel E: The hippocampal tissue section of Group E (Heinsia crinita-treated group) shows mild astrocyte activation characterized by increased expression of GFAP and slight increase in astrocyte size and morphology.



Panel F

Panel F: The hippocampal tissue section of Group F (Heinsia crinita + tender coconut water -treated group shows mild astrocyte activation characterized by increased expression of GFAP and Changes in astrocyte morphology, such as increased process length and branching.

GFAP staining method x 40.

Astrocytes = Arrow

3.3 Assessment of Melandialdehyde (MDA) Levels and Glutathione Peroxidase (GPx) Activity

The results of the biochemical analysis revealed the differences in the Melandialdehyde levels and GPx activity among the experimental groups. Positive control group (Group B) show that scopolamine induced biochemical alterations associated with Alzheimer's diseases in Albino Wister rats with increase in MDA level and decrease in GPx activity which suggests increased oxidative stress and lipid damage in the cells and depreciated antioxidant defense in the cells. Negative control group (Group A) show not show the biochemical alterations associated with Alzheimer's diseases in Albino Wister rats. Treatment with Heinsia crinita + tender coconut water (Group F) show that it may have similar effect in reducing oxidative stress and lipid damage in the cells and enhancing antioxidant defense in the cells compared with the treatment with Donepzil alone with reduction in MDA level and increase in GPx activity. Treatment with Heinsia crinita (Group E) show that it may be more efficient in reducing oxidative stress and lipid damage

in the cells and enhancee antioxidant defense in the cells compared with the treatment with treatment with tender coconut water (Group D) with reduction in MDA level and increase in GPx activity. Treatment with Heinsia crinita + tender coconut water (Group F) show that it may be more efficient in reducing oxidative stress and lipid damage in the cells and enhance antioxidant defense in the cells compared with the treatment with tender coconut water alone with reduction in MDA level and increase in GPx activity

Table 4: Melandialdehyde (MDA) Levels and Glutathione Peroxidase (GPx) Activity in the Experimental Groups

Group	MDA (nmol/µL)	GPx (U/L)
A	3.20 ± 0.47	1988.00 ± 300.10
B	6.40 ± 1.64	353.10 ± 49.0[*]
C	3.62 ± 0.60	1822.00 ± 213.00 [a]
D	6.19 ± 1.45	632.30 ± 212.00 [*b]
E	1.82 ± 0.18[a,c]	1361.00 ± 127.40 [c]
F	3.22 ± 0.47	1796.00 ± 489.40 [a,c]

Values are expressed as mean ± SEM, n = 5, P < 0.05

*significantly different from Group 1at p<0.05;

a = significantly different from Group 2 at p<0.05;

b = significantly different from Group 3 at p<0.05;

c = significantly different from Group 4 at p<0.05.

Group A: Negative control group

Group B: Positive control group

Group C: Donepzil treated group

Group D: Tender coconut water (TCW) - treated group

Group E: Heinsia crinita-treated group

Group F: TCW + Heinsia crinita-treated group

3.4 Discussion

The study investigated the potential neuroprotective effects of *Heinsia Crinita* and Tender Coconut water against scopolamine-induced Alzheimer's disease in Wister rats. The results of the phytochemical analysis revealed significant differences in the phytochemical profiles of *Heinsia Crinita* and Tender Coconut water, with *Heinsia Crinita* showing a more potent profile with higher concentrations of alkaloids, saponins, and tannins. This is consistent with previous studies that have shown that *Heinsia Crinita* has a rich phytochemical profile with potential medicinal properties (11).

The results of the histological evaluation of hippocampal tissues using Hematoxylin and Eosin staining revealed that treatment with *Heinsia Crinita* was more effective in reducing inflammation, cellular damage, or stress and neuronal cell death compared to treatment with tender coconut water. This is consistent with previous studies that have shown that *Heinsia Crinita* has anti-inflammatory and antioxidant properties that can help protect against oxidative stress and cellular damage (11, 9, 11). The reduction in inflammation, cellular damage, or stress and neuronal cell death suggests that *Heinsia Crinita* may have potential neuroprotective effects against scopolamine-induced Alzheimer's disease.

The results of the GFAP staining of hippocampal tissues also revealed that treatment with *Heinsia Crinita* was more effective in ameliorating the immunochemical alterations associated with scopolamine-induced

Alzheimer's disease model in Wister rats compared to treatment with tender coconut water. This is consistent with previous studies that have shown that *Heinsia Crinita* has anti-inflammatory and antioxidant properties that can help protect against neurodegeneration (29, 11). The reduction in immunochemical alterations suggests that *Heinsia Crinita* may have potential neuroprotective effects against scopolamine-induced Alzheimer's disease.

The results of the biochemical analysis revealed differences in Melandialdehyde (MDA) levels and glutathione peroxidase (GPx) activity among the experimental groups. Treatment with *Heinsia Crinita* + tender coconut water showed similar effects in reducing oxidative stress and lipid damage in cells and enhancing antioxidant defence in cells compared to treatment with Donepzil alone. This suggests that the combination of *Heinsia Crinita* and tender coconut water may have synergistic effects in reducing oxidative stress and lipid damage in cells and enhancing antioxidant defence in cells.

The study's findings suggest that *Heinsia Crinita* may have potential neuroprotective effects against scopolamine-induced Alzheimer's disease in Wister rats. The study's results are consistent with previous studies that have shown that *Heinsia Crinita* has anti-inflammatory, antioxidant, and neuroprotective properties (19). The study's findings also suggest that the combination of *Heinsia Crinita* and tender coconut water may be a more effective treatment option than either agent alone, which is consistent with previous studies that have shown that combination of *Heinsia Crinita* and Tender Coconut water showed improvements in the spinal cord histopathtology following induction of Alzheimer’s disease (19).

The study's results are also supported by previous studies that have shown that *Heinsia Crinita* has potential therapeutic applications in the treatment of neurological disorders such as Alzheimer's disease (29, 30, 11, 19). And also, the study supported by previous studies that have shown that medicinal plants has potential therapeutic applications in the treatment of neurological disorders (31, 32, 33).

The study's findings suggest that further research is needed to fully understand the mechanisms by which *Heinsia Crinita* exerts its neuroprotective effects and to determine its potential therapeutic applications in the treatment of Alzheimer's disease.

4.0 Conclusion

The study's findings provide evidence for the potential neuroprotective effects of *Heinsia Crinita* against scopolamine-induced Alzheimer's disease in Wister rats. The results of the phytochemical analysis, histological evaluation, and biochemical analysis suggest that *Heinsia Crinita* has a more potent profile with higher concentrations of alkaloids, saponins, and tannins, which may contribute to its anti-inflammatory and antioxidant properties. The study's findings are consistent with previous studies that have shown that *Heinsia Crinita* has potential therapeutic applications in the treatment of neurological disorders such as Alzheimer's disease.

The combination of *Heinsia Crinita* and tender coconut water may be a more effective treatment option than either agent alone, as it showed synergistic effects in reducing oxidative stress and lipid damage in cells and enhancing antioxidant defence in cells. Further research is needed to fully understand the mechanisms by which *Heinsia Crinita* exerts its neuroprotective effects and to determine its potential therapeutic applications in the treatment of Alzheimer's disease.

Overall, this study provides new insights into the potential therapeutic effects of *Heinsia Crinita* and tender coconut water against scopolamine-induced Alzheimer's disease in Wister rats. The findings suggest that *Heinsia Crinita* may be a promising natural compound for the treatment of Alzheimer's disease, and further research is needed to explore its potential therapeutic applications.

ETHICAL CONSIDERATION

Approval was given by the Faculty of Basic Medical Sciences Committee on animal use and care, University of Calabar to carry out this research work following laid down rules and guidelines of the institution in the use of medicinal plants and animal models.

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