Niger. J. Physiol. Sci. 40 (June 2025): 119 – 126
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full-Length Research Article

Hesperidin Nanoparticles Prevent Scopolamine-Induced Cognition Impairment Through Amplification of Antioxidant Defense System and Cholinergic Neurotransmission in Mice

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Summary: The rapid increase in the aging population and age-linked cognitive impairment, as well as dementia of Alzheimer's type, are becoming more prevalent globally. Genetic and environmental interactions played a key role in dementia pathology. Oxidative stress and cholinergic disruptions are well-linked with dementia. Hence, phytochemicals with neuroprotective and antioxidant properties could help ameliorate mitochondrial dysfunction and toxic effects misfolded amyloid-beta and tau proteins in dementia of Alzheimer's type. Previous studies have alluded to the beneficial action of hesperidin in mild cognitive impairment, but delivery could be better enhanced in nanoparticulate form. Hence, this study sought to investigate the memory-enhancing ability of hesperidin nanoparticles (HES_) on scopolamine-induced memory impairment in mice. Mice were randomly assigned into 6 groups (n=6) and treated as follows; vehicle only, vehicle + SCOP (1mg/kg, i.p.), HES (1,10 and 50mg/kg, p.o., respectively) + SCOP and donepezil (1mg/kg; p.o.) + SCOP for 14 consecutive days followed by behavioral assessment for memory function using open field test, Y-maze, novel object recognition and Morris water maze for locomotion, working, cognition and spatial learning, respectively. The animals were euthanized and brain samples were collected for biochemical assays (oxidative stress markers and acetylcholinesterase activity). SCOP or HES administration did not affect locomotor activity; however, SCOP reduced the percentage of alternation behaviour in the Y-maze and discrimination index in NOR tests with no significant change in escape latency time in the MWM task, indicative of working memory, cognition and spatial learning impairment. In contrast, the pre-administration of HES produced a dose-dependent and significant increase in working memory, cognition and spatial learning abilities. Similarly, HES_ pretreatment reduced scopolamine-induced increases in lipid peroxidation and acetylcholinesterase activity and deficit in antioxidant enzyme activity in the hippocampus and prefrontal cortex caused by SCOP. The results of the present study further showed the potential of hesperidin in nanoparticle form in the enhancement of memory formation through the amplification of antioxidant defense and cholinergic neurotransmission.

Keywords: *Alzheimer of dementia type; hesperidin nanoparticles; oxidative stress; scopolamine; memory.*

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Manuscript received- November 2024; Accepted: March 2025

DOI: https://doi.org/10.54548/njps.v40i1.14

INTRODUCTION

Cognitive impairment is a decline in memory and other cognitive functions and can also manifest as an underlying condition including Alzheimer's disease (AD) and aging. Cognition is a mental action and the part of the central nervous system that plays a pivotal role in normal cognition is the brain's cholinergic system. The cholinergic system has also been implicated in the pathology of AD, age-related cognitive decline, and mild cognitive impairment (Ishola *et al*., 2020; Sultzer *et al*., 2022). AD, the most common form of dementia, is a genetic and sporadic neurodegenerative disease characterized by the progressive loss of neurons, leading to severe impairments in cognitive functions (Briggs *et al*., 2016). According to WHO (2023), more than 20 million people worldwide between the ages of 65 years and above have AD and over 60% of them reside in low and middle-income countries (WHO, 2023). Furthermore, evidence from various studies has linked oxidative stress to brain aging and AD as oxidative stress mediates an increase in amyloid precursor protein (APP) expression and secretase activities, which contribute to the pathological hallmark of AD: amyloid-β peptide accumulation and associated neurodegeneration (Ishola *et al*., 2017; Cheignon *et al*., 2018; Ionescu-Tucker *et al*., 2021; Chae *et al*., 2022). The general pathology of cognitive deficits is damage to neuronal tissue, of which oxidative stress plays a significant role. AD continues to pose a global healthcare challenge,

placing heavy economic and social strains on families and societies, despite progress in understanding its underlying mechanisms.

Despite significant pharmacological advancements, definitive cures for these diseases are still lacking, prompting increased interest in naturally-derived substances for potential preventive benefits. The potential of antioxidants (especially from natural origin) to mitigate cognitive aging has caused an emerging interest in their therapeutic potential for disease management (Morris *et al*., 2002; Tamilselvam *et al*., 2013; Almukainzi *et al*., 2024). Flavonoids, found in various plant-based foods (fruits, vegetables and leaves), offer medicinal benefits, including antioxidant, and anti-inflammatory properties. Reactive oxygen species (ROS)-driven oxidative stress in the hippocampus and prefrontal cortex underlies aging-related cognitive decline and AD development, making antioxidants a potential therapeutic target (Ishola *et al*., 2020; Chae *et al*., 2022). Hence, this study was designed to investigate the potential benefit of hesperidin in scopolamine-induced memory impairment in mice. Hesperidin is a flavonoid found in citrus fruits such as oranges and possesses a variety of biological activities including antioxidant properties (Almukainzi *et al*., 2024), anti-inflammatory properties (Tamilselvam *et al*., 2013), and anti-apoptotic activities (Ikram *et al*., 2019). Thus, making hesperidin a potential and promising neuroprotective agent (Hong and An, 2018; Moghaddam and Zare, 2018; Almukainzi *et al*., 2024). Despite hesperidin's therapeutic potential, its limited bioavailability and solubility restrict its absorption, emphasizing the need for a targeted delivery mechanism to optimize its therapeutic efficacy. Hence, in this study, hesperidin nanoparticles were used to improve its potential therapeutic benefits in scopolamine-induced cognitive impairment.

Scopolamine acts by blocking muscarinic receptors, impairing learning and memory in animal studies, making it a useful research tool for investigating the memory-enhancing properties of new therapeutic agent (Ishola *et al*., 2020; Chae *et al*., 2022; Ishola *et al*., 2023; Al-Tawarah *et al*., 2023). Evidence from previous studies has shown that scopolamine disrupts short-term or long-term spatial working memory, however, acetylcholinesterase inhibitors (AChEIs), like donepezil, reverse its activity (Sheng *et al*., 2018; Ishola *et al*., 2020; Chae *et al*., 2022). This underscores the urgent need for safe and effective AD treatments.

MATERIALS AND METHODS

Materials: Donepezil, scopolamine, ethanol, and hesperidin were obtained from Sigma Aldrich (MO, USA), and other reagents used in this experiment are of analytical grade.

Synthesis of water-dispersible Hesperidin Nanoparticles: The micro-emulsion method described by Ali *et al* was employed to synthesise hesperidin nanoparticles (HES) with some modifications. In a typical synthesis, 1 g (0.012 mol) of polyvinyl acetate (PVAc) was added to 15 ml of distilled water and heated to about 65 °C to dissolve the polyvinyl acetate. Then, 0.25 g of the PVAc solution was measured, added to 30 mL of dichloromethane, followed by 20 mL of acetone to form homogeneous solution and lastly 0.25g (0.00041 mol) of bulk hesperidin was added. The mixture was then sonicated for 30 mins to form an emulsion. The primary emulsion was then injected into 10 ml of the prepared Bovine serum albumin (BSA) solution (0.015mmol) and sonicated for about 15 mins. To disperse the final oil/water emulsion and remove the residual organic solvent in the solution, 15 ml of deionized water was added and stirred overnight. The mixture was centrifuged at 6000 rpm for 30 mins. The supernatant was discarded and the obtained nanoparticles were washed with deionized water and centrifuged at 6000 rpm for 5 mins. The resulting nanoparticles were dried and kept at room temperature.

Laboratory animals: Adult mice used in this study were purchased from the Laboratory Animal Centre, College of Medicine, University of Lagos, Lagos State, Nigeria. The animals were housed in well-aerated plastic cages and well-fed (Livestock Feeds, Lagos, Nigeria) and water ad libitum. This study was carried out following ethical approval obtained from the Animal Care and Use Research Ethic Committee of the College of Medicine, University of Lagos (CMUL/ACUREC/01/20/7810). In accordance with the ARRIVE (Animal Research: Reporting of in vivo experiments) guidelines for reporting animal research.

Experimental Procedure: Mice were randomly assigned into 6 groups (n=6) and treated as follows; groups 1 and 2 received normal saline (10 ml/kg, p.o.), respectively, group 3- donepezil (1mg/kg; p.o.) and groups 4-6 received graded doses of HES (1,10 and 50mg/kg, p.o., respectively) for fourteen (14) days. Scopolamine (SCOP) administration commenced from day 3 to day 14, mice in groups 2 to 6 received SCOP (1mg/kg, i.p) one hour after pretreatment with vehicle or graded doses HES. Thereafter, animals were subjected to behavioral tests.

Behavioral Assessments

Open field Test (OFT): The Open Field Test (OFT) assesses locomotion, anxiety, and exploration in laboratory animals using a 96cm × 96cm × 45cm wooden arena divided into 16 squares (18 × 18cm) by black lines (Ishola *et al*., 2019). Each mouse placed at the centre point of the apparatus was allowed an acclimatization period of 60 s. Afterwards, the total number of rearing, line crosses and grooming behaviours were recorded for 5 mins. The apparatus was cleaned with 10 % ethanol and allowed to dry after each mouse.

Y-Maze Test: The Y Maze Test is a behavioral assay used to measure spontaneous exploration and short-term memory capabilities (Ishola *et al*., 2020). The Y-maze apparatus is Y- shaped (wooden) with arms labelled A, B, and C. Each animal was placed in the mid-point of the maze, and the number of arm entries and spontaneous alternations (sequence of entries [ABC, BAC, CBA]) were observed and recorded.

$$\% \text{ Spontaneous alternation: } \frac{\text{Number of alternation}}{\text{Number of entries- 2}} \times 100$$

Novel Object Recognition test (NORT): The Novel object recognition test is used to evaluate memory performance in mice based on the natural tendency of the mice to explore novel objects. This test was carried out using an open field area (60 cm x 50cm x 40cm). The animals were allowed to interact with the familiar object for 5 minutes before testing. This test was in two phases, namely, the trial phase and the test phase. During the trial phase, each mouse was positioned in the middle of two identical objects (A and B) in the open field arena for 5 minutes. Afterwards, the animals were returned to their cages for 1 hour. In the test phase, object B was replaced with object C, which was novel to the mice and different from objects A and B. Then, the mouse was left to explore objects A and C for a period of 5 minutes. The apparatus was cleaned after each test, and the time spent exploring each object was recorded in both phases. The preference index was calculated as the difference in exploration time of novel objects and familiar objects divided by the total amount of time spent with both objects (Ennaceur, 2010)

$$\text{Preference index (PI)} = \frac{T1}{T1 + T2}$$

Morris Water Maze Task (MWM): MWM is a behavioral test protocol used to assess spatial memory and learning in laboratory animals. The apparatus is made of a circular black water tank (110 cm diameter and 60 cm height) to a depth of 30 cm. The tank was partitioned into four sections (North, East, West, and South) with a submerged platform in the Southwest quadrant serving as an escape route. The mice were allowed a maximum of 60 s to find the hidden/submerged platform and were allowed to stay on it for 10 s. The escape latency time (ELT), defined as the time it takes for the mouse to locate the escape platform, was recorded. Mouse unable to locate the platform within 1 minute were gently guided to it and allowed a 10-s recovery time. Three trials were conducted each day for four days (days 10-13). On the 5th day (day 14), a probe test was done, during which the escape platform was removed from the tank, before placing the animal into it and each animal was given 30 s to search for the platform. The time spent around the area where the platform was initially located was recorded (Ishola *et al*., 2013; Ishola *et al*., 2019).

Dissection: After the probe test, the animals in each group were anaesthetized with ketamine, then perfused with cold normal saline and brain was rapidly removed, and hippocampus was then dissected on iced pack, weighed and kept in 0.1× PBS (pH 7.4) at −20 °C until biochemical analysis.

Biochemical Analysis: The extent of lipid peroxidation was evaluated by measuring MDA levels through the TBA spectrophotometric assay procedure as described by Ishola *et al*. (2017). The content of reduced glutathione (GSH) in brain tissue was determined as non-protein sulphydryl, following a previously described protocol by Sedlak and Lindsay (1968). The activity of superoxide dismutase (SOD) was assayed according to the method described by Nauseef *et al*. (2014). Catalase activity was also determined according to the method described by Sinha *et al*. (1972) while the acetylcholinesterase (AChE) activity in the hippocampal homogenate was quantified using the protocol of Ellman *et al*. (1959).

RESULTS

Characterization of Hesperidin: The FTIR spectra of hesperidin nanoparticles capped with PVAc is shown in Figure 1. The absorption band at 3414 and 1644 cm-1 correspond to O–H stretching vibration, C–H stretching, respectively while 1603 and 1517cm-1 are attributed to aromatic C=C stretch. At 1439, 1297, and 1096 cm-1, the peaks observed are attributed to C-H bend, C-O-C stretching of aryl ether, and C-O stretching of secondary alcohol respectively. The peak at 2919 cm-1 was attributed to C–H stretching from the PVAc. The SEM images revealed that the nano hesperidin is composed of agglomerated granules in the 2 to 10 µm size, as shown in Plate 1.

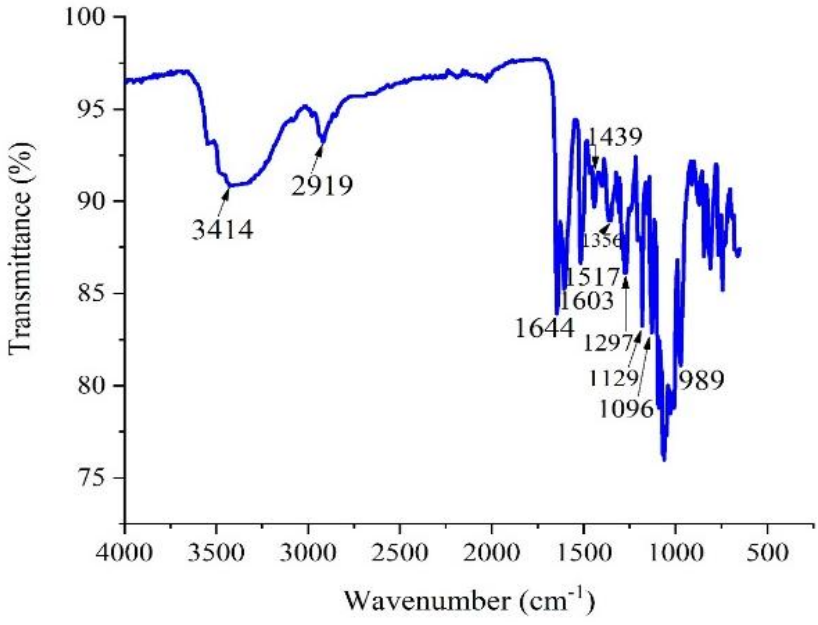

Figure 1:
FTIR spectrum of hesperidin nanoparticles capped with polyvinyl acetate.

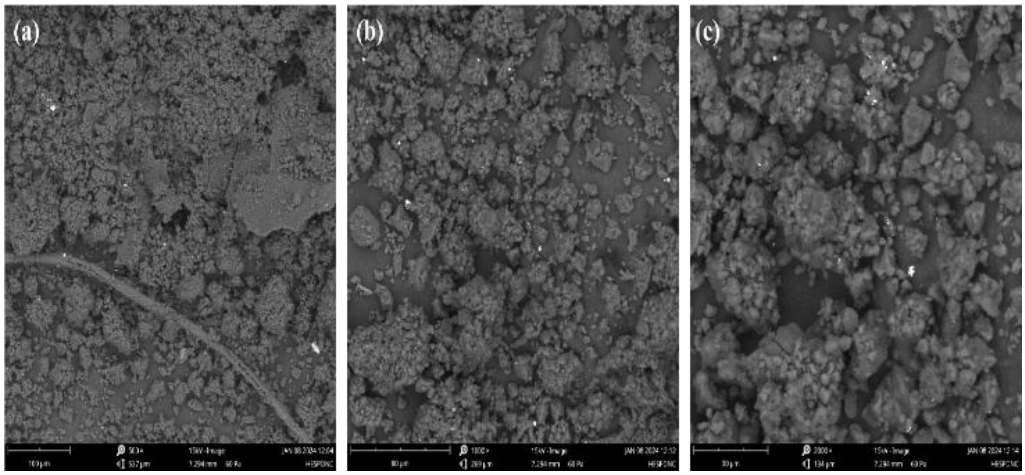

Plate 1:
SEM images at different magnifications (a) 100 µm, (b) 80 µm and (c) 30 µm

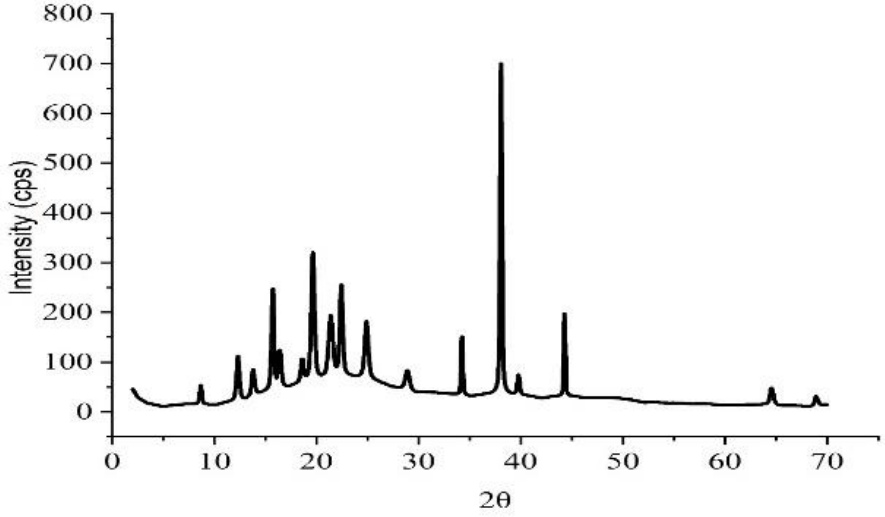

Plate 2
The p-XRD patterns of hesperidin nanoparticles

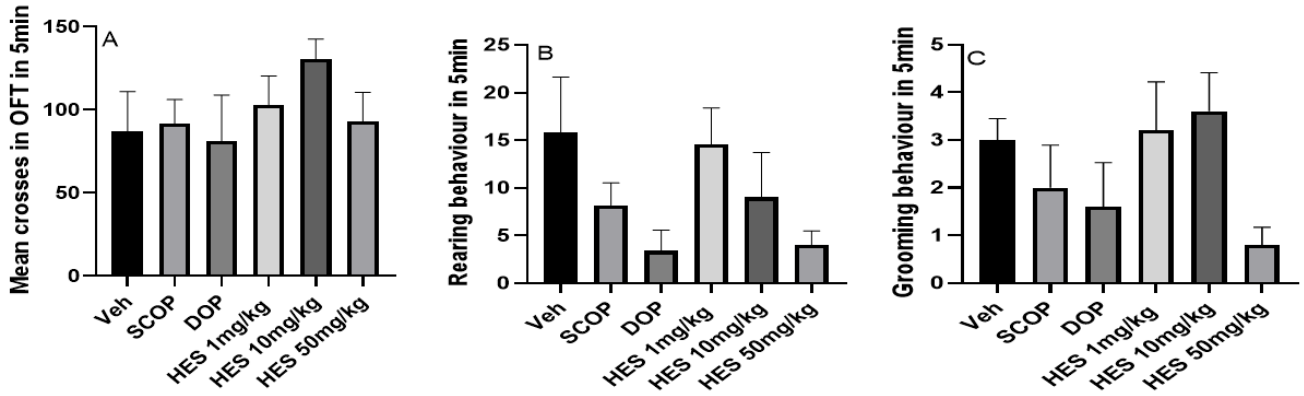

Figure 2A-C:
Effect of hesperidin nanoparticles on spontaneous motor activity (a) number of crosses, (b) number of rearing behaviour, and (c) number of grooming behaviour in open field test. Values are presented as mean±SEM (n=5). P>0.05 versus vehicle normal control, *p>0.05* versus vehicle-scopolamine treated group

Plate 2 shows the p-XRD patterns characterized by prominent peaks appearing at 12,1 15.5, 17.2, 19.6, 22.4, 23.4, 25,6, and 29.2 indicating the crystallinity of the compound. This result is similar to those reported by Ali *et al*., (2019).

Effect of HES on Locomotion: One way ANOVA showed no significant effect of treatment on mean number of crosses [F(5,30)=0.82,P=0.55] (Fig. 2A), rearing behaviour [F(5,30)=1.92,P=0.13] (Figure 2B) and grooming [F(5,30)=1.88,P=0.14] (Figure 2C) in the open field test. Dunnet post hoc multiple comparison tests showed no significant difference between scopolamine treated and control, as well as HES treated mice.

Effect of HES on Working memory of Mice: One way ANOVA showed significant [F(5,30)=17.37,P<0.0001] effect of vehicle, scopolamine and HES treatment on percent alternation behaviour in Y-MAZE task. Vehicle treated showed significant discriminative percent compared

to scopolamine treated (p<0.001). the decrease in spatial working memory caused by scopolamine was dose dependently reversed by HES (1, 10 and 50mg/kg) as shown in Figure 3.

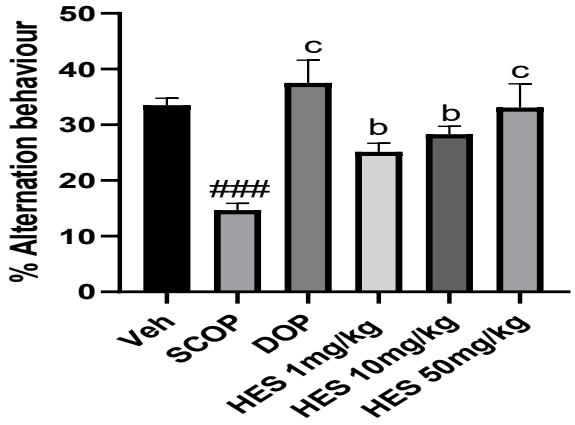

Figure 3:
Effect of hesperidin nanoparticle on working memory in Y-maze. Values are presented as mean±SEM (n=5). ###p<0.001 versus vehicle normal control, bp<0.01; cp<0.001 versus vehicle-scopolamine treated group

Effect of HES on exploratory time in mice subjected to NORT: One way ANOVA and multiple comparison tests revealed no significant effect of treatments on time spent interacting with the three similar objects in familiarization phase [F(5,24)=2.19,P=0.0885] (Figure 4a) indicative of no preference for all the objects. In the test trial, scopolamine significantly reduced the time spent by the animals exploring the novel object compared with the familiar objects indicative of a reduced preference index. However, the pretreatment of mice with HES (1, 10 and 50mg/kg) or donepezil significantly increased the preference index when compared with scopolamine-vehicle treated [F(5,24)=3.98,P=0.009] (Fig. 4b) in NORT.

Effect of HES on Escape latency (MWM): Two-way ANOVA results showed that the number of training days [F (3, 96)=51.70, p= 0.0001), the treatments [F(5,96)=7.30, p< 0.0001), and their interaction [F(15,96)=2.67,p=0.002) had a significant impact on the escape latency (Figure7a). On days 3-4 of training, the scopolamine-vehicle treated group exhibited significant prolongation of escape latency compared to the vehicle only treated animals. Donepezil administration significantly reduced escape latency during training days 3 and 4 compared to scopolamine-vehicle treated. HES (1, 10 and 50mg/kg) administration significantly decreased the time spent locating the escape platform. In the probe trial, HES (1, 10 and 50mg/kg) significantly increased the time spent by the animals at the target quadrant in comparison to scopolamine-vehicle treated [F(5,24)=3.17,P=0.02] (Figure 7b). Interestingly, scopolamine-vehicle treated mice exhibited a reduced time spent in the target zone in comparison with the vehicle control group. Donepezil significantly prolonged the time spent by the animals in the target zone in the probe test.

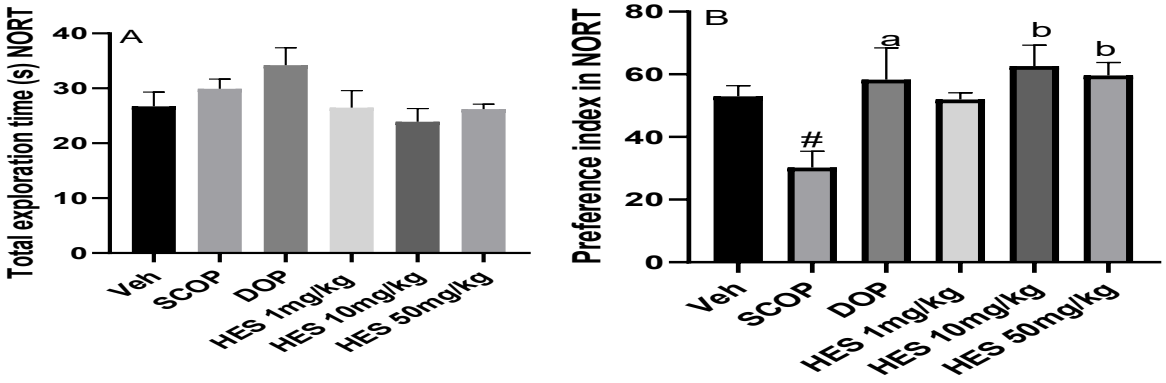

Figure 4A-B:
Effect of hesperidin nanoparticle on objects exploration time in (a) familiarization phase and (b) preference index in test phase in NORT in mice. Values are presented as mean±SEM (n=5). ###p<0.001 versus vehicle normal control, bp<0.01; cp<0.001 versus vehicle-scopolamine treated group.

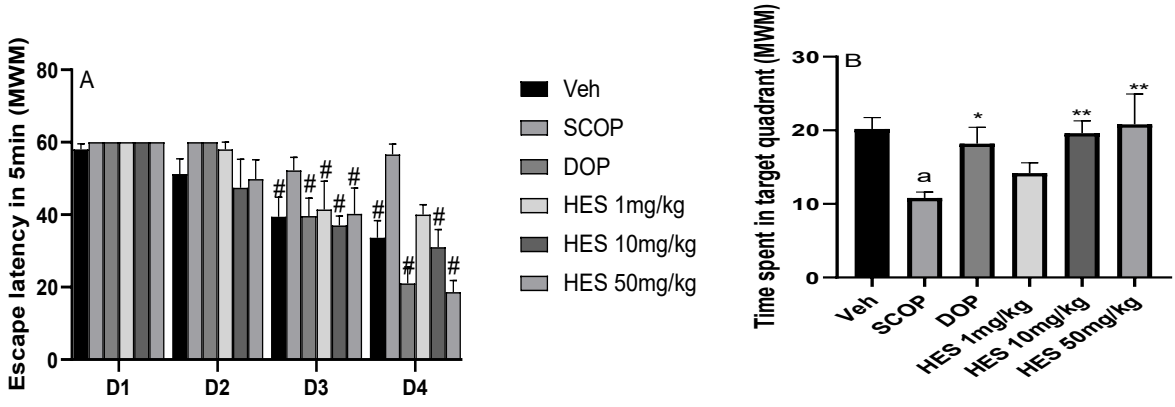

Figure 5A-B:
Effect of hesperidin nanoparticles on (A) escape latency and (b) time spent at target quadrant in MWM test. Values are presented as mean±SEM (n=5). #p<0.05 versus session 1, ap<0.01; cp<0.05 versus vehicle-control treated group, **p<0.01 versus vehicle-scopolamine treated.

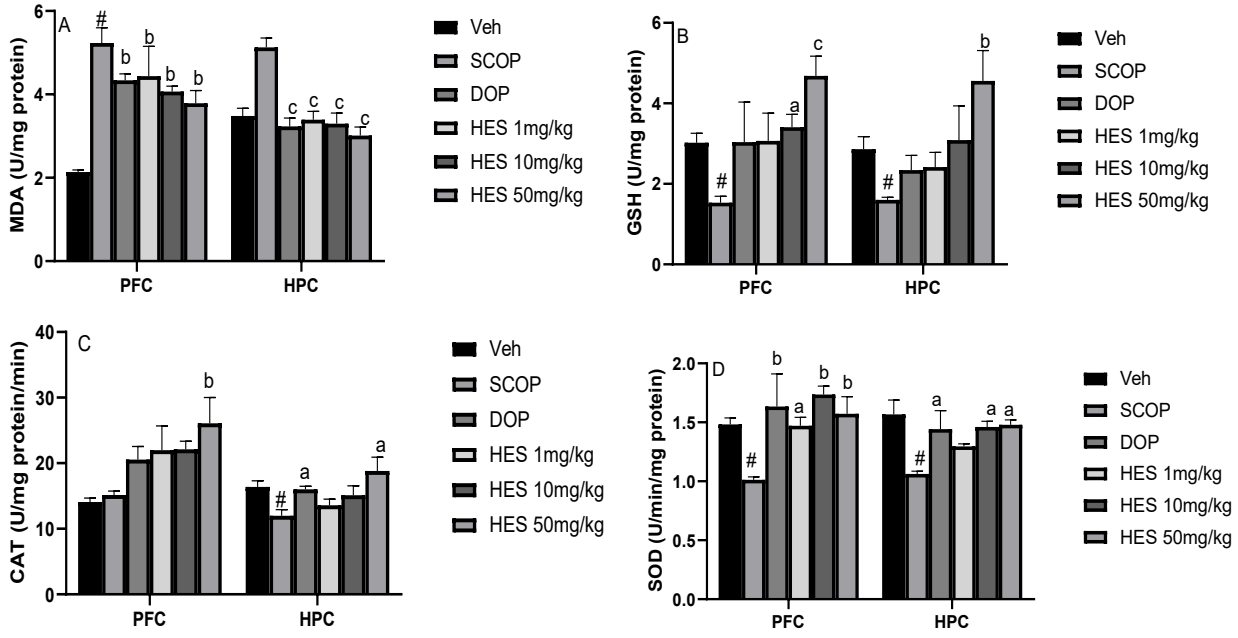

Figure 6A-D:
Effect of hesperidin nanoparticle on scopolamine-induced oxidative stress markers (A) MDA, (B) GSH, (C) catalase and (D) SOD in discrete brain regions of the prefrontal cortex (PFC) and hippocampus (HPC). Values are presented as mean±SEM (n=5). $^{\#}p<0.05$ versus vehicle-control treated, $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$ versus scopolamine-vehicle treated.

HES protected against Scopolamine-induced oxidative stress in the hippocampus and PFC of mice: Two-way ANOVA results showed the effect of treatment on MDA levels in the PFC and hippocampus [F (1,48)=5.73, p= 0.02), the treatments [F(5,48)=14.05, p< 0.0001), and their interaction [F(5,48)=4.94,p=0.001) had a significant impact on MDA level (Figure 8a). Scopolamine-vehicle treated caused significant increase in MDA level. However, HES_ (1, 10 and 50mg/kg) or donepezil administration significantly attenuated MDA generation in the PFC and HPC. Figure 8b showed the effect of treatments on GSH activity in the PFC and HPC [F (1,48)=0.99, p= 0.32), the treatments [F(5,48)=6.62, p< 0.001), and their interaction [F(5,48)=0.15,p=0.97) had a significant impact on GSH activity. Scopolamine-vehicle treated caused a significant decrease in GSH activity in the PFC and HPC. However, HES (1, 10 and 50mg/kg) or donepezil administration significantly reversed the deficit in GSH activity caused by scopolamine in the PFC and HPC.

Two-way ANOVA revealed significant effect of treatment on catalase activity in the PFC and hippocampus [F (1,48)=17.56, p= 0.001), the treatments [F(5,48)=5.03, p= 0.0009), and their interaction [F(5,48)=2.08,p=0.08) (Figure 8c). Scopolamine-vehicle treated caused significant reduction in catalase activities in the PFC and HPC. However, HES_(1, 10 and 50mg/kg) or donepezil administration significantly improved catalase activities in the PFC and HPC.

Also, a significant decrease in SOD activities in the PFC and HPC following scopolamine treatment was observed when compared with vehicle treated group. Conversely, HES_ (1, 10 and 50mg/kg) or donepezil administration significantly reversed the decrease in SOD activity in the PFC and HPC caused scopolamine administration. Moreover, two-way ANOVA revealed significant effect of HES_ pretreatment and scopolamine treatment on SOD activity in the PFC and hippocampus [F (1,48)=2.28,p=0.14), the treatments [F(5,48)=5.03, p= 0.0009), and HES × scopolamine interaction [F(5,48)=0.78,p=0.57) (Figure 8d).

Effect of HES on AChE activities in the hippocampus and PFC of mice: In this study, scopolamine administration significantly increased AChE activities in the PFC and HPC when compared with vehicle-treated group. However, HES (1, 10 and 50mg/kg) or donepezil administration significantly reversed the increase in AChE activities in the PFC and HPC caused scopolamine treatment. Moreover, two-way ANOVA revealed significant effect of HES pretreatment and scopolamine treatment on AChE activities in the PFC and HPC [F (1,48)=42.57,p<0.0001), the treatments [F(5,48)=8.04, p<0.0001), and HES × scopolamine interaction [F(5,48)=1.17,p=0.33) (Figure 7).

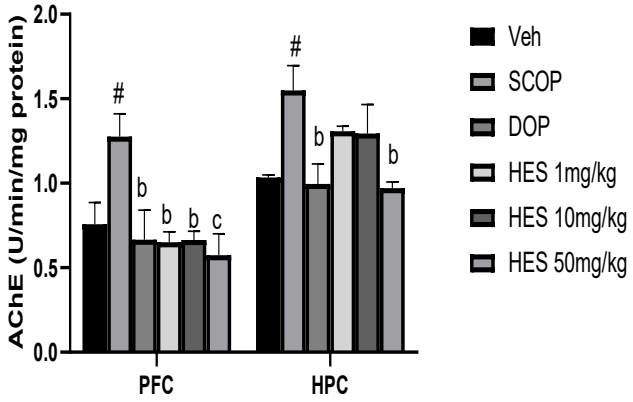

Figure 7:
Effect of hesperidin nanoparticles on scopolamine-induced acetylcholinesterase activities in in the prefrontal cortex (PFC) and hippocampus (HPC). Values are presented as mean±SEM (n=5). $^{\#}p<0.05$ versus vehicle-control treated, $^{b}p<0.01$; $^{c}p<0.001$ versus scopolamine-vehicle treated.

DISCUSSION

Results from the behavioral and biochemical analysis of the mouse hippocampus and prefrontal cortex showed that hesperidin nanoparticles used in this study ameliorated scopolamine-induced memory impairment, cholinergic dysfunction, oxidative stress, and lipid peroxidation in mouse models of AD (Ishola *et al*., 2023; Amoah *et al*., 2023). HES (10, 50mg/kg) increased spontaneous alternation in Y-maze, decreased escape latency time in MWM, and increased preference index in NORT which were altered by scopolamine. In addition, HES ameliorated the scopolamine-induced increase in AChE activities in the hippocampus and prefrontal cortex of mice.

In AD, short-term memory deficit occurs at the early stages and then progresses to loss of long-term memory (Götz *et al*., 2018). Animal models of AD, including Y-maze, Morris water maze, NORT are used to measure spatial memory deficit.

The Y-maze test is used to assess spatial recognition and spontaneous alternation behaviour in rodents. This spontaneous alternation behaviour reflects the rodents' innate tendency to explore novel environments (Kraeuter *et al*., 2019). This test also provides insights into hippocampal integrity, cognitive deficits, and the therapeutic potential of novel cognitive-enhancing agents (Choi *et al*., 2023). In line with previous studies, findings from this study showed that scopolamine (1mg/kg) reduced spontaneous alternation behaviour of mice subjected to Y-maze test which denotes spatial working memory impairment (Choi *et al*., 2023; Ishola *et al*., 2020). However, pretreatment with HES (10 and 50mg/kg) inhibited this effect, evidenced by an increase in percentage alternation, indicative of the cognitive enhancing properties of HES. Also, to rule out the locomotor activity of scopolamine on the mice, the animals were subjected to open field test, and the results showed that scopolamine treatment had no significant effect on the number of line crosses.

To further investigate the possible memory enhancing property of HES, the mice were subjected to NORT. The NORT is more relatable to human memory studies, making it a valuable tool for understanding human cognition as it relies on the rodents' natural predisposition for exploring the unfamiliar (Lueptow, 2017). The time taken to explore the new object serves as an index of recognition memory (Leger *et al*., 2013). Mice treated with scopolamine only, showed decrease in time spent exploring the novel object evidenced by a reduced preference index which was similar to findings from other studies (Wahid *et al*., 2022; Cheon *et al*., 2021). But, pretreatment with HES_ (10 and 50mg/kg) ameliorated this effect, increasing the preference index. Interestingly, the effect of HES_ was similar to that of donepezil (anticholinesterase inhibitor) which served as the standard reference drug, further suggestive of the cognitive-enhancing property of hesperidin nanoparticles.

In addition, the effect of HES_ on scopolamine-induced decline in spatial learning and memory function was evaluated by carrying out Morris water maze test. In MWM, the time spent locating the hidden platform (escape latency) and the time spent exploring the platform area are used to assess spatial learning and memory function (Lee *et al*., 2018). Scopolamine treatment has been reported to cause an increase in escape latency and reduced time spent in the escape platform area, which implies spatial learning and memory impairments (Hindam *et al*., 2020; Ishola *et al*., 2020). Similarly, in this present study, Scopolamine treated group spent more time swimming (increased escape latency) and a reduced time in the escape platform area during the probe test. But, pretreatment with HES ameliorated this effect, suggestive of the potential of HES_ in improving spatial learning and memory in mice.

Furthermore, the effect of hesperidin nanoparticles on scopolamine induced oxidative damage in mice hippocampal and PFC brain region was investigated. Findings from literature have reported that oxidative stress is one of the hallmark of neurodegenerative diseases pathogenesis and it can further exacerbate neurodegenerative disorder. In AD, oxidative stress contributes to AD's progression by fostering amyloid-β (Aβ) accumulation, tau protein hyperphosphorylation, and neuronal damage (Chen and Zhong, 2014). Results from this study showed that GSH, SOD and catalase activities were decreased and the levels of MDA were increased in the hippocampal and PFC region of mice exposed to scopolamine. However, pretreatment with HES reversed these effects leading to an increase in GSH, SOD and catalase levels with a marked reduction in MDA level, indicative of the neuroprotective and antioxidant properties of HES.

We further evaluated the effect of HES_ against scopolamine-induced acetylcholine hydrolysis as increased acetylcholinesterase activity would invariably lead to a cascade of events leading to cholinergic dysfunction (Garabadu *et al*., 2019). Cholinergic system dysfunction, particularly ACh depletion, disrupts cognitive processes (learning and memory) and this has been implicated in AD. Scopolamine is used to induce loss of cholinergic function in the hippocampal and Prefrontal cortex brain region characterized by increased acetylcholinesterase activity (Ishola *et al*., 2023; Ishola *et al*., 2020). By previous findings, scopolamine treatment caused a surge in AChE activities in the himpocampus and PFC of mice. However, HES pretreatment attenuated this effect resulting in the inhibition of ACh hydrolysis in the hippocampus and PFC, which further suggest the neuroprotective effect and memory-enhancing effect of hesperidin nanoparticles.

In conclusion, HES showed promising potential as a memory-enhancing agent against scopolamine-induced learning and memory deficits possibly through the enhancement of antioxidant defense mechanisms and cholinergic signaling.

Acknowledgements

Authors are grateful for the technical assistance from Mr. Chijioke MC of the Department of Pharmacology, Therapeutics and Toxicology, College of Medicine, University of Lagos, Lagos state, Nigeria.

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