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Memory Enhancing Activity of *Spondias mombin (Anarcadiaceae)* and *Pycanthus angolensis (Myristicaceae)* on Scopolamine induced Amnesia in Mice

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: In traditional medical practices, several plants have been used to treat cognitive disorders associated with aging as well as neurodegenerative diseases such as Alzheimer's. *Spondias mombin* and *Pycnanthus angolensis* were found among recipes used ethnomedicine in Nigeria as memory enhancer.
Objective: The present study seeks to evaluate the memory enhancing ability of extracts of *Spondias mombin* and *Pycanthus angolensis* in a mice model with scopolamine induced amnesia.
Materials and Methods: *Spondias mombin* and *Pycanthus angolensis* leaves were extracted with 100% ethyl acetate by maceration. Memory enhancing activities of both extracts were evaluated in scopolamine induced amnesic mice in Morris water maze test at various doses by determining the escape latency. The histopathology of the brain was also carried out to assess any change to the hippocampus that might have effects on memory.
Results: The escape latency time of *Spondias mombin* and *Pycanthus angolensis* treated group decreased through days 1 to 3 when compared with the scopolamine group showing their memory enhancing potential There was no visible lesion on the brain of the mice in all the groups. However, the CAI region of the hippocampus of the brain of mice treated with the ethyl acetate extracts of both plants showed increased cell number and cell density when compared to that of brain cells of scopolamine pretreated groups who received no treatment
Conclusion *Spondias mombin* and *Pycanthus angolensis* may hold some promise in the management of memory related disorders since the ethylacetate extract of both plants showed considerable enhancement of memory in the treated animals.

Keywords: Memory enhancement, *Spondia mombin*, *Pycanthus angolensis*, Amnesia

INTRODUCTION

Normal aging is known to be associated with a slow decline in the function of the brain such as sensory and motor performance (Seidler *et al.,* 2010) The brain cells are slowly lost once people reach their 20's and the number of brain cells lost increases with age. The body also begins to make fewer of chemicals needed by the brain cells to function. This decline results in progressive memory loss, dementia and other cognitive dysfunctions (Panpandreous *et al*., 2011).

Memory impairment and dementia are on the increase due to an increase in ageing population apart from pathological memory loss of neurodegenerative diseases. In both aged humans and rodents, cognitive impairment has been correlated to the accumulation of oxidative damage to lipids, proteins nucleic acids and the vulnerability of some neurotransmitters to oxidative stress (Lau *et al.,* 2005). Brain areas involved in cognitive functions especially the neocortex and hippocampus are usually affected in memory disorders. Memory loss is considered a major problem in many traditional settings including Nigeria. Many people seek memory enhancers either to alleviate sufferings associated with old age or to enhance their performances in examinations. (Elufioye *et al.,* 2013).

An age related reduction in cholinergic function has been thought to be partially responsible for memory disorders (Nebes *et al.,* 2000) and one major marker of cholinergic function is the activity of the enzyme acetylcholinesterase. This is known to be decreased with aging in cerebral areas and synaptic plasma membranes (Perry1980). Acetylcholinesterase activity is also known to be decreased by free radicals and increased oxidative stress (Ranjbar *et al.,* 2002). Thus, a reduction in this enzyme activity correlates to a reduction in cholinergic functions and hence memory performance.

Alzheimer disease is not well defined in many ethnomedical practices including Nigeria. However senile conditions are observed among the aged which could be due to neurodegeneration. Several studies have been done on antioxidant supplements and phytochemical

components that might be useful in preserving brain functions and forestalling age related deficits (Shukitt-Hale, *et al.*, 2005, Joseph, *et al.*, 2009, Krikorian, *et al.*, 2010). Also, botanical have been useful in the improvement of cognitive function by modulating factors such as oxidative stress, inflammation and neurotransmission (Darvesh *et al.*, 2010). Extracts of some medicinal plants have good potential and many of medicinal plants and medicines derived from them have shown memory enhancing properties as a result of their medicinal constituents (Howes and Houghton 2003, Russo and Borrelli 2005, Meena *et al.*, 2012). These plant extracts enhance the memory as well as increase blood circulation in the brain thus improving some aspects of brain function (Youdim and Joseph 2001). Such enhancers involve interactions between slow acting chemical communication in the nervous system (neurohumoral signaling, responses) and cholinergic system (Soreq and Seidman 2001, Sarter *et al.*, 2009)

In this study, we examined the memory enhancing potential of *Spondias mombin* and *Pycanthus angolensis* in scopolamine induced amnesic mice so as to validate their inclusion in remedies traditionally used as memory enhancers.

MATERIALS AND METHODS

Collection of *Spondias mombin* and *Pycanthus angolensis* leaves:

Leaves of *Spondias mombin* with DPHUI number 1426 were collected at Staff School University of Ibadan, Oyo state during the month of August 2014 while the leaves of *Pycanthus angolensis* with DPHUI number 1424 were collected along Sankore Road University of Ibadan Oyo State during the month of September 2014. The two plants were authenticated at the Department of Pharmacognosy Herbarium University of Ibadan.

Preparation of *Spondias mombin* and *Pycanthus angolensis* extract:

Leaves of *Spondias mombin* and *Pycanthus angolensis* were air dried and blended. 500g of the blended leaves of *Spondias mombin* and *Pycanthus angolensis* were weighed separately and macerated for 48 hours with 2.5 L of ethyl acetate. This was then filtered and concentrated using a rotator evaporator. The extracts were transferred into a clean glass container and stored in a cool place till further use.

Behavioral study

Animals:

Albino mice were purchased from Institute of Advance Medical Research and Training (IMRAT).

Administration of doses for the various groups:

The mice were labeled, weighed and divided into 7 groups of 5 animals each. Thereafter, all animals were pretreated with 3mg/kg scopolamine intraperetoneally. Groups 1-3 were administered 0.2 ml equivalent doses of 4mg/kg, 6mg/kg and 8mg/kg of the extract of *Spondias mombin* while groups 4-6 were given same doses of *Pycanthus angolensis* and group 7 was given 0.2 ml of distilled water for 3 consecutive days.

Morris Water Maze Test Procedure:

This is a spatial memory test performed according to the method of Morris (1984) with slight modifications according to Kim *et al.*, 2003 and *Lee et al.*, 2009. The Morris water maze is made of a circular pool (90 cm in diameter and 45 cm in height). The pool was filled with water to a height of 30 cm mixed with 157 mL of evaporated milk. The pool was divided into four equal quadrants and a platform submerged at 1 cm below water level in one of the quadrants such that it was invisible from the surface.

On the first day of the experiment, the animals were trained in swimming for 60 sec in the absence of the platform. For four subsequent days after the initial training, the animals were given two trial sessions each day with the platform in place. The location of each animal from start position to the platform was monitored. During the trials, the escape latencies of each animal were measured with a stop watch and an average calculated for each trial session. Each mouse was allowed a period of 10 sec on the platform after locating it. However, any animal which could not locate the platform after 120 sec was also placed on the platform for 10 sec and then removed from the pool.

The interval between daily trials was 30 min while the point of entry into the pool for the animals and the location of the platform remained unchanged between daily trials. The point of entry and the platform location however was changed on a daily basis. Any changes in the escape latency from day to day represents long term or reference memory while changes from trial 1 to trial 2 on the same day represent short-term or working memory.

Five mice were used per treatment and control group. Amnesia was induces in all animals with 3 mg/kg scopolamine dissolved in water/DMSO and administered intraperitonially. All animals were tested for spatial memory 24 hours after the administration of scopolamine. Treatment with different doses (0.2 mL of 4 mg/kg, 6 mg/kg and 8mg/kg) of extract of *S. mombin* and *P. angolensis* commensed after establishing amnesia in the animals. The control group received 0.2 mL distilled water.

Histopathology

At the end of the experiments, the animals were sacrificed by cervical dislocation, their brain removed and preserved in phosphate formalin buffer for histopathology. Slides of the hippocampus and forebrain were prepared and observed under the light microscope. Photomicrographs were taken and the number of cells in, and area in the CA1 region of the hippocampus was estimated and density of each slide was calculated as follows:

All area values were multiplied by a constant value of 0.088899

Density = Number of cells
Area× 0.088899

RESULTS AND DISCUSSION

The muscarinic cholinergic antagonist scopolamine is capable of inducing transient memory impairment in normal people (Broks *et. al.* 2010). Normal young human subjects treated with scopolamine exhibit memory dysfunctions similar to those observed in patients with dementia and scopolamine induced memory impairment is related to reduce cholinergic transmission (Flood *et. al.* 1998). Thus scopolamine induced amnesia in animal models is a widely accepted method for screening compounds with potential therapeutic value in treatment of dementia.

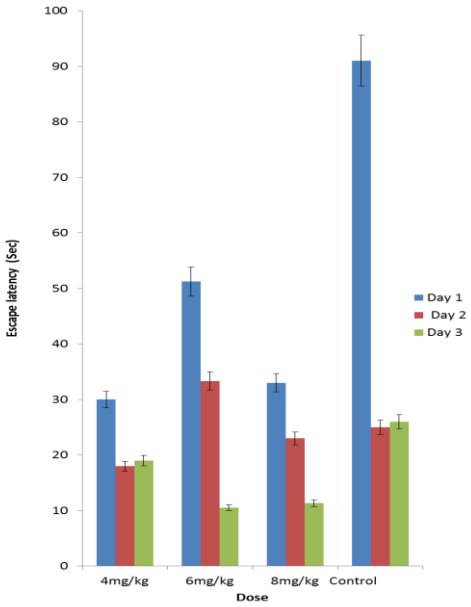


Figure 1: Escape latency time of *Spondias mombin* extract

Morris water maze test was used to evaluate the effects of *Spondias mombin* and *Pycanthus angolensis* on scopolamine induced memory deficit. Morris water maze is a test that evaluates spatial memory and can also be used to assess for working or reference memory (Lee et al., 2009). The animals used for this study quickly learned the location of the platform during the training period as demonstrated by the reduction of swimming time from both first to second trial on day 1 and reaching stable latencies on subsequent days of training. Cognitive flexibility was assessed using the water maze paradigm in which the hidden platform is continually relocated. Escape latency time (the time it takes for the mice to find the platform) was longer for mice induced with scopolamine who received 0.2 mL of distilled water as against the treated group. The scopolamine treated group exhibited a characteristic swimming behavior of circling round the pool of water and this may be attributed to impairment of spatial memory. The time it took the animals to locate the platform was reduced by administering ethyl acetate extracts of both *Spondias mombin* and *Pycanthus angolensis*. The escape latency time of *Spondias mombin* and *Pycanthus angolensis* treated group reduced gradually through days 1 to 3 thus inferring the ability of these plants to enhance memory and cognition due to the activity of their constituents.

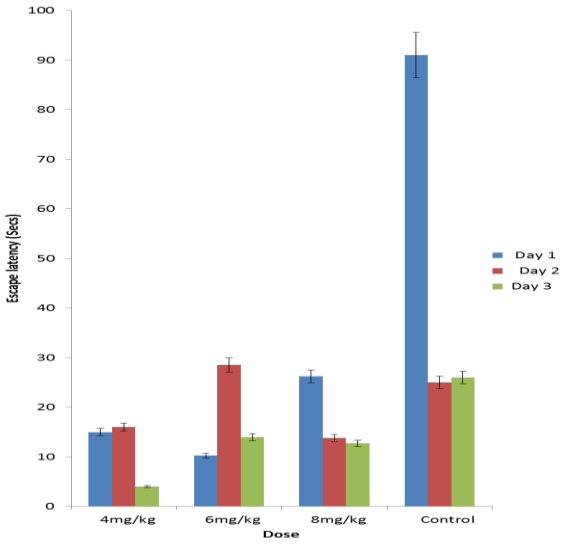


Figure 2: Escape Latency Time of *Pycanthus angolensis* extract

The cognitive enhancing property of the extract was found to be dose- dependent. *Pycanthus angolensis* with escape latency of 17.55 ± 4.3 at 8 mg/kg appears to have better cognitive enhancing ability when compared with *Spondias mombin* with escape latency of 22.43 ± 6.2 at a similar dose.

TABLE 1: Effect of *Spondias mombin* extract on Morris water maze test showing the escape latency time as Mean ± SEM values n= 5

Dose	Escape Latency (sec) (Mean ± SEM)
4mg/kg	22.33±3.844
6mg/kg	31.70±11.79
8mg/kg	22.43±6.270
Scopolamine	47.33±21.85

TABLE 2: Effect of *Pycanthus angolensis* extract in Morris water maze test showing the escape latency time as Mean±SEM values. n= 5

Dose	**Escape Latency (sec) (Mean±SEM)**
4mg/kg	11.66±3.844
6mg/kg	17.58±5.546
8mg/kg	17.55±4.335
Scopolamine	47.33±21.85

TABLE 3: Effect of *Spondias mombin* on place cell density in rats with scopolamine induced amnesia

Dose	Cell	Area	Density
4mg/kg	73	731850.0	0.00112222
6mg/kg	43	454331.2	0.01064
8mg/kg	50	555675.0	0.0010123
-control	35	456875.0	0.00086183

TABLE 4: Effect of *Pycanthus angolensis* on place cell density in rats with scopolamine induced amnesia

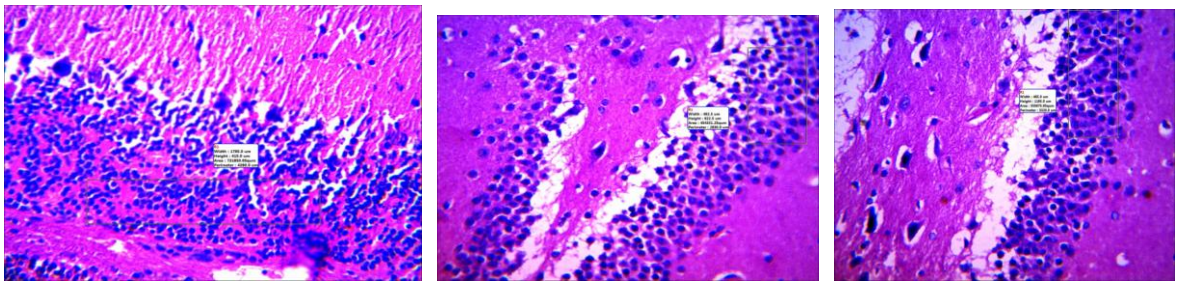
Dose	Cell	Area	Density
4mg/kg	38	610681.2	0.0007003
6mg/kg	58	504075.0	0.0012944
8mg/kg	39	313706.2	0.0013986
-control	35	456875.0	0.00086183

Morris water maze test allows the simultaneous analysis of a distinction between reference and working memory. In this study, both extracts significantly reduced the cognitive deficit in both working and reference memory induced by scopolamine.

Also, acetyl cholinesterase inhibitors are known to counteract scopolamine induced amnesia (Lee *et al.*, 2009). The cholinesterase inhibitory activity of these plants has been previously reported (Elufioye *et al.*, 2009). This may be providing some proof to the cognitive enhancing activity observed in this study.

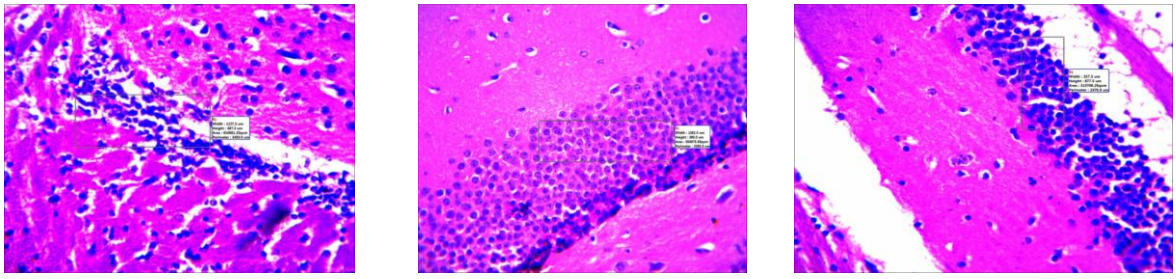
The histopathological assessment of the hippocampus of the mice used for the Morris water maze test was carried out. This revealed that there was a reduction in the density of cells of the brain of the control group that received only distilled water. There was however an increase in the density and number of cells in mice injected with the ethyl acetate extract of *Spondias mombin* and *Pycanthus angolensis* at the different doses which may also account for the cognitive enhancing activity of the plant extracts (Tables 2 and 3).

In conclusion, the Morris water maze test showed that *Spondias mombin* and *Pycanthus angolensis* extracts have potent memory and cognition enhancing. Thus they have promise in the treatment of neurodegenerative illnesses such as Alzheimer's disease and other forms of dementia.



A (4 mg/kg) **B (6 mg/kg)** **C (8 mg/kg)**

Figure 3: Histopathology of the brain of mice injected with *Spondias mombin*



A (4 mg/kg) **B (6 mg/kg)** **C (8 mg/kg)**

Figure 4: Histopathology of the brain of mice injected with *Pycanthus angolensis*

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