Quest Journals
Journal of Research in Pharmaceutical Science
Volume 9 ~ Issue 11 (2023) pp: 24-39
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

Dichloromethane fraction of the methanolic extract of *Pycnantusangolensis* stem bark ameliorates memory impairment in mice.

Chinwuba, Ekwutosi Patricia* Iwalewa, Ezekiel Olugbenga.
**Corresponding Author:*
Address; Department of Pharmacology and Toxicology, College of Pharmacy, Afebabalola University (ABUAD)Ado-Ekiti, NIGERIA.

ABSTRACT
Numerous health benefits have been ascribed to the use of different parts of Pycnanthusangolensis (Welw) Warb.,Myristicaceae,inethnomedicine including its role in cognition enhancement and inflammation. This study was undertaken to investigate the stem bark of the plant for memory enhancing activity in mice.
The plant material was extracted by maceration with 80% methanol.This was subsequently fractionated using N-hexane, Dchloromethane(DCM), and Ethyl acetate.The Dichloromethane (DCM) fraction-the most potent fraction-(25, 50, and 100mg/kg) was evaluated for memory enhancing activity using the Y maze, Morris Water Maze (MWM) and the Novel Object Recognition Test (NORT) , on the scopolamine and lipopolysacharide (LPS)induced amnesia model. The antioxidant markers and acetyl cholinesterase inhibiting effect of DCM were also evaluated.
The results obtained from the study indicate that the DCMsignificantly ($p<0.05$) increased the alternation behaviour of the mice in the Y maze, increased the discrimination index in the NORT and decreased the escape latency in the MWM paradigm. Biochemically, DCM increasedGlutathione (GSH), andsuperoxide dismutase (SOD), but decreasedMalondialdehyde(MDA) and acetylcholinesterase (AChE) activity in the brain. The histopathological results revealed that the DCM protected against the decreased neuronal density induced by scopolamine and LPS.
The study therefore suggests that the DCM fraction may possess significant memory enhancing activity which could be through enhancing cholinergic action, combating inflammatory responses by reducing influence of LPS and increasing anti-oxidant status of the brain.Also DCM protected against the decreased neuronal density induced by scopolamine and LPS.

Key words
Memory, percentage alternation, escape latency, discrimination index.

***R**eceived 28 Oct., 2023; **R**evised 06 Nov., 2023; **A**ccepted 08 Nov., 2023 © The author(s) 2023.*
P**ublished with open access at **www.questjournals.org

I. Introduction

Alzheimer's disease (AD) is a specific neurodisintegrative illness ranking as the highest reason of dementia in the aged. It is a condition that involves disorder in protein conformation, two main neuropathological features typicalizes this disease: amyloid-β peptides and neurofibrillary tangles (NFT). Amyloid-β peptides occur as accumulation of extracellular plaques, NFT consist of intracellular tangles resulting from high-phosphorylation of tau proteins. (Prince et. al., 2011).

Memory loss, cognitive impairment and behavior dysfunction to death are the characteristic symptoms of the disease. People with AD develop physiological malfunctions like bladder control, swallowing and balance. Depression and other psychological symptoms may also be seen.Alzheimer's disease is a chronic, neurodegenerative and fatal disease currently affecting 35 million people in the world. The prevalence of the disease shows an age dependent pattern with 3% of people in the age group 60-69 years, 5% in that of 70-79 and 30-50% in the age group 80-89 years being affected. [1].

Suggested causes of AD include increased levels of aggregated proteins (amyloid plaques and neurofibrillary tangles), mitochondria dysfunction, decreased production of neurotransmitters (acetylcholine (ACh), norepinephrine (NE), and serotonin) [2, 5] inflammation and oxidative imbalance [1].

In AD a substantial number of postsynaptic cholinergic receptors are left undamaged even with the loss of cholinergic neurons [3]. Cognitive decline is related to degeneration of cholinergic neurons which results in cholinergic neurotransmission deficits consequently research on cognitive improvement have focused on elevating cholinergic activity in the brain (4, 5).Cholinesterase inhibitors, have been employed in an effort to enhance cholinergic activity in the treatment of AD (4, 6). Several compounds that affect behavior (antidepressants, anxiolytics and antipsychotics) are also involved in the management of the disorder.

Currently five medications (Donepezil (Aricept^{R}), Galantamine (Razadyne^{R}), Memantine (Namenda^{R}), Rivastigmine (Exelon^{R}) and Tacrine) are approved for the management of AD. Notwithstanding, they do not effect cure but only slows the disease progression or produce a short term improvement in cognitive functions [1].Though, AD is currently managed with orthodox drugs,ethnomedicinal plants have not been extensively evaluated for natural product. Plants have a reservoir of potential compounds for managing many diseasesincluding those involving cognitive abilities like AD. Resort to plants for drugs is not misplaced, hence the study of *Pycnanthusangolensis* for the evaluation of its effect on neurodegenerative diseases.

Pycnanthusangolensis (Welw) Warb, Myristicaceae, belongs to the order Magnonliflorae and is popularly known as 'African nutmeg' or 'false nutmeg'. The plant is widely used in ethnomedicineas a memory enhancing agent,antimicrobial, analgesic, anthelmintic, antidote for poisoning, antihaemorrhagic, anti-inflammatory and ashyoglycaemic agent [7, 8]. It has been identified as having the following major chemical constituents; Fatty acids, Steroids, Cerobrosides (Pycnangloside), Allantoin, Lignans, Plastiquinones and Ubiquinones, Glyceryl-1,3-ditetradecanoate, Terpenes and Sesquiterepenes[9],and is now being evaluated for its effects on amnesia.

II. Materials And Method

Collection

Fresh stem bark of *Pycnanthusangolensis*was collectedfrom Opa villagein Ile-Ife.A specimen was identified by Mr. Ibhanesebhor Gabriel and was deposited at the IFE Herbarium, Department of Botany, ObafemiAwolowo University, Ile-Ife, with the voucher number; IFE 17635.

Extraction

After collection, the stem bark was further cut into smaller pieces and air driedat room temperature for three weeks. The air dried material (2.3 kg) was grinded into powder using amilling machine, extracted thrice by maceration with 80%methanol at roomtemperature for 72 h. The combinedmethanolic extract was filteredwith double-layered muslin cloth and concentrated on a water bathat 40◦C to yield a blackish-brown solid (64.4 g).

Acute toxicity

The method of acute toxicity test described by Jaijoy*et al*.[10] was employed in the acute toxicity study of *P. angolensis.* The procedure involved the administration of a single oral dose of 5000 mg/kg to seven male mice which had fasted for 12h. The behavioral parameters monitored, over a period of seven days include; convulsion, hyperactivity, sedation, grooming, increased or decreased respiration.

Partitioning of the crude extract into fractions

The separation funnel was set up by placing it in a ring clamp attached to a ring stand. Since funnels are easy to break, it was cushioned in the metal clamp using pieces of slit rubber. 80% methanol was added to the dried extract to obtain a suspension, this was slowly pour into the separating funnel with its stopcock closed. N-hexane (250 ml) was added to the separating funnel following which the funnel was hand shaken gently several times. The top stopper was removed, the solvents were then allowed to settle until two immiscible phases were evident. The aqueous phase (bottom layer) and the hexane phase (top layer) were recovered in separate Erlenmeyer flasks. This was done by slowly opening the stopcock and closing it just before the curved meniscus between the two liquids reached the stopcock. The aqueous phase was placed back inside the separating funnel, and the extraction was repeated twice using fresh hexane. The above procedure using n-hexane was repeated with dichloromethane and ethyl acetate.

Some sodium anhydrous sulfate was sprinkled into the recovered n-hexane, dichloromethane and the ethyl acetate phases while gently manually rotating the Erlenmeyer flasks this was continued until the liquids were completely clear. Each organic phase was then filtered into a round-bottom flask using a glass filter funnel overlaid with filter paper. The insoluble material (sodium sulfate) remaining on the filter paper was discarded. The filtered extracts were clear solutions; which were concentrated to dryness under reduced pressure at ≤40 °C using a rotary evaporator.

Preparation of the extractfor administration
A suspension of the extract of *P. angolensis* was prepared fresh on each day of the experiment, using 5% Tween 80 and distilled water as vehicle. Dilutions of the standard drugs were made with distilled water.

Chemicals
Scopolamine (Embassy Nigeria Ltd), Donepezil, Tween 80

Animals
Male and female mice weighing 20-25 g were selected for the study. They were randomly divided into groups of six animals and were housed in clean cages laden with fresh wood shavings. They were allowed to acclimatize for a period of one week before the commencement of the experiments. They were fed with growers mash (manufactured by UAC Foods Nigeria Plc.) and water was provided *ad libitum*. This research work was carried out in compliance with the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC) regulations for animal use with ethical number: UI- ACUREC/16/0041

Behavioural studies
(1) Y-maze Test
Sixgroups of six mice each were used for this experiment. Group 1-3 received 25, 50 and100mg/kg of the DCM fraction per oral. The fourthgroup wasalso administered orally with 5% Tween 80 in normal saline which served as the negative control. Group (5) received Donepezil (1 mg/kg)ip, while group six received scopolamine.Thirty (30) minutes later, scopolamine(scop) (3 mg/kg) ip was administered to each mouse. The treatment was done acutely and daily for seven days.One hour after the administration of the extract, the vehicle or the standard, each mouse was placed at the center of the Y- maze whose arms were labelled A, B and C and was allowed to roam the different arms of the maze for six minutes, records of the arms visited and the sequence of the arms the animal entered were manually recorded. An arm entry is defined as the entry of the body of the mousecompletely into an arm compartment except for its tail whilean alternation consists of entering the three different arms consecutively.

(2) Morris Water Maze test
The Morris water maze (MWM) procedure was carried out according to the method described by Logue *et al.*[11].In this experiment, six groups of six mice each were used. Group 1-3 received 25, 50 and100mg/kg of the DCM fraction. The fourth group was administered 5% Tween 80 in normal saline which served as the control, all test compounds and vehicle were given orally. Group (5) received Donepezil (1 mg/kg)ip, while group six received scopolamine.Thirty (30) minutes later, scopolamine (3 mg/kg) ip was administered to each mouse. The treatment was done acutely and daily for seven days. After 30 minutes of the administration of Scopolamine each mouse was gently placed in the water with the tail-end lower, so the head does not dip under water (dropping them in head-first is stressful) and in such a way thatit faces the pool side (to minimize bias). The time taken for the mouse to reach the platform and climb it (escape response) was measured.

(3) The Novel object recognition test. (NORT)
The effect of *P. angolensis* was further evaluated using the NORT[12].In which the open field chamber (60cm x 50cm x 40 cm) was used. Also identically sized (4.5cm diameter and 11.5cm height) cylindrical bottles labeled A, B and C were used as the discriminated objects. Objects A and B were white, while object C had a multi-colour pattern. The test consist of two phases: the trial and the test phases with six groups of six mice each used for each experiment. Group 1-3 received 25, 50 and100 mg/kg of the DCM fraction. The fourth group was administered 5% Tween 80 in normal saline which served as the control, all test compounds and vehicle were given orally. Group (5) received Donepezil (1 mg/kg, i.p), while group six received scopolamine.Thirty (30) minutes thereafter, scopolamine (3 mg/kg, i.p) was administered. The treatment was done acutely and daily for seven days. Each mouse was first placed in the open field chamber for five minutes in order to acclimatize to the experiment set up.The trial phase commenced with the identical objects (A and B) placed on opposite sides(at a distance of 8 cm from the walls and 34 cm from each other). To start the trial phase each mouse was then individually placed in the open field chamber in the middle of the two objects for five minutes andthereafter, returned to their home cages for an interval of 30 mins. In the test phase object B was replaced with object C, (which was novel to the mice and different from objects A and B) and mice were left to explore objects A and C for five minutes, the result was recorded.

Preparation for histological and biochemical assays

After the last treatment the animals were sacrificed by cervical dislocation and the brains were immediately removed and kept in a cooler with ice blocks for 30mins. Thereafter whole brains were weighed and divided into two portions. One portion was place in formalin and used for histo-morphology while the otherwas homogenized with 10% phosphate buffer (0.1M, pH 7.4), cold centrifuged and the supernatant was separated and divided into portions for the different biochemical assays as follows:

The biochemical assays

1. Determination of acetylcholineterase (AChE) activity in mice brain

Acetylcholnesterase activity, which is a marker for cholinergic neurotransmission was assayed in the mouse using the method of Ellman*et al.*[13]. Briefly, 0.1ml of acetylthiocholineidodide solution was added to a reaction mixture containing 2.6ml of 1M phosphate buffer at a pH 7.4, 0.1ml of 5,5'-dithio-bis(2-nitrobenzoic acid) (DNTB) and 0.4ml of the supernatant. The absorbance was read in a spectrophotometer atthe wavelength of 412 nm, at two min intervals for ten minutes. The change in colour produced when thiocholine reacts with DNTB was used as a measure of the rate of acetylcholinesterse activity. The change in absorbance per minute was determined and the rate of acetylcholinesterse activity was calculated and expressed as µmoles/min/g tissue.

2. Determination of reduced glutathione (GSH) concentration

The concentration of reduced glutathione (GSH) was determined using the method of Moron *et al.* [14]Briefly, 0.4ml of brain supernatant of individual mouse in the respective treatment groups was each mixed with 20% trichloroacetic acid (TCA (0.4ml) the mixture was cold centrifuged at 10,000 rpm at 4^{oC} for 20 min. 2ml of 0.6mM DNTB reagent was added to resulting the supernatant (0.25ml) and phosphate buffer (0.2M, pH 8.0) was used to make up the final volume to 3ml. The absorbance was, read at 412nm against a blank in the spectrophotometer. Subsequently the concentrations of reduced GSH in the brain tissues were calculated and expressed as micromoles pergram tissue (µmoles/g tissue).

3. Determination of lipid peroxidation

The assay of the MDA level was done according to the method of Adam-Viziand Seregi[15].A mixture of 0.4ml of the sample and 1.6ml of Tris –kcl buffer to which 0.5ml of 30% TCA was added was incubated in a water bath at 80ºC for 45 mins. Following which it was cooled in ice and centrifuged at 3000rpm for 15 minutes. The absorbance of the clear supernatantwas measured against a blank at 532nm in spectrophotometer. The MDA was calculated using a molar extinction coefficient of $1.56x10^5$ $M^{-1}cm^{-1}$ and values were expressed as µmoles of MDA per gram tissue.

4. Determination of superoxide dismutase (SOD) activity

The assay of the level of SOD activity in mice brain was done according to the method of Misra and Fridovich[16].A1 in 10 dilution of the sample was made with distilled water, 0.2ml of the diluted sample was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) and allowed to equilibrate in the spectrophotometer.This was followed by the addition of 0.3ml freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml substrate (adrenaline) and 0.2ml of water. The absorbance was read at 480nm every 30 seconds for 150 seconds.

Statistical analysis

The data were analyzed using the GraphPad® prism 4.0 statistical software.The test doses were compared with control by one way analysis of variance (ANOVA) followed by post hoc analysis using StudentNewman-Kuels multiple comparison tests. Test doses were also compared with the standard drug. All results were expressedas mean±standard error of mean (SEM). P values less than 0.05 were taken as significant (i.e. $p< 0.05$). Appropriate tables and figures were used to display the data.

III. RESULTS

Acute toxicity

Following a single dose administration of 5000mg/kg of the extract of *Pycnanthusangolensis*nomortality or visible signs of toxicity or abnormal behavior were observedat the end of 24hours and through seven days, indicating that the extract might have a reasonably low toxicity profile, this is corroborated by the works of Gregory *et al.*[17].

Effect of dichloromethane (DCM)fraction on memory performance

(a) Effect of the DCM fraction of *P. angolensis* on spatial working memory

The effect of the DCM fraction of *P. angolensis* on spatial working memory as measured by percentage alternation and escape latency using the y maze and Morris water maze are shown in figures 1 and 2. Figure 1 shows that the DCM fraction($F_{(5, 30)} = 5.56$; $p<0.05$) at all the dose levels used, 25, 50 and 100mg/kg produced a significant increase in the percentage alternation when compared with the scopolamine group. The positive

control, Donepezil (DZP) produced a significant increase in percentage alternation when compared with both the scopolamine and the vehicle group. A similar pattern was repeated in the Morris water maze result (figure 2) with all doses producing a significant decrease in escape latency when compared with the scopolamine group. The DPZ group had a significant difference with the scopolamine group.

(b) Effect of the dichloromethane (DCM) fraction of*P. angolensis* on recognition memory

The result for the effect of the DCM fraction on recognition memory is as shown in figure 3.It shows that when compared with the scopolamine group the 25mg/kg and the vehicle group produced a significant increase in the discrimination index.

Effect of the dichloromethane (DCM) fraction of *P. angolensis* given for seven days on scopolamine induced amnesia

The result for the effect of the DCM fraction given for seven days on scopolamine induced amnesia is as shown in figure 4 and 5, all the doses of the DCM used (25, 50 and 100mg/kg) significantly ($F_{(5, 30)} = 5.86$; $p<0.05$) increase the level of alternation in mice thereby countering the amnesia induced by scopolamine.While in the MWM test figure 5, the 25 and 50mg/kg doses reversed the amnesia induced by scopolamine by significantly reducing the escape latency. DPZ group produced a significant decrease in escape latency

Effect of the dichloromethane (DCM) fraction of *P. angolensis* given for seven days on lipopolysacharide (LPS) induced amnesia

The results of the effect of the DCM fraction given for seven days on LPS induced amnesia in mice are as shown in figures 6 and 7. The three dose of the DCM fraction used (25, 50 and 100mg/kg) effectively reversed the amnesia induced by LPS by significantly($F_{(5, 30)} = 18.46$; $p<0.05$) increasing the percentage alternation. In the MWMfigure 7, the three dose of the DCM fraction used (25, 50 and 100mg/kg) effectively reversed the amnesia induced by LPS by significantly decreasing the escape latency.

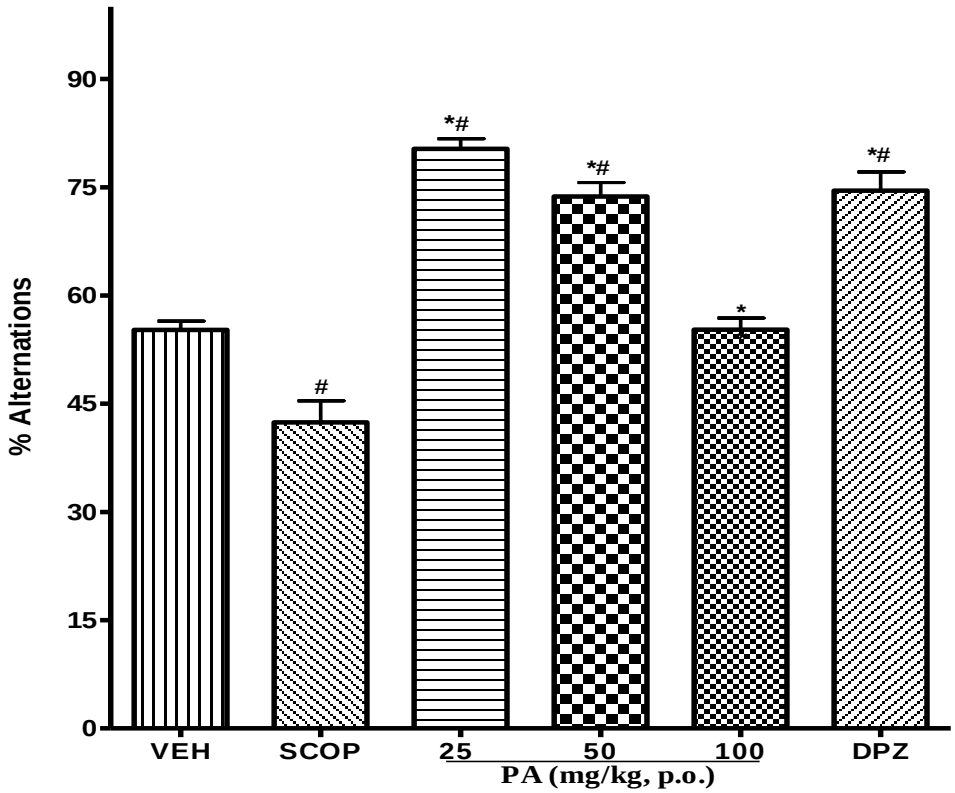

Figure 1: Effect of the DCM fraction ofPAon memory performance in the y-maze paradigm.
Each bar represents the Mean ±S.E.M for 6 animals per group**.** These values are significant at$^{*}p < 0.05$ when compared with scopolamine (SCOP) group (ANOVA followed by Newman Keul post hoc test). $^{\#}p < 0.05$ when compared with control group (VEH) (ANOVA followed by Newman Keul post hoc test). And the treated 25, 50 and 100 mg/kg of the DCM fraction of PA, Donepezil (DPZ) 1mg/kg- a cholinesterase inhibitor.

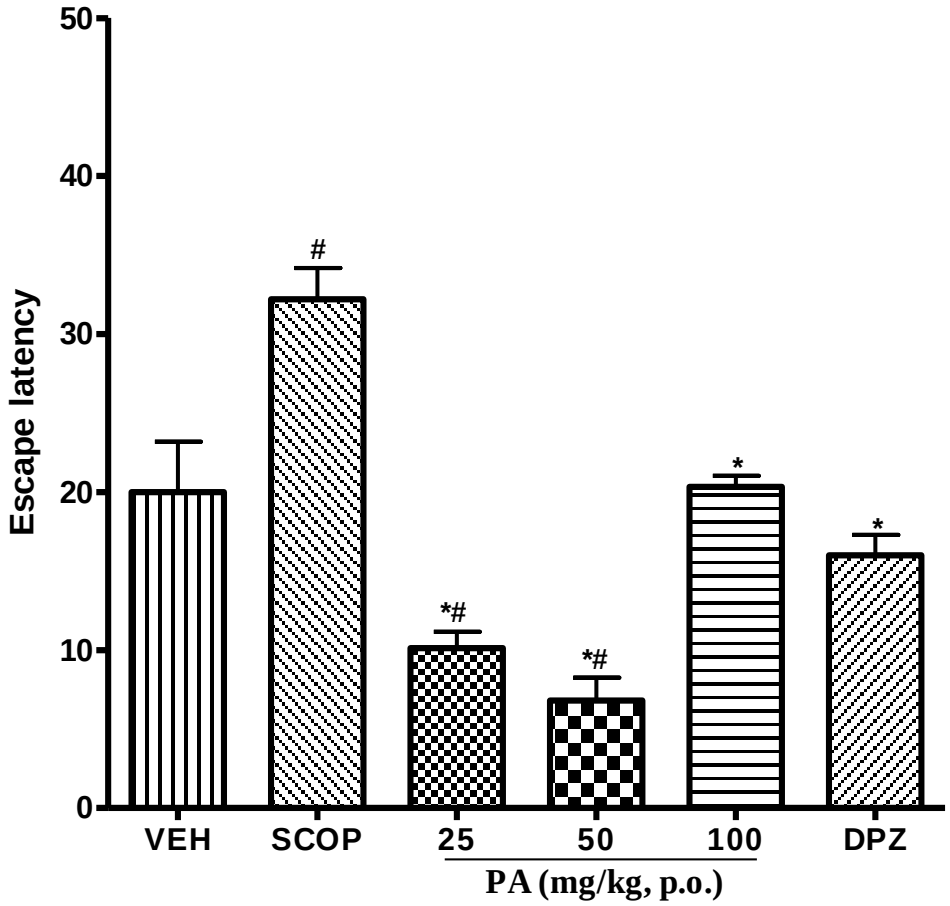

Figure 2: Effect of the DCM fraction of PA on memory performance in the Morris Water Maze paradigm
Each bar represents the Mean ±S.E.M for 6 animals per group**.** These values are significant at $^{*}p < 0.05$ when compared with scopolamine (SCOP) group (ANOVA followed by Newman Keul post hoc test). $^{\#}p < 0.05$ when compared with control group (VEH) (ANOVA followed by Newman Keul post hoc test). And the treated 25, 50 and 100 mg/kg of the DCM fraction of PA, Donepezil (DPZ) 1mg/kg- a cholinesterase inhibitor.

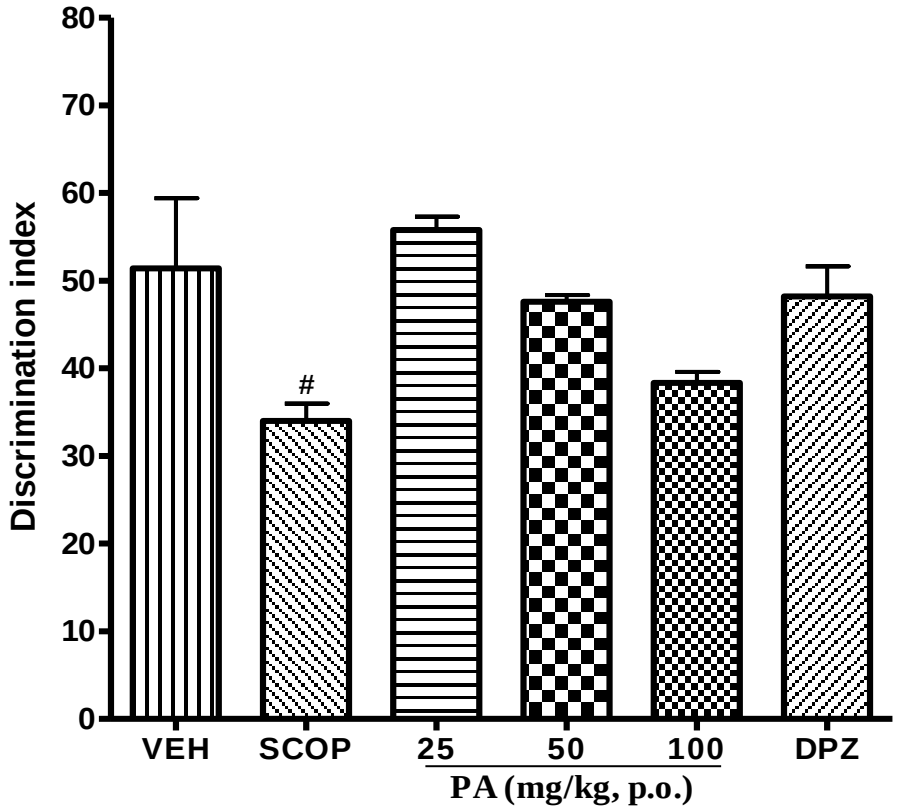

Figure 3: Effect of the DCM fraction of PA on memory performance in the novel object recognition test paradigm

Each bar represents the Mean ±S.E.M for 6 animals per group**.** These values are significant at$^{*}p < 0.05$ when compared with scopolamine (SCOP) group (ANOVA followed by Newman Keul post hoc test). $^{\#}p < 0.05$ when compared with control group (VEH) (ANOVA followed by Newman Keul post hoc test). And the treated 25, 50 and 100 mg/kg of the DCM fraction of PA, Donepezil (DPZ) 1mg/kg- a cholinesterase inhibitor.

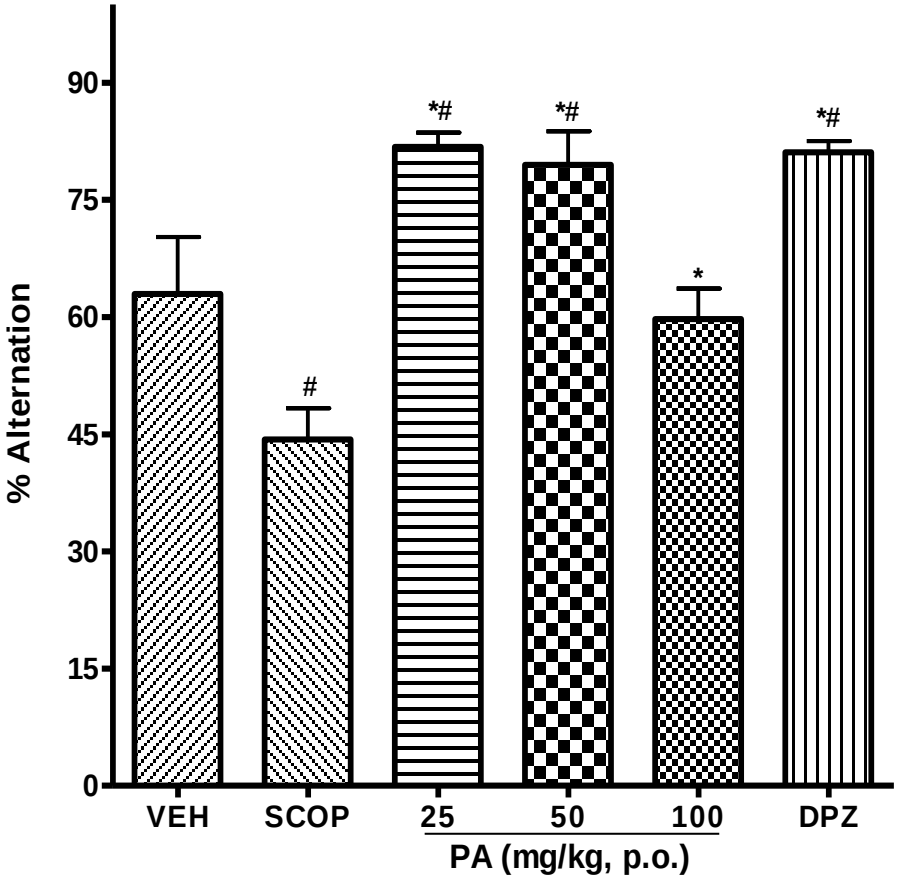

Figure 4: Effect of the DCM fraction of PA given for seven days on scopolamine induced amnesiain the Y-maze paradigm

Each bar represents the Mean ±S.E.M for 6 animals per group**.** These values are significant at $^{*}p < 0.05$ when compared with scopolamine (SCOP) group (ANOVA followed by Newman Keul post hoc test). $^{\#}p < 0.05$ when compared with control group (VEH) (ANOVA followed by Newman Keul post hoc test). And the treated 25, 50 and 100 mg/kg of the DCM fraction of PA, Donepezil (DPZ) 1mg/kg- a cholinesterase inhibitor.

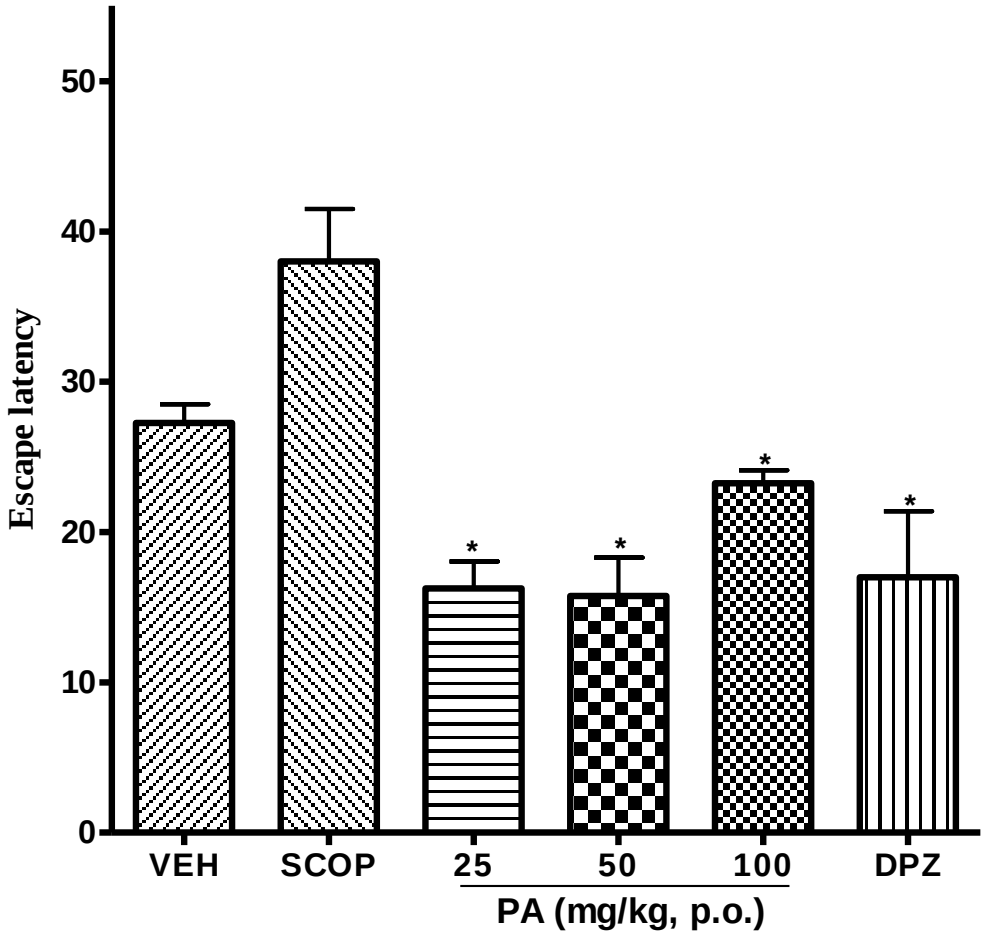

Figure 5: Effect of the DCM fraction of PA given for seven days on scopolamine induced amnesiain the Morris water maze paradigm

Each bar represents the Mean ±S.E.M for 6 animals per group**.** These values are significant at $^{*}p < 0.05$ when compared with scopolamine (SCOP) group (ANOVA followed by Newman Keul post hoc test). $^{\#}p < 0.05$ when compared with control group (VEH) (ANOVA followed by Newman Keul post hoc test). And the treated 25, 50 and 100 mg/kg of the DCM fraction of PA, Donepezil (DPZ) 1mg/kg- a cholinesterase inhibitor.

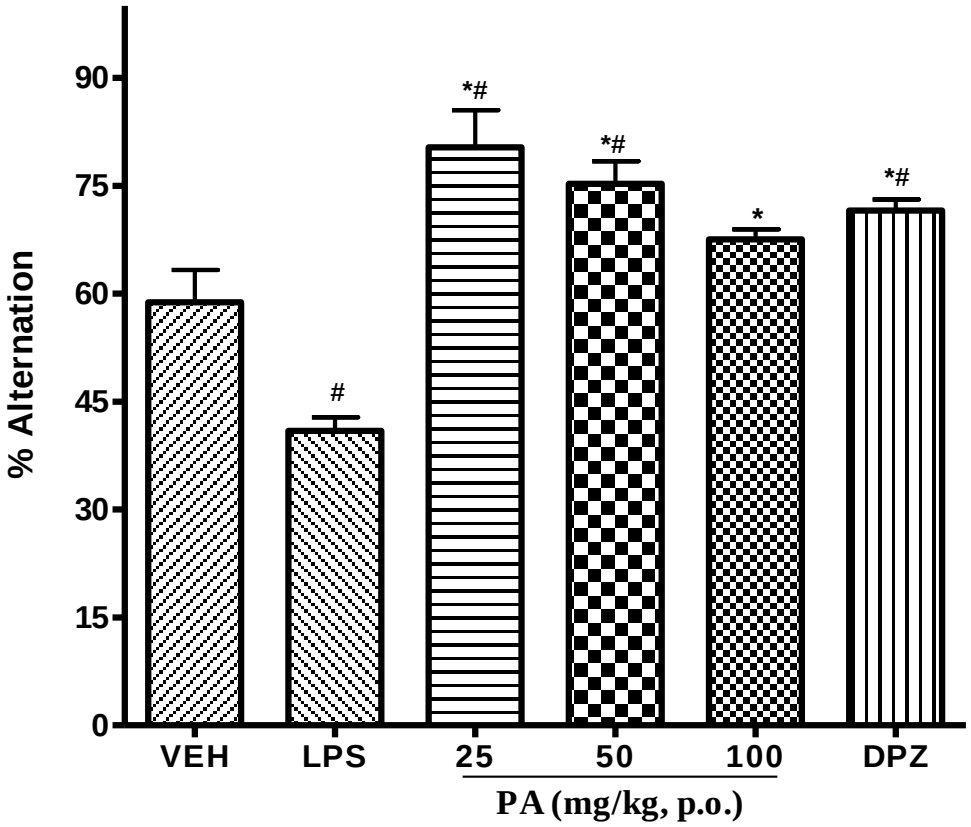

Figure 6: Effect of the DCM fraction of PA given for seven days on LPS induced amnesiain the Y- maze paradigm

Each bar represents the Mean ±S.E.M for 6 animals per group**.** These values are significant at$^{*}p < 0.05$ when compared with scopolamine (SCOP) group (ANOVA followed by Newman Keul post hoc test). $^{\#}p < 0.05$ when compared with control group (VEH) (ANOVA followed by Newman Keul post hoc test). And the treated 25, 50 and 100 mg/kg of the DCM fraction of PA, Donepezil (DPZ) 1mg/kg- a cholinesterase inhibitor.

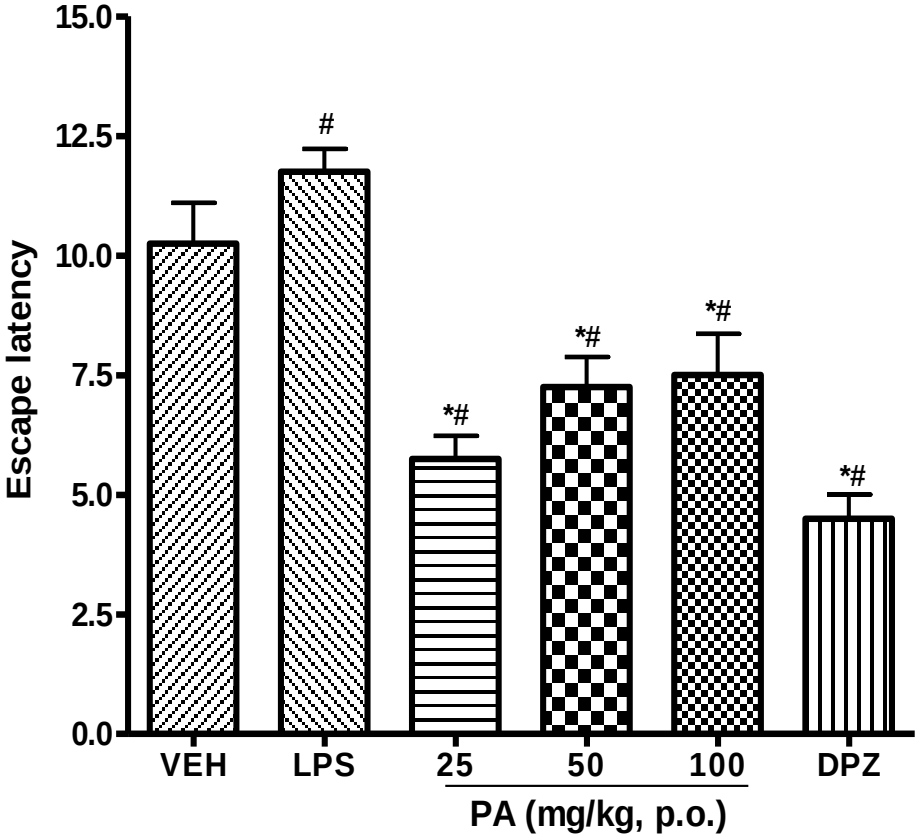

Figure 7: Effect of the DCM fraction of PA given for seven days on LPS induced amnesiain the Morris water maze paradigm

Each bar represents the Mean ±S.E.M for 6 animals per group**.** These values are significant at$^{*}p < 0.05$ when compared with scopolamine (SCOP) group (ANOVA followed by Newman Keul post hoc test). $^{\#}p < 0.05$ when compared with control group (VEH) (ANOVA followed by Newman Keul post hoc test). And the treated 25, 50 and 100 mg/kg of the DCM fraction of PA, Donepezil (DPZ) 1mg/kg- a cholinesterase inhibitor.

Biochemical Assays

Scopolamine Model

(a) Effect of DCM fraction of *P. angolensis* on acetylcholinesterase activity (AChE) in mice brains

The effect of DCM fraction of *P. angolensis* administered daily for seven days in scopolamine treated mice is shown in table 1, whereas scopolamine (3 mg/kg, i.p.) significantly ($p<0.05$) increased the activity of the enzyme when compared to the vehicle, the DCM fraction of *P. angolensis* (25-100mg/kg p.o.) significantly ($p<0.05$) suppressed AChE activity caused by scopolamine. DPZ a standard anti-amnesic drug, also demonstrated a significant inhibition of AChE activity in mice brains (Table 1)

(b) Effect of the DCM fraction of *P. angolensis* on malondialdehyde (MDA) content in mice brains

Scopolamine (3mg/kg i.p) significantly ($p = 0.05$) increased the concentration of MDA in the brain of mice in comparison with the vehicle (table 1). The increased concentration of MDA induced by scopolamine was significantly suppressed by the DCM fraction of *P. angolensis* (25-100mg/kg o.p.) in mice brains. Also DPZ (1mg/kg i.p) the positive control significantly decreased the concentration of MDA in mice brain.

(c) Effect of the DCM fraction of *P. angolensis* on reduced glutathione concentration in mice brains.

Table 1 shows the effect of the DCM fraction of *P. angolensis* on GSH concentration in the brains of mice treated with scopolamine. Scopolamine (3mg/kg) significantly ($p= 0.05$) decreased the concentration of GSH in mice brains. This decrease was however significantly ($p=0.05$) attenuated by the DCM fraction of *P. angolensis*. Similar effect was also observed in the group treated with DPZ.

Table 1: The effect of the DCM fraction of *P. angolensis* on the concentration of the biochemical markers; AChE MDA, and GSH in the brains of mice treated with scopolamine.

Treatment	AChE activity (mmol/min/g tissue)	MDA level (nmol/g tissue)	GSH conc. (µmol/g tissue)
Control	0.495 ± 0.048	0.101 ± 0.003	7.450 ± 0.574
SCOP	0.718 ± 0.068$^{\#}$	0.311 ± 0.009$^{\#}$	2.931 ± 0.560$^{\#}$
25mg/kg	0.167 ± 0.032*	0.130 ± 0.006*	9.606 ± 0.312*
50mg/kg	0.205 ± 0.054*	0.152 ± 0.004*	7.427 ± 0.860*
100mg/kg	0.247 ± 0.047*	0.243 ± 0.24$^{*\#}$	6.716 ± 0.369*
DPZ	0.263 ± 0.009*	0.102 ± 0.004*	7.725 ± 0.504*

Values represent the Mean ±S.E.M for 6 animals per group**.** These values are significant at;$^{*}p < 0.05$ when compared with scopolamine (SCOP) group (ANOVA followed by Newman Keul post hoc test), $^{\#}p < 0.05$ when compared with control group (VEH) (ANOVA followed by Newman Keul post hoc test). And the treated 25, 50 and 100 mg/kg of the DCM fraction of PA, Donepezil (DPZ) 1mg/kg- a cholinesterase inhibitor.

Lipopolysacharide Model

(a) Effect of DCM fraction of *P. angolensis* on acetylcholinesterase activity (AChE) in mice brain

The effect of DCM fraction of *P. angolensis* administered daily for seven days in LPS treated mice is shown in table 2. Whereas LPS (3 mg/kg, i.p.) significantly ($p<0.05$) increased the activity of the enzyme when compared to the vehicle, the DCM fraction of *P. angolensis* (25-100mg/kg p.o.) significantly ($p<0.05$) suppressed the increase in AChE activity caused by LPS (Table 2). DPZ a standard anti-amnesic drug also demonstrated a significant inhibition of AChE activity in mice brains.

(b) Effect of the DCM fraction of *P. angolensis* on malondialdehyde (MDA) content in mice brains

LPS (3mg/kg i.p) significantly ($p = 0.05$) increased the concentration of MDA in the brain of mice in comparison with the vehicle (table 2). The increased concentration of MDA induced by LPS was significantly suppressed by the DCM fraction of P. angolensis (25-100mg/kg o.p.) in mice brains. Also DPZ (1mg/kg i.p) the positive control significantly decreased the concentration of MDA in mice brain.

(c) Effect of the DCM fraction of *P. angolensis* on reduced glutathione concentration in mice brains.

Table 2 shows the effect of the DCM fraction of *P. angolensis* on GSH concentration in the brains of mice treated with LPS. LPS (3 mg/kg) significantly ($p= 0.05$) decreased the concentration of GSH in mice brains. This decrease was however, significantly ($p=0.05$) attenuated by the DCM fraction of *P. angolensis* (25-100mg/kg). Similar effect was also observed in the group treated with DPZ.

(d) Effect of the DCM fraction of *P. angolensis* on superoxide dismutase (SOD)

The effects of the DCM fraction of *P. angolensis* on SOD activity in the brain of mice treated with LPS are as shown in Table2. LPS significantly decreased the activity of SOD in mice brains. Whereas the DCM fraction of *P. angolensis* (25-100mg/kg p.o.) significantly ($p=0.05$) increased the Sod activity in mice brain. DPZ demonstrated similar effect.

Table 2: The effect of the DCM fraction of *P. angolensis* on the concentration of the biochemical markers; AChE, MDA, and GSH in the brains of mice treated with LPS.

Treatment	AChE activity (mmol/min/gtissue)	MDA level (nmol/g tissue)	GSH conc. (µmol/g tissue)	SOD activity (units/mg protein)
Control	0.495 ± 0.048	0.101 ± 0.003	7.519 ± 0.627	22.26 ± 2.904
LPS	0.737 ± 0.041$^{\#}$	0.261 ± 0.002$^{\#}$	2.060 ± 0.627$^{\#}$	8.11 ± 1.149$^{\#}$
25mg/kg	0.204 ± 0.021 *	0.143 ± 0.010*	8.000 ± 0.290*	41.81 ± 1.663*
50mg/kg	0.187 ± 0.017 *	0.192 ± 0.001*	7.358 ± 0.307*	35.59±3.715*
100mg/kg	0.237 ± 0.026*	0.241 ± 0.024*	6.808 ± 0.215*	26.73± 6.012*
DPZ	0.194 ± 0.013*	0.192 ± 0.013*	8.229 ± 0.691*	22.99 ± 1.246*

Values represent the Mean ±S.E.M for 6 animals per group**.** These values are significant at;$^{*}p < 0.05$ when compared with scopolamine (SCOP) group (ANOVA followed by Newman Keul post hoc test), $^{\#}p < 0.05$ when compared with control group (VEH) (ANOVA followed by Newman Keul post hoc test). And the treated 25, 50 and 100 mg/kg of the DCM fraction of PA, Donepezil (DPZ) 1mg/kg- a cholinesterase inhibitor.

Effect of the DCM fraction of*P. angolensis* on hippocampal neuronal cell density in Scopolamine treated and lipopolysacharide (LPS) treated mice.

The result of the effect of the DCM fraction of *P. angolensis*on hippocampal neuronal cell density in scopolamine treated mice is as shown in figure 8 while figure 9 shows that of LPS. In both cases the DCM fraction countered the amnesic activities of scopolamine and LPS

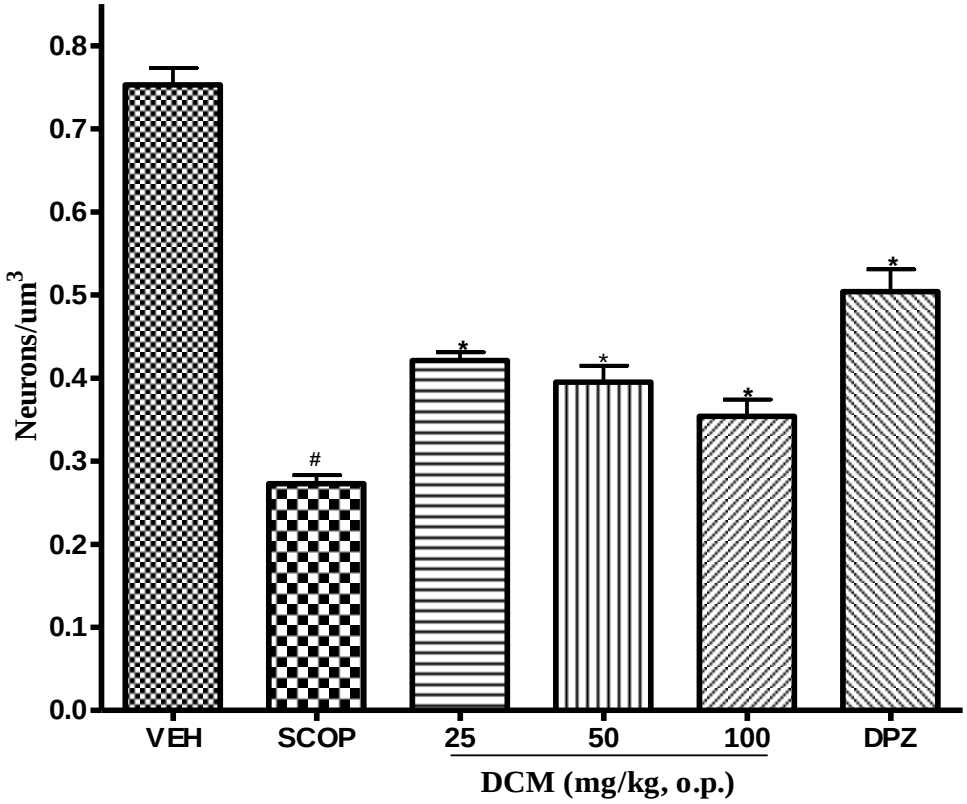

Figure 8: Effect of the DCM fraction ofPA on hippocampal neuronal cell density in scopolamine treated mice. Each bar represents the Mean ±S.E.M for 6 animals per group. These values are significant at$^{*}p < 0.05$ when compared with scopolamine (SCOP) group (ANOVA followed by Newman Keul post hoc test). $^{\#}p < 0.05$ when compared with control group (VEH) (ANOVA followed by Newman Keul post hoc test). And the treated 25, 50 and 100 mg/kg of the DCM fraction of PA, Donepezil (DPZ) 1mg/kg- a cholinesterase inhibitor.

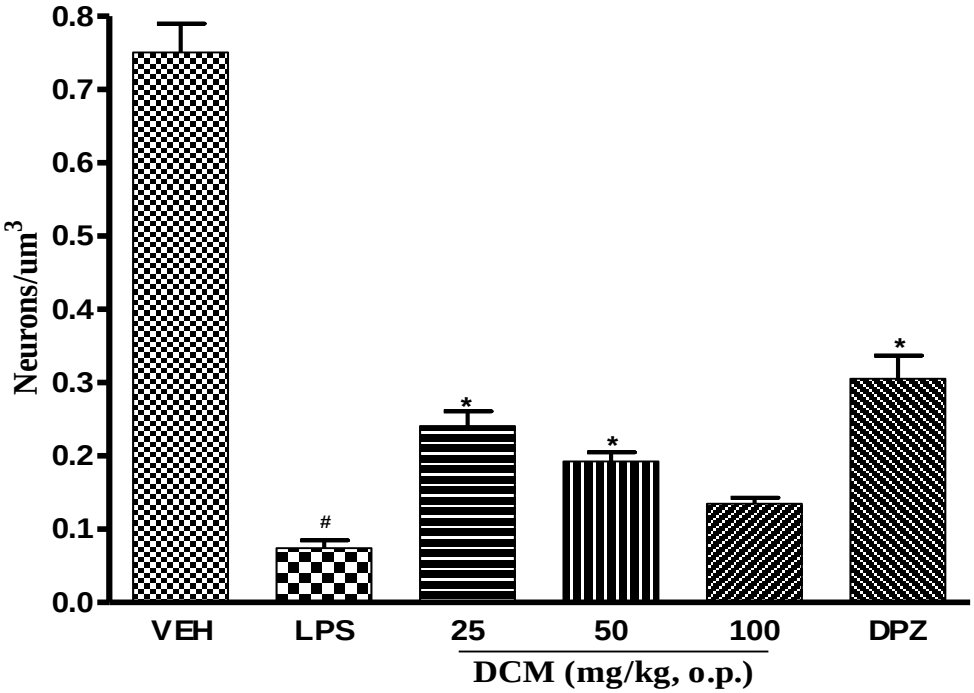

Figure 9: Effect of the DCM fraction ofPA on hippocampal neuronal cell density in LPS treated mice.
Each bar represents the Mean ±S.E.M for 6 animals per group. These values are significant at $^{*}p < 0.05$ when compared with scopolamine (SCOP) group (ANOVA followed by Newman Keul post hoc test). $^{\#}p < 0.05$ when compared with control group (VEH) (ANOVA followed by Newman Keul post hoc test). And the treated 25, 50 and 100 mg/kg of the DCM fraction of PA, Donepezil (DPZ) 1mg/kg- a cholinesterase inhibitor.

IV. Discussion

The present study evaluated the effect of the methanolic extract of *P angolensis* on memory in Scopolamine and LPS treated mice, using threebehavioural models namely Y-maze, Morris water maze and the Novel object recognition paradigms.

One of the widely accepted entry screening test for cognitive enhancers is continuous spontaneous alternation in a symmetrical Y maze which measures spatial working memory rodents. The ability of rodents to choose alternate arms in a Y maze is referred to as spontaneous alternation. This behavior was first described about 80 years ago by Tolman[18].It measures the ability of rodents to remember the sequence of arms entry in the Y maze[19]. The list of arms visited have been proposed to be held in working memory and this enables it to avoid making revisits to previously visited arm [19, 20]. The inclination of rodents to correctly alternate the arm choice is dependent on their ability to usually remember the last recently visited arm and this serves as a measure of short term memory [20, 21, 22].In this study the DCM of *P. angolensis* significantly ($p<0.05$) increased, the percentage alternation in the Y-maze paradigm. This suggests enhanced spatial memory. Scopolaminewhen administered intraperitoneally would generate deficits of performance in the Y maze. The resulting deficit could be prevented by acetylcholine esterase inhibitors and sigma-1 agonists[23, 24]. The increase in alternation behavior produced by the treatment in the present study may be an indication that it may possess acetylcholinesterase inhibitory activity this is corroborated by the work of Elufioye*et al*.,2010 which demonstrated that the crude stem bark extract of Pycnanthusangolensis has acetylcholinesterase inhibitory activity [25].

The Morris Water Maze (MWM) test assesses spatial learning in rodents. In this test the animals swims from a start location and uses distal cues to find a submerged platform which is the only escape route in the swimming arena. The animals are trained several times to locate the hidden platform before the test trial is performed to assess spatial learning[26]. If an agent is amnestic it will produce escape latency that is not significantly different between the first training and the test sessions or this figure could be significantly higher than the control but still smaller than its training value (partial amnesia). A facilitatory treatment will produce a test latency that is significantly smaller than the control- group test value [26]. The results of the Morris water maze test show that theDCM of *P. angolensis* significantly ($p<0.05$) reduced the escape latency, this is collaborated by the work done by Elufioye and Oyeludein which it was found that the escape latency in

the*Pycanthusangolensis* treated group decreased through days 1 to 3 when compared with the scopolamine group showing their memory enhancing potential [28].

The effect of the extract of *P. angolensis* on recognition memory was evaluated using the novel object recognition test. Naturally rodents would spend more time exploring a novel object than a familiar one however, any impairment in memory may cause a deviation from this normal pattern. An absence of any difference in the time spent exploring the two objects in the test phase could be interpreted as a memory deficit or, if the drug being tested is an anti- amnesic drug, then it could be interpreted as a non-functioning drug. The results from this study show that the DCM fraction of *P. angolensis* increased the exploration time of the novel objects by the mice, implying memory enhancing activity.

The outer membrane of gram-negative bacteria contain the endotoxin, LPS, which is a very potent activator of both peripheral immune cells (macrophages and monocytes) and brain glia (microglia and astrocytes) thereby causing the release from these cells of various pro-inflammatory and immuniregulatory cytokines and free radicals[29, 30], inflammatory processes have detrimental effects on brain cells. The up regulation of pro-inflammatory factors lead to neuronal death in the brain [31, 32].The neuronal death in-turn induces memory impairment. In the present study theDCM of *P. angolensis* reversed the amnesia induced by LPS this suggests that the extract may possess anti-inflammatory activity which must have countered the neuronal death produced by LPS and the resulting memory impairment.

The accumulation of reactive oxygen species (ROS) to toxic levels in cells otherwise known as oxidative stress results in injury to DNA, lipids and protein. The buildup of ROS is due either to excessive production or inadequate degradation[33]. Brain tissue contain small amount of protective antioxidant defense system than other tissues this makes them more susceptible to the damaging effects of ROS[34]. Findings from the present study show that the brain levels of the antioxidant enzymes superoxide dismutase and gluthathione were significantly ($p<0.05$) increased by the DCM fraction of *P. angolensis* this may have reversed the oxidative imbalance thereby, improving neuronal function by protecting the neuron against further attack and damage by ROS. Furthermore, the brain level of lipid peroxidation (malonidildehyde) was decreased by the plant under investigation validating its positive effects on the oxidative imbalance.

References

[1]. YoungSoo Kim, Yunkyung Kim, Onyou Hwang and Dong Jin Kim (2012). Pathology of Neurodegenerative Diseases, Brain Damage - Bridging Between Basic Research and Clinics, Dr. Alina Gonzalez-Quevedo (Ed.), ISBN: 978-953-51-0375-2, InTech, 16, March, 2012

[2]. Barner EL, Gray SL. Donepezil use in Alzheimer's disease. Ann Pharmacother 1998; 32:70–77.

[3]. Avery EE, Baker LD, Asthana S. Potential role of muscarinic agonists in Alzheimer's disease. Drugs Aging 1997;11:450–459.

[4]. Brodaty H. Realistic expectations for the management of Alzheimer's disease. EurNeuropsychopharmacol 1999; 9 (Suppl 2): S43–52.

[5]. Bryne GJA. Treatment of cognitive impairment in Alzheimer's disease. Aust J Hosp Pharm 1998; 28: 261–266.

[6]. Schachter AS. Guidelines for the appropriate use of cholinesterase inhibitors in patients with Alzheimer's disease. CNS Drugs 1999;11:281–288.

[7]. Wiart C. Family Myristicaceae. In Medicinal plants of the Asia- Pacific: Drugs for the future? : Singapore: World Scientific Publishing Co. Pte. Ltd, 2006; 27-31.

[8]. Sofidiya MO, Awolesi AO. Antinociceptive and antiulcer activities of PycnanthusangolensisRevistaBrasileira de Farmacognosia 2015; 25: 252–257

[9]. Achel DG, Alcaraz M, Kingsford-Adaboh R, Nyarko AK, Gomda Y. A review of the medicinal properties and applications of Pycnanthusangolensis (welw) warbPharmacolofgyOnLine Archives 2012; 2: 1-22

[10]. Jaijoy K, Soonthornchareonnon N, Lertprasertsuke N, Panthong A, Sireeratawong S. Acute and chronic oral toxicity of standardized water extract from the fruit of Phyllanthusemblica Linn. Int. J. Appl. Res. Nat. Prod. 2010; 3: 48–58

[11]. Logue SF, Paylor R, Wehner JM.Behav Neurosci.1997; 111:104–113

[12]. Wang W, Li S, Dong HP, Lv S, Tang YY. Differential impairment of spatial and nonspatial cognition in a mouse model of brain aging. Life Sci. 2009; 85:127–135

[13]. Ellman GL, Courtney KD, Andre JV, Featherstone RM, A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. Biochemical Pharmacology 1961; 7: 88-95.

[14]. Moron MS, Depierre JW,Mannervik B. Levels of Glutathione, Glutathione Reductase and Glutathione S-Transferase Activities in Rat Lung and Liver. BiochimicaetBiophysicaActa, 1979; 582: 67-78.

[15]. Adam-Vizi V, Seregi M. Receptor dependent stimulatory effect of noradrenalin on Na+-K+ 393 ATPase in rat brain homogenate. Role of lipid peroxidation. Biochemical Pharmacology1982; 31: 2231-2236

[16]. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a sample assay for superoxide dismutase. Journal of Biological Chemistry1972; 247: 3170-3175

[17]. Gregory KP, Franklin G, Kalaichelavan V. Anti-ulcer (ulcerpreventive) activity of FicusarnottianaMiq. (Moraceae) leaf methanolic extract.Am. J. Pharmacol. Toxicol. 2009; 4: 89–93

[18]. Tolman EC. Purpose and cognition: the determiners of animal learning. Psychol. Rev.1925; 32: 285–97.

[19]. Blokland A. Scopolamine-Induced Deficits in Cognitive Performance: A Review of Animal Studies. Scopolamine Review 2005; 1: 1-76.

[20]. Hooper N, Fraser C, Stone TW. Effects of purine analogues on spontaneous alternation in mice. Psychopharmacol1996; 123: 250–7.

[21]. Lee M, Yun B, Zhang D, Liu L, Wang Z, Wang C, Gu L, Wang C, Mo E, Ly S, Sung C. Effect of aqueous antler extract on scopolamine induced memory impairment in mice and antioxidant activities. Food Sci. Biotechnol. 2010; 19: 655–661
[22]. Heo HJ, Kim M, Lee J, Choi S, Cho H, Hong B, Kim H, Kim E, Shin D. Naringenin from Citrus junos has an inhibitory effect on acetylcholinesterase and a mitigating effect on amnesia. Dementia and Geratric Cognitive Disorders 2004; 17: 151–157
[23]. Maurice T, Su TP,Privat A. Sigma1 receptor agonists and neurosteroids attenuate beta25–35-amyloid peptideinduces amnesia in mice through a common mechanism. Neuroscience1998; 83:(2) 413–428.
[24]. Meunier J, Ieni J, Maurice T. The anti-amnesic and neuroprotective effects of donepezil against amyloid beta25-35 peptide-induced toxicity in mice involve an interaction with the sigma1 receptor. Br. J. Pharmacol. 2006; 149:(8) 998-1012.
[25]. Elufioye TO, Obuotor EM, Sennuga AT, Agbedahunsi JM, Adesanya SA: Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some selected Nigerian medicinal plants. Rev Bras Farmacogn 2010;20:472- 477
[26]. Vorhees CV, Williams MT. Morris water maze: Procedures for assessing spatial and related forms of learning and memory.Nature Protocols.2006;I: 848-858
[27]. Quillfeldt JA. Behavioral Methods to Study Learning and Memory in Rats.V21 - May 24, 2006.
[28]. Elufioye TO, Oyelude FO. Memory enhancing activity of SpondiasmombinAnarcadiaceae) (and Pycanthusangolensis (Myristicaceae) on scopolamine induced amnesia in mice Nigerian Journal of Pharmaceutical Research 2015; 11: (1)
[29]. Dentener MA. Von Asmuth EJ. Francot GJ. Marra MN. Buurman WA. Antagonistic effects of lipopolysaccharide binding protein and bactericidal/permeability-increasing protein on lipopolysaccharide-induced cytokine release by mononuclear phagocytes. Competition for binding to lipopolysaccharide. J. Immunol. 1993; 151, 4258–4265.
[30]. Medvedev AE. Kopydlowski KM. Vogel SN. Inhibition of lipopolysaccharide-induced signal transduction in endotoxin-tolerized mouse macrophages: Dysregulation of cytokine, chemokine, and toll-like receptor 2 and 4 gene expression. J. Immunol. 2000; 164: 5564–5574
[31]. McGuire SO. Ling ZD. Lipton JW. Sortwell CE. Collier TJ. Carvey PM. Tumor necrosis factor α is toxic to embryonic mesencephalic dopamine neurons. Exp. Neurol. 2001; 169: 219–230.
[32]. Ramesh G. MacLean AG. Philipp MT. Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. Mediators Inflamm. 2013; 480739
[33]. El-Sherbiny DA. Khalifa AE.Attia AS. Eldenshary ES. Hypericumperforatum extract demonstrates antioxidant properties against elevated rat brain oxidative status induced by amnestic dose of scopolamine. Pharmacology Biochemistry and Behaviour2003; 76: 523–533
[34]. Jimenez-Jimenez FJ. Alonso-Navarro H. Avuso-Peralta L. Jabbour-Wadih T.Oxidative stress and Alzheimer's disease. Review Neurol., 2006; 42: 419–427