COGNITIVE ENHANCING AND EX-VIVO ANTIOXIDANT EFFECTS OF THE PHYTEXPONENT OF SELECTED MEDICINAL PLANT PREPARATION.

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DECLARATION

I hereby declare that this research project is my original work and it has not been presented to any institution as a credit work for academic purposes. I also declare that any other information and material cited in this paper has been duly acknowledged

SIGN: ______________________

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SUPERVISOR'S APPROVAL

I confirm that this research projected has been conducted and submitted with my approval as the student supervisor.

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Sign: ______________________

Date: ______________________

DEDICATION

I would like to dedicate this research project to my parents Mr. and Mrs. Kimwere, my sister Ms. Ann and Ms. Charity, my brother Mr. John and my fiancée Ms. Wairimu for their endless financial, emotional support and prayers.

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May God blessings be with you all the time

ABSTRACT

Alzheimer's disease, characterized by the dysfunction in cognitive function of the brain such as loss of memory accounts for the largest disease in the dementia condition. The management of this disease involves the use of the conventional drugs that inhibit the activity of the acetylcholine esterase enzyme or some antagonists of an N-methyl D-aspartate (NMDA). On addition to the high cost, the side effects and toxic effects have been associated with these drugs. Also, these drugs have been reported to only manage the symptoms since the cure for the Alzheimer's disease is not yet known. Alzheimer's disease (AD) has been linked with aberrant extracellular proteins misfolding oxidative stress, acetylcholine deficiency in the brain, and inflammatory processes. Some attention has been laid on plants as source of the natural antioxidants that have the ability to reduce the effects of oxidative stress and in increase antioxidant defense levels. In this study the cognitive enhancing and ex-vivo antioxidant effects of the phytexponent preparation in the scopolamine induced memory impairment in swiss albino mice was investigated. The passive avoidance task was used to evaluate the cognitive enhancing activity while the standard biochemical protocols were adopted for determination of the antioxidant enzymes activity. Donepezil was used as the standard drug. The results showed that administration of the scopolamine (1mg/kg bw) significantly affected learning in the swiss albino mice reducing the latency time for entering the shock stimuli compartment. The phytoexponent preparation reduced the effect of scopolamine in a dose depended manner with the higher dose (50 mg/kg) restoring the learning in the swiss albino mice more among the treatment dose levels. The normal control recorded the highest learning ability as compared to all the other groups. The administration of the scopolamine significantly affected the activity of catalase in the brain tissues of the mice in the negative control by reducing the activity of the catalase to degrade hydrogen peroxide ($p<0.05$). The administration of the phytoexponent at the three study dose levels significantly reduced the concentration of the hydrogen peroxide in the brain tissues of the swiss albino mice in a dose depended manner. The phytoexponent preparation at dose level 50 mg/kg bw significantly increased the catalase activity by recording the least concentration of hydrogen peroxide in the brain tissues ($p<0.05$). Similarly, the administration of the phytexponent at the different study dose levels increased the glutathione levels in the brain tissues of the swiss albino mice in a dose depended manner. The phytexponent at the higher dose (50 mg/kg bw) significantly recorded higher levels of glutathione ($p<0.05$). The administration of the scopolamine significantly reduced the glutathione levels in the brain tissues. In conclusion the phytexponent can be used as a potential cognitive enhancer and as well as an antioxidant agent.

TABLE OF CONTENT

LIST OF TABLES

ABBREVIATION AND ACRONYMS

ACHE	Acetylcholine esterase
AD	Alzheimer's disease
BW	Body weight
KG	Kilograms
MDA	Malondialdehyde
MG	Milligrams
NMDA	N-methyl-d-aspartate
PUFAS	Polyunsaturated fatty acids
ROM	Reactive oxygen metabolites
ROS	Reactive oxygen species
WHO	World health organization

CHAPTER ONE: INTRODUCTION

1.1 Background Information

The impairment of cognitive and emergency of dementia has been reported to be the major challenges to mankind mostly among the elderly in the present years. The WHO 2017 report has prioritized dementia as a public health problem and outlined the action plan for the 2017-2025(World Health Organization (WHO), 2017). The report indicated that about 47 million people globally were affected by dementia in the year 2015. These numbers were projected to double to about 75 million people by 2030 or even triple to 132 million people by the year 2050(World Health Organization (WHO), 2017);(Zabel et al., 2019) as the frequency of development of this condition is increasing at ahigh rate as the majority of the world population is aging. Dementia is a neuro-cognitive disorder that presents itself with slower and progressive deterioration in multiple cognitive domains that is severe enough to affect the daily functioning (Benhalla et al., 2019).

The dementia disorders such as AD diseases are currently managed by conventional drugs that include the acetylcholine esterase inhibitors such as donepezil or rivastigmine that help in improving the cognitive function that is important in memory and learning(Akram & Nawaz, 2017). Also, the NMDA (N-methyl-d-aspartate) antagonist memantine is used in improving cognitive function in the severe AD patients (Citron, 2010). Nimodipine, an L-type calcium current blocker or piracetam, a nootropicum are as well used to prevent the AD (Evans et al., 2004). However, these conventional drugs have been associated with side effects and in some instances are toxic. The acetylcholine esterase inhibitor has been associated with many side effects such as diarrhea and nausea while the use of tacrine has been reported to cause hepatotoxicity (Akram & Nawaz, 2017).These drugs are as well costly or even cumbersome to adapt. This resulted into many patients suffering from dementia and their care givers preferring the use of complementary medicines such as plants(Chang et al., 2016).

Medicinal plants have been part and parcel of human beings as they have been the main source of therapy for the many diseases. The use of plants in the developing countries has been rise with WHO report indicating that about 75 % of the population depend on them(Cathrine & Nagarajan, 2011). The history for the use of plants in the management of cognitive impairment and boosting of memory dates many years back (Chang et al., 2016). These plants contain various bioactive such as the alkaloids, sterols, triterpenes, polyphenols, tannins, flavonoids and lignin which have been identified via the phytochemical studies. These compounds have been reported to have pharmacological activities such as the anti-cholinesterase and anti-amyloidogenic(Akram & Nawaz, 2017). Plants as well have been the source of the various conventional ant-dementia products that are used. Many plants have been used such as the *Curcuma longa* has been used in Southern Asia countries as ant-dementia agent. It has been reported to reduce the oxidative stress as well as the amyloid pathology (Akram & Nawaz, 2017). Phytexponent preparation is aherbal product that is made from five medicinal plants; *Viola Tricolor, Echinacea purpurea, Allium sativum, Triticum repens and Matricaria chamomilla.* The individual plants have been reported to have various bioactive compounds with antioxidant and anti-inflammatory activities. Its marketed globally as an immune-modulator. The cognitive enhancing and antioxidant activities of the phytexponent was investigated in the scopolamine induced amnesia in the swiss albino mice.

1.2 Problem statement and justification.

The neurodegenerative diseases that include AD the most preference type of dementia among many people and whose genesis is linked to oxidative stress in the brain and dysfunction in the cognitive function has proven to be a burden both at the individual level and even at the community level (Chen et al., 2017). This disease in its severe stages it affects the individuals leaving them helpless hence entirely depend on family members and friends as care givers. This affects the friends and family members psychologically. The cost of managing the AD patient is very high making it difficult for many people to afford. Conventionally western medicines that target the acetylcholine esterase enzymes such as donepezil, the regulators of the activity of glutamate such as memantine are used in the management of cognitive impairment diseases symptoms (Stella et al., 2015). However, these drugs have been associated with side effects after use. Additionally, the high cost of these drugs is a challenge to the many people under economic constrains and all these drugs only reduce the symptoms(Galimberti & Scarpini, 2017). The increase in the side effects that are incurred when these drugs are used, the high cost and reduced potency, has necessitated the search for an alternative natural agent that is a potential cognitive enhancing and antioxidant agent.

Medicinal plants are a hub of many phytochemicals that are associated with various pharmacological properties. The medicinal plants have been used by many for many years in the management of many ailments. Many of them have been used as antioxidants, anti-inflammatory agents, anti-analgesics'. plants have been used due to their low or no toxicity effects, fewer side effects and less expensive that make them easily available. The medicinal plants have as well given rise to the present allopathic medicines such as aspirin. However, the many plants that have been reported to be potential agents in management of the diseases, have no data to back up their claims. There are very few plants that have been screened and clinical trials done. This has left many plants unexplored hence their healing potential not being validated. In this study the cognitive enhancing and antioxidant activities of the phytexponent preparation were investigated.

1.3 Objective

1.3.1 General objective

To investigate the passive avoidance learning and the antioxidant activities of the phytexponent preparation in the scopolamine induced amnesia in swiss albino mice.

1.3.2 Specific objective

I. To investigate the effect of phytexponent preparation on the Passive a voidance learning ability of the swiss albino mice in scopolamine induced amnesia.

II. To determine the effect of phytoexponent preparation on catalase activity and glutathione in the brain tissues of the scopolamine induced amnesia swiss albino mice.

1.4 Research question

I. Does the phytexponent have effect on the passive a voidance learning in the scopolamine induced amnesia in swiss albino mice?

II. What is the effect of the phytexponent preparation on the activities of catalase and glutathione in the scopolamine induced amnesia in swiss albino mice brain tissues?

CHAPTER TWO: LITERATURE REVIEW

2.1 Cognition

Cognition is the collective set of abilities that comprises of the various information processing, storage and retrieval on need. It hubs the concept of intelligence or metacognition which is capacity to learn from the previous experiences and the ability to acclimatize to the new surrounding environment or situations (Moriasi et al., 2020).

Decline in cognition has been on the rises with an increase in the age of an individual. However, other factors such as genetics have been linked to cause dementia hence the reason why cognitive function decline with age in many people but not in others. The cognition abilities that been interfered with as the age increases includes attention, language skills, executive cognitive function, visuospatial abilities such as perceiving spatial orientation of objects and visuoperceptual judgement and certain types of memory such working memory and future memory. Also, a well-defined and gradual decline in cognition that involves learning capacities has been noted (Kent, 2016). The decline in cognition is as well varied depending on the genetic and environmental factors. These factors have an impact on the characteristic nature of the cells to withstand the damage as a result of the stressors of aging(Barter & Foster, 2018). In regards to memory the most affected is the episodic memory which consist of the memories of the events which becomes difficult in retrieval with aging. The second memory which is the sematic memory that comprises of the general knowledge of vocabulary, facts and many others that does not need quick remembrance not affected by aging (Kinugawa et al., 2013).

The impairment in the cognition function has been reported to be the leading cause of mental disability and dependency among the older people and those suffering from the Alzheimer's disease and other dementia related conditions worldwide. This condition is not only a burden to the affected individuals abut as well to the dependents of these individuals comprising of both the families and the caregivers (Moriasi et al., 2020). the inadequate information regarding the cognitive disorders among many people has raised the level of stigma and inability of the affected people to seek treatment. The cognitive impairment has as well been a challenge to the caregivers, families and society at larger physical and psychological problems (Ren et al., 2018).

2.2 Causes of cognitive impairment

The cause of cognitive impairment has been attributed to many factors including oxidative stress. Oxidative stress arises due to the increased population of the reactive oxygen species in body as a result of the dysfunction of the antioxidant system or reduced level of the antioxidants in the body. These excess reactive oxygen species results into the damage on the brain cells causing cognitive impairment (Simpson et al., 2015). The high population of the lipids mostly the polyunsaturated fatty acids (PUFAs) in the brain makes the brain vulnerable to oxidative damage by reactive oxygen species hence triggering the brain damage leading to cognitive impairment (Luca et al., 2015);(Kurutas, 2016). The reaction of the reactive oxygen species on lipids results into lipid peroxidation which is one of the most notable destructive form of oxidative degradation that generates the secondary products such as reactive oxygen metabolites (ROM) that have been linked to neurotoxicity (Sajjad N et al., 2019). One of these reactive oxygen metabolites is the malondialdehyde (MDA) whose high levels is an indicator and marker of lipid peroxidation and oxidative stress respectively (Cervellati et al., 2014);(Zhou et al., 2016).

2.3 conventional management of cognitive impairment disorders

The total treatment of the cognitive related disorders such AD is an area of concern in the current years. no conventional drugs that are able to manage these disorders are available since the one in the market only manage the symptoms. These drugs usually focus on the improving the memory by inhibiting the acetylcholine esterase enzyme (Ghumatkar et al., 2015). The mostly used drugs include donepezil, galantamine and rivastigmine which elicit their activity by inhibiting the catabolic enzyme acetylcholine esterase (AChE) in order to maintain or even delay the decreases of the levels of acetylcholine in the synaptic clefts (Stella et al., 2015). Even though they have been the most preferred drug, they only manage the symptoms and their use is as well limited to the mild conditions(Galimberti & Scarpini, 2017).

Donepezil is a highly selective inhibitor of catabolic enzyme AChE and its inhibition ability is reversible. It elicits its mechanism of action at dose level of 5-10 mg daily. However, to the patients that are in the severe and moderate stages of the AD, a higher dose of 23 mg is administered(Ehret & Chamberlin, 2015). The rivastigmine inhibits both the AChE and butyryl cholinesterase and the prescriptions begins with the dose level of 1.5 mg which progressively increases to the dose level of 6 mg two times a day. The administration via the transdermal path has been characterized with short half life hence this allows single prescription each day. This is as well associated with mild gastrointestinal side effects(Galimberti & Scarpini, 2017). Galantamine is also a reversible and at the same time a competitive inhibitor of AChE and an allosteric modulator through binding to the cholinergic nicotinic receptors at an additional site. The galantamine has an extended daily prescription that range from 16-24 mg(Cutler, 2015).

The side effects associated with these drugs include the gastrointestinal manifestation such as nausea, vomiting, diarrhea and lose of weight are the most observed while the less common effects include insomnia, headache, agitation, bradycardia and syncope (Selkoe & Hardy, 2016).

2.4 Use of plants as cognitive enhancers

Herbal medicines generally derived from medicinal plants have their origin in ancient cultures such as the Egyptian, Indians and the Chinese culture (Akram & Nawaz, 2017). The use of medicinal plants in managing various disease has increased in the recent years. this has been triggered by the side effects and cost associated with the conventional medicine. Additionally, the safety associated with the medicinal plants has been an added advantage. Herbal drugs medication is as well cheap and easily available to the majority of the population more so the developing countries such as sub-Saharan Africa where a huge percentage of pupation about 75 % depend on them. Medicinal plants have played an enormous role in the management of the cognitive impairment condition. Various plants via the available literature have been studied and found to be potential memory enhancers (Akram & Nawaz, 2017). These include the *Valeriana officinalis, Punica granatum L., Salvia officinalis, Myristica fragrans Bacopa monnieri Linn, Centella asiatica Linn and Evolvulus alsinoides Linn*

2.5 Phytoexponent preparation

Phytoexponent preparation is an herbal preparation of Belgium origin that comprises of five different medicinal plants. The plants are first extracted with ethanol by soaking individual plant material into an extraction vessel for two days and then filtered on the third day. The different extracts solution of the respective plants is then mixed in specific percentages. The mixing is as follows; *Viola Tricolor* - 3.77% *Echinacea purpurea*- 26.42% Allium sativum- 11.32% *Triticum repens*- 26.42% *Matricaria chamomilla*- 32.08%. The final product then comprises of 62.1 % ethanol with the rest of the percentage representing the various plant extract.

2.5.1 Composition of the phytexponent

Phytexponent comprises of five different medicinal plants that are mixed in different proportions to make the final product.

Viola tricolor also commonly known as Heartsease, Johnny Jump up, Call-me-to-you, or Bird's Eye (Hellinger et al., 2015). Its taxonomically placed in the family Violaceae. This plants has been used as anti-inflammatory agent and skin remedy in Europe (Hellinger et al., 2015). These plants contain various phytochemicals such as flavonoids, polysaccharides, phenylcarbonic acids, cumarins, catechins, and salicylic acid derivatives. The plant has as well been reported to have macrocyclic peptides such as cyclotides that are potent immunosuppressive peptides that have the ability to inhibit T-cell proliferation(Hellinger et al., 2015).

Echinacea purpurea is a perennial medicinal herb that is commonly identified as eastern purple coneflower or generally the purple coneflower and its native to North America. It has been reported to be an anti-inflammatory and an immunostimulatory agent (Manayi et al., 2015). Additionally, it has as be used in managing g cold symptoms and as well it has shown antimutagenicity, cytotoxicity, antidepression, and antianxiety effects. Due to its multiple uses it has gained much attention from many researchers. However it has not be reported to have certain side effects such as urticaria, erythema, rash, prutitus, nausea, dyspnea, angioedema, and abdominal pain (Manayi et al., 2015).

Allium sativum also known as garlic is the most widely used spices in most of the countries and the most sold herbal product globally (Majewski, 2014). It is as well used as an aphrodiciac and belongs to the family alliaceae in the onions are the members (Moutia et al., 2018). Various bioactive compounds summing up to 200 have been identified garlic. The aqueous garlic extracts have been reported to have antioxidant activity by inhibiting reactive oxygen species (ROS) and enhancing enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (Arreola et al., 2015).

Matricaria chamomilla is amedicinal plant found in the family *Asteraceae* . the componds isolated from this plant have been used in about 26 drugs. It has as well been used as antiinflammtory and antispasmodic drug. It has as well been used in traeting pain that results from the disturbace from the stomach. Its has as well been used extensivel as a tea or tonic and treating hysteria, anxiety, insomnia, and nightmares(Satyal et al., 2015).

Tricum vulgare commonly identified as cough grass is an invasive weed whose roots and leaves are used medicine. Its is used in managing constipation,swollen bladder, cough, fever, hypertension, kidney stones and inflammation (Sanguigno et al., 2018). It has as well been incoparated in some pharmaceuticl formulations for treatment of burns, skin lesions and decubitus ulcers. The antiinflammatory properties of this weed has been an area of corncern.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Source of the phytexponent

The phytexponent preparation was purchased from Maendeleo pharmacy in Nairobi and then kept until the experimental day.

3.2 Experimental animals

Healthy 30 swiss albino mice weighing 25-30 grams were used in this study. They were sourced from Kenya agricultural and livestock research organization Muguka animal breeding housed and transported to Mount Kenya university in propylene transparent cages fitted with stainless steel grill to prevent them from escaping. They then kept in standard animal house in Mount Kenya university at 12-hour day/night cycle. They were fed with standard a laboratory rodent pellets and allowed free access to clean water. All the mice were allowed to acclimatize to the laboratory conditions for a period of seven days prior to the day. All the guidelines regard the handling of laboratory animals were followed to the end.

3.3 Experimental design

In this study 30 experimental swiss albino mice were used. The swiss albino mice were first ly segregated into six groups with each group consisting of five animals (n=5) and then treated as outlined in table 3.1.

Table 3. 1 Treatment protocol of the passive avoidance test

Group	Category	Treatment
I	Normal control	Normal saline
II	Negative control	Normal saline + Scopolamine
III	Positive control	Normal saline + Scopolamine + DNP 0.5mg/kg bw
IV	Experimental group A	Normal saline + Scopolamine (1 mg/kg bw) + 25 mg/kg bw phytexponent
V	Experimental group B	Normal saline + Scopolamine (1 mg/kg bw) + 50 mg/kg bw phytexponent
VI	Experimental group C	Normal saline + Scopolamine (1 mg/kg bw) + 75 mg/kg bw phytexponent

3.3.1 Behavioural experiments (cognitive measurements)

Passive Avoidance Tasks method (G. Y. Lee et al., 2017) was adopted with minor modifications in this study. It was used to assess the effects of the phytoexponent preparation on the learning and memory ability scopolamine induced amnesia in the swiss albino mice. The locally made passive avoidance box was used in this study. The box consisted of two compartments of similar measurements (25 cm × 20 cm × 20 cm), the bright lit and dark compartments partitioned with carton wall, fitted with a sliding door of measurements (3 cm × 3 cm). The test was conducted into two phases, the first phase involved training of the mice and the second phase acquisition trail.

3.3.1.1 Training

On the first day all the mice were trained to be able to get into the non-illuminated compartment. Each mouse was placed into the illuminated compartment with the head facing away from the entrance to the dark side and then allowed to explore. After the mouse completely gets into the dark chamber with its four paws the chamber door was closed and mild electrical shock applied for about 1-2 seconds and then lifted. The mouse was then allowed to stay in the chamber for about 10 seconds before being allowed to get out. If the moose did not enter into the dark compartment for a period of 60 seconds it was guided and then allowed to stay there for 20 seconds. The training was conducted for two days and on the third day amnesia was then induced in the mice in all the groups expect the normal control by administering of scopolamine (1mg/kg bw).

3.3.1.2 Learning acquisition

On the final day, 30 minutes after receiving the final dose of treatment, each mouse was placed in the lighted compartment, facing away from the dark compartment. After 10 seconds, the guillotine door was lifted. When the mouse entered the dark compartment with all four paws, the guillotine door will be closed, and the latency time to enter the dark compartment was recorded up to a maximum of 300 seconds. The mouse was then removed and returned to its home cage.

3.3.2 Antioxidant assays

Following the passive avoidance test, mice in all the five study groups were decapitated by cervical dislocation, the head dissected and the whole brains scoped off. The brain samples were then kept in into clean vials followed by addition of the phosphate buffer. The brain samples were then vortexed to make the homogenate and then centrifuged at 3000 rpm for 10 minutes. The resultant supernatant was then separated and then used to determine the antioxidant by evaluating the levels of the enzymatic antioxidant levels.

3.3.2.1 catalase activity assay

The catalase activity was determined by following the modified method of Ghumatkar et al.(2015). This briefly involved use of three test-tubes labeled as 1,2, and 3 in which 100ul sample was added followed by 250ul phosphate buffer and 200ul of 1mM hydrogen peroxide to initiate the reaction. The reaction was then stopped after 0,30 and 60 seconds for the tube 1,2 and 3 respectively by addition of 1ml of dichromate - acetic acid mixture. The tubes containing the reaction mixtures were then incubated in a hot water bath set at 100^0C for 10 minutes. All the tubes were then allowed to cool to room temperature and the absorbance measured at 620nm using UV-vis spectrophotometer and the catalase activity calculated following the equation.

3.3.2.1 Glutathione peroxidase assay

The glutathione peroxidase activity was evaluated by the method Ghumatkar et al. (2015) with minor modifications. The reaction mixtures consisted of 1.49 ml of sodium phosphate buffer (0.1M ph.7.4) 0.1ml EDTA 1mm, 0.1ml sodium azide (1mm), 0.1ml 1mm GSH, 0.1ml of NADPH (0.02mm), 0.01ml or 1mm $H_2 0_2$ and 0.1ml of brain supernatant .the oxidation of

NADPH was monitored recorded spectrophotometrically at 340nm every 30 seconds for 2 minutes using a UV-vis spectrophotometer.

3.4 Data management and statistical analysis

The antioxidant activity and the passive avoidance learning data was first tabulated in an excel spread sheet (office 365) and then imported in the mini-tab software for descriptive statistics. The data was then expressed as mean ± standard error of the mean. This data was then further analyzed by one-way anova followed by fishers' test to determine the level of significance ($p<0.05$) and the data presented in form of tables.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Passive avoidance Learning-enhancing effects of the Phytexponent preparation of selected medicinal plants.

The results for the passive avoidance learning enhancing effects of the phytexponent are presented in table 4.1. the results showed on day one (training day) the normal control group took significantly higher latency time to enter the non-illuminated compartment as compared to all the other groups($p>0.05$). however, the experimental group two those that received the phytoexponent at dose of 10 mg/kg bw significantly recorded least latency time ($p<0.05$). generally, there was significantly difference in the latency time recorded by the different groups in day one of learning acquisition trial ($p<0.05$).

On the second day of acquisition learning, the mice in the experimental group three those that received the phytexponent at a dose of 50 mg/kg bw and those in the negative control group significantly recorded the highest and least latency time respectively as compared to all the other groups ($p<0.05$).However, the experimental groups two and one those that received the phytexponent at doses 10 mg/kg bw and 2 mg/kg bw respectively did not show any significantly difference in the latency time recorded ($p>0.05$). similarly, the negative control and the positive control groups recorded significantly similar latency time ($p>0.05$).

On day three the last day of learning acquisition, there was no significant difference in the latency time recorded by the mice in the experimental group three (50 mg/kg bw) and the normal control group ($p>0.05$). similarly, the mice in experimental group two (10 mg/kg bw) and negative control group recorded no significant difference in their latency time ($p>0.05$). However, there was significant difference in the latency time recorded by mice in experimental group one (2 mg/kg bw) and positive control group ($p<0.05$).

The Colum wise comparisons of the latency time recorded between the three days per treatment group revealed that for the normal control, positive control and all the experimental groups that were treated with the phytexponent at three different doses shown significant in the recorded latency time for all the three days ($p<0.05$). however, the negative control group showed no significant difference in the latency time recorded in day one and two ($p>0.05$).

Table 4. 1 Passive avoidance Learning-enhancing effects of the Phytexponent preparation of selected medicinal plants.

Treatment	Latency (seconds)		
	Day 1	Day 2	Day 3
Normal control	$37.68 \pm 0.88^{a}_{c}$	$83.33 \pm 1.76^{b}_{b}$	$112.00 \pm 1.15^{a}_{a}$
Negative control	$21.67 \pm 1.20^{c}_{b}$	$18.67 \pm 1.20^{d}_{b}$	$26.00 \pm 0.57^{d}_{a}$
Positive control	$15.00 \pm 0.58^{de}_{c}$	$81.33 \pm 0.88^{b}_{b}$	$93.33 \pm 0.88^{b}_{a}$
$Phyt^{exponent}$ 2 mg/Kg bw	$25.67 \pm 0.33^{b}_{c}$	$63.67 \pm 2.33^{c}_{b}$	$75.67 \pm 1.76^{c}_{a}$
$Phyt^{exponent}$ 10 mg/Kg bw	$12.33 \pm 1.45^{e}_{c}$	$65.00 \pm 0.58^{c}_{b}$	$95.00 \pm 1.00^{b}_{a}$
$Phyt^{exponent}$ 50 mg/Kg bw	$17.00 \pm 1.00^{d}_{c}$	$89.67 \pm 0.88^{a}_{b}$	$114.33 \pm 2.33^{a}_{a}$

Values are expressed as $\bar{X} \pm SEM$; means that have the same superscript letter within the same column and the same subscript within the same row are not significantly different using one-way Anova followed by Fisher's list significance difference.

4.2 The effects of phytoexponent of the catalase activity in the scopolamine induced amnesia in Swiss albino mice

The results for effects of the phytexponent on the levels of catalase in the scopolamine induced amnesia in swiss albino mice are presented in table 4.2. the results showed that there was no significant difference in the levels of catalase recorded at zero seconds in the normal control, positive control and experimental group two (10 mg/kg bw) and three (50 mg/kg bw) ($p>0.05$). however, three was significant difference in the levels of catalase recorded in the negative control and experimental group one (2 mg/kg bw) ($p<0.05$).after 30 seconds, the levels of catalase recorded in all the treatment groups except the negative control group were significantly not different from each other ($p>0.05$). similarly, after 60 second there was no significant difference in the catalase levels in all the treatment groups ($p>0.05$).

The column-wise comparison of the levels of catalase recorded in each treatment group for the tested time interval showed that in the normal and positive control groups no significant difference in the levels of catalases were recorded for the tested time ($p>0.05$).similarly, there was no significant difference in the catalase levels recorded at 30 seconds and 60 seconds for the negative control and experimental groups two (10 mg/kg bw) and one (2 mg/kg bw) respectively ($p>0.05$). however, for the same groups (negative control and experimental groups two (10 mg/kg bw) and one (2 mg/kg bw)) significantly different catalase levels were recorded ($p<0.05$).

Table 4. 2 Effects of phytexponent of the catalase activity in the scopolamine induced amnesia in swiss albino mice

Treatment	Catalase level		
	0 seconds	30 seconds	60 seconds
Normal control	$8.43\text{x}10^{-4} \pm 3.62\text{x}10^{-4}\,{}^{c}_{a}$	$1.91\text{x}10^{-3} \pm 6.37\text{x}10^{-4}\,{}^{b}_{a}$	$9.64\text{x}10^{-4} \pm 4.63\text{x}10^{-4}\,{}^{b}_{a}$
Negative control	$9.54\text{x}10^{-2} \pm 7.96\text{x}10^{-4}\,{}^{a}_{b}$	$8.97\text{x}10^{-1} \pm 4.71\text{x}10^{-2}\,{}^{a}_{a}$	$1.09\text{x}10^{0} \pm 1.83\text{x}10^{-1}\,{}^{a}_{a}$
Positive control	$1.30\text{x}10^{-3} \pm 1.30\text{x}10^{-4}\,{}^{c}_{a}$	$1.51\text{x}10^{-3} \pm 4.60\text{x}10^{-5}\,{}^{b}_{a}$	$1.22\text{x}10^{-3} \pm 6.2\text{x}10^{-5}\,{}^{b}_{a}$
Phyt^{exponent} 2 mg/Kg bw	$1.12\text{x}10^{-2} \pm 7.21\text{x}10^{-4}\,{}^{b}_{a}$	$2.13\text{x}10^{-3} \pm 5.18\text{x}10^{-4}\,{}^{b}_{b}$	$2.04\text{x}10^{-3} \pm 4.45\text{x}10^{-4}\,{}^{b}_{b}$
Phyt^{exponent} 10 mg/Kg bw	$1.11\text{x}10^{-3} \pm 4.1\text{x}10^{-5}\,{}^{c}_{a}$	$9.20\text{x}10^{-5} \pm 1.0\text{x}10^{-6}\,{}^{b}_{b}$	$1.23\text{x}10^{-4} \pm 1.0\text{x}10^{-5}\,{}^{b}_{b}$
Phyt^{exponent} 50 mg/Kg bw	$1.93\text{x}10^{-4} \pm 4.0\text{x}10^{-6}\,{}^{c}_{b}$	$1.25\text{x}10^{-4} \pm 4.0\text{x}10^{-6}\,{}^{b}_{c}$	$8.15\text{x}10^{-4} \pm 1.7\text{x}10^{-5}\,{}^{b}_{a}$

Values are expressed as $\bar{X} \pm SEM$; means that have the same superscript letter within the same column and the same subscript within the same row are not significantly different using one-way Anova followed by Fisher's list significance difference.

4.3 The effects of phytexponent of on glutathione levels in the scopolamine induced amnesia in swiss albino mice level results.

The results for the effect of the phytexponent preparation at varied doses on the glutathione levels in the scopolamine induced amnesia in swiss albino mice are presented in table 4.3. The results indicated that at 30 seconds there was significant difference in the glutathione levels in all the treatment groups ($p<0.05$). similarly, the experimental group three (50 mg/kg bw) and group one (2 mg/kg bw) recorded higher and least levels of glutathione respectively. At 60 seconds the normal control group and experimental group two (10 mg/kg bw) recorded no significant levels of glutathione ($p>0.05$). However, at the same time all the other groups recorded significant differences in the levels of glutathione ($p<0.05$).

Table 4. 3 effects of phytexponent of on glutathione levels in the scopolamine induced amnesia in swiss albino mice level results.

Treatment	Glutathione level			
	30 seconds	60 seconds	90 seconds	120 seconds
Normal control	$7.087 \pm 0.338^{d}_{a}$	$7.087 \pm 0.338^{c}_{a}$	$7.044 \pm 0.295^{c}_{a}$	$7.108 \pm 0.359^{c}_{a}$
Negative control	$3.518 \pm 0.170^{f}_{a}$	$2.581 \pm 0.103^{e}_{b}$	$2.163 \pm 0.109^{e}_{bc}$	$2.024 \pm 0.166^{e}_{c}$
Positive control	$11.403 \pm 0.059^{b}_{a}$	$8.530 \pm 0.334^{b}_{b}$	$9.154 \pm 0.418^{b}_{b}$	$9.006 \pm 0.448^{b}_{b}$
$Phyt^{exponent}$ 2 mg/Kg bw	$4.339 \pm 0.078^{e}_{b}$	$4.239 \pm 0.052^{d}_{b}$	$5.573 \pm 0.061^{d}_{a}$	$5.679 \pm 0.127^{d}_{a}$
$Phyt^{exponent}$ 10 mg/Kg bw	$8.647 \pm 0.074^{c}_{b}$	$7.650 \pm 0.125^{c}_{c}$	$8.732 \pm 0.025^{b}_{ab}$	$8.900 \pm 0.0412^{b}_{a}$
$Phyt^{exponent}$ 50 mg/Kg bw	$23.213 \pm 0.146^{a}_{b}$	$20.416 \pm 0.239^{a}_{d}$	$24.501 \pm 0.256^{a}_{a}$	$22.411 \pm 0.264^{a}_{c}$

Values are expressed as $\bar{X} \pm SEM$; means that have the same superscript letter within the same column and the same subscript within the same row are not significantly different using one-way Anova followed by Fisher's list significance difference.

In this study, scopolamine was used to induce animal models for amnesia, such as Alzheimer's disease to compare the response and mechanism of learning in the swiss albino mice. Scopolamine has been shown to competitively inhibit the binding of acetylcholine to muscarine receptors and the outcome being cause of cholinergic depression (Kwon et al., 2010);(Y. K. Lee et al., 2009). Additionally, it has been reported that scopolamine stimulates the generations of the reactive oxygen species (ROS) inducing the oxidative stress (Balaban et al., 2017);(Wong-Guerra et al., 2017). Oxidative stress has been linked to the pathogenesis of Alzheimer's disease this has been through the various clinical trials. Similarly, scopolamine has been reported to results into the reduction of activity of the enzymatic antioxidants such as SOD and catalase which are the two vital antioxidant enzymes (Balu et al., 2005);(Mann et al., 1997).

Scopolamine administration model for animals has been identified as the best approach of exploring the basic knowledge of learning and memory response. It has as well been identified as best option for assessing the newly developed drugs for neurodegenerative disorders such as AD that is characterized by impairment in the learning and memory (Lin et al., 2016). In human beings it has been reported to be very easy in assessing clinically the learning and memory abilities and no specific methods needs to be used. But with animals, specific methods such as passive avoidance test and the Morris water maze are employed (John et al., 1997).

In the current study the ability of the phytexponent revert the effects of scopolamine on the learning ability of the swiss albino mice was evaluated by the passive avoidance test.in this test donepezil was used as the positive ant amnesia drug. Scopolamine was intravenously administered while donepezil was orally administered. The administration of the scopolamine significantly reduced the latency time the mice took to enter the non-illuminated compartment as compared to the normal group ($P>0.05$). This was an indication the dysfunction in memory and learning acquired during the learning period. The oral administration of the phytexponent preparation at a dose of 50 mg/kg bw significantly reversed the effects of scopolamine by increasing the latency time as compared to the negative control and all the other groups. Day three of the acquisition learning, the administration of the phytexponent at dose level of 50 mg/kg bw significantly ameliorated the effects of scopolamine in the swiss albino mice. However, in all the three days of acquisition learning the positive control drug (donepezil at 1mg/kg bw) was not effective in the reverting of the effects of scopolamine on learning and memory in swiss albino mice.

The levels of the antioxidant enzymes in this study were as well evaluated in the brain tissues of the swiss albino mice. The investigation revealed that the administration of the scopolamine significantly reduced the levels of glutathione at 30, 60, 90 and 120 seconds as compared to the normal mice. This was an indication that scopolamine has effect on the antioxidant levels in the brain tissues and it participates in the generation of the reactive oxygen species that deplete the brain antioxidant enzymes. However, the administration of the phytexponent at the different studied doses significantly increased the levels of glutathione at all study time. The phytexponent at dose level of 50 mg/kg significantly recorded higher glutathione levels followed by the dose level of 10 mg/kg bw and 2 mg /kg bw. The positive control significantly increased the levels of glutathione in the brain tissue as compared to the brain tissues in the mice that received the 10 mg/kg bw and 2 mg/kg bw and the normal and negative control.

To determine the activity of the catalase in the brain tissues the hydrogen peroxide concentration was determined and then used to conclude on the activity of the catalase enzyme. The administration of the scopolamine showed significantly higher concentration of hydrogen peroxide as compared to all the other groups. This was an indication of low catalase activity. However, the administration of the phytexponent at different study doses resulted into reduction in the concentration of the hydrogen peroxide in the brain tissues in a dose dependent manner an indication of an increase in the activity of the catalase enzyme in degrading of the hydrogen peroxide. Similarly, the administration of the phytexponent at all the studied dose levels reduced the concentration of hydrogen peroxide more as compared to the positive control group.The phytoexponent at a dose level of 50 mg/kg bw significantly reduced the hydrogen peroxide concentration at zero seconds as compared to the other two experimental groups that were administered the phytoexponent at dose levels 2 mg/kg bw and 50 mg/kg bw. Similarly, the administration of the The positive control group that were administered donepezil at dose level 1 mg/kg bw significantly reduced the concentration of hydrogen peroxide more as compared to the normal control group that was not induced with amnesia. catalase activity did not in result into the increase in the levels of catalase in the brain tissues. Hence this is an implicartion that the phyotxponent did not counteract the effects of the scopolamine.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, the findings from the current study suggests that the phytexponent can be a potential cognitive enhancing agent. Its ability to revert the effects of scopolamine on learning in the swiss albino mice showed that it has cognitive properties. Similarly, its ability to increase the antioxidant enzymes in the brain showed that is a potential antioxidant that can be used to clear the excess reactive oxygen species reducing the chances of occurrence of oxidative stress which is an etiology in the cognitive impairment.

5.2 Recommendation

From the current study the following recommendation were made

I. The antioxidant activity of the phytexponent preparation to be evaluated by other methods

II. The guided isolation of the active compounds from the phytexponent preparation to be conducted.

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