

Neurochemical Impact of the Aqueous Extract of *Vernonia amygdalina* and *Talinum triangulare* on Learning and Memory in Male Wistar Rats

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Abstract Background: Nutrition affects virtually everything in the life of an organism, including cognitive abilities, at the level of learning and memory. The brain being a metabolically active tissue is prone to lipid peroxidation which affects cognitive functions. Despite the well documented nutritional and medicinal values of *Vernonia amygdalina* (bitter leaf) and *Talinum triangulare* (water leaf), their neurochemical impact through learning and memory are yet to be fully elucidated. **Objective:** The aim of the present study was to evaluate the neurochemical impact of the aqueous extracts of *Vernonia amygdalina* and *Talinum triangulare* on cognitive function in male Wistar rats. **Methods:** Twenty one male Wistar rats ($175.83 \pm 6.92\text{g}$), were randomly assigned into three groups A, B, and C ($n=7$ per group); *Vernonia amygdalina*, *Talinum triangulare* and Control (Saline) groups respectively. They were treated with 2ml/100g BW of the extracts every other day for thirty days. Twenty four (24) hours after the last administration, rats were subjected to learning and memory assays for one week, thereafter, brain tissue was homogenized and assayed for cholinergic activity, while blood samples were taken and used for biochemical parameters. **Results:** There was a significant increase in body weight of treated rats relative to control. *Vernonia* group A; 176.5 ± 4.6 , *Talinum* group B; 161.8 ± 2.9 and the Control group C; $150.8 \pm 16.5\text{g}$ respectively ($p < 0.05$). The treated rats spent more time in the light compartment of the Shuttle box in the learning and memory assays. Both extracts exhibited hypoglycemic effect, however, only *Vernonia* enhanced a significant increase in brain acetylcholinesterase (AChE) activity. **Conclusion:** *Vernonia amygdalina* and *Talinum triangulare* enhanced learning and memory via modulation of brain cholinergic neurotransmission. Hence, as a future directed study, their neuroprotective effect in brain cell death inhibition is recommended.

Keywords *Vernonia amygdalina*, *Talinum triangulare*, Cognitive function, Learning, Memory, Acetylcholinesterase, Rats

1. Introduction

The role that nutrition plays in cognitive function has remained an area of keen interest over the past few decades, with the emergence of natural products of neurochemical importance. The brain, which is the seat of cognitive function in variety of living organisms, is a metabolically active organ [1]. Its membrane is rich in lipids, as a result, it is a very vulnerable organ, when it comes to lipid peroxidation, a process which impairs brain function. Plants, fruits and vegetables are the main source of biologically active compounds and molecules of nutritional and medicinal importance [1, 2]. Despite the well-established

versatile usage of *Vernonia amygdalina* (bitter leaf) and *Talinum triangulare* (water leaf), their neurochemical impact is yet to be fully elucidated. Therefore, the aim of the present study was to evaluate the neurochemical impact of the aqueous extracts of *Vernonia amygdalina* and *Talinum triangulare* on cognitive function in albino Wistar rats.

In general terms, cognitive function is the ability of the brain to retrieve and make use of simple to complex information to meet daily life challenges. Nutrition plays a very important role in brain function, certain nutrients provides key chemical compounds that are important in brain development and function, such that, deficiency in these nutrients, could lead to impaired cognitive functions [2]. Cellular processes and organism development are equally influenced by nutrition [2, 3]. Certain nutrients found in plants, fruits and vegetables are main precursors of neurotransmitters, needed by the central nervous system in conveying chemical signals through neural pathways. These

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chemical messengers which include serotonin, epinephrine, acetylcholine, and dopamine, could be synthesized from certain nutritional amino acids [2]. *Talinum triangulare* is a rich source of proteins which could possess some of these essential amino acids.

The beneficial effects of plants and natural products, is in their ability to contribute immensely to the general antioxidant defense system in the body, basically in the brain, thereby influencing brain function via learning and memory. Certain plant compounds modulates the activity of the acetylcholinesterase enzyme, in the process influences the availability of the neurotransmitter acetylcholine which is highly implicated in learning and memory [4]. Furthermore, vegetables and fruits are major sources of vitamins and minerals which are needed in cellular free radical scavenging pathways. Endogenous antioxidants enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX), among others, require certain minerals which serve as coenzymes in this free radical scavenging pathway [5], these antioxidants equally reduce inflammatory reactions in the brain. Exogenous antioxidants from fruits and vegetables have been shown to increase the activity of brain kinase enzymes, which are involved in signaling pathways of learning and memory. Antioxidant potency of certain vegetables has equally been shown to influence brain cognitive functions [6]. The brain which is very vulnerable to oxidative stress and inflammation, requires an efficient steady supply of potent antioxidants to neutralize the lipid peroxidation chain reaction, which induces free radical damage. Therefore, exogenous antioxidants obtained from fruits, vegetables and other natural products does influences brain function and health, hence, nutrition plays a crucial role in brain function [7]. With respect to the present study is the neurochemical impact of *Vernonia amygdalina* and *Talinum triangulare* on cognitive function at the level of learning and memory.

Vernonia amygdalina commonly known as bitter leaf is a native of the tropical regions of the world including Nigeria. It is characterized by its deep-greenish foliage and bitter taste, which is one of the characteristics of natural products with enriched bioactive compounds. The HPLC fingerprint of *Vernonia amygdalina* revealed the presence of potent antioxidants [8] and bioactive compounds, such as catechin, chlorogenic acid, quercitrin, quercetin, caffeic acid and luteolin [9]. It has nutritive, antioxidant, anticancer, anti-inflammatory, hepatoprotective, haematological properties, and other pharmacological properties [10], which endorses it, as a unique plant whose usage is versatile. The plant has equally been shown induce weight loss [11] by its cholesterol lowering properties. A particular fraction (F6) of the plant has been shown to have hypoglycemic effect, that is in its ability to reduce blood glucose levels, which endorses its efficacy in the treatment of diabetes mellitus [12]. It is locally used for the treatment of malaria and it has been scientifically proven to have anti-malarial, anti-helminthic, anti-diabetic and laxative effects via its anti-inflammatory activity [13]. The aqueous extract has also been shown to be

very mild with minimal side effects and toxicity, up to 5000mg/kg concentration was shown to be non-toxic [14].

Talinum triangulare (Jacq.) Willd, commonly known as water leaf is equally common in the tropical regions of the world including Nigeria and India. It is characterized by its light-greenish and very soft foliage, it is commonly consumed in West Africa [15]. It is equally a plant with very rich nutritional and medicinal value. *Talinum triangulare* is a very potent source of proteins [15, 16], which makes it a very good source of essential amino acids, which could serve as precursors for potent brain neurotransmitters. It is equally a very potent source of antioxidants such as flavonoids and phenolic compounds, it also contains saponin and alkaloids [17]. *Talinum triangulare* possess immuno-modulatory properties, its active components are of ethno-medicinal importance and it has been shown to be effective against skin infections [18]. It has been shown to enhance proteomic and ascorbate-glutathione metabolism for its significance in detoxification of lead-induced oxidative stress [19]. Furthermore, despite the well documented multi-dimensional usage of the above mentioned plants, their role in cognitive function is still an emerging area of interest. Therefore, the present study revealed the neurochemical impact of the aqueous extracts of the two plants in albino Wistar rats.

2. Materials and Methods

Experimental procedure and animal handling were in compliance with standard measures and the reagents and chemicals used in the present research work were of analytical grade.

2.1. Plant Collection Authentication and Extraction

Fresh mature leaves of *Vernonia amygdalina* (bitter leaf) and *Talinum triangulare* (water leaf) were procured from a nearby garden in the premises of the Lagos University Teaching Hospital (LUTH), Lagos State, Nigeria. Identification and verification was made by Prof. J.D. Olowokudejo, of the Department of Botany, University of Lagos, Lagos, and assigned Voucher Specimen Nos. and deposited in the University Herbarium. The fresh leaves were sun-dried leaves were pulverized, soaked and extracted in distilled water. Thereafter, the extract was sieved using a Muslin cloth and filtered using Whatman No. 1 paper, the crude plant extracts were then stored at 4°C for further use.

2.2. Experimental Animal and Design

Twenty one (21) albino Wistar rats (175.83 ± 6.92g) were obtained from the National Institute of Medical Research (NIMR), Lagos. They were acclimatized for two weeks, and maintained at the Laboratory Animal Centre, College of Medicine, University of Lagos, Lagos, under standard laboratory conditions on commercial pellet diet and water *ad libitum*. The rat pellets were obtained from Livestock Feed Ltd. Ikeja, Lagos State, and the composition comprises of all

the essential nutrients needed for good health, growth and mental well-being. Animals were randomly assigned into groups A, B and C, two treatment groups and a control group (n=7 per group). Groups A and B were administered aqueous extract of *Vernonia amygdalina* (AEVA) and *Talinum triangulare* (AETT) respectively, 2ml per 100g body weight, an equal volume of normal saline was given to group C (control), every other day for thirty [30] days. Twenty Four [24] hours after the last administration, rats in each group were made to undergo some training (learning) and memory tests after which they were sacrificed by cervical dislocation. The cerebrum were excised and assayed for acetylcholinesterase activity and total protein, blood samples were collected for biochemical assay.

2.3. Brain Homogenate Preparation

The brain tissue was quickly removed, washed in ice-cold saline solution. One gram of the brain tissue was homogenized with 10ml of ice cold 0.05M phosphate buffer pH 7.4 using a mini mechanical approach. The homogenate obtained was centrifuged at 7,000rpm for 15 min., in the cold medium, the supernatant obtained was stored at 4°C for further analysis.

2.4. Blood Chemistry

Rat blood samples were taken and centrifuged at 2000 rpm for 10 min, serum obtained was used for biochemical analysis. The following biomarkers were assayed for; aspartate amino transferase (AST), alanine amino transferase (ALT) activities, urea, total protein, total cholesterol, tri acyl glycerol, glucose, high density lipoproteins, low density lipoproteins and albumin concentration.

2.5. Determination of Blood Biochemical Parameters

Liver biomarker enzymes and the other biochemical parameters were determined spectrophotometrically using commercially Randox Kits, with respect to the manufacturer's instruction.

2.6. Determination of Brain Acetylcholinesterase (AChE) Activity

The acetylcholinesterase activity was assayed using a modified method [20]. The assay reaction mixture consisted of brain homogenate (0.2 ml) in a cuvette containing 2.0 ml phosphate buffer (0.1M, pH 8) and 100 µl of 5, 5-dithiobis 2-nitrobenzoic acid. A basal reading was obtained, the reaction was started by adding 20µl of the substrate acetylthiocholine and the change in absorbance was monitored at 412 nm for 10 min at 2 min interval. The specific enzyme activity was expressed as moles of substrate hydrolyzed/min/mg of the brain protein. Total protein in brain homogenate was determined using the Biuret method.

2.7. Behavioural Assay

A Shuttle box, used in the assessment of short term memory in rats was used in the present study according to the

modified method of Ebuehi and Akande [21]. The apparatus comprises of two wooden compartments of identical dimensions (28 by 15cm) separated by a door in the middle. One of the two compartments was illuminated (the first compartment) and the other was dark (second compartment). Under normal circumstances, the dark compartment is usually loved by the rats, because it is the "safe" compartment. The rats could enter into any of the two compartments once the door is raised. The floor consisted of 6mm diameter wire rods (spaced 1.7 cm apart at the center) through which 1.5mA of scrambled foot shock was administered. The rods were connected to a set down transformer with a regular dimmer which could be switched on and off to deliver an instant scrambled foot shock to either compartment. The wooden door allows the rats to cross to the other compartment, in order to avoid or escape foot shock [21].

2.8. Acclimatization

On the first day of training, all rats in the three groups were placed in the Shuttle box and allowed to have access to both the light and dark compartment for a period of 30 min up to an hour, in order for them to get familiar with the two compartments.

2.9. Learning

Following the acclimatization, the next day, rats from each group were placed in the illuminated compartment for about 30 seconds after which the door was raised. As soon as the rats entered the dark compartment, the middle door was closed and a 1.5mA instant current shock was applied for about 20 seconds, after 20 seconds, the rats were removed from the dark compartment and placed in their home cages, this learning task was done repeatedly at different intervals on the same day. The learning skills acquired, determined their memory [21].

2.10. Memory Assay

Twenty four (24) hours after training, each rat from each group was placed in the illuminated compartment and 30 seconds later, the door was raised, the time spent in the light compartment before entering the dark compartment was recorded, as a measure of their memory. The memory testing procedure was repeated for a period of one week [21].

2.11. Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD), with the aid of SPSS version 24.0 and GraphPad Prism version 6.0 statistical software. Significant differences were analyzed using one-way ANOVA with Dunnet's multiple comparison tests. The results obtained were considered as statistically significant if $p < 0.05$.

3. Results

There was a significant increase in general body weight

of treated rats relative to the control. *Vernonia* group A; 176.5 ± 4.6 , *Talinum* group B; 161.8 ± 2.9 and the Control group C; 150.8 ± 16.5 grams respectively ($p < 0.05$). Considering the weights before and after extract administration, there was a significant increase in body weight of AEVA treated rats after the experiment ($p = 0.0028$), however, there were no significant changes in the body weight of the AETT treated rats after the experiment ($p = 0.1729$) compared to the control group (Fig. 1A).

In the learning and memory assay, there was a significant improvement in the memory of both AEVA and AETT treated rats on days 1, 2, 3 and 6 compared to the control group C ($p < 0.05$). Both treated groups had an increased memory output with respect to the longer time spent in the light compartment, having recalled the foot shock experienced in the dark compartment, even in the absence of a shock (Fig. 1B).

There was a significant decrease ($p < 0.05$) in blood glucose levels in the treated groups compared to the control, most especially in the AEVA treated group (Fig. 1C).

In the brain cholinergic activity assay, *Vernonia* treated rats showed a significant increase in acetylcholinesterase

activity relative to control ($p = 0.0242$), however, there were no significant changes in the acetylcholinesterase activity of the *Talinum* treated group relative to control ($p = 0.1987$), (Figure 1D).

The blood biomarkers revealed an increased levels of AST in the treated rats relative to control, however, the blood ALT was significantly reduced in the *Vernonia* treated group, while there were increased levels in the *Talinum* treated groups. There were increased levels of albumin and urea in the treated groups, HDL (High density lipoprotein; the good cholesterol) was significantly increased in the *Talinum* treated group, while there were no significant changes in the LDL (Low density lipoprotein; the bad cholesterol). Total cholesterol was increased in the *Talinum* group. Triglycerides were significantly reduced in the *Vernonia* treated group relative to control as shown in table 1. As for the concentration of protein in brain homogenate, the brain protein concentration in the *Vernonia* treated group was significantly reduced while there were no significant changes in protein concentration in the *Talinum* treated group compared to the control group as shown in Table 2.

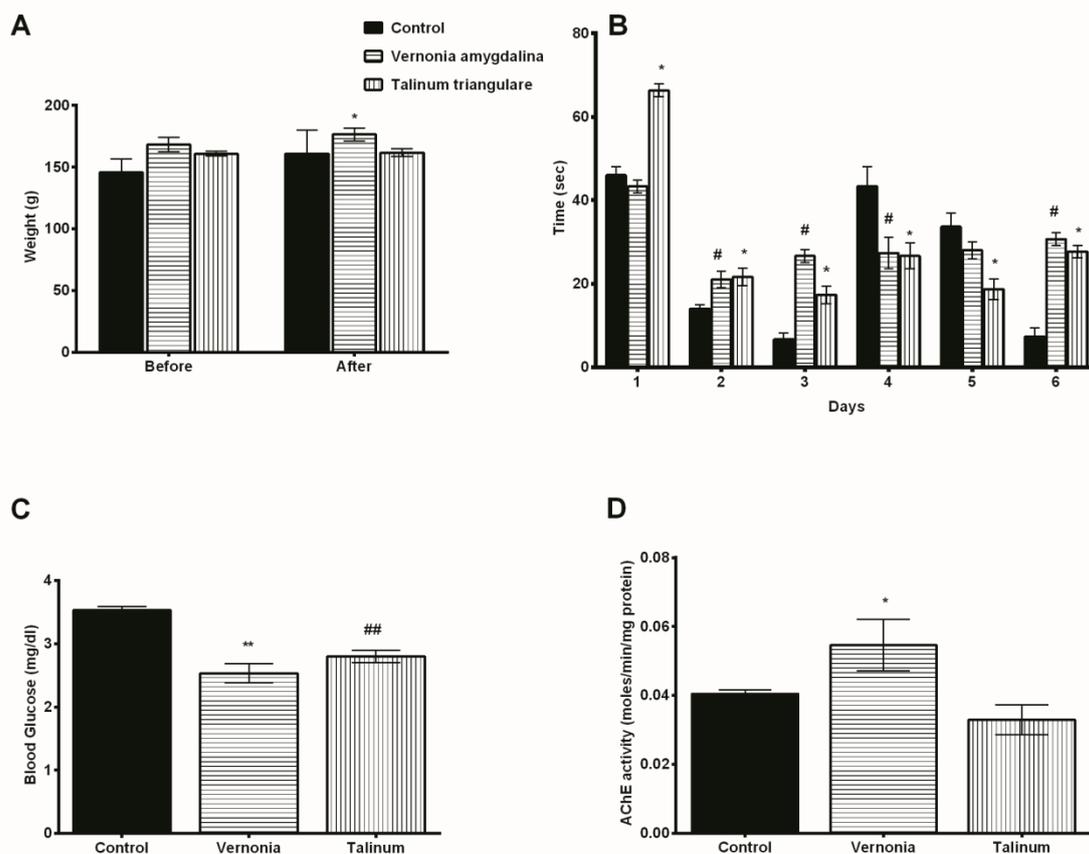


Figure 1. *Vernonia amygdalina* and *Talinum triangulare* enhanced learning and memory and brain acetylcholinesterase activity in rats. A. There was a significant increase in body weight of treated rats relative to control. B. There was a significant increase in memory of treated rats relative to control with respect to the longer time spent in the light compartment of the shuttle-box. C. The blood glucose levels of treated rats were significantly reduced relative to control. D. There was a significant increase in brain acetylcholinesterase activity in *Vernonia* treated group, but there were no significant changes in the activity of *Talinum* treated group relative to control. [Significant values; $p < 0.05$, as evaluated by one-way ANOVA, Dunnett's multiple comparison test]

Table 1. Blood biochemical parameters

Group	AST (U/L)	ALT (U/L)	ALB (mg/dl)	Urea (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	T.CHO (mg/dl)	T.G (mg/dl)
Control	95.2±5.5	49.2±7.8	33.3±0.7	6.6±0.5	1.3±0.3	0.3±0.2	0.9±0.3	1.2±0.4
<i>Vernonia</i>	103.9±13.8*	37.8±7.6*	35.8±2.9	7.1±0.2	1.2±0.1	0.2±0.1	1.1±0.1	0.7±0.1*
<i>Talinum</i>	111.3±10.2*	63.3±5.1*	35.2±0.6	7.4±0.5*	1.7±0.3*	0.4±0.2	1.6±0.4*	1.3±0.5
Values are expressed as Mean ± SD, p < 0.05 (One-way ANOVA, Dunnett's multiple comparison test)								

Table 2. Total protein concentration in rat brain homogenate

Group	Total Protein Conc. (mg/ml)
Control	10.34±1.93
<i>Vernonia amygdalina</i>	7.75 ± 1.83*
<i>Talinum triangulare</i>	11.89 ± 1.27
Values are expressed as mean ± SD. p < 0.05	

4. Discussion

The increase in body weight experienced by the AEVA and AETT treated groups could be as a result of their packaged nutrients, both supplements are very rich in vitamins and minerals which are key players in most metabolic pathways, these nutrients could have contributed to their body weight enhancing effect. *Talinum triangulare* has been reported to be a potent store of proteins, vitamins [16], nutritive antioxidants and bioactive compounds of nutritional importance [21, 22]. It has been reported to enhance the activities of endogenous antioxidant enzymes, as such, it could be helpful in the phyto-therapeutic management of common diseases associated with the hypoactivity of the enzymes, when it comes to diet and nutrition [23]. Furthermore, a study on the mineral profile of *Talinum triangulare* reveals that it contain essential minerals such as Na, K, Zn and Mn, therefore, the vegetable efficiently contribute to nutritional requirement and food security [22].

The present study equally revealed the hypoglycemic properties of *Vernonia amygdalina* and *Talinum triangulare*, the current findings are in line with previous study which showed that *Vernonia amygdalina* has hepatoprotective and hypoglycemic effect [24], coupled with the fact that the aqueous extract of *Vernonia* has been reported to be non-toxic [14]. The anti-diabetic effect of *Vernonia* was equally reported by Onasanwo *et al.*, [13], the plant was able to exhibit this effect via its anti-inflammatory mode of action [13]. Cognitive dysfunction associated with diabetes was shown to be ameliorated by *Vernonia amygdalina* [24]. *Talinum triangulare* has equally been shown to have an ameliorative effect on diabetic induced cognitive impairment, the plant has anti-diabetic effect just like *Vernonia amygdalina* [25, 26].

The vitamins and minerals in *Vernonia amygdalina* and *Talinum triangulare* provides the body with essential protective antioxidants, these antioxidants require the help of coenzymes for efficient metabolic activities, and these coenzymes could be obtained from orally consumed vitamins and minerals in vegetables and fruits. Antioxidants offer protection to the brain cells from the havoc of oxidative stress. It was previously reported that waterleaf consumption has benefiting effects on the neurons of the cerebrum and may probably enhance the cognitive ability [15], therefore, the present study has revealed the ability of *Talinum triangulare* (water leaf) to enhance cognitive ability through learning and memory. This study equally revealed the memory enhancing ability of *Vernonia amygdalina*, this is in-line with the work of Ademosun *et al.*, (2017) [9], they have reported that *Vernonia amygdalina* may offer neuroprotective effects through the inhibition of AChE, BChE and MAO in the process, exerting oxidative stress inhibition, therefore, *Vernonia amygdalina* may possess memory enhancing properties [27]. Some medicinal plants have been effectively used in the management of Alzheimer's' disease and memory deficit disorders.

In order to understand the possible mechanism by which these plants may have enhanced learning and memory in the present study, their effect on acetylcholinesterase activity revealed that, they could modulate the availability of the neurotransmitter Acetylcholine through the activity of the enzyme acetylcholinesterase as shown in Figure 1. Acetylcholine is highly implicated in the modulation of learning and memory [4]. Certain plant extracts have been shown to inhibit acetylcholinesterase activity. This cholinergic enzyme is highly implicated in cognitive dysfunction and pathogenesis of Alzheimer's disease, because clinical studies have shown that the sufferers of

cognitive dysfunction, exhibited some behavioral improvements when cholinesterase inhibitor therapy commenced [4]. The present study showed that *Vernonia amygdalina* increased the activity of the acetylcholinesterase enzyme, while there was no significant changes of enzyme activity observed in the brain of *Talinum triangulare* treated group of animals. The memory enhancing effect of these two plants shown in the present study could be as a results of their neurochemical activity, their ability to enhance the bio-availability of the neurotransmitter, Acetylcholine.

Moreover, Ekong *et al.*, (2016) reported that there were no significant changes in the learning and memory of animals treated with a combination of *Vernonia amygdalina* and *Rauwolfia vomitoria*, they equally reported that the extract have no significant effect on the activities of liver enzymes; AST, ALT, ALP cholesterol and triglyceride [28]. Aspartate amino transferase AST, Alanine amino transferase ALT, are important enzymes involved in protein metabolism, they are liver function enzymes and efficient biomarkers of liver diseases. In the present study, there were elevated levels of AST in the blood of treated rats relative to control, this could be as a result of possible metabolic stress effects, however, blood ALT was significantly lowered in the *Vernonia* treated group, relative to control. Nevertheless, *Vernonia amygdalina* is well documented to have hepatoprotective effects [10], that is, they offer protection to the liver cells.

We equally report a significant reduction in the blood triglycerides and LDL of rats treated with *Vernonia amygdalina*, this reveals the ability of the plant to modulate blood cholesterol level, this is in line with the work of Egedigwe *et al.* [11], that the aqueous and methanol leaves extract of *Vernonia amygdalina* induces weight loss and can alleviate the burden of obesity [11].

The ability of the two plant extracts to enhance cognitive function in rats at the level of learning and memory could be associate with their high antioxidative capacities, neurochemical modulatory effects and nutritive values. There are some plant products which have been reported to enhance cognitive performance due to their enhancement of the brain acetylcholinesterase activities [6]. Wet and dry samples of *Talinum* has revealed appreciable amount of phytoconstituents such as flavonoids, which are potent antioxidants [29], the aqueous and ethanol extract of *Talinum* have equally been shown to enhance the activities of endogenous antioxidant enzymes SOD, CAT, reduced glutathione [22], key biomarkers involved in glutathione metabolism has been identified in the same plant, *Talinum* [19].

Their antioxidative property endorses their immuno-modulatory effects, for instance, pre-biotics extracted from *Vernonia amygdalina* has been shown to possess immuno-modulatory properties. Reducing power by electron donating effect is an indicator of antioxidant activity of the bioactive compounds assessed in *Talinum triangulare* [30]. In other terms, the ethanolic extract of *Talinum* was efficiently used in the management of Urinary schistosomiasis; snail fever [31]. The studied plant extracts

were able to mediate brain and body biochemical homeostasis, in the process enhancing brain function through learning and memory. Nutrition influences virtually everything in the life of an organism such as cognitive function, locomotion and also ageing [32].

5. Conclusions

In conclusion, in the evaluation of the neurochemical impact of the aqueous extracts of *Vernonia amygdalina* (AEVA) and *Talinum triangulare* (AETT) on cognitive function in albino Wistar rats, the two aqueous extracts have been shown to exhibit memory enhancing effect through the modulation of the cholinergic activities in the brain which endorses their efficacy through learning and memory as neuroprotective agents. Equally, we reported their blood glucose and lipid lowering effect, which endorses their efficacy in the management of certain metabolic disorders such as diabetes and obesity. Further work should be carried out to evaluate their effect on long term memory, neuronal cell death inhibitory effects in the brain tissue, and extensive screening of the plants for the identification and isolation of their actual bioactive components, for characterization purposes.

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Abbreviations

AEVA; Aqueous leaf extract of *Vernonia amygdalina*, **AETT**; Aqueous leaf extract of *Talinum triangulare* (Jacq.) Willd, **AChE**; Acetylcholinesterase enzyme, **ALT**; Alanine amino transferase, **AST**; Aspartate amino transferase, **ALB**; Albumin, **HDL**; High density lipoprotein, **LDL**; Low density lipoprotein.

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