Lipid Peroxidation and Glutathione Level Following Scopolamine-Induced Cognitive Dysfunction in Rats: Potentials of *TelfairiaOccidentalis*Seeds and *TaliniumTriangulare*Leaves Aqueous Extracts

ABSTRACT

Background: Oxidative stress augmented with progressive age, causes changes in mitochondrial DNA, mitochondrial disruption and more oxidative trouble. This process is facilitated in Alzheimer's disease by the pathology of αβ amyloid and activated microglia.

Objectives: The present study compared the potentials of aqueous extract of *Telfairiaoccidentalis*(TO) seeds and *Talinumtriangulare*(TT)on the malondialdehyde (MDA) and glutathione (GSH) levels following scopolamine hydrobromide (SHB)-induced Alzheimer's type cognitive dysfunction in rats.

Methods: Forty-two Wistar rats were arrayed into seven arrays (I-VII). Alzheimer's type cognitive dysfunction was induced in arrays II-VII by administering intraperitoneally (IP) 1 mg/kg body weight (BW) of SHB for seven days before aqueous extracts of TO (850 mg/kg and 1750 mg/kg), TT (850 mg/kg and 1750 mg/kg) and donepezil (1 mg/kg) administrations for 14 days. The rats' blood serums were taken and analyzed.

Result: The MDA estimation in group II (672±39.65), VI (707.67±24.99) and VII (671.64±32.07) increased significantly compared with others ($P<0.05$). Glutathione level was significantly increased in arrays I (0.44±0.05), IV (0.41±0.08), V (0.46±0.07) and VII (0.44±0.05) compared to others.

Conclusion: Aqueous extract of TOseeds reduced MDA levels and both extracts increased glutathione levels in Alzheimer's type cognitive dysfunction rats; though the effect of TT was dose-dependent.

Keywords: Alzheimer's disease, Glutathione, Lipid peroxidase, Oxidative stress,Wistar rats.

INTRODUCTION

Lipid peroxidation is an act through which oxidants such as free radicals or non-radical species fight lipids with carbon-carbon double bonds, such as polyunsaturated fatty acids[1]. At sub-toxic conditions (low lipid peroxidation), the cells actuate their maintenance and survival through antioxidant defense mechanisms or signaling pathways activation that up-regulate antioxidant proteins resulting in an adaptive stress response. At medium or elevated lipid α peroxidation states (toxic condition), the extent of oxidative stress overpowers repair capacity and causes cells' apoptosis or necrosis; both processes eventually lead to molecular cell destruction which may hasten the development of different disease states and accelerate ageing[2].

On the other hand, Glutathione directly removes different oxidants such as superoxide anion, hydroxyl radical, nitric oxide and carbon radicals. Glutathione catalytically neutralizes hydroperoxides, peroxynitrites and lipid peroxides[3]. In health, accumulation of glutathione disulphide due to oxidative stress is directly poisonous to cells, causing programmed cell death by activation of the stress activated protein kinase/mitogen-activated protein kinase (SAPK/MAPK) pathway[4]. Glutathione depletion triggers apoptosis[3]. The disease associated with glutathione depletion includes neurodegenerative diseases such as Alzheimer's (AD), Parkinson's and others[5].

Oxidative stress augmented with progressive age, causes changes in mitochondrial DNA, mitochondrial disruption and more oxidative trouble. This process is facilitated in AD by the act of αβ and actuated microglia[6]. Oxidative trouble underlies the gradual neurodegenerative characteristics of AD[7]. Reports have it that oxidative stress elevates the expression and turns on β and γ secretase and inhibits the actions of α-secretase[8]. High formation and absence of Aβ peptides removal can

sediment αβ which energizes different cell signaling networks, hence, causing degradation of synapses, loss of neurons and reduced cognitive function[9].
In vitro experiments revealed αβ elevated H_2O_2 and lipid peroxidase statuses. Age-associated αβ sedimentation elevates hydrogen peroxide, nitric oxide formation and oxidative mutation of proteins and lipids deducing that αβ causes oxidative stress[10,11]. In hippocampal neuron cultures, the cause of oxidants by Aβ needs the stimulation of N-methyl diaspartate (NMDA) receptor and quick elevated neuronal Ca^{2+} status[12].The externally generated antioxidants are mainly from food and herbs[13]. These antioxidants abet to counteract the surplus enlarged radicals, guard the cell against envenom effects and contribute to ailment aversion[14] However, comparing the effects of aqueous extracts of *Telfairiaoccidentalis*(TO) seeds and *Taliniumtriangulare* (TT) leaves on the lipids and glutathione levels using Alzheimer's type cognitive dysfunction rats may provide in-depth knowledge to their physiology, thus, thenecessity for this research.

MATERIALS AND METHODS

Experimental animals

With ethical approval number: FAREC-FBMS 042ANA3719, forty-two adult Wistar rats with both sexes and weight ranging from 180-200g were bought from the animal farm and kept in the Departmental animal room for two weeks. Before the experiment, the experimental animals were kept for acclimatization under standard conditions of temperature (27^oC – 30^oC), given rat chow and water *ad libitum*. After two weeks of adjustment, the rats were arbitrarily arrayed into seven arrays; each having six rats designated I-VII.

Plant extract preparation

Fresh TOseeds and TTleaves were bought from the market in Cross River State, Nigeria. These seeds and leaves were registered with voucher numbers: HERB/BOT/UCC/322 and HERB/BOT/UCC/120 in Botany Department, University of Calabar, Calabar. The TO seeds were exposed from the husks, washed alongside with the TT leaves, reduced into tiny parts and dehydrated (air dried) in the laboratory. The dehydrated specimens were pulverized into powdered form (1600g) with ablender (model number Bravo3JARS Mixer grinder) and then soaked in 1000 mL of distilled water for 24 hours. The admixture was purified with Whatman No.1 filter paper and chess cloth. The products were collected and made potent to a gooey remnant at 40-50° and kept in a cool dry place for later use.

Alzheimer's type cognitive dysfunction induction

1.0 mg/kg bw of SHB (bought from Bristol Scientific Company, Bristol Road, Apapa, Lagos- Nigeria) was intraperitoneally (IP) injected to adult Wistar rats in arrays II-VII for seven days to establish Alzheimer's type cognitive dysfunction.

LD_{50} determination

Using Lorke's method[15], LD_{50} of aqueous extracts of TO seeds and TTleaves were both established to be >7000 mg/kg and doses were determined using 12.5% and 25% of the established LD_{50}.

Plants extract and donepezil administration.

Array I served as the negative control and received animal feed and water *ad libitum*; array II served as the positive control and received 1.0 mg/kg body weight of SHB only; array III received 1.0 mg/kg body weight of SHB and 1.0 mg/kg body weight of Donepezil (bought from Bez Pharmacy, Etta AgboCalabar-Nigeria); array IV received 1.0 mg/kg body weight of SHB and 875 mg/kg body weight of aqueous TOseeds; array V received 1.0 mg/kg body weight of SHB and 1750 mg/kg body weight of aqueous TO seeds; array VI received 1.0 mg/kg body weight of SHB and 875 mg/kg body weight of aqueous TTleaves while array VII received 1.0 mg/kg body weight of SHB and 1750 mg/kg body weight of aqueous TT leaves. These extracts and drugs were administered for fourteen days.

Determination of MDA and glutathione

24 hours after the last extracts and drug administration, the experimental animals were sacrificed and the blood serums were taken through cardiac puncture for analysis. Serum levels of Lipid peroxidation and glutathione peroxidase were used as markers for oxidative stress. Lipid peroxidation was estimated by measuring 0.25ml of serum and 1.25ml of 10% trichloroacetic acid and added to a clean centrifuge tube and allowed for 10 minutes. 1.25mls of 0.05 M H_2SO_4 and 1.5ml of 0.67 TBA (thiobarbituric acid) were placed in aboiling water tap and 2ml of butanol was added. Thiobarbituric acid reactive material was extracted, and absorbance wasread at 532nm wavelength.

Glutathione was estimated using 40µL of the reaction mix and summated with the specimen, positive control and reagent control wells, properly combined and was incubated at room temperature for 15 minutes to deplete all glutathione disulphite (GSSG) in the samples. 10µL of cumene hydroxide solution was added to start the glutathione peroxidase reaction and mixed well. The output (A1) was measured on a microplate reader at OD340nm at T1 and incubated at 25^{o}C for 5 minutes (protected from light). The output (A2) was then measured on a microplate reader at OD340nm at T2.

Statistical analysis

Data were analyzed using astatistical package for social science version 21.0 The student t-test was used with data represented as mean ± standard error of themean (SEM) and statistically significant at $p < 0.05$.

RESULTS

SHB administered to array II elevated MDA level (672± 39.65) when analogized to the negative control arrayl (336± 39.21) and Donepezil treated array III (446±78.33). TO(875mg/kg and 1750 mg/kg) administration to arrays IV and V meaningfully decreased MDA level (487±78.33; 477±29.50) when compared to SHB array II and TT(875mg/kg and 1750 mg/kg BW) treated arrays VI and VII (707.67±24.99; 671.64 ±32.07) at P<0.05 (figure 1). Rats in TTarrays VI and VII revealed minimal elevation compared to the SHB array II. Array V givenan elevated dose of TOshows a meaningful reduction (P<0.05) in MDA levels as analogized to negative control array I (figure 1).

Increased glutathione is a label of cellular antioxidant and givesdefenseagainst oxidative trouble. SHB treated array II rats (0.15±0.56), TT treated array VII(0.22±0.29) and Donepezil treated array III (0.20±0.00) revealed a meaningful declined glutathione status when analogized to control array I (0.49±0.21), TO treated arrays IV and V (0.41±0.08 and 0.46±0.07) and low dose TT treated array VI(0.44±0.05). The rats in array V treated with 1750mg/kg of TOrevealed aslight reduction (P<0.05) of GSH levels analogized to thearray I and significantly increased compared to Donepezil treated array III (0.46±0.18) and SHB treated array II. Rats in array VII treated with high dose TT(1750 mg/kg) showed a meaningful decrease (0.22±0.29) at P>0.05 (figure 2).

Table 1: showing Malondialdehyde (MDA) and Glutathione (GPX) concentrations in diverse experimental arrays

Arrays	MDA (Mean ± SEM)	GSH (Mean ± SEM)
I	336±39.21	0.49±0.21
II	672±39.65	0.15±0.56
III	446±78.33	0.20±0.00
IV	487±78.33	0.41±0.08
V	477±29.50	0.46±0.07
VI	707.61±24.99	0.44±0.18
VII	671.64±32.07	0.22±0.29

Data are represented as mean±SEM, n=6.
* = Meaningfully dissimilar control at P < 0.05
a = Meaningfully dissimilar scopolamine hydrobromide at P < 0.05.

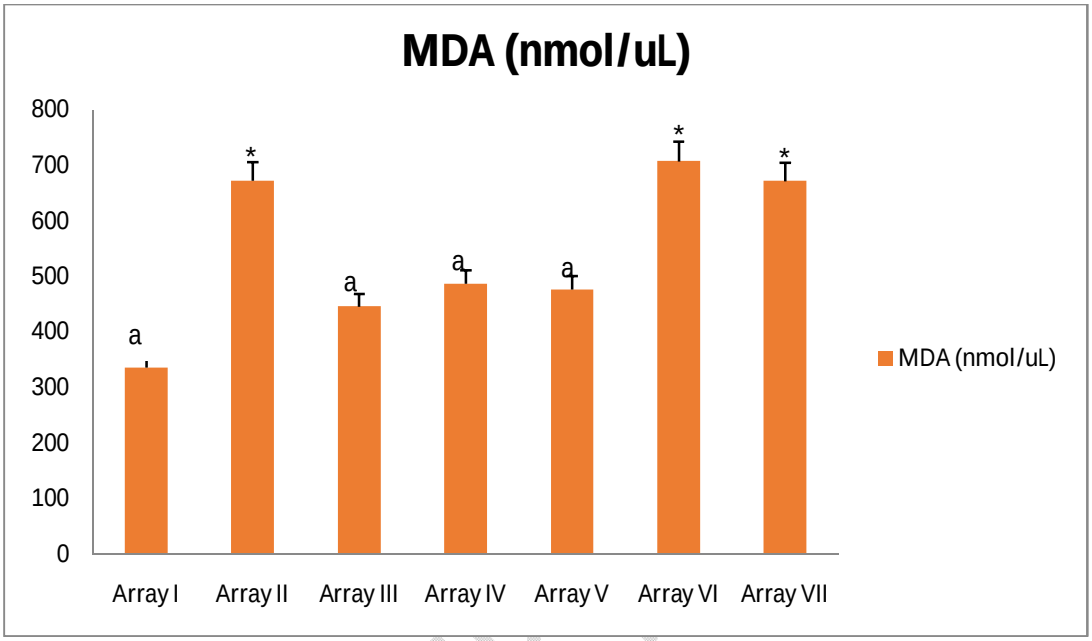

Figure 1: Malondialdehyde concentrations in diverse experimental arrays
Data are represented as mean±SEM, n=6.
* = Meaningfully dissimilar control at P < 0.05
a = Meaningfully dissimilar scopolamine hydrobromide at P < 0.05.

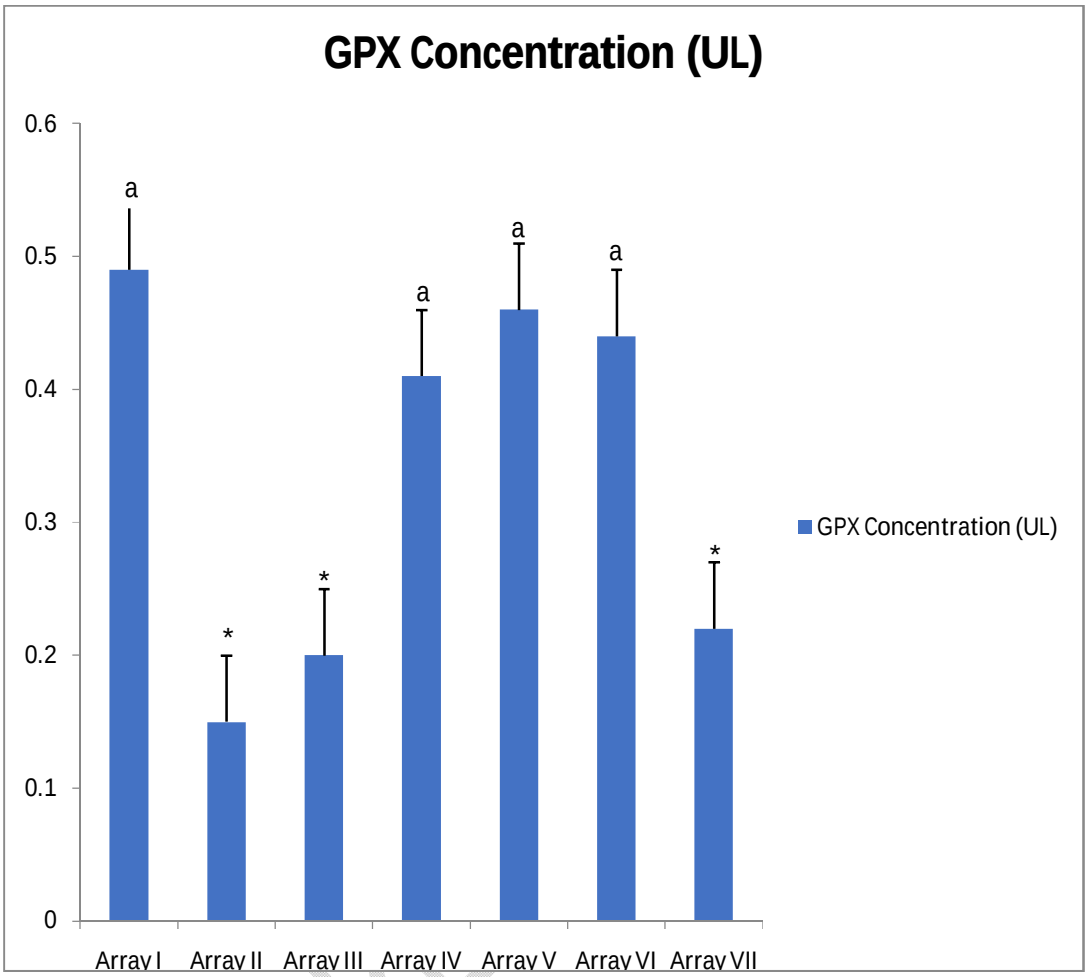

Figure 2: Glutathione (GPX) activity in the dissimilar experimental arrays.
Data are represented as mean±SEM, n=6
* = Meaningfully dissimilar control at P < 0.05
a = Meaningfully dissimilar scopolamine hydrobromide at P < 0.05.

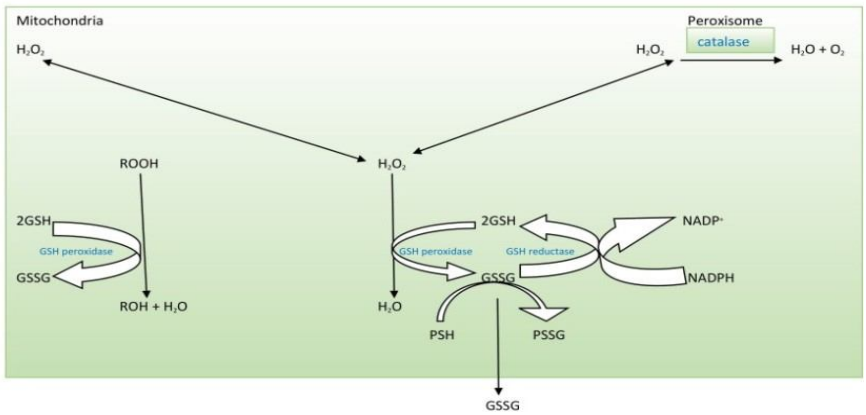

Figure 3: Biosynthesis of glutathione

DISCUSSION

The generation of free radicals associated with enlarged oxidative stress is attributed to the pathogenesis of Alzheimer's disease resulting in ageing and cell apoptosis[16]. Studies suggested the involvement of oxidants in the neurological and neurodegradative ailments, thus directing the way of free radicals in progression of Alzheimer's ailment. The use of herbal and natural extracts for neurological, psychiatric and neurotoxicological ailments has been elevated greatly because of their no or reduced side effects[17]. *Citrus esculentus* extract reversed radiation-induced damage on the ultrastructure of albino Wistar rats' testes[18], *Averrhoa carambola* aqueous fruit extract ameliorates diazepam-caused poison of the hippocampus in rats[19], *Musa paradisiaca*stem juice limit the extent of status epilepticus, increased neuronal protein synthesis, reduced cytoarchitectural damage and astriogliosis as well as enhancing long term recognition memory in rats[41,42]while*Ziziphusjujuba* fruit protects oxidative brain damage in rats [20]. Also, while Agbor and Anyanwu reported that β-D-Glucagon polysaccharide supplementation ameliorates hypoglycaemic-induced nephrotoxicity in diabetic Wistar rats[21], Cennet*et al* linked bulbs of *Muscarimuscarimi*extract having more marked anticancer activities against H1299 cell line than other cell lines[22] and Nema*et al*demonstrated that erythropoietin is cytoprotective and blocked neurodegeneration by inhibiting caspase activity and apoptosis[23]; *Talinumtriangulare*leaves possess antioxidant activities which was linked to amelioration of the atrophied hippocampal microstructures as well as aiding learning and recall in Alzheimer's type cognitive dysfunction rats[24].

In this study, there was a meaningful elevation of serum MDA levels in arrays II (treated witha SHB alone),VI and VII (treated with TT)analogous to the negative control in array I with a reduction in MDA level in arrays III, IV and V (figure 1). This result is in line with research that documented that TOseeds reduce lipid peroxidation at lower doses[25]. The SHB has been found to increase oxidative stress and improve the antioxidative defense system[26,27]. This is similar to reports that SHB significantly increased MDA levels in rats compared to the control[28]. Lipid peroxidation is a major pointer of neuron degradation of thebrain. Unlike other body envelops, neuronal envelops encompass a very elevated percentage of long chain polyunsaturated fatty acids and oxidants are continually formed during metabolism[29]. Lipids and proteins, the important anatomical and physiological constituents of the cell membranes are the mark of oxidative alteration by free radicals in neurons degrading ailments[30]. A broad fact documented on lipid peroxidation and protein oxidation resulting to loss of envelop structure, a good agent to increase aging and age-related neurodegenerated diseases. Oxidative trouble has been enlaced in the pathogenesis of AD in humans[31]. This still lends credence to the fact that neurodegenerative changes in histological, histochemical, immunohistochemical and neurobehavioral observations of the hippocampus could be attributed to activities of free radicals generated by SHB; hence, aqueous extract of TOseedsand TT leaves were able to ameliorate the effects[32,33,34,35,40].

Glutathione plays an important part in many metabolic and biochemical reactions such as DNA mixture and repair, protein mixture, prostaglandin mixture, amino acid movement and enzyme actuation[36]. According to Penninckx[37], glutathione also called y-L-glutamyl-L-cysteinylglycerine is a redox-action tripeptide thiol found in the cells of a living organism. This bio-molecule serves as adetoxicant and acts as an antioxidant. During acute oxidative stress, the cells may not have the ability to reduce glutathione disulphite (GSSG) to GSH which may lead to accumulation of GSSG in the cell. This may be averted either by ingestion of diet containing antioxidants(such as TO seeds and TT leaves) or removing the accumulated GSSG from the cytosol. Hence, all system in the body can be influenced by the state of the glutathione system including the nervous system. In this study, alow level of glutathione was observed in arrays II, III and VII analogous to the negative control array I (figure 2). This correlates with theresult fromthe MDA assay which showed increased levels of free radicals. This still showed a trend that scopolamine generates free radicals and increases oxidative stress (figure 1). A research work revealed that scopolamine caused behavioral and biochemical alterations in rats[39]. Their result showed that scopolamine-induced memory impairment increases MDA and decreases glutathione levels in hippocampal homogenate. Animals treated with TOseed

and TTleavesshowed increased levels of glutathione peroxidase compared to the SHB treated array II though the effect of TTwas dose dependent (figure 2). The changes could be linked to the antioxidant capabilities of TOseeds and TT leaves. Consumption of antioxidants via diet and supplements is expected to turn off reactive oxygen species from the living system and give health benefits. The antioxidant potentials of TOseeds observed in biochemical analysis correlate with the Morris water maze test where extract of TO seeds aid learning and recall[35]. Glutathione is important antioxidant according to Lu[38] where free radicals (such as hydrogen peroxide) generated as a result of aerobic metabolism are metabolized by GSH peroxidase in the cytoplasm and mitochondria and by catalase in the peroxisome of a cell. The formed GSSG is reduced back to GSH by GSSG reductase at the expense of NADPH (figure 3). The organic peroxidase (ROOH) can be reduced by GSH peroxidase or GSH S-tranferase. Under acute oxidative stress as seen in array II only (figure 2) may result to theinability of the cell to reduce GSSG to GSH, hence causing GSSG accumulation in the cell. To avoid a shift in the redox equilibrium, GSSG can be actively transported out of the cell or react with a protein sulfhydryl (PSH) to form a mixed disulfide (PSSG) (figure 3)[38]. In all the analysis, there is still a trend of protection observed that could be due to the plants' antioxidant potentials that may activate the biosynthesis of the glutathione leading to either the transport of GSSG out of the cell or forming PSSG by reacting with PSH.

CONCLUSION

In conclusion, aqueous extracts of TOseeds reduced MDA, TTleaves increased MDA and increase glutathione levels which may provide an enabling environment for cell survival and behavior using SHB-induced Alzheimer's type cognitive dysfunction rats.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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