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ORIGINAL ARTICLE

Protective Effect of Afang (*Gnetum africanum*) Vegetable Inclusive Diet in Cyclophosphamide-Induced Oxidative Stress in the Brain of Male Wistar Rats

Esther Emem Nwanna

Authors' Affiliation

Department of Biochemistry, Federal University of Technology. P.M.B 704 Akure, Nigeria

Corresponding author

Esther Emem Nwanna

Department of Biochemistry, Functional Food and Nutraceutical Unit. Federal University of Technology. P.M.B 704 Akure, Nigeria

Email:
eenwanna@futa.edu.ng
esthernwanna@gmail.com

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Abstract

Afang (*Gnetum africanum*) leafy parts are used in fore lore medicine and in soup preparation. The protective effect of dietary inclusion of *Afang* (2 and 4%) inclusive diet on cyclophosphamide (75 mg /kg body weight) induced oxidative stress in rat's brain was assessed on endogenous enzymes parameters. The dietary inclusion caused a significant ($p<0.05$) decrease in malondialdehyde (MDA) produced in rat's brain while the key enzymes linked to neurodegeneration such as acetylcholinesterase, butylcholinesterase and monoamine oxidase activities decreased significantly when compared to cyclophosphamide-induced and the Donepezil groups as a result of the diet-inclusion. Also, there were reduction in alkaline phosphatase and aspartate amino transferase activities with an increase in catalase activity and vitamin C content, which indicates its high antioxidant properties. *Afang* leafy vegetable is rich in bioactive compounds and could be used to manage neurodegenerative diseases.

Practical application

Afang which is indigenous edible leafy vegetables from the Southwestern part of Nigeria scientifically known as *Gnetum africanum* which is used in soups preparation and in folklore for the management and treatment of human ailments. The leaf is a rich source of alkaloids, phenolic compounds and antioxidant vitamins which makes the leafy vegetable diet a potential plant food for healthy living and in the management of neurological stress.

Keywords : *Afang, neurodegeneration, antioxidant, endogenous enzymes, malondialdehyde*

1. Introduction

Neurodegeneration describes the gradual deterioration in a person's cognitive abilities, such as memory. This loss is due to either structural change that prevents neurons (brain cells) from functioning normally, or to cell death. Neurodegeneration is a key feature of several diseases that are referred to as "neurodegenerative diseases" (Nwanna *et al.,* 2020). Mitochondrial dysfunction and the consequent increase in reactive oxygen species (ROS) also trigger a sequence of events that leads to cell demise (Young Soo *et al.*, 2012).

The high lipid content of nervous tissue, coupled with its high aerobic metabolic activity, makes it particularly susceptible to oxidative damage (Bauer *et al.*, 1999). There is substantial evidence that oxidative stress is a causative or at least ancillary factor in the pathogenesis of major neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS, "Lou Gehrig's disease") as well as in cases of stroke, trauma, and seizures (Ghadge *et al.*, 1997). The brain is highly sensitive to oxidative stress because this 1300 g organ consumes about 20–30 % of inspired oxygen, contains high levels of polyunsaturated fatty acids

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(PUFAs), ideal target of free radical attack, and high levels of redox transition metals. In particular, markers of lipid peroxidation have been found to be elevated in brain tissues and body fluids in several neurodegenerative diseases, including Alzheimer disease (AD), Parkinson disease (PD), amyotrophic lateral sclerosis (ALS), Huntington disease (HD) and Down syndrome (DS) (Butterfield *et al.*, 2010). Currently, there is no therapy clinically available that delays the neurodegenerative process, and therefore modification of the disease course via neuroprotective therapy is an important unmet clinical need. Donepezil was developed by Eisai and Pfizer is used to improve cognition and behavior of people with Alzheimer, but does not slow the progression of or cure the disease (Knowles, 2006). Common side effects include loss of appetite, gastrointestinal upset, diarrhea, difficulty sleeping, vomiting, or muscle cramping. Donepezil acts as a centrally acting reversible acetylcholinesterase inhibitor (Grossberg, 2003). The side effects of the synthetic drugs have increased the search for medicinal plants with antioxidant and neuroprotective properties.

Gnetum africanum is a leafy vegetable desired in different African countries and especially south-eastern Nigeria (where it is commonly referred to as "*afang*" for its nourishment and medicinal properties (Nwanna *et al.*, 2015). In Nigeria, the leaf of *G. africanum* is used in the treatment of an enlarged spleen, sore throats and as a cathartic and it was also reported that flavonoids, phenols anthocyanins have been shown to be present in the leaves (Akintola *et al.*, 2012). This study was undertaken to investigate the protective properties through the inclusion of the *afang* leaves in a formulated diet and its modulating ability in cyclophosphamide-induced oxidative stress in wistar rat's brain.

2. Materials and methods

2.1. Sample Collection and Preparation

Gnetum africanum leafy vegetable was purchased from a local market in Akure, Ondo state Nigeria and authenticated at the Crop, Soil and Pest Department FUTA. The leaves were washed clean in tap water and allowed to dry overnight at room temperature. The leaves were further subjected to oven dry at the temperature of 40°C for 8 h. The sliced dried leaves of the plant were cut to bits and ground into powder using an electrical blender.

Afang (*Gnetum africanum*) leaves (**Source:** BehealthyAfrica.com)

2.2. Feed Formulation and Treatment Groups

Forty-two (42) male albino rats of the Wistar strain weighing 150 - 200 were obtained from the animal breeding unit, Department of Biochemistry, Federal University of Technology Akure, Nigeria. The rats were allowed to acclimatize in the Laboratory for a period of 4 weeks, during this period, they were maintained *ad libitum* on commercial growers. The rats were subsequently divided into 7 groups of 6 rats per group.

The experimental diet was prepared using the method of Nwanna *et al.* (2016). It consists of corn flour which served as a source of carbohydrate, skimmed milk served as protein

source and fat and oil were obtained from groundnut oil. The mineral and vitamins mixtures were from mineral premix. They were thereafter kept in air tight container and stored at 4℃ until needed for use.

At the end of acclimatization, the rats were divided into 7 groups with 6 rats each.

Groupings

- Group A: normal control rats, fed with basal diet
- Group B: cyclophosphamide induced (75 mg/kg body weight) rats, fed with basal diet
- Group C: cyclophosphamide induced (75 mg /kg body weight) incubated with Donopezil (1 mg/kg body weight) rats, fed with basal diet
- Group D: cyclophosphamide induced (75 mg /kg body weight) rats, fed with basal diet supplemented with 2% *afang*
- Group E: cyclophosphamide induced (75 mg /kg body weight) rats, fed with basal diet 4% *afang*
- Group F: normal rats fed with basal diet supplemented with 2% *afang*
- Group G: normal rats fed with basal diet supplemented with 4% *afang*

Note: 1 g of the mineral and vitamin premix contains; 3200 i.u vitamin A, 600 i.u vitamin D3, 2.8 mg vitamin E, 0.6 mg vitamin K3, 0.8 mg vitaminB1, 1 mg vitamin B2, 6 mg niacin, 2.2 mg pantothenic acid, 0.8 mg vitamin B6, 0.004 mg vitamin B12, 0.2 mg folic acid, 0.1 mg biotin h2, 70 mg choline chloride, 0.08 mg cobalt, 1.2 mg copper, 0.4 mg iodide, 8.4 mg iron, 16 mg manganese, 0.08 mg selenium, 12.4 mg zinc, 0.5 mg antioxidant.

Feeding Pattern

Animals in group A, B and C were fed the basal diet, while animals in group D and F were fed basal diet containing 2% supplementation of *afang* leaves and those in group E and G were fed the basal diet containing 4% supplementation of *afang* leaves. The rats in group E were given donepezil (1mg/kg body weight) orally and once daily. The experiment lasted for 21 days during which the animals were allowed access to food and water ad libitum. The rats in groups B-E were injected intraperitoneally with cyclophosphamide (75 mg/kg body weight) 24 hours before termination of the experiment, while group A served as control. At the completion of the feeding period, animals were fasted overnight and sacrificed by decapitation. The cerebral tissue was rapidly dissected; the brain was removed, placed in ice and weighed.

2.3. Cyclophosphamide induced brain damage bioassay

2.3.1. Preparation of Brain Homogenate

The brain was weighed and subsequently homogenized in cold saline (1/10, w/v) with about 10 up and down strokes at approximately 1200 rev/min in a Teflon glass homogenizer. The homogenate was centrifuged for 10 minutes at 3000 g to yield a pellet that was discarded. The supernatant was used for the following assays lipid peroxidation, determination of catalase, vitamin C, acetylcholinesterase activity, butylcholinesterase activity, monoamine oxidase activity, alkaline phosphatase activity, Aspartate Aminotransferase Activity AST.

2.3.2. Determination of Tissue Total Protein Content

Total protein assay was carried out using commercially available kit (RANDOX Laboratories).

2.4. Determination of Tissue Malondialdehyde (MDA) Content

This was carried out by the modified method of Ohkawa *et al.* (1979). 100 ml of tissue homogenate was mixed with a reaction mixture containing 30ml of 0.1 M pH 7.4 Tris–HCl buffer, extract (0–100 ml) and 30ml of 7mM freshly prepared sodium nitroprusside. The volume was made up to 300 ml by water before incubation at 37°C for 1 hour. The color reaction was developed by adding 300 ml 8.1% Sodium dodecyl sulfate to the reaction mixture containing the homogenate this was subsequently followed by the addition of 600 ml of acetic acid/HCl (pH 3.4) mixture and 600 ml 0.8% thiobarbituric acid. This mixture was incubated at 100 °C for 1 hour. Thiobarbituric acid-reactive species (TBARS) produced were measured at 532 nm in the spectrophotometer (JENWAY 6305) and the absorbance was compared with that of a standard curve using malondialdehyde (MDA).

2.5.Determination of Aspartate Aminotransferase activity (AST)

The method described in Agappe kit was used. 100 µl of the sample and 1000 µl of the AST reagent kit were mixed in a test tube, and the initial absorbance was taken at 340 nm between 0-1 min thereafter the readings were taken after 1, 2 and 3 min the results were pooled to get the average which was used for the calculation.

ALT activity (nm/min) = 1746 × ΔA 340 nm/min

ΔA 340 nm/min = change in absorbance per minute for the homogenate sample, 1745 = Extinction coefficient.

2.6. Determination of Alkaline phosphatase Activity

The substrate *p*-nitrophenyl phosphate is hydrolyzed by alkaline phosphatase from the sample in the presence of magnesium ions, to form nitrophenol which is yellow and can be read at 405 nm. The intensity of color produced is proportional to the activity of alkaline phosphatase. The procedure described in Randox kit was used for the assay. In a cuvette, 10 µl of sample was mixed with 500 µl of the reagent. The initial absorbance was read at 405 nm, and subsequently over 3 min.

2.7. Determination of Vitamin C Content

Vitamin C content was determined using the method of Benderitter *et al.* (1998). Briefly, 75 µl DNPH were added to 500 µl reaction mixture, 300 µl of brain homogenate with 100 µl 13.3% trichloroacetic acid (TCA) and water. The reaction mixture was subsequently incubated for 3 h at 37 °C , then 0.5 ml of H_2SO_4 (V/V) was added to the medium and the absorbance was measured at 520 nm in a UV-Visible spectrophotometer. The vitamin C content of the homogenate was subsequently calculated using ascorbic acid as standard.

2.8. Cholinesterase activity assay (ChE)

The effect of the *afang* supplementation diet on cholinesterase [acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)] activities was assessed by a modified colorimetric method (Perry *et al.*, 2000). The cholinesterase activity was determined in a reaction mixture containing 200 µl of the homogenate in 0.1 M phosphate buffer (pH 8.0), 200 µl solution of 5,5′-dithio-bis(2-nitrobenzoic) acid (DTNB 3.3 mM), sample extracts (0–100 µl) and phosphate buffer, pH 8.0. After incubation for 20 min at 25 °C, acetylthiocholine iodide (for AChE activity assay)

or butyrylthiocholine iodide (for BChE activity assay) was added as the substrate and enzyme activity were determined in a UV/visible spectrophotometer (Jenway 6305 model) at 412 nm. The AChE and BChE activities were thereafter expressed as percentage inhibition

2.9. Determination of Monoamine 0xidase Activity (MAO)

MAO activity was measured according to with slight modification of (Turski *et al.,* 1972). In brief the reaction mixture containing 0.025 M phosphate buffer of pH 7, 0.0125 M semicarbazide, 10 mM benzylamine (pH adjusted to 7), and 0.67 mg of MAO enzyme and the tissue homogenate (100 μg/ml) in a total reaction volume of 2 ml. After 30 min 1 ml of acetic acid was added and boiled for 3 min in boiling water bath followed by centrifugation. The resultant supernatant (1 ml) was mixed with equal volume of 0.05% of 2, 4-DNPH and 2.5 ml of benzene this was allowed to incubate for 10 min under room temperature After separating the benzene layer, equal volume of 0.1 N NaOH was added. Alkaline layer was decanted and heated at 80°C for 10 min. The orange–yellow color developed was measured at 450 nm in a spectrophotometer.

2.10. Determination of catalase Activity

Catalase activity in the brain homogenate sample was determined according to the method of Sinha (1972). In brief, 0.1ml of tissue homogenate was reacted with 0.4 ml 2 M H_2O_2 in the presence of 0.1ml 0.01M phosphate buffer (pH 7.0). The reaction was stopped by the addition of 2.0 ml dichromate acetic acid. The absorbance of the reaction mixture was taken at 620 nm in a spectrophotometer. A standard curve was prepared by reacting 0.4 ml 2 M H_2O_2 with 2 ml dichromate acetic acid in the presence of 1.0 mL 0.01M sodium phosphate buffer (pH 7.0) to generate a straight line equation. CAT activity was expressed as mole of H_2O_2 consumed /min/g protein.

2.11. Data Analysis

The results of three replicate experiments were pooled and expressed as mean standard deviation (SD). One-way analysis of variance (ANOVA) was used to analyze the mean and the post hoc treatment was performed using Duncan multiple test. Significance was accepted at ($p< 0.05$) (Zar, 1984).

3. Results and Discussion

3.1. Results

The result of the lipid peroxidation level is shown in figure 1. The result revealed that cyclophosphamide administration caused a significant ($p<0.05$) increase in the MDA content of the brain in the control rats when compared to the normal rats, while the groups with dietary inclusion of *afang* leaves (2% and 4%) experienced a significant ($p<0.05$) decrease in the MDA level when cyclophosphamide was given, compared to negative control group where cyclophosphamide was induced but the rats fed with basal diet. Figures 2 and 3 present the results of alkaline phosphatase, aspartate aminotransferase activities, which were elevated ($p<0.05$) in cyclophosphamide administered rats while the presence of *afang* inclusion (2% and 4%) caused a significant ($p<0.05$) decrease in the level of these enzymes activities in cyclophosphamide induced rats. Figures 4 and 8 show the effect of dietary inclusion of *afang* (2% and 4%) on the brain antioxidant enzyme (catalase) status and vitamin C content. The results revealed significant ($p< 0.05$) reduction in the brain activities of the antioxidants following cyclophosphamide administration in control rats compared with normal rats. However, there was significant (p<

0.05) improvement in the brain antioxidant activities in the groups fed with diets supplemented with *afang* (2% and 4% inclusion).

In addition, the results of the key enzymes acetylcholinesterase(AChE), butyrylcholinesterase (BChE) and monoamine oxidase linked to neurodegenerative diseases as presented in figures 5, 6 and 7 respectively. The results revealed that cyclophosphamide administration caused significant ($p < 0.05$) increase in the activities of these enzymes in the control rats with compared to normal rats. However, this cyclophosphamide induced elevated brain cholinesterase and monoamine activities were significantly lower in the groups fed with diets supplemented with *afang* (2% and 4% inclusion).

Note: Key words in the figures

Group A: normal control rats, fed with basal diet;

Group B: cyclophosphamide induced (75 mg/kg body weight) rats, fed with basal diet

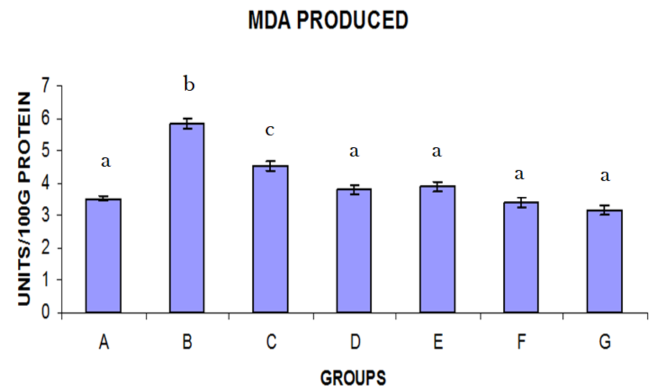
Group C: cyclophosphamide induced (75 mg /kg body weight) incubated with Donopezil (1 mg/kg body weight) rats, fed with basal diet

Group D: cyclophosphamide induced (75 mg /kg body weight) rats, fed with basal diet supplemented with 2% *afang* leaves

Group E: cyclophosphamide induced (75 mg /kg body weight) rats, fed with basal diet 4% *afang* leaves

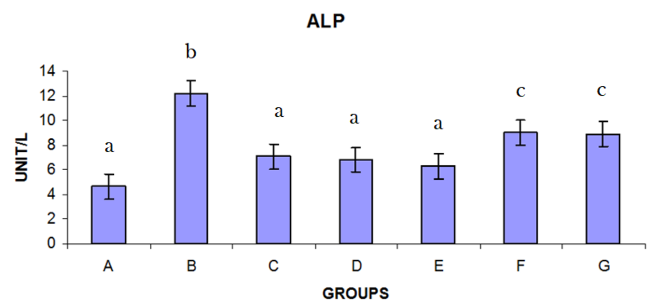
Group F: normal rats fed with basal diet supplemented with 2% *afang* leaves

Group G: normal rats fed with basal diet supplemented with 4% *afang* leaves



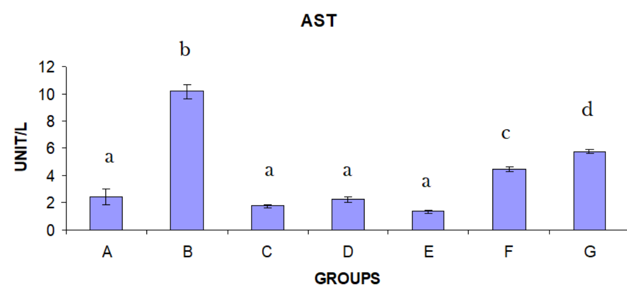
Values represent mean standard deviation (n=6). [a-b]Data are presented as Mean ± Standard deviation. Values of the same storage day with different superscripts are significantly different at p<0.05

Figure 1. Effect of Dietary Inclusion of *Afang* leaves on the level of MDA produced in rats brain



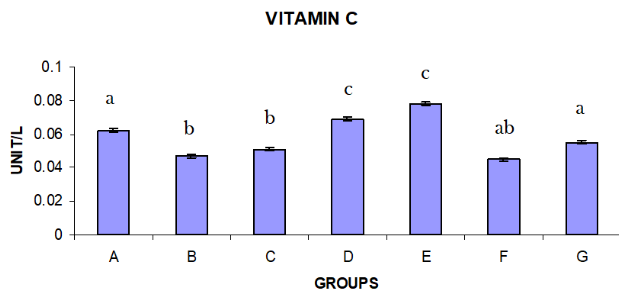
[a-c]Data are presented as Mean ± Standard deviation. Values of the same storage day with different superscripts are significantly different at p<0.05

Figure 2: Effect of Dietary Inclusion of *Afang* leaves in the Brain Alkaline Phosphatase in Normal and Cyclophosphamide induced rats



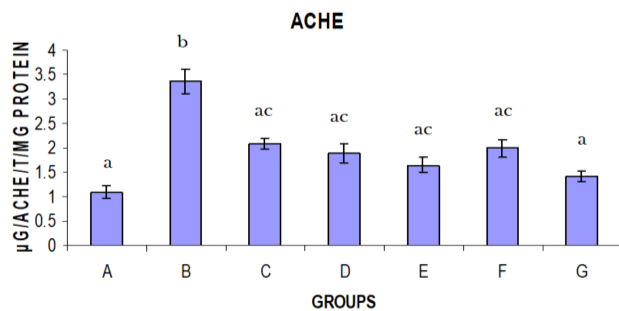
[a-d]Data are presented as Mean ± Standard deviation. Values of the same storage day with different superscripts are significantly different at p<0.05

Figure 3: Effect of Dietary Inclusion of Afang leaves iin the Brain Aspartate Aminotransferase in Normal and Cyclophosphamide induced rats



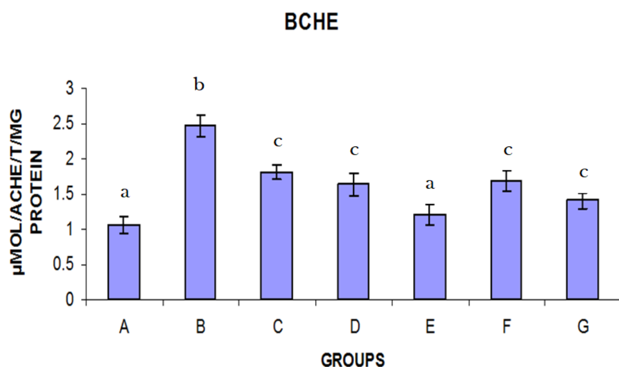
[a-c]Data are presented as Mean ± Standard deviation. Values of the same storage day with different superscripts are significantly different at $p<0.05$

Figure 4: Effect of Dietary Inclusion of *Afang* leaves on the Vitamin C Content in Normal and Cyclophosphamide induced rats brain



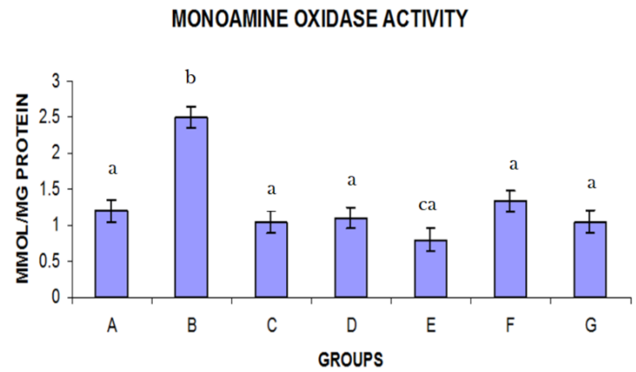
[a-c]Data are presented as Mean ± Standard deviation. Values of the same storage day with different superscripts are significantly different at $p<0.05$

Figure 5: Effect of Dietary Inclusion of *Afang* leaves on the Acetylcholinesterase Activity in Normal and Cyclophosphamide induced rats brain



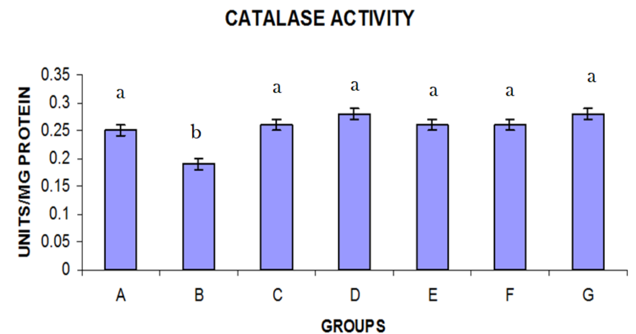
[a-c]Data are presented as Mean ± Standard deviation. Values of the same storage day with different superscripts are significantly different at $p<0.05$

Figure 6: Effect of Dietary Inclusion of *Afang* leaves on the Butylcholinesterase Activity in Normal and Cyclophosphamide induced rats brain



[a-c]Data are presented as Mean ± Standard deviation. Values of the same storage day with different superscripts are significantly different at $p<0.05$

Figure 7: Effect of Dietary Inclusion of *Afang* leaves on Monoamine Oxidase Activity in Normal and Cyclophosphamide induced rats brain



[a-c]Data are presented as Mean ± Standard deviation. Values of the same storage day with different superscripts are significantly different at $p<0.05$

Figure 8: Effect of Dietary Inclusion of *Afang* leaves on the Catalase Activity in Normal and Cyclophosphamide induced rats brain

3.2. Discussion

The most abundant antioxidants in our diets are polyphenols and they are widely distributed and found in several food products such as fruits, vegetables, cereals, olive, dry legumes, chocolate and beverages (Scalbert *et al*., 2005). *Afang* leaves have very high nutritional value and constitute an important source of mineral elements (Ekpo *et al*., 2012).

While flavonoids, phenols anthocyanins have been shown to be present in the leaves of *Gnetum africanum* (Akintola *et al.,* 2012). These

could be the reason why the dietary supplementation of *afang* supplemented diet was able to modulate cyclophosphamide-induced neurotoxicity in rats' brain.

Lipid peroxidation is thought to proceed via radical abstraction of hydrogen atoms from methylene carbon in polyunsaturated fatty acids (Rael *et al.*, 2004). Malondialdehyde (MDA) is one of the end products of lipid peroxidation and the extent of lipid peroxidation is estimated by measuring MDA level. MDA is a reactive aldehyde and is one of the many reactive electrophilic species that cause toxic stress in cells and form advanced glycation end products. The production of MDA is used as a biomarker to measure the level of oxidative stress in an organism (Nwanna *et al.*, 2020). The ability of *afang* leaves to inhibit lipid peroxidation was determined in rats' brain tissues *in-vivo*. The results revealed that *afang* diet caused a concentration dependent inhibition on MDA production in the brain tissue. This indicates that *afang* exerted an antioxidant effect. Dietary inclusion of *afang* leaves 2 and 4% caused a significant ($p<0.05$) decrease in the brain MDA content in a concentration dependent manner although no significant effect was observed between 2 and 4%. This inhibition of MDA production may be due to the fact that *afang* is rich in bioactive phytochemicals with antioxidants property such as phenolic compounds. The presence of these phytochemicals agrees with previous work. Iweala *et al.* (2009) and Nwanna *et al.* (2015) elucidated the presence of phenolic compounds such as flavonoids, anthocyanidins, phytosterols, tannins, saponins, alkaloids in *G. africanum* leaves. The administration of a single dose of cyclophosphamide (75 mg/kg body weight) prior to the termination of the experiment caused a significant ($p< 0.05$) increased in the MDA content of the rat brain in group B. This increase in the Malondialdehyde content following intraperitoneal administration of cyclophosphamide is in line with the results of Nwanna *et al.* (2018). Cyclophophamide administration was observed to significantly ($p<0.05$) increase aspartate aminotransferase, alkaline phosphatase in the brain tissue. Increased activities of these enzymes in the brain are well known diagnostic indicators of liver disease or injury. The results show that the inclusive diet with basal diet followed by cyclophosphamide administration protected the brain. This indicated that cyclophosphamide induced oxidative stress on the liver which consequently resulted in the significant rise in AST and ALP but were ameliorated with the formulated diet.

Vitamin C is an important water soluble antioxidant in biological fluids (Gillman, 1996). The vitamin C level was significantly lower ($p<0.05$) especially in the cyclophosphamide treated group, which may be as a result of increase in free radical production mediated by cyclophosphamide metabolites. The rats treated with *afang* supplemented diet induced with cyclophosphamide presented significantly higher ($p<0.05$) vitamin C level in a concentration dependent manner when compared with the donepezil treated group. The normal rats fed with *afang* supplement also showed significant ($p<0.05$) increase in vitamin C content in a concentration dependent manner. This result agrees with earlier report by Lawal *et al.* (2015) that shows that *afang* has high vitamin C content and this protects *in-vivo* oxidation of lipids and DNA by scavenging free radical.

Furthermore, cyclophosphamide administration caused a significant ($p<0.05$) increase in BChE and AChE activities in the rat brain of group B compared to control A and *afang* treated groups as shown in Figure 4 and 5. This shows that the

the enzymes linked to neurodegenerative diseases was not inhibited. Increased activity of brain acetylcholinesterase facilitates rapid hydrolysis of ACh neurotransmitter, thus impairing the cholinergic neuronal function, which leads to neurodegenerative diseases. However, there was a significant ($p < 0.05$) decrease in the AChE and BChE activities of those rats fed with diet containing *afang* leaves. This decrease was also dose dependent, indicating that *afang* was able to impair cholinergic neuronal function. This agrees with earlier report by Nwanna *et al.* (2015) on the ability of *afang* extract to inhibit AChE and BChE activities as a result of their constituent polyphenols. The result also showed that *afang* extract inhibited AChE and BChE activities in concentration dependent manner (Nwanna *et al.*, 2016; Ademosun *et al.*, 2016). Since cholinesterase inhibitors are effective therapy for the management of neurodegenerative diseases especially AD and PD, then dietary source of cholinesterase inhibitors such as *afang* leafy vegetable diet could serve as complementary approach in the prevention/management of neurodegenerative diseases.

Monoamine oxidases (MAO) are a family of enzymes that catalyze the oxidation of monoamines (Tipton *et al.*, 2004). Rapid oxidation of monoamine neurotransmitter by MAO leads to impairment of the monoaminergic neurotransmission which causes pathogenesis and progression of several neurodegenerative diseases, especially AD and PD (Young Soo *et al.*, 2012). The result of monoamine oxidase assay reveals that cyclophosphamide administration caused a significant ($p<0.05$) increase in monoamine oxidases activity in the brain tissue of rats fed with basal diet compared to the control. This may be as a result of free radical mediated by the cyclophosphamide metabolism. Though, there was a significantly ($p<0.05$) decrease in monoamine oxidases activity of rat fed with *afang* supplement diet. This decrease was also dose dependent, indicating that *afang* was able to inhibit the activity of monoamine oxidase thereby leading to less oxidation of monoamine enzyme. Ability of *afang* to inhibits the activity of monoamines oxidase activity could also be a strategy for its neuroprotective properties and it can therefore be used in the management and prevention of neurodegenerative diseases.

Catalase activity was significantly lower ($p < 0.05$) in the cyclophosphamide treated rats fed with basal diet compared to control group and this could be as a result of more free radicals produced during cyclophosphamide metabolism which can cause reduction in endogenous antioxidant system. There was a significant increase ($p<0.05$) in the catalase activity of rats fed with *afang* supplemented diet. This could be due to the phytochemical constituents such as flavonoid, phenolics acids which has high antioxidant property (Iweala *et al.*, 2009) with protective property on biological system against free radicals.

4. Conclusion

Dietary inclusion of *Afang* leaves protects the brain from the negative assault of cyclophosphamide a neurotoxicant. It also modulate the activities of endogenous antioxidant and neuroprotective enzymes which makes the supplemented diet a good source of functional food for the management and treatment of neurodegenerative diseases.

Conflict of Interest

The authors declare that there are not conflicts of interest.

Ethics

The rats received humane care according to the criteria outlined in the Guide for the care and use of Laboratory animals prepared by the National Academy of Sciences and published by the National Institutes of Health (USA). The handling and the use of animal conforms to the prepared guideline by the Ethical Committee of the University, with approval number FUTA/ SOS/1411.

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