Original Article

Antioxidant Properties of the Extracts of *Talinum Triangulare* and its Effect on Antioxidant enzymes in Tissue Homogenate of Swiss Albino Rat

Olakunle Bamikole Afolabi, Omotade Ibidun Oloyede[1]

Department of Biochemistry, Afe-Babalola University, Ado-Ekiti, [1]Department of Biochemistry, Ekiti State University, Ado-Ekiti, Nigeria

ABSTRACT

Objectives: This study was designed to put into consideration both the in vitro and in vivo investigations on Talinum triangulare (Tt), an herbaceous perennial plant that is a native of tropical America and one of the most important vegetables in Nigeria. **Methods:** Total phenolic contents in (mg GAE/100 g), flavonoid contents, the ferric reducing antioxidant properties (FRAP), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl free radical scavenging ability (OH-) and iron chelating ability were carried out in vivo using standard described methods while GSH, GPx, catalase and SOD were determined in vivo using standard described methods. **Results:** In the three different solvents extraction of T. triangulare that were studied in vitro, it was noted that ethyl acetate and ethanolic fractions of T. triangulare showed potent antioxidant activity against DPPH and iron chelating property with high phenolic content except Hydroxyl free radical scavenging ability that showed highest value in the aqueous extract, while the Reduced GSH indicated the highest in the parameter determined in vivo. **Conclusion:** The antioxidant properties showed in this solvent extractable component probably could have been the basis for the enhanced activities of antioxidant enzymes at very lower dose in the examined tissue homogenates. Therefore, T. triangulare can thereby serve as a means of Preventing some of major degenerative diseases challenging Humans

Key words: Antioxidant, free radicals, homogenate, *in vitro*, *Talinum triangulare*

INTRODUCTION

Plants are rich innumerous endogenous antioxidants such as polyphenols, carotenoids, ascorbic acids, tocopherol, and flavonoids.[1,2] These valuable antioxidants which are plant secondary metabolites whose primary functions are defensive and protective in nature against oxidative stress caused by the formation of reactive oxygen species (ROS) are widely recognized and annexed by humans as ingredient and supplement in dietary process in the hope of maintaining health and occasioning prevention to diseases such as cancer, coronary diseases, and even altitude sickness.[3-6] These phytochemicals which are sometimes referred to as phytonutrients most of which are vitamin-based antioxidant.[7]

Free radicals are generated via several endogenous metabolic mechanisms and actions such as enzymatic activities of NADPH oxidase, xanthine oxidase, and peroxidases in the cells. Once formed, they participate in several reactions yielding various ROS such as hydrogen peroxide, OH radical, hypochlorous acid, and so on.[8,9] When an antioxidant destroys a free radical, this antioxidant itself becomes oxidized. Therefore, the antioxidant sources must be constantly restored in the body.[10] Thus, while in one particular system an

Access this article online

Quick Response Code:

Website: www.toxicologyinternational.com

DOI: 10.4103/0971-6580.155377

Address for correspondence: Mr. Olakunle Bamikole Afolabi, College of Chemical Sciences, Biochemistry Unit, Afe Babalola University, Ado-Ekiti, Nigeria. E-mail: afolabioblessed10@yahoo.com

antioxidant is effective against free radicals; in other systems the same antioxidant could become ineffective.

The antioxidant process can function in any of the two ways: Chain-breaking or prevention. For the first, when a radical releases or acquires an electron, a second radical is initiated and thus exerts the same action on another molecule and continues until either the free radical formed is stabilized by a chain-breaking antioxidant (vitamin C, E, carotenoids, etc.) or it simply disintegrates into an harmless product, such could be found in lipid peroxidation chain reaction.[10,11] For the preventive way, an antioxidant enzyme like superoxide dismutase, catalase (CAT), and glutathione (GSH) peroxidase (GPx) can prevent oxidation by reducing the rate of chain initiation either by scavenging initiated free radicals such as superoxide and hydrogen peroxide, the most reactive free radical *in vivo* or by stabilizing transition metal radicals such as Fe^{2+} or Cu^{+}.[8] *Talinum triangulare*, popularly called water leaf with wide range of biological and pharmacological activities has been concisely studied in the present study to know the level of antioxidant contents and the activities on the endogenous antioxidant enzymes in tissue homogenates of an albino rats.

MATERIALS AND METHODS

Plant materials and preparation

Fresh water leaves, *T. triangulare* were bought from popular area called Olorunda in Ado-Ekiti, Ekiti State, Nigeria. Sample was taken to the Department of Plant Science in Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria for identification, it was identified with Herbarium number UHAE 2013/76, after proper taxonomic investigations from the data base.

Preparation of samples

Preparation of 70% ethanolic extract

The leaves (edible) parts of the plant were air-dried in a ventilated place at ambient temperature of 30 ± 2°C for 15 days pulverized using a laboratory blender and the fine powders obtained stored at moderate temperature until further use. A 120 g of the powdered sample was weighed and used for the extraction with 1l solvent combination (via maceration) of 70% ethanol (700 ml ethanol with 300 ml distilled water) for 72 h. The mixture was filtered using sterile Whatman product filter paper grade 1. The filtrate was later reconcentrated for the assays.

Preparation of ethylacetate extract

A5gethanolic extract was weighed and reconstituted in 50 ml of distilled water, to this was added 25 ml of petroleum ether (Pet. ether). This was mixed thoroughly, turned into separating funnels (250 ml) and left to stand 30 min. The extract has very low fat content. The aqueous top layer was removed and to the fraction was added 33.3 ml of ethyl acetate to wash. This procedure was repeated for the second time and the mixture was evaporated to dryness.

Preparation of aqueous extract

A 50 g of the blended air-dried leaves (edible parts) of *T. triangulare* was soaked in 500 ml distilled water for 48 h. The mixture was filtered and reconcentrated, the crude extract was later subjected to bioassay analyses.

Invitro antioxidant properties of *T. triangulare*

Determination of OH radical scavenging ability

The ability of the extract to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge.[12] Briefly, freshly prepared extract (100 µl) was added to a reaction mixture containing 120 µl, 20 mM deoxyribose, 400 µl, 0.1 M phosphate buffer pH 7.4, 40 µl, 20 mM hydrogen peroxide and 40 µl, 500 µM $FeSO_4$, and the volume was made to 800 µl with distilled water. The reaction mixture was incubated at 37°C for 30 min and the reaction was stopped by the addition of 0.5 ml of 2.8% trichloroacetic acid (TCA); this was followed by the addition of 0.4 ml of 0.6% thiobarbituric acid (TBA) solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was read at 532 nm.

Determination of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging ability

The free radical scavenging ability of the extract against DPPH (2, 2-diphenyl-1-picryhydrazyl) using Gyamfi *et al*., described method.[13] One milliliter of the extract was mixed with 1ml of the 0.4 mM methanolic solution of the DPPH, the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm.

Percent inhibition was calculated using the following expression:

$$\% \text{ Inhibition} = (Abs_{blank} - Abs_{sample}/Abs_{blank}) \times 100$$

Where Abs_{blank} and Abs_{sample}stand for absorption of the blank samples and absorption of tested extract solution, respectively.

Determination of ferric reducing property

The reducing property of the extract was determined by assessing the ability of the extract to reduce a $FeCl_3$ solution as described by Pulido *et al*., 2000.[14] 2.5 ml aliquot was mixed with 2.5 mL, 200 mM sodium phosphate buffer (pH 6.6), and 2.5 ml, 1% potassium ferricyanide (KFC). The mixture was incubated at 50°C for 20 min, and then 2.5 ml, 10% TCA was added. This

was then centrifuged at 2,000 rpm for 10 min. A 5 ml of the supernatant was mixed with an equal volume of water and 1 ml, 0.1% ferric chloride. The same treatment was performed to a standard ascorbic acid solution and the absorbance taken at 700 nm. The reducing power was then calculated and expressed as ascorbic acid equivalent.[15]

Determination of Fe^{2+} chelating ability

The ability of the extract to chelate iron II was determined using a modified method of Minotti and Aust, 1987.[12] A 150 µl of 150 mM $FeSO_4$ was added to a reaction mixture containing 165 µl of 0.1M Tris HCl pH 7.4, 215 µl saline with the extract and the volume is made up to 1 ml with distilled water. The reaction mixture was incubated for 5 min, before the addition of 13 µl of 1, 10-phenantroline and the absorbance read at 510 nm.

Determination of total phenol

The total phenol content of the extract determine by the method described by Singleton *et al*., 1999.[16] 0.2 ml of the extract was mixed with 2.5 ml of 10% Folin–Ciocalteu's reagent and 2 ml of 7.5% sodium carbonate. The reaction mixture will be subsequently incubated at 45°C for 40 min, and the absorbance was measure at 700 nm in the spectrophotometer, garlic acid would be used as standard phenol.

Determination of total flavonoid

The total flavonoid content of the extract was determined using a colorimeter assay developed by Bao *et al*., 2005.[17] 0.2 ml of the extract was added to 0.3 ml of 5% $NaNO_3$ at zero time. After 5 min, 0.6ml of 10% $AlCl_3$ was added and after 6 min, 2 ml of NaOH was added to the mixture followed by the addition of 2.1 ml of distilled water. Absorbance was read at 510 nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

In vivo antioxidant enzymes activity of *T. triangulare*

Animal treatment

Healthy adult male and female Swiss albino rats (Wister rat), 8 weeks of age and body weight between 90 and 150 g were used in this study. The principles of laboratory animal care (National Institutes of Health (NIH)) were followed. They were maintained with 12 h dark/light cycle in a conducive atmospheric temperature and 50–70% humidity with free access to pelleted feed and distilled water.

Preparation of the tissue homogenates

After sacrifice by cervical dislocation, the rats were dissected in order to isolate tissues of interest (i.e. the liver, kidney, and brain). The isolated tissues were cleansed in the washing buffer to remove blood stains, weighed, and immediately stored in ice cold 0.25 M sucrose solution.

The tissues were then subjected to homogenization using Teflon homogenizer in ice-cold 0.25 M sucrose solution (1:5 w/v), the homogenates were as same time centrifuged. The supernatants were stored in the freezer (−5°C).

Determination of reduced GSH

The level of reduced GSH in the samples was determined by the method described by Jollow *et al*., 1974.[18] 0.2 ml of the sample was added to 1.8 ml of distilled water and 3 ml of the precipitating reagent was mixed with the sample. The mixture was then allowed to stand for 5 min and then filtered. At the end of the 5th min, 1ml of the filtrate was added to 4 ml of 0.1M phosphate buffer and finally, 0.5 ml of the Ellmans' reagent was added. A blank was prepared with 4 ml of the 0.1M phosphate buffer, 1 ml of diluted precipitating solution and 0.5 ml of the Ellman's reagent. The optical density was measured at 412 nm. GSH concentration was proportional at that wavelength and the estimate was obtained from the GSH standard curve.

Determination of GPx activity

GPx activity in the sample was determined according to the method adopted by Rotruck *et al*., 2005.[19] The reaction mixture containing 200 µl of ethylenediaminetetraacetic acid (EDTA) (0.8 mM, pH 7.0), 400 µl of phosphate buffer (10 mM), 200 µl of tissue homogenate was incubated with 100 µl of H_2O_2, and 200 µl of GSH for 10 min at 37°C. Oxidation of GSH by the enzyme was read at 420 nm. The activity of GPx was expressed as l µmol GSH oxidized/min/g protein.

Determination of superoxide dismutase activity

The superoxide dismutase activity was determined by the method described by Misra and Fridovich, 1972.[20] An aliquot of the sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction was initiated by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline), and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 s for 150 s.

Calculation

$$\text{Increase in absorbance (per min)} = \frac{A_3 - A_0}{2.5}$$

A_0 = absorbance after 30 s
A_3 = absorbance after 150 s

$$\%\ \text{Inhibition} = \frac{\text{Increase in absorbance of substrate}}{\text{increase in absorbance of blank}} \times 100$$

One unit of superoxide dismutase (SOD) activity was defined as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 min.

Determination of CAT activity

CAT activity was determined by the method described by Beers and Sizer, 1952.[21] Sample (70 µl) was mixed with 920 µl 0.1 M sodium phosphate buffer (pH 7) containing 0.1mM EDTA. The reaction started by adding 10 µl of H_2O_2. The decrease in H_2O_2 concentration was taken by reading the absorbance at 240 nm (10 s intervals) for 180 s. H_2O_2 extinction coefficient (E) = 39.4 $mM^{-1}cm^{-1}$ at 240 nm and 1 mmole. $H_2O_2ml^{-1}$ min^{-1} is defined as 1 unit of CAT.

Data analysis

The results of replicate readings were pooled and expressed as mean ± standards deviation. One way analysis of variance was used to analyze the results and Duncan multiple tests was applied for the *post hoc* by Zar, 1984.[22] Statistical Package for Social Sciences (SPSS) 10.0 for Windows was used for the analysis.

RESULTS AND DISCUSSION

Figure 1 clearly shows that ethanolic extract of *T. triangulare* has the highest total phenolic content with 1.044 ± 0.01 mg GAE/100 compared to that of ethylacetate and aqueous extract with total phenolic content of 0.292 ± 0.01 and 0.264 ± 0.01 mg GAE/100 g, respectively. Figure 2 below revealed the percentage ferric reducing ability of the extracts in $FeCl_3$ solutionand it was found out that ethylacetate and aqueous extract of *T. triangulare* had the highest ability and almost the same percentage ferric reducing property were observed in ethylacetate with 2.99 ± 0.01 and aqueous extract with 2.988 ± 0.01 compared to that of ethanolic extract with the lowest percentage ferric reducing property of 1.606 ± 0.013, respectively. Also, Figure 3 shows the free radical scavenging ability of the extract against DPPH (2, 2-diphenyl-1-picryhydrayl). DPPH which is a free radical donor that accepts an electron or hydrogen to become a stable diamagnetic molecule when incubated with 1ml of 0.4 mM methanolic solution of the DPPH, from the figure the percentage inhibition shows that ethylacetate with 72.09 ± 0.04 has the highest inhibitory potential against DPPH over that of ethanolic and aqueous extracts with 70.64 ± 0.37 and 53.07 ± 0.04, respectively. The Figure 4 below shows the OH radical scavenging ability of ethylacetate, ethanolic and aqueous extracts of *Talinum triangulre*, that is, the ability of the extract to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose in the presence of $FeSO_4$ shows that aqueous extracts of the plant

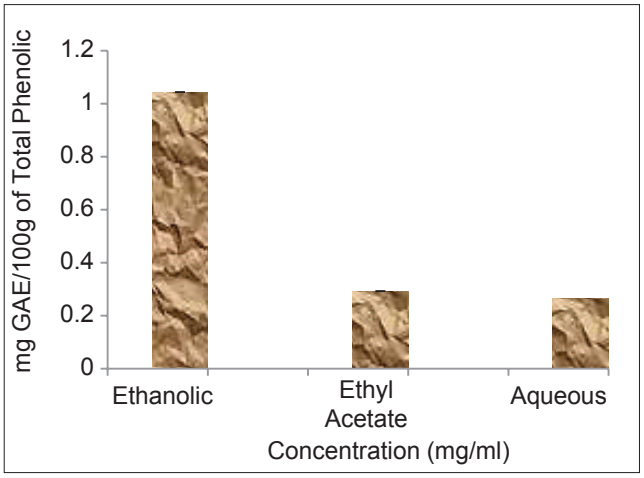

Figure 1: Total Phenolic (mg GAE/100 g)

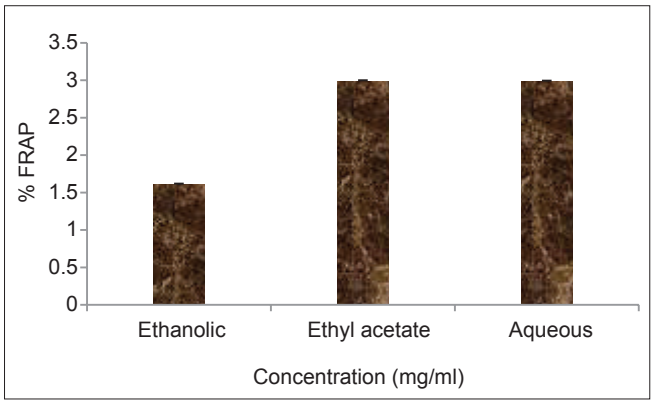

Figure 2: Ferric reducing antioxidant properties

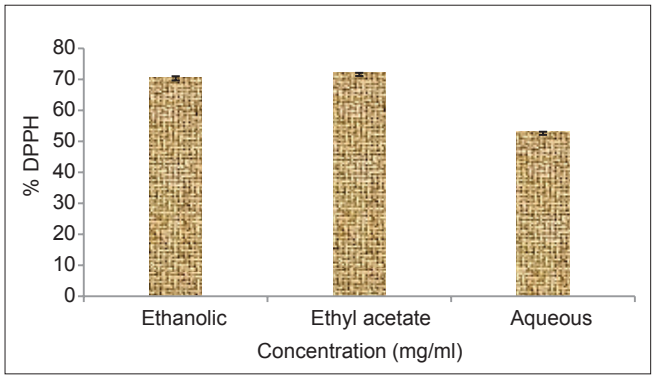

Figure 3: 1, 1- Diphenyl-2-picrylhydrazyl (%)

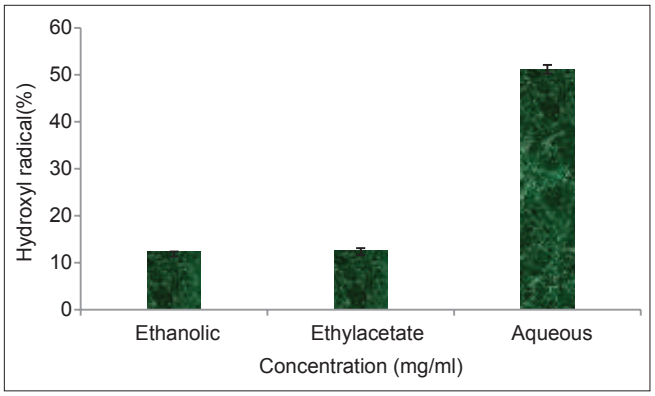

Figure 4: (%) Hydroxyl radical

contained the highest percentage of 51.24 ± 0.873 with ethyl acetate and ethanolic extracts having almost the same % OH scavenging ability.

The ability of the extracts to chelate Fe^{2+} when incubatedwith 150 mM $FeSO_4$ is shown in Figure 5. Ethylacetate showed the highest percentage chelating ability with 60.23 ± 0.07%; with ethanolic extract showing 36.24 ± 1.62%, and the least which is the aqueous extract with 14.49 ± 0.01%. The extracts when incubated with 5% $NaNO_3$ to determine the flavonoid content as shown in the Figure 4. It is clearly shown that ethylacetate extract with 1.146 ± 0.01 has the highest percentage flavonoid content with ethanolic and aqueous extracts showing almost equal flavonoid values with 1.044 ± 0.01 and 1.037 ± 0.01%, respectively [Figure 6].

Figures 7-9 relates the antioxidant enzymes parameters that were carried out with 100 mg/kg of the aqueous extract of *T. triangulare* to determine the effect (s) of the extract at this relatively minimum dosage on the antioxidant enzyme activities in the hepatic, renal, and the cerebral tissues. Antioxidants carry out their protective roles on cells in one of the ways either by preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the cells or by reducing/chelating the transition metal composition of vegetable.[6,23] Aerobic cells are made up of extensive cellular antioxidant defense mechanisms which includes both low molecular weight scavengers which prevent the damaging effects of toxic oxygen species,[24] and high molecular weight endogenous antioxidant enzyme such as SOD which converts the superoxide free radical anion to hydrogen peroxide and which is the most effective when its activity is followed by that of CAT and GPx. CAT is capable of scavenging the hydrogen peroxide radical that is formed during various metabolic reactions in the cells. The GSH is involved in many important cellular functions, ranging from the control of physiochemical properties of cellular proteins and peptides to the detoxification of free radicals.[25] However, when there occurs imbalance between these parameters and ROS, a state of oxidative stress is occasioned, possibly leading to establishment of cellular impairment. From Figures 7-9 below, the values of the enzyme activities were analyzed and from the table the correlation among GSH, GPx, SOD, and CAT activities on the tissues were relayed and clearly shown from the figures how the extract has proven to cause increase in GPx in the organs (i.e. the kidney, liver, and brain) compared to that of the normal

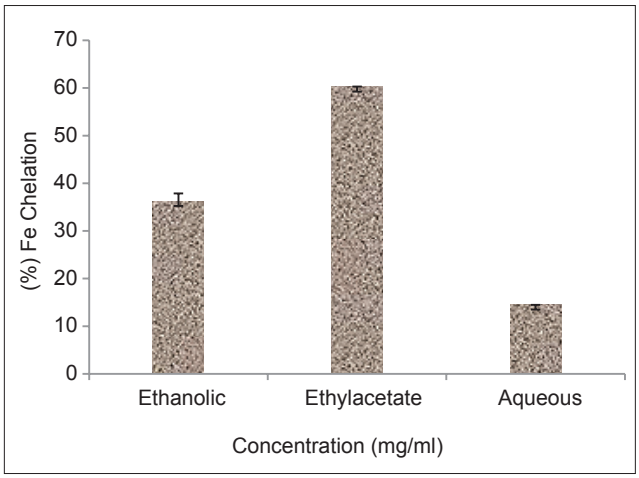

Figure 5: Fe chelation (%)

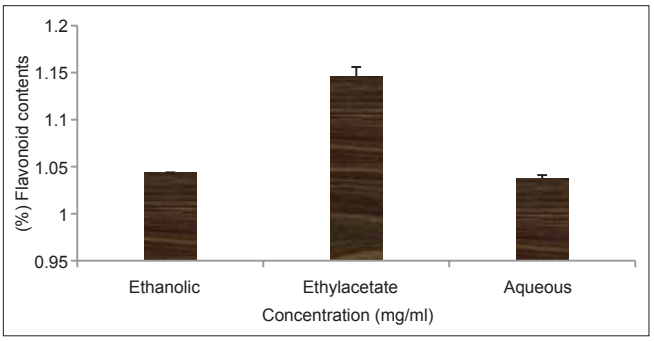

Figure 6: Total flavonoid (%)

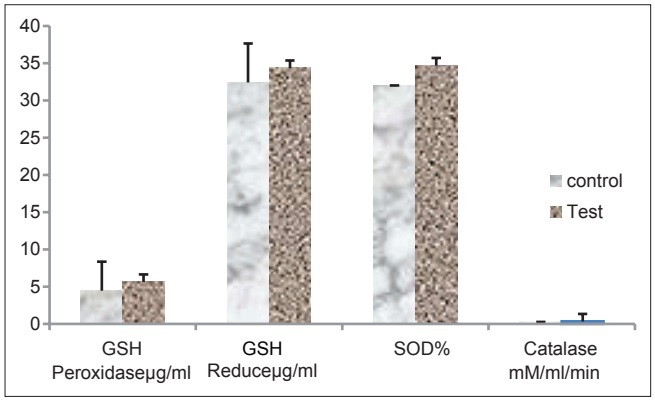

Figure 7: Effect of aqueous extract of T. triangulare on liver antioxidant enzymes ± SE and *n* = 4. GSH = Glutathione, SE = Standard error, SOD = Superoxide dismutase

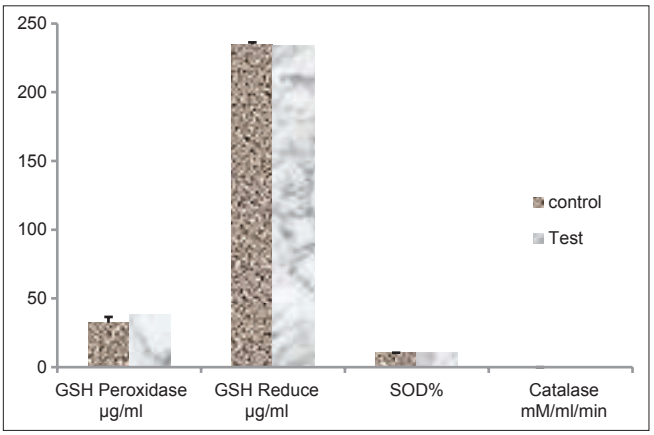

Figure 8: Effect of aqueous extract of *T. triangulare* on brain antioxidant enzymes ± SE and *n* = 4. GSH = Glutathione, SE = Standard error, SOD = Superoxide dismutase

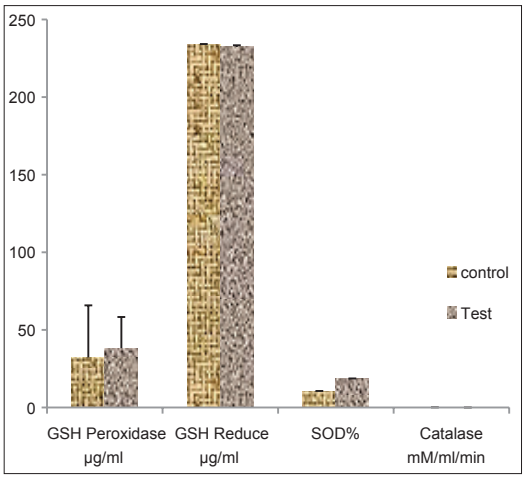

Figure 9: Effect of aqueous extract of *T. triangulare* on kidney antioxidant enzymes ± SE and *n* = 4

group. Control and the tests results observed for GPx in the brain were 4.46 ± 3.88 < 5.63 ± 70.18 µg/ml, liver 32.39 ± 4.18 < 38.44 ± 40.2 µg/ml, and kidney 32.22 ± 33.59 < 38.11 ± 20.19 µg/ml, respectively. Also from the figures, reduced GSH was observably increased in the brain (32.36 ± 5.25 < 34.36 ± 2.53), but much stable in the liver after the administration of the extract (234.9 ± 1.31 > 234.4 ± 0.64), but decrease was observed in reduced GSH in the kidney (233.7 ± 0.48 > 232.4 ± 0.95), this really proved the effectiveness of the extract on the organs.

Superoxide dismutase was stable in the liver (10.6 = 10.6) also that of GSH, but significantly increase of SOD was observed in the kidney (10.6 < 18.67) and in the brain (32 < 34.7) homogenates. This is an indication that aqueous extract of *T. triangulare* has an antioxidant and protective properties on the organs when the values observed are put into consideration. Also CAT activities was reduced in the liver compared to that of control ($2.0 \times 10^{-2} > 1.7 \times 10^{-2}$) and increased in the kidney ($9.5 \times 10^{-3} < 1.3 \times 10^{-2}$) and also in the brain ($2.7 \times 10^{-2} < 3.4 \times 10^{-2}$) this aligned with work described by Vani and Reddy, 2000.[26] This probably makes the brain more fortified against any biochemical damage (s).

CONCLUSION

The current study shows an increase in the activities of CAT as implicated in the brain tissue homogenate of rat treated with the extract of *T. triangulare* beyond other enzymes assayed for, in regards to this it is clearly shown that aqueous extract of *T. triangulare* could be cerebral and hepatic protective, and thereby serve as a means of preventing some of the major degenerative diseases clearly stated above.

ACKNOWLEDGEMENT

Authors which to acknowledge the efforts of Mr. Festus (Department of Biochemistry, Federal University of Technology, Akure, Ondo State Nigeria) for his enormous contribution to the success of this work.

REFERENCES

1. Olajire AA, Azeez L. Total antioxidant activity, phenolic, flavonoid and ascorbic acid contents of Nigerian vegetables. Afr J Food Sci Tech 2011;2:22-9.
2. Oboh G, Akinyemi AJ, Ademiluyi AO. Inhibition of α-amylase and α–glucosidase activities by ethanolic extract of *Telfairiaoccidentalis* (fluted pumpkin) leaf. Asian Pac J Trop Biomed 2012;2:733-8.
3. Afolabi OB, Oyeyemi AO, Obafemi TB, Awe JO. Phytochemical Screening of the Bark of *Vernonia Amygdalina*. J Nat Sci Res 2014;7:49-50.
4. Oloyede OI, Aluko OM. Determination of antioxidant potential of *Momordica Foetida* leaf extract on tissue homogenate. Sci J Med Clin Trial 2012;4:225.
5. Bahorun T, Soobrattee MA, Lux, Mon-Ramma V, Aruoma OI. Free radicals and antioxidants in cardiovascular health and diseases. Int J Medupdates 2006;1:25-41
6. Alia M, Horcajo C, Bravo L, Goya L. Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. Nutr Res 2003;23:1251-67.
7. Oboh G, Rocha GBT. Antioxidant in foods: A new challenge for food processors: In: Panglossi HV, editor. Leading edge antioxidants research. Nova Science Publishers Inc: New York; 2007. pp. 35–64.
8. Valko M, Leibfritz D, Moncola J, Cronin MD. Free radicals and antioxidants in normal physiological functions and human disease. Rev Int J Biochem Cell Biol 2007;39:44-84.
9. Droge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47-95.
10. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci 2008;4:89-96.
11. Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol 2001;54:176-86.
12. Minotti G, Aust SD. The requirement for iron (III) in the initiation of lipid peroxidation by iron (II) and hydrogen peroxide. J Biol Chem 1987;262:1098-104.
13. Gyamfi MA, Yonamine M, Aaniya Y. Free radical scavenging action of medicinal herbsfrom Ghana. Thonningia sanguine on experimentally induced liver injuries. Gen Pharmacol 1999;32:661-7.
14. Pulido R, Bravo L, Sauro-Calixo F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J Agric Food Chem 2000;48:3396-402.
15. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Japanese J Nutr 1986;44:307-15.
16. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxi- dation substrates and antioxidants by means of Follin-Ciocalteu reagent. Met Enzymol 1999;299:152-78.
17. Bao JY, Cai M, Sun G, Wang G, Corke H. Anthocyanins, flavonoid and free radical scavenging activity of myrialrubia extracts and their colour properties and stability. J Agric Food Chem 2005;53:2327-32.

18. Jollow DJ, Mitchell JR, Zampaglione N, Gillete JR. Bromobenzene-induced liver necrosis, Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacol 1974;11:151-69.
19. Rotruck JT, Pope AL, Ganther HE, Swanson DG, Hafeman DG, Schneider C. Chemistry and biology of vitamin E. Mol Nutr Food Res 2005;49:7-30.
20. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; 247:3170-5.
21. Beers RF Jr, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 1952; 195:133-40.
22. Zar JH. Biostatistical analysis. 2nd ed. Englewood Cliffs: Prentice-Hall; 1984. p. 718.
23. Amic D, Davidovic-Amic D, Beslo D, Trinajstic N. Structure-radical scavenging activity relationship of flavonoids. Croat Chem Acta 2003; 76:55-61.
24. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. 2nd ed. Oxford: Clarendon press; 1989. p. 188-276.
25. Meister A, Anderson ME. Glutathione. Ann Rev Biochem 1983; 52:711-60.
26. Vani ML, Reddy KP. Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. Fluorid 2000; 33:17-26.

How to cite this article: Afolabi OB, Oloyede OI. Antioxidant Properties of The Extracts of *Talinum Triangulare* and its Effect on Antioxidant Enzymes in Tissue Homogenate of Swiss Albino Rat. Toxicol Int 2014;21:307-13.

Source of Support: Nil. **Conflict of Interest:** None declared.