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In vitro Inhibitory Effects of *Cyperus esculentus* L. (Tiger Nut) Tubers on some Enzymes Associated with Neurodegeneration and Iron-Induced Lipid Peroxidation in Rats' Brain Tissue Homogenate

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ABSTRACT

This study investigated the inhibitory effect of *Cyperus esculentus* L. (raw and processed) tubers on Iron (Fe^{2+}) induced lipid peroxidation and key enzymes linked with neurodegeneration *in vitro*. Ten grams of tiger nut tubers was weighed and added to 100 mL of distilled water (1:10 w/v), left for 24 h, filtered, centrifuged to obtain a clear supernatant and lyophilized. Activities of acetyl cholinesterase (AChE), butyryl cholinesterase (BChE), Fe^{2+}-induced lipid peroxidation as well as antioxidants status as indicated by 2,2-azino-bis (3-ethylbenthiazoline-6-sulphonic acid radical scavenging ability and Fe chelation were evaluated. Raw tiger nut extracts inhibited activities of AChE and BChE in the brain tissue of rats (0 – 11.54 mg /mL) with EC_{50} = 3.599 mg /mL and 2.71 ± 0.01 mg /mL respectively, and increased antioxidant activity when compared with the processed tuber. Inhibition on these enzymes as well as prevention of Fe^{2+} induced lipid peroxidation may be the possible mechanism of action by which tiger nut prevents neurodegeneration.

Key words: *Tiger nut; Acetyl cholinesterase; Butyryl cholinesterase; Neuroprotection; Antioxidant*

INTRODUCTION

In recent years, studies have implicated oxidative stress role in neurodegenerative disease such as Alzheimer's disease (AD), via lipid peroxidation of cell membranes of neurons (Pratico and Delanty 2000; Saliu et al. 2017). Of particular importance, the brain is an organ extremely susceptible to free radical damage because of its high oxygen demands and its relatively low concentration of antioxidant enzymes and free radical scavengers (Kaul and Forman 2000). AD was first described by the German neurologist Alois Alzheimer, as a neurodegenerative disease affecting the brain which was an irreversible, progressive brain disease that slowly destroys memory and thinking skills and eventually leading to inability to carry out simple task (Cumming and Cole 2002) such as, finding the right words for a situation, vision/spatial issues and impaired reasoning. The human brain is one of the most metabolically active organs in the body and metabolizes a large amount of glucose to produce cellular energy in the form of adenosine triphosphate (ATP) (Cunnane et al. 2011). If the brain is unable to produce ATP, synapses cannot be maintained and cells cannot function, ultimately leading to impaired cognition (Costantini et al. 2008).

Cholinergic deficit has been well documented in AD and therefore, cholinesterase enzyme family has become imperative in neuropathology of AD, as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) hydrolyze the neurotransmitters; acetylcholine and butyrylcholine respectively (Giacobini 1998). AChE is the primary cholinesterase in the body, hydrolyzing the neurotransmitter acetylcholine and other choline esters at the neuromuscular junctions and brain cholinergic synapses, thus, terminating signal transmission. AChE exists in multiple molecular forms which

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possess similar catalytic properties, but differ in their oligomeric assembly and mode of cell attachment to the cell surface. The major form of AChE is found in the brain, muscle and other tissues in the hydrophilic species, which forms disulphide linked oligomers with collagenous, or lipid containing structural subunits. Alzheimer's medications work to preserve acetylcholine by inhibiting AChE. BChE produced by the liver, circulates in the blood is involved in the breakdown of certain drugs, including muscle relaxants called choline esters used during general anaesthesia. BChE also breaks down toxic substances before they reach the nerves and is found in significantly higher quantities in AD plaques than in plaques of age related non demented brains (Schneider 2001).

Tiger nut is valued for it's highly nutritious starch content, dietary fibre and digestible carbohydrate (Oladele et al. 2009), and has been cultivated as a livestock feed and for human consumption. It can be eaten raw, roasted, grated, baked or used for ice cream and beverage amongst others (Belewu and Abodunrin 2006; Sanful 2009). For many years, tiger nut tubers have been considered to have adequate properties to fight respiratory infections and some stomach illnesses (Belewu and Abodunrin 2006). Tiger nut is known to be very rich in phytochemicals and has been an important ingredient in folklore medicine for the treatment/management of AD with limited scientific basis. This study therefore seeks to assess its effect on the key enzymes (AChE and BChE) linked with AD as well as Fe^{2+}-induced lipid peroxidation.

MATERIALS AND METHODS

Materials

Sample Collection

Tiger nut (*Cyperus esculentus* L.) was purchased June 2016 from a local market in Akure, Nigeria. The nuts were thoroughly washed under running tap to remove stones and other dirt. Thereafter, a portion of the nuts was roasted in an electric oven for 30 min at 120 ℃. The remaining portion was oven dried at 45 ℃ to a constant weight, and the two samples were pulverized, milled, defatted in cold n-hexane and kept prior to analysis. A voucher specimen of the nut was deposited at the Federal University of Technology, Akure Centre for Research and Development (CERAD) herbarium.

Aqueous Extract Preparation

Ten grams (10g) each of the milled samples were subjected to aqueous extraction at room temperature of 25 ℃ for 24 h in 100 mL of distilled water (1:10 w/v). The mixture was filtered, centrifuged (model KX3400C) to obtain clear supernatant which was then freeze-dried (Lab-Kit FD-10-MR model; Lab-Kits, Utherm International, China) and stored in the refrigerator for subsequent analysis (Oboh et al. 2007).

Chemicals and Reagents

Thiobarbituric acid (TBA), 1,10-phenanthroline, deoxyribose, gallic acid, Folin–Ciocalteau's reagent were obtained from Sigma-Aldrich, Inc., (St Louis, MO), trichloroacetic acid (TCA) was sourced from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany), dinitrophenyl hydrazine (DNPH) from ACROS Organics (New Jersey, USA), hydrogen peroxide, n-hexane, methanol, acetic acid, thiourea, copper (II) tetrasulphate (VI) pentahydrate ($CuSO_4.5H_2O$), sulphuric acid (H_2SO_4), sodium carbonate, aluminium trichloride ($AlCl_3$), potassium acetate, Tris-HCl buffer, sodium dodecyl sulphate, Iron (II) tetrasulphate (VI) ($FeSO_4$), potassium ferricyanide and ferric chloride were sourced from BDH Chemicals Ltd., (Poole, England) while the water was glass distilled.

Methods

Determination of Total Phenol Content

The total phenol content was determined according to the method of Singleton et al. (1999). Briefly, appropriate dilutions of tiger nut extracts (raw and processed) were oxidized with 0.5 mL (500 µL) 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 0.4 mL (400 µL) of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45 ℃ and the absorbance was measured at 765 nm in the JENWAY UV-visible spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

$(Abs_{ref} – Abs_{sample}) / Abs_{ref} \times 100$ - Formula 1.

where Abs_{ref} = absorbance of the reference (reacting mixture without the test sample) and Abs_{sample} = absorbance of reacting mixture with the test sample.

Determination of Total Flavonoid Content

The total flavonoid content was determined using a slightly modified method reported by Meda et al. (2005). Briefly 0.5 mL of tiger nut tuber aqueous extract was mixed with 0.5 mL methanol, 50 µL 10% $AlCl_3$, 50 µL 1M potassium acetate and 1.4 mL water, and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm in the JENWAY UV-visible spectrophotometer; the total flavonoid content was calculated with quercetin as standard using equation 1.

Determination of Vitamin C (Ascorbic Acid)

Vitamin C content of the tiger nuts (raw and processed) was determined using the method of Benderitter et al. (1998). Briefly, 75 µL DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO_4.5H_2O$ in 100 mL of 5 M H_2SO_4) were added to 500 µL reaction mixture [300 µL of the extracts with

100 µL 13.3% TCA and 100 µL water]. The reaction mixture was subsequently incubated for 3 h at 37 ℃, then 0.5 mL (500 µL) of 65% H_2SO_4 (v/v) was added to the medium and the absorbance was measured at 520 nm in the UV-visible spectrophotometer. The vitamin C content of the raw and processed tiger nut was subsequently calculated using ascorbic acid as standard.

In vitro **Antioxidant Studies**

2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) Radical Scavenging Ability

The ABTS radical scavenging ability' (ABTS*) scavenging ability of the extracts were determined according to the method described by Re et al. (1999). The ABTS* was generated by reacting an ABTS aqueous solution (7 mmol /L) with potassium persulphate ($K_2S_2O_8$) (2.45 mmol /L, final concentration) in the dark for 16 h and adjusting the Abs734 nm to 0.700 with ethanol. Tiger nut extract (0.2 mL) was added to 2.0 mL ABTS* solution and the absorbance were measured at 734 nm after 15 min in the UV-visible spectrophotometer. The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated.

Iron (Fe^{2+}) Chelation Ability

The Fe^{2+} chelating ability of the tiger nut extracts were determined using the method of Puntel et al. (2005). Freshly prepared 500 µM $FeSO_4$ (150 µL) was added to a reaction mixture containing 168 µL 0.1M Tris-HCl (pH 7.4), 218 µL saline and the extracts (20, 40, 60 and 80 µL). The reaction mixture was incubated for 15 min at room temperature, before the addition of 13 µL 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in the UV-visible spectrophotometer.

Table 1: Total Phenolics, Total Flavonoid and Vitamin C Contents of Aqueous Extracts of *Cyperus esculentus* L.

Samples	Total phenolics (mg/g)	Total flavonoid (mg/g)	Vitamin C (mg/g)
TIG 1	2.00±0.14[a]	0.32±0.12[a]	1.040±0.100[b]
TIG 2	2.72±0.15[b]	0.47±0.03[b]	0.97±0.035[a]

Values represent mean ± standard deviation of triplicate readings. Values with the same superscript letter along the same column are not significantly different (P>0.05). TIG 1- processed tiger nut; TIG 2 - raw tiger nut

Fenton Reaction (Degradation of Deoxyribose)

The method of Halliwell and Gutteridge (1981) was used to determine the ability of the extract to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose. The extract ranging between 0-100 µL was added to a reaction mixture containing 120 µL of 20 mM deoxyribose, 400 µL of 0.1M phosphate buffer, 40 µL of 500 µM of $FeSO_4$, and the volume were made up to 800 µL with distilled water. The reaction mixture was incubated at 37 ℃ for 30 min and the reaction was then stopped by the addition of 0.5 mL of 2.8% trichloroacetic acid. This was followed by addition of 0.4 mL of 0.6% TBA solution. The test tubes containing the reaction mixtures were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in the JENWAY UV-Visible spectrophotometer.

Determination of Ferric Reducing Antioxidant Property

The reducing property of the tiger nut extracts was determined by assessing their ability to reduce iron (III) chloride ($FeCl_3$) solution as described by Oyaizu (1986). Exactly 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. The mixture was incubated at 50 ℃ for 20 min, and then 2.5 mL 10% TCA was added. This mixture was centrifuged at 650 rpm for 10 min. About 5 mL of the supernatant was mixed with an equal volume of water and 1 mL 0.1% ferric chloride. The absorbance was measured at 700 nm in the UV-visible spectrophotometer.

Lipid Peroxidation Assay

Animals and Preparation of Tissue Homogenates

Wistar rats (n = 8) were obtained and house at the Afe Babalola University animal house (25 ℃), fed with standard feed and given water *ad libitum*. The use of the animals was approved by Afe Babalola Ethical Committee with the reference number ABUAD/EC/2017/202. The rats were decapitated under mild diethyl ether anaesthesia and the brains rapidly isolated, placed on ice and weighed. The tissue was 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 homogenates were centrifuged for 10 min at 3000 xg to yield pellets that were discarded, and a low-speed supernatant (S1) was kept for lipid peroxidation assay (Belle et al. 2004).

Lipid Peroxidation and Thiobarbibutric Acid Reactions

The lipid peroxidation assay was carried out using the modified method of Ohkawa et al. (1979). Briefly, 100 µL S1 fraction was mixed with a reaction mixture containing 30 µL of 0.1M at pH 7.4 Tris-HCl buffer, extract (0-100 µL) and 30 µL of 250 µM freshly prepared $FeSO_4$. The volume was made up to 300 µL by water before incubation at 37 ℃ for 2 h. The colour reaction was developed by adding 300 µL 8.1% sodium dodecyl sulphate to the reaction mixture containing S1. This was subsequently followed by the addition of 500 µL of acetic acid/HCl (pH 3.4) mixture and 500 µL 0.8% TBA. This mixture was incubated at 100 ℃ for 1 h. Thiobarbituric acid reactive species

(TBARS) produced were measured at 532 nm in the UV-visible spectrophotometer and the absorbance was compared with that of standard curve using malondialdehyde (MDA).

Cholinesterase Inhibition Assay
Cholinesterase inhibition was assessed by a modified colorimetric method (Ellman et al. 1961). The AChE and BChE activities were determined in a reaction mixture containing 200 µL of a solution of 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) (3.3 mM in 0.1 M phosphate-buffered solution, pH 7.0) containing sodium hydrogen carbonate ($NaHCO_3$) (6 mM), aqueous extracts of tiger nut (raw and processed) and 500 µL of phosphate buffer (pH 8.0). After incubation for 20 min at 25 °C, 100 µL of 0.05 mM acetylthio-choline iodide and butyrylthio-choline iodide solution were added as the substrates for AChE and BChE respectively, and their activities were determined as changes in absorbance reading at 412 nm for 3 min at 25 °C in a spectrophotometer. The inhibitory activities were expressed as percentage inhibition using the equation 1.

Statistical Analysis
The results of the three replicates were pooled and expressed as mean ± standard deviation (Zar 1984). One way analysis of variance was used to analyse the results and Duncan multiple test was used as post hoc test.

Table 2: ABTS Radical Scavenging Ability and Ferric Reducing Antioxidant Properties of *Cyperus esculentus* L. Aqueous Extracts

Samples	ABTS (mmol.TEAC/g)	FRAP (mg.GAE/100g)
TIG 1	462.560±330.400[a]	0.70±0.10[a]
TIG 2	1079.305±770.935[b]	1.30±0.10[b]

Values represent mean ± standard deviation of triplicate readings. Values with the same superscript letter along the same column are not significantly different (P>0.05). TIG 1- processed tiger nut; TIG 2 - raw tiger nut; FRAP - ferric reducing antioxidant properties

RESULTS

The results revealed total phenolic content of the raw tiger nut (2.715 mg/g) and that of the processed tiger nut (2.00 mg/g). The total flavonoid content in same manner showed raw tiger nut (0.470 mg/g) higher than processed tiger nut (0.320 mg/g). The ascorbic acid (vitamin C) content of raw and processed tiger nut showed 1.04 mg/g and 0.97 mg/g, respectively (Table 1).
The ABTS scavenging ability is presented as trolox equivalent antioxidant capacity (mmol.TEAC/100g). The results showed that both extracts scavenged ABTS radical; however, the raw sample (1079.305 mmol.TEAC/g) had a significantly higher ABTS scavenging ability compared to the processed sample (462.560 mmol.TEAC/g). Ferric reducing antioxidant properties of the aqueous extract of the tiger nut showed that the raw sample aqueous extract (1.3 mg.GAE /100g) had a higher reducing power than the processed (0.7mg.GAE /100g) (Table 2).
The hydroxyl radical ($OH^{\cdot}$) scavenging ability of the aqueous extracts of tiger nut showed that all the extracts were able to scavenge $OH^{\cdot}$ produced from the decomposition of deoxyribose in fenton reaction (Fig. 1). Furthermore, the tiger nut extracts chelated Fe^{2+} considering their EC_{50} (concentration of extracts required to inhibit 50% of activity) value for raw (4.22 ± 0.01 mg /mL) and processed (4.81 ± 0.04 mg /mL) tiger nut extract (Fig. 2; Table 3). The incubation of rats' brain tissue in the presence of 250 µM $FeSO_4$ caused a significant increase in the MDA content of the brain (Fig. 3). However, the introduction of the tiger nut extracts inhibited MDA production in a dose dependent manner (0 - 33.33 mg /mL).
Both extracts inhibited AChE activity. However, raw tiger nut extract (EC_{50} = 3.599 ± 0.03 mg /mL) had a higher AChE inhibitory activity than the processed tiger nut extract (EC_{50} = 7.000 ± 0.08 mg /mL) (Fig. 4; Table 4). Both extracts also inhibited BChE activity *in vitro*. Nevertheless, the raw tiger nut extract (EC_{50} = 2.71 ± 0.01 mg /mL) had higher BChE inhibitory activity than the processed tiger nut extract (EC_{50} = 4.76 ± 0.03 mg /mL) (Fig. 5; Table 4).

DISCUSSION

Cyperus esculentus L. tubers have long been recognized for their health benefits as they have high content of soluble glucose, rich in minerals for bone, tissue repairs, muscle and blood stream. They are also found to be rich in antioxidant vitamins (vitamin E and C) and vitamin B1 which assist in balancing the central nervous system by providing support and encourage the body to adapt to stress (Belewu and Abodunrin 2006; Sanful 2009).
Free radicals have an important role in pathogenesis of a wide range of disease including AD. Phenolic compounds can protect the body from free radicals, whose formation is associated with the normal metabolism of aerobic cells. Flavonoids are widely found in food products derived from plant source and they have been shown to possess significant antioxidant activities that could lower cellular oxidative stress and act as a natural radical scavenger (Oboh et al. 2007; 2013). Ascorbic acid is a good reducing agent and exhibit antioxidant activities by electron donation. It helps the immune system to fight off foreign invaders and tumour cells, and supports the cardiovascular system by facilitating fat metabolism and protecting tissue from free

radicals damage, while also assisting the nervous system convert certain amino acids to neurotransmitters. The ascorbic acid content of raw and processed tiger nut (1.04 ± 0.01 mg/g and 0.97 ± 0.035 mg/g) from our study was lower than that reported for *Solanum anguivi* Lam fruits (Elekofehinti and Kade 2012).

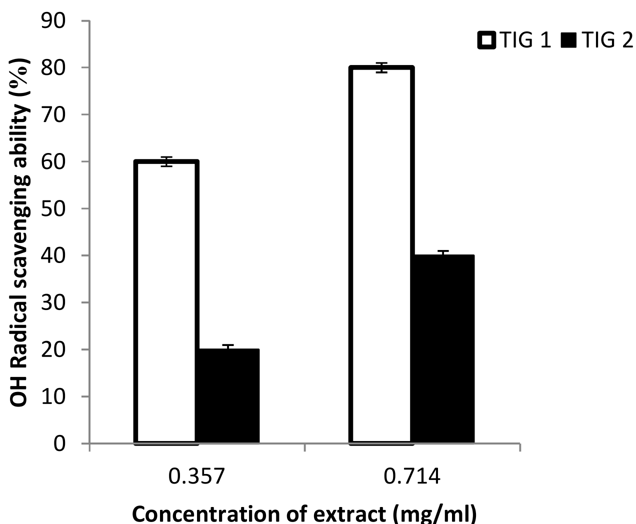

Fig. 1: OH* radical scavenging ability of aqueous *Cyperus esculentus* L. extracts.. $OH^{\cdot}$ - hydroxyl radical; TIG 1- processed tiger nut; TIG 2 - raw tiger nut

Antioxidant can prevent biological and chemical substances from free radical-induced oxidative damage and stress. Consequently, antioxidants have gained a great attention for their potential in management of many diseases (Zhang-Numes et al. 2006). Since deregulation of metal ions such as Fe^{2+}, Cu^{2+} and Zn^{2+} and consequential induction of oxidative stress have been reported to be associated with AD (Matinez (2003), antioxidant mechanism demonstrated by the tiger nut (raw and processed) could play some part in the prevention of oxidative-stress-induced neurodegeneration. Iron (Fe^{2+}) chelating ability may also be one of the possible mechanisms through which antioxidants phytochemicals in tiger nut extract prevents lipid peroxidation in tissues by forming a complex with Fe, thus preventing the initiation of lipid peroxidation (Oboh et al. 2007).

The incubation of rat brain tissues in the presence of 250 µM $FeSO_4$ caused a significant increase in the MDA content of the brain (185.7%). This finding agrees with the report of Butterfield and Lauderback (2002), where significant increase in MDA production was observed in the presence of Fe^{2+}. The MDA in the presence of Fe^{2+} could be attributed to the fact that Fe^{2+} can catalyze one-electron transfer reactions that generate reactive oxygen species, such as the reactive $OH^{\cdot}$, which is formed from H_2O_2 through the Fenton reaction (Zago et al. 2000). Elevated Fe^{2+} content in the brain have been linked to a host of neurodegenerative disease which include AD, a finding demonstrated in animal models of disease by Matinez (2003). However, the introduction of the tiger nut extracts inhibited MDA production in a dose dependent manner (0 - 33.33 mg /mL) and this finding is consistent with the report of Butterfield and Lauderback (2002), where plant extracts inhibited Fe^{2+}-induced lipid peroxidation in rat brain *in vitro*. The tiger nut tuber extracts probably had a high inhibitory effect on Fe^{2+}-induced lipid peroxidation in brain tissue probably due to its high phenolic, flavonoid and vitamin C contents. It could be speculated that tiger nut, being a good iron chelator must have prevented the oxidation of iron (II), thereby preventing the generation of hydroxyl radical and inhibiting oxidative stress in the process.

Acetylcholine (Ach) is one of the neurotransmitters in the autonomic nervous system. It acts on both the peripheral and central nervous systems, and is the only neurotransmitter used in the motor division of the somatic nervous system. In the central nervous system, acetylcholine and the associated neurons from the cholinergic system, tends to cause anti-excitatory actions. ACh has an important role in enhancing sensory perception when one wakes up (Jones 2005) and in sustaining attention (Himmelheber et al. 2000). AChE is an important regulatory enzyme that controls the transmission of nerve impulses across cholinergic synapses by hydrolysing the excitatory transmitter acetylcholine (ACh) (Milatovic and Dettburn 1996). Normally, in the healthy brain AChE is predominant, however, in AD brain BChE activity rises while AChE activity remains unchanged or diminished (Gray et al. 1987). Inhibition of AChE is considered as a promising

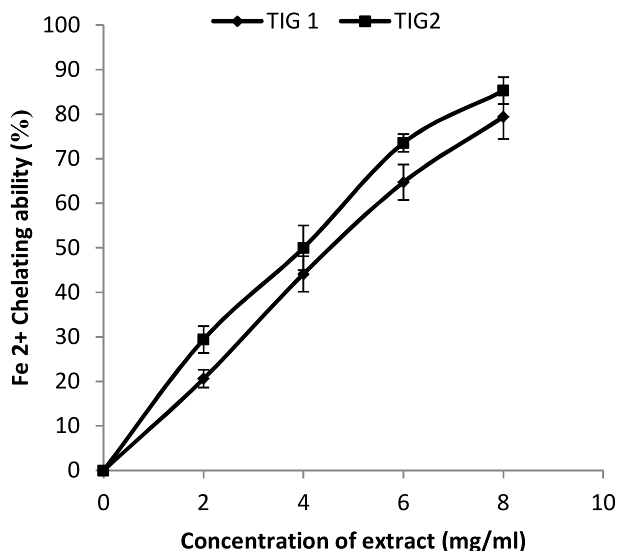

Fig. 2: Iron chelating ability of aqueous of *Cyperus esculentus* L. extracts. TIG 1- processed tiger nut; TIG 2 - raw tiger nut

approach for the treatment of AD (Quinn 1987). BChE on the other hand has been considered to be directly associated with the side effects of the AChE inhibitors and the existing drugs of AD (Tong et al. 1996). More recent studies have shown that BChE is found in significantly quantities in AD plaques than in plaques of age related non-demented brains. Aqueous extract of *Cyperus esculentus* L. inhibited both AChE and BChE. The inhibition of these cholinesterases could be as a result of important phytochemicals in these extracts. Phenolic acids such as caffeic acid, chlorogenic acids and catechin have been reported to be a potent inhibitor of AChE and BChE (Anwar et al. 2012; Oboh et al. 2013). Therefore, inhibition of both AChE and BChE by the tiger nut extracts is an indication that the nut could have potential therapeutic benefits. However, the processed tiger nut extract showed a reduced effect on the antioxidant and key enzymes inhibitory abilities compared to the raw extract.

Table 3: EC_{50} of Aqueous *Cyperus esculentus* L. Extracts on Fe^{2+} Chelating Ability and Inhibition of Fe^{2+}- induced Lipid Peroxidation in Rats' Brain

Samples	EC_{50} of Fe^{2+} chelating ability (mg/mL)	EC_{50} of Fe^{2+} induced lipid peroxidation (mg/mL)
TIG 1	4.81 ± 0.04^b	25.78 ± 1.2^b
TIG 2	4.22 ± 0.01^a	20.19 ± 0.9a

Values with the same superscript letter along the same column are not significantly different (P>0.05). EC_{50} - concentration of extracts required to inhibit 50% of activity; TIG 1- processed tiger nut; TIG 2 - raw tiger nut

Conclusion

The aqueous extract of tiger nut (*Cyperus esculentus* L.) from our findings showed significant anticholinesterase properties as well as antioxidant activity. The nut extracts were also able to protect the brain homogenates from Fe^{2+} induced lipid peroxidation in vitro. Hence, the tiger nut has potential usefulness as functional food in the management of neurodegenerative disease such as Alzheimer's disease as it exhibited inhibitory activity on key enzymes (AChE and BChE) linked to this disease.

Conflict of Interest

None declared

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Table 4: EC50 of Aqueous Cyperus esculentus L. Extracts on AChE and BChE in Rats' Brain

Samples	EC_{50} of AChE (mg/mL)	EC_{50} of Fe^{2+} BChE (mg/mL)
TIG 1	7.000 ± 0.08^b	4.76 ± 0.03^b
TIG 2	3.599 ± 0.03^a	2.71 ± 0.01^a

Values with the same superscript letter along the same column are not significantly different (p>0.05). AChE – acetylcholinesterase; BChE – butyrylcholinesterase; EC_{50} - concentration of extracts required to inhibit 50% of activity; TIG 1- processed tiger nut; TIG 2 - raw tiger nut

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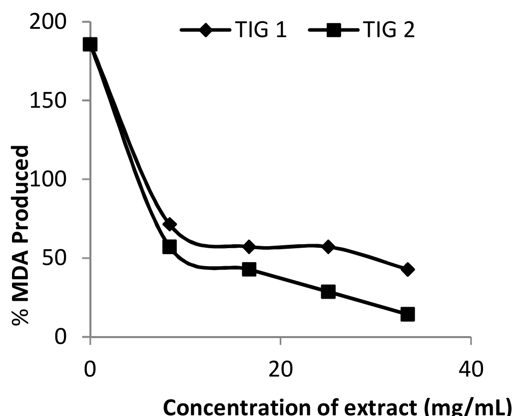

Fig. 3: Iron-induced lipid peroxidation in rat's brain by aqueous *Cyperus esculentus* L. extracts. TIG 1- processed tiger nut; TIG 2 - raw tiger nut

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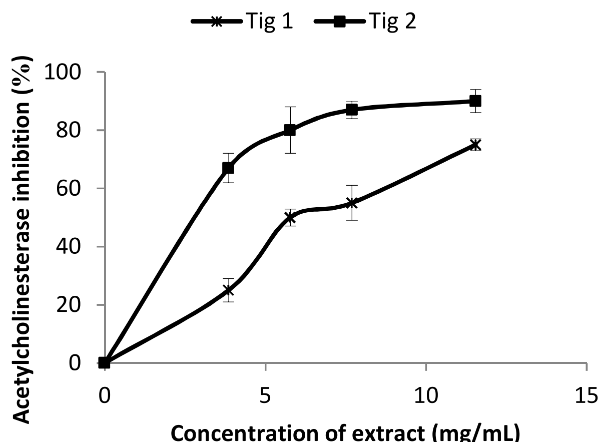

Fig. 4: Acetylcholinesterase activity of aqueous *Cyperus esculentus* L. extracts. TIG 1- processed tiger nut; TIG 2 - raw tiger nut

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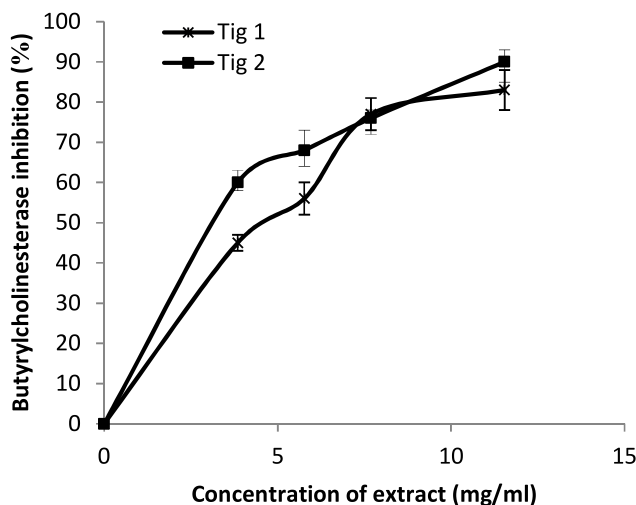

Fig. 5: Butyrylcholinesterase activity of aqueous *Cyperus esculentus* L. extracts. TIG 1- processed tiger nut; TIG 2 - raw tiger nut

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