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The Protective Effect of Polyphenol-Rich Extract of *Syzygium cumini* Leaves on Cholinesterase and Brain Antioxidant Status in Alloxan-Induced Diabetic Rats

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Abstract

Syzygium cumini leaves are used locally especially in Nigeria for the treatment\ management of diabetes mellitus and Alzheimer's disease. This study was designed to investigate the effects of polyphenols extracted from *Syzygium cumini* leaves on the occurrence of oxidative stress in the brain of rats with diabetes, which can trigger Alzheimer's disease by determining both *in vitro* and *in vivo* cholinesterase, the antioxidant defense system, and the extent of oxidative damage. The effect of polyphenols extracted from *Syzygium cumini* leaves was investigated on *in vitro* cholinesterase. Thereafter, the extract (400 mg/kg body weight) of both free and bound polyphenols was administered orally to alloxan-induced rats, and the effect were monitored on *in vivo* cholinesterase, superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione, lipid peroxidation and hydroperoxides. The extract demonstrated inhibitory effects against *in vitro* cholinesterase. A significant reduction in the cholinesterase activities increased the activities of superoxide dismutase, catalase, glutathione peroxidase, and reduced glutathione. A reduction in lipid peroxidation and hydroperoxide concentrations was observed in the brain of diabetic rats treated with polyphenols extracted from *Syzygium cumini* leaves. This study suggests that the polyphenols of *Syzygium cumini* leaves have anti-Alzheimer and antioxidant boosters, as well as antiperoxidative activities. Therefore, the plant is recommended for both diabetic and Alzheimer's disease patients worldwide.

Keywords: *Syzygium cumini*, Diabetic mellitus, Antioxidant enzymes, Alzheimer's disease

1. Introduction

Functional foods are characterized by bioactive constituents, including the polyphenolic compounds, which form a defensive mechanism against oxidative insults (Khan, 2012). These polyphenolic compounds are antioxidant, anti-diabetic, anti-cancer, anti-microbial, and anti-inflammatory. Also, polyphenols are an integral part of the human diet, and are available in functional foods that are utilized in alternative medicine (Kazeem *et al*., 2013). One among the key complications of diabetes mellitus is the neurological disturbance in the central nervous system. Pari and Latha (2004) reported that cognitive deficits and the morphological and neuro-chemical alterations demonstrate neurological complications in patients with diabetes mellitus.

Persistent hyperglycaemia as well as the high levels of reactive oxygen species (ROS) could trigger central nervous system disorders, particularly Alzheimer's disease. Alzheimer's disease patients are characterized by an elevated activity of acetylcholinesterase (AChE) (EC 3.1.1.7) primarily in the brain, which is responsible for the breakdown of acetylcholine (ACh). According to Chang *et al*. (2014), ACh is a neurotransmitter that plays a significant role in the correct functioning of the central cholinergic system. Also, butrylcholinesterase (BChE) (EC 3.1.1.8) is responsible for the breakdown of certain drugs, toxins (before reaching the nerves), including the choline esters used in anaesthesia. BChE is found in a higher level in the Alzheimer's disease plaques than in the plaques of age-related non-demented brains (Schneider, 2001). Currently, more than 30 million individuals suffer from this disease, and it has been projected that by 2050 more than 115 million people will have dementia, if necessary actions were not taken (Querfurth and LaFerla, 2010).

Moreover, the neuronal network, particularly of the brain, is more liable to oxidative damage because of the elevated levels of polyunsaturated lipids in neuronal cell membranes. The high oxygen consumption, the high metabolic rate of transitional metals, and the poor antioxidative defense contribute to the prevalence of Alzheimer's disease (Lane *et al*., 2006). Therefore, antioxidant defenses of the brain are unpretentious. Uddin *et al*. (2016) define antioxidants as exogenous or endogenous substances that prevent oxidation, and act against the oxidative stress associated with deleterious effects on the cellular system. They are also coupled with

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free radical scavenging preventing free radical chain-reactions and improving the antioxidant status in Alzheimer's disease patients (Uttara *et al*., 2009). The antioxidant defense enzymes in the brain, including catalase (EC 1.11.1.6), superoxide dismutase (EC 1.15.1.1), and glutathione peroxidase (GSH-Px) (EC 1.11.1.9) among others, play an important role to prevent the progression of free radical mediated oxidative stress, (Sharma *et al*., 2013).

Recently, the use of functional foods is increasing worldwide probably because of their no or less side effects in comparison with modern available drugs. Several plants have been documented in this regard. One example is *Syzygium cumini* (L.) Skeels (popularly called jamun in India, and black plum in Europe etc.) [Fam.: Myrtaceae; Syn.: *Syzygium jambolanum* (Lam.) DC, *Eugenia jambolana* Lam., *Eugenia cumini* (L.) Druce] which has been used for years by the Indian Ayurvedic medicine practitioners for various therapeutic reasons (Sanches *et al.,* 2016). Therefore, this study was conducted to evaluate the role of the polyphenolic-rich extract of *Syzygium cumini* leaves on cholinesterase and the antioxidant efficacies on the brains of rats with alloxan-induced diabetes.

2. Materials and Methods

2.1. Drugs and Chemicals

All the drugs and chemicals used in this study were obtained from Sigma Chemical Company Inc., St. Louis, Mo, USA. Also, all reagents used were of analytical grades.

2.2. Plant Material

Fresh leaves of *Syzygium cumini* were collected from the vicinity of the Zamani College in Kaduna, Nigeria in September, 2016. The plant was identified and authenticated at the Department of Botany of Ahmadu Bello University in Zaria. The leaves were washed under tap water, and were rinsed with distilled water. The leaves were then air-dried for seven days using paper towels. Thereafter, they were ground into powder by a blender, and were stored at 10 ^{0}C to be used later in the analysis.

2.3. The Extraction of Phenolics

2.3.1. The Extraction of Free Phenolics

One hundred grams of powdered *Syzygium cumini* leaves were extracted with 80 % acetone (1:5 w/v), and were filtered using Whatman (Number 4) filter paper. The filtrate was then evaporated by a rotary evaporator under vacuum at 45 ^{0}C until almost 90 % of the filtrate had been evaporated and lyophilized to obtain a dry extract. The extract was then kept at -4 ^{0}C for subsequent analyses while the residue was kept for the extraction of bound phenolics (Chu *et al*., 2002).

2.3.2. The Extraction of Bound Phenolics

The residue obtained from the extraction of free phenolics was flushed with nitrogen and hydrolyzed with 20 mL of 4 M NaOH solution at room temperature (24 ±1 ^{0}C) for a one-hour shaking. After that, the pH of the mixture was adjusted to two using concentrated HCl. Then the bound phytochemicals were extracted with ethylacetate six times. The ethylacetate extracts were evaporated to dryness at 45 ^{0} C using a rotary evaporation as described by Chu *et al.* (2002).

2.4. Animals

Male Wistar albino rats (seven weeks old), weighing between 150 to 170 gram were obtained from the Central Animal House of Afe Babalola University in Ado-Ekiti, Ekiti State in Nigeria. The animal experiments were approved by the ABUAD Animal Ethical Committee. The animals were acclimatized for fourteen days. They were fed with normal rat pellet chow and were kept under constant light and dark cycles for twelve hours, with an environmental temperature of 21 to 23 ^{0}C.

2.5. Induction of Diabetes

Diabetes was induced in the albino rats by a single intraperitoneal injection of freshly-prepared alloxan of 150 mg/kg body weight in normal saline. Forty-eight hours (two days) following the alloxan induction, blood samples were obtained from the tips of the rats' tails and the fasting blood glucose levels were determined using the OneTouch Ultra glucometer (LifeScan, USA) to confirm diabetes. The diabetic rats exhibiting blood glucose levels greater than or equal to 200 mg/mL were used in this study (Akanksha *et al*., 2010; Ajiboye and Ojo, 2014).

2.6. Animal Grouping

Only male rats were used in this study to avoid differences in the hormonal actions and to have a proper comparison within the same-sex group. Also, using both sexes in one group may lead to the impregnation of the female rats by male rats, which might affect the experimental outcome. Accordingly, a total of thirty male Wistar albino rats were randomly divided into five groups with six rats in each and were treated as follows:

Group 1: normal rats administered with distilled water;
Group 2: alloxan-induced diabetic rats (without treatment);
Groups 3 and 4: alloxan-induced diabetic rats administered with 400 mg/kg of the free and bound polyphenolic extract of *Syzygium cumini* leaves respectively.
Group 5: alloxan-induced diabetic rats administered with 5 mg/kg of metformin.

The extract was suspended in distilled water, and was orally administered to rats daily for fourteen days by an orogastric tube. In addition, 400 mg/kg of both free and bound phenolics was used based on the oral glucose tolerance test (OGTT) previously determined by the authors.

2.7. The Preparation of Tissue Homogenates

The experiment lasted for fourteen days, after which the rats were sacrificed by cervical dislocation. Their brains were immediately removed, and rinsed in ice-cold 1.15% KCl, then blotted and weighed. The brains were then minced with scissors in three volumes of ice-cold 100 mM potassium phosphate buffer, pH 7.4, and were homogenized in a teflon glass homogenizer. The homogenates were centrifuged for ten minutes at 12,000 × *g* to yield a pellet that was discarded. A low speed supernatant (S1) was used to assess the activities of different enzyme studied assays (Belle *et al.* 2004).

2.8. In vitro Determination of Acetylcholinesterase and Butyrylcholinesterase Inhibitory Activities

These were determined by a modified colorimetric method of Ellman *et al*. (1961). The AChE activity was determined in a reaction mixture containing 200 µL of a solution of AChE (0.415 U/mL in 0.1 M phosphate buffer, pH 8.0), 100 µL of a solution of 5, 5'-dithiobis (2-nitrobenzoic acid) (3.3 mM DTNB in 0.1 M phosphate buffered solution of pH 7.0, containing 6 mM $NaHCO_3$), the plant extract and 500 µL of phosphate buffer (pH 8.0). After incubation for twenty minutes at 25 ^{0}C, 100 µL of 0.05 mM acetylthiocholine iodide solution was added as the substrate, and AChE activity was determined as changes in the absorbance reading at 412 nm for three minutes at 25 ^{0}C using a spectrophotometer. Also, 100 µL of butyrylthiocholine iodide was used as a substrate to assay butyrylcholinesterase activity, while all the other reagents and conditions being the same. The AChE and BChE inhibitory activities were expressed as percentage inhibition.

2.9. Determination of in vivo Acetylcholinesterase and Butyrylcholinesterase Inhibitory Activities

The method of Ingkaninan *et al.* (2003) was employed in this determination. Briefly, 140 µL of sodium phosphate (0.1 M, pH 6.8) was pipette into a test tube, and 20 µL of the brain homogenate and 20 µL of acetylcholinesterase or butyrylcholinesterase (0.09 unit/mL) were added as the case may be. The mixture was incubated for fifteen minutes at room temperature. Thereafter, 10 µL of 10 mM DTNB and 10 µL of acetylthiocholine chloride (14 mM) or s-butyrylthiocholine chloride (substrate) as the case may be were added. Finally, the mixture was allowed to stand for fifteen minutes, and the absorbance was read at 417 nm using a microplate reader.

2.10. Determination of Catalase (CAT) Activity

The catalase activity of the brain homogenates was determined according to the method described by Sinha (1972). One millimeter of the supernatant fraction of the brain homogenate was mixed with 19 mL distilled water to give a 1:20 dilution. The assay mixture contained 4 mL of hydrogen peroxide solution (800 mmol) and 5 mL of phosphate buffer at pH 7.0 in a 10-mL flat bottom flask. Thereafter, 1 ml of the properly diluted sample was rapidly mixed with the reaction mixture by a gentle swirling motion at room temperature. Then 1 mL portion of the reaction mixture was withdrawn and blown into 2 mL dichromate/acetic acid reagent at 60-second intervals. The hydrogen peroxide contents of the withdrawn sample were determined by reading the absorbance at 570 nm.

2.11. Determination of the Superoxide Dismutase (SOD) Activity

The SOD activity was determined according to the method described by Misra and Fridovich (1972). An aliquot of the brain homogenate was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reactions were initiated by the addition of 0.3 mL 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 adrenaline, and 0.2 mL of water. The increase in absorbance at 480 nm was monitored every thirty seconds.

2.12. Determination of Reduced Glutathione Concentration

The reduced glutathione (GSH) level was determined using Ellman's reagent, 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) as described by Jollow *et al*. (1974). This method is based on the development of a relatively-stable yellow complex formed as a result of the reaction between Ellman's reagent and free sulphydryl groups. The chromophoric product, 2–nitro–5– thiobenzoic acid, resulting from the reaction of Ellman's reagent with GSH. The absorbance of the complex formed was read at 412 nm, which is proportional to the level of GSH in the brain homogenate sample. Thereafter, the activity of GSH was determined.

2.13. Glutathione Peroxidase (GPx) Assay

This assay was carried out using the Rotruck *et al.* (1973) method. The reaction mixture containing 500 mL phosphate buffer, 100 mL sodium azide, 200 mL of GSH, and 100 mL of H_2O_2 was added to 500 mL of the brain homogenate, after which 600 mL of the sample (brain homogenate) was added and thoroughly mixed. The whole reaction mixture was incubated at 37 ^{0}C for three minutes, after which 0.5 mL TCA (trichloroacetic acid) was added and thereafter centrifuged at 3000 g for 5 minutes. Subsequently, to 1 mL of each of the supernatants, 2 mL of K_2HPO_4 and 1 mL DTNB (Ellman's Reagent) was added and the absorbance was read at 412 nm against a blank.

2.14. Determination of Lipid Peroxidation

Lipid peroxidation was determined according to the method described by Adam-Vizi and Seregi (1982). An aliquot of 0.4 mL of the brain homogenate was mixed with 1.6 mL of Tris-KCl buffer to which 0.5 mL of 30% TCA was added. Then 0.5 mL of 0.75% TBA was added and placed in a water bath for fort-five minutes at 80 ^{0}C. This was then cooled in ice, and centrifuged at 3000 g for five minutes. The clear supernatant was collected, and the absorbance was measured against a reference blank of distilled water at 532 nm. The malondialdehyde level was then calculated.

2.15. Determination of Hydroperoxides

The method described by Jiang *et al.* (1992) was used for this determination. Briefly, 0.1 mL of brain homogenate was added to 0.9 mL of Fox reagent (contained 88 mg of butylated hydroxytoluene, 7.6 mg of xylenol orange and 9.8 mg of ammonium ion sulphate were added to 90 mL of methanol and 10 mL of 250 mM sulphuric acid). This was then incubated at 37 ^{0}C for thirty minutes. The color developed was read at 560 nm calorimetrically. The hydroperoxides were expressed as mM/100 g tissue.

2.16. Data Analysis

Data were expressed as the mean of six replicates ± standard error of mean. The statistical evaluation of data was performed by SPSS (version 20). Using the one-way analysis of variance, followed by Dunett's post hoc test for multiple comparisons, values were considered statistically-significant at $p < 0.05$ (Zar, 1984).

3. Results

Figure 1 and 2 show the effects of the free and bound polyphenolic extract of *Syzygium cumini* leaves on the acetlycholinesterase and butyrylcholinesterase inhibitory activities respectively. The extracts demonstrated a significant increase ($p < 0.05$) in dose dependent manner in the inhibitory activities of acetlycholinesterase and butyrylcholinesterase. However, the bound polyphenolic extract of the *Syzygium cumini* leaves showed more inhibitory activities in dose dependent manner than the free polyphenolic extract on both cholinesterases.

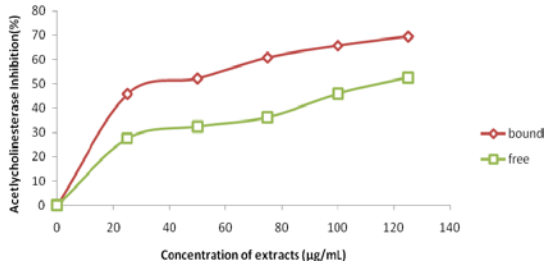

Figure 1. Effect of polyphenolic extract of Syzygium *cumini* leaves on acetylcholinesterase (AChE) activity *in vitro*. Each value is a mean three replicates ± standard error of mean.

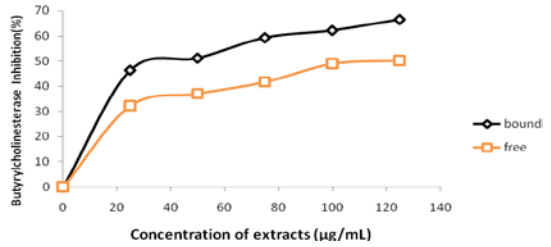

Figure 2. Effect of polyphenolic extract of Syzygium cumini leaves on butyrylcholinesterase (BChE) activity in vitro. Each value is a mean three replicates ± standard error of mean.

The brain of diabetic control rats demonstrated a significant ($p < 0.05$) increase in cholinesterases inhibitory activities when compared with normal control (Figure 3). But by the end of the fourteen-day treatment with the polyphenolic extract of the *Syzygium cumini* leaves, the activities of these enzymes were significantly reduced ($p < 0.05$), and compared favorably with normal control rats in the metformin-treated groups.

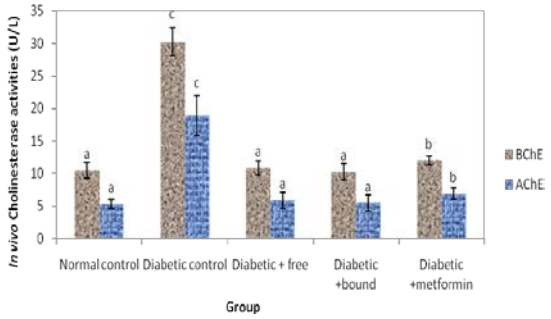

Figure 3. Effect of administration of polyphenols from *Syzygium cumini* leaves on *in vivo* cholinesterases activities in brain of normal and alloxan-induced diabetic rats. Each value is a mean of six replicates ± standard error of mean.

The activities of catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and glutathione peroxidase (GPx) were significantly decreased ($p < 0.05$) in the diabetic control rats when compared with the normal rats (Table 1). Also, the treatment of diabetic rats with the free and bound polyphenolic extract of the *Syzygium cumini* leaves significantly increased ($p<0.05$) the activities of all the antioxidant enzymes studied and compared favorably with the normal control.

Table 1. Effect of administration of polyphenols from *Syzygium cumini* leaves on antioxidant enzymes activities (U/mg protein) in the brain of normal and alloxan-induced diabetic rats.

Group	Catalase (CAT)	Superoxide dismutase (SOD)	Glutathione Peroxidase (GPx)	Reduced glutathione (GSH)
Normal Control	86.23±3.56^a	43.56 ±4.80^a	64.78 ± 8.34^a	30.21 ±5.56^a
Diabetic control	15.23 ± 2.12^c	9.69 ± 2.12^c	12.89 ± 3.65^c	11.67 ±6.80^c
Diabetic + free polyphenols	84.89 ± 7.34^a	40.58 ±5.48^a	63.84 ± 5.29^a	28.76 ±9.24^a
Diabetic + bound polyphenols	85.65 ± 4.38^a	42.39 ± 6.28^a	63.97 ± 6.87^a	29.56 ±7.00^a
Diabetic + metformin	75.29 ± 2.14^b	35.38 ±3.45^b	57.86 ± 4.80^b	25.56 ±3.11^b

Values are mean ± SEM of six rats. Values down the vertical column carrying different superscripts for each parameters are significantly different at $p<0.05$.

There was a significant increase ($p < 0.05$) in the brain lipid peroxidation and hydroperoxides of the diabetic control rats in comparison to the normal control and metformin- treated diabetic rats (Table 2). The fourteen-day administration of the polyphenolic extract of the *Syzygium cumini* leaves on diabetic rats significantly decreased ($p < 0.05$) the level of lipid peroxidation and hydroperoxides in the brains of rats, compared favorably with normal control rats.

Table 2. Effect of administration of polyphenols from *Syzygium cumini* leaves on lipid peroxidation (MDA) and hydroperoxides concentrations in the brain of normal and alloxan-induced diabetic rats**.**

Group	MDA (X 10^9 mmol/mL)	Hydroperoxides (mM/100g tissue)
Normal control	1.34 ± 0.14^a	100.21 ± 4.28^a
Diabetic control	6.42 ± 0.34^c	150.45 ± 6.90^c
Diabetic + free polyphenols	1.36 ± 0.34^a	100.34 ± 5.23^a
Diabetic + bound polyphenols	1.35 ± 0.45^a	102.58 ± 7.32^a
Diabetic + metformin	2.13 ± 0.67^b	110.12 ± 4.21^b

Values are mean ± SEM of six rats. Values down the vertical column carrying different superscripts for each parameters are significantly different at $p<0.05$

4. Discussion

Persistent/chronic hyperglycaemia might cause an imbalance in the oxidative status of the nervous tissue. Thus, the resulting free radicals could injure the brain (a key organ in the nervous system) by a process referred to as peroxidative mechanism (Pari and Latha, 2004). This

might lead to a disease condition known as Alzheimer's diseases which suggested that continuous hyperglycemia in diabetes mellitus patients may interact with the acetylcholine/butyrylcholine receptors affecting the binding efficiency and leading to an increase in the AChE/BChE activity, and the decomposition of the higher levels of the neurotransmitter. According to Wacker *et al*. (2005) acetylcholine, the principal neurotransmitter of the cholinergic neurons, is one amongst many neurotransmitters responsible for neurodegenerative diseases. It is also related to cognitive functions concerned with learning and memory processes (Blockland, 1995). Moreover, cholinergic neurons correspond to about 25% of the brain cells, which are represented mainly by cortical and hippocampal neurons (Wacker, 2005). The synaptic cholinergic transmission depends on the acetylcholinesterase (AChE) activity, since this enzyme promotes the hydrolysis of the neurotransmitter acetylcholine in choline and acetic acid, resulting in the terminus of the transmission of the nervous impulse within the synapses (Taylor, 1996). During this study, the polyphenols extracted from the *Syzygium cumini* leaves demonstrated inhibitory activities of AChE and BChE in dose dependent manner (Figures 1 and 2). This is an indication that the extract can be useful in the ameliorating AChE/BChE activity, and may therefore serve as a neurotransmitter booster. Also, this was supported by the *in vivo* inhibitory activities of AChE and BChE (Figure 3), which was in agreement with the findings in Gill *et al.* (1991) and Gallegos *et al*. (2001).

In addition, the likely mechanism, by which the persistent hyperglycemia causes neurotoxicity, is probably by targeting the learning and memory processes of the brain by inhibiting the N-methyl-D-aspartate receptor (NMDAR), essential for hippocampus-mediated learning and memory (Akinyemi *et al*., 2015). Furthermore, Orhan *et al*. (2004) reported that the enhanced activities of AChE and BChE are detrimental to patients suffering from Alzheimer's disease. Accordingly, the consumption of polyphenols extracted from *Syzygium cumini* leaves can be very functional to health as it could restructure the neurodegeneration by inhibiting the AChE and BChE activities.

Moreover, the treatment with antioxidants is a promising approach for reducing the progression of Alzheimer's disease, associated with chronic hyperglycemia coupled with oxidative stress. Grundman *et al.* (2002) reported a link between antioxidants and reduced incidence of dementia. The vulnerability of the brain to oxidative stress induced by oxygen free radicals might be attributed to the utilization of one fifth of the total oxygen demand of the body; the brain is not enriched with enough antioxidant enzymes when compared with other organs (Bayes and Thrope, 1999). Antioxidant enzymes play an important role in the maintenance of physiological concentrations of oxygen and hydrogen peroxides by enhancing the dismutation of oxygen radicals and mopping up organic peroxides generated by the exposure to alloxan (Kazeem *et al*., 2013). Superoxide dismutase (SOD) may mop up superoxide radicals by converting them into H_2O_2 and oxygen while both CAT and GPx are useful in the elimination of H_2O_2 (Vincent *et al*., 2004). In this study, a significant reduction was noted in the activities of CAT, SOD and GPx (Table 1) in the brain of diabetic rats. This can be attributed to the rise in the generation of reactive oxygen species like superoxide and the hydroxyl radical by alloxan (Kaleem *et al*., 2006). Also, the free radicals produced may inactivate the activities of these antioxidant enzymes (Soon and Tan, 2002).Accordingly, this may be accountable for the dearth of antioxidant defenses in justifying the ROS mediated damage (Pari and Latha, 2005). The administration of polyphenols extracted from *Syzygium cumini* leaves enhanced the activities of these antioxidant enzymes probably by ameliorating the imbalance between the ROS production and the antioxidant enzyme-activity in diabetic rats. This may be responsible for the AChE and BChE inhibitory activities observed in Figure 3.

In the same context, GSH is an antioxidant and its reduction has been documented in diabetes mellitus, which may be attributed to its increased utilization by oxidative stress (Pari and Latha, 2004). In the current study, the decreased GSH activity was observed in diabetic rats, but at the end of the fourteen-day experimental period of the administration of the polyphenols extracted from the *Syzygium cumini* leaves, the brain GSH activity increased. The elevated levels can protect cellular proteins against oxidation through the glutathione redox cycle (Yu, 1994).

In addition, the elevated levels of MDA and hydroperoxides were observed in brains of diabetic rats, because the brain contains a high concentration of easily peroxidizable fatty acids (Carney *et al*., 1991); this was in agreement with the present study (Table 2). However, the administration of polyphenols extracted from *Syzygium cumini* leaves reversed their concentrations. This may be attributed to the free radical scavenging ability of the extract. The extract was found to be anti-cholinesterase, an antioxidant enzyme booster, anti-lipid peroxidation, and anti- hydroperoxides on the treated diabetic rats. This can be attributed to the beneficial bioactivity and antioxidant nature of the extracts.

5. Conclusion

The brain demonstrates a series of morphological and functional alterations during diabetes mellitus which can trigger diseases including Alzheimer's disease, probably as a result of the oxidative stress. The treatment of diabetic rats with polyphenols extracted from *Syzygium cumini* leaves significantly inhibit the activities of AChE and BChE, increased the antioxidant enzymes activities, and decreased the levels of lipid peroxidation and hydroperoxides. This may be attributed to the readily absorbable and antioxidant nature of the polyphenols of *Syzygium cumini* leaves.

Conflict of Interests

The authors declare no conflict of interests.

Ethical Approval

This study was approved by ABUAD Ethical Committee.

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References

Adam-Vizi V and Seregi A. 1982. Receptor independent stimulatory effect of noradrenaline on Na, K-ATPase in rat brain homogenate. Role of lipid peroxidation. *Biochem Pharmacol.*, **34**: 2231-2236.

Ajiboye BO and Ojo OA. 2014. Effects of aqueous leaf extract of *Senecio biafrae* on hyperglycaemic and haematological parameters of alloxan induced diabetic rats. *PharmacolOnline*, **3**:163 169.

Akanksha A, Srivastava A and Maurya R. 2010. Antihyperglycemic activity of compounds isolated from Indian medicinal plants. *Ind J Exp Biol*., **48**:294-298.

Akinyemi AJ, Oboh G and Ademiluyi AO. 2015. Local salt substitutes "Obu-otoyo" activate acetylcholinesterase and butyrylcholinesterase and induce lipid peroxidation in rat brain. *Interdiscip Toxicol.*, **8(3)**: 139–145.

Bayes JW and Thrope SR. 1999. Role of oxidative stress in diabetic complications. *Diabetes*, **48**:1-9.

Belle NAV, Dalmolin GD, Fonini G, Rubim MA and Rocha JBT. 2004. Polyamines reduces lipid peroxidation induced by different prooxidant agents. *Brain Res.*, **1008**:245-251.

Blockland A. 1995. Acetylcholine: a neurotransmitter for learning and memory? *Brain Res Rev.*, **21**: 285–300.

Carney JM, Strake-Reed PE, Oliver CN, Landum RW, Chang MS, Wu JF and Floyd RA. 1991. Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity and loss in temporal and spatial memory by chronic administration of the spin trapping compound N-tert-buty-alfa-pheynitrone. *PNAS*, **88**:3633-3636.

Chang YT, Chang WN, Tsai NW, Huang CC, Kung CT and Su YJ. 2014. The Roles of biomarkers of oxidative stress and antioxidant in Alzheimer's disease: A Systematic Review. *BioMed Res Inter.*, **2014:** 1-11.

ChuY, Sun J, Wu X and Liu RH. 2002. Antioxidant and antiproliferatve activity of common vegetables. *J Agric Food Chem*., **50 (23**): 6910-6916.

Ellman GL, Courtney KD, Andres V and Featherstone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*, **7**:88–95.

Gallegos MEH, Zannatha MMI, Osornio EG, Sanches AS and Rio FAP. 2001. Immediate and delayed effects of lead on AChE, GSH-T and thiols in the substantia nigra, neostriatum and cortex of the rat brain. *J Appl Toxicol*.,**21**:397–401.

Gill TS, Teware H and Pande J. 1991. *In vivo* and *in vitro* effects of cadmium on selected enzymes in different organs of the fish *Barbus conchonius* Ham. (Rosy barb). *Comp Biochem Physiol Part C*,**100**: 501–505.

Grundman M, Grundman M and Delaney P. 2002. Antioxidant strategies for Alzheimer disease. *Proc Nutr Soc*., **61**: 191-202.

Ingkaninan K, Temkitthawon P, Chuenchon K, Yuyaem T and Thongnoi W. 2003. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. *J Ethnopharmacol*., **89**, 261–264.

Jiang ZY, Hunt JV and Wolff SP. 1992. Ferrous ion oidationin the presence of xylenol orange for detection of lipid hydroperoxide in low–density lipoprotein. *Anal Biochem*, **202**:384-387.

Jollow DJ, Mitchell JR, Zampaglione N and Gillete JR. 1974. Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenze oxide as a hepatotoxic metabolite. *Pharmacol.*, **22**:151-169.

Kaleem M, Asif M, Ahmed QU and Bano B.2006. Antidiabetic and antioxidant activity of *Annona squamosa* extract in streptozotocin-induced diabetic rats. *Singapore Med J*., **47(8):** 670–675.

Kazeem MI, Akanji MA, Yakubu MT and Ashafa AOT. 2013. Protective effect of free and bound polyphenol extracts from ginger (*Zingiber officinale* Roscoe) on the hepatic antioxidant and some carbohydrate metabolizing enzymes of streptozotocin-induced diabetic rats. *Evidence-Based Complement Alternative Med*.,**2013,** article ID 935486.

Khan RA.2012. Effects of *Launaea procumbens* on brain antioxidant enzymes and cognitive performance of rat. *BMC Complement Alternative Med.*, **12**:219.

Lane RM, Potkin SG and Enz A.2006. Targeting acetyl-cholinesterase and butyrylcholinesterase in dementia. *Intl. J. Neuropsycopharmacol*, **9**:101-124.

Misra HP and Fridovich I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*., **247**:3170-3175.

Orhan I, Sener B, Choudhary MI and Khalid A. 2004. Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some Turkish medicinal plants. *J Ethnopharmacol*, **91**: 57–60.

Pari L and Latha M. 2005. Antidiabetic effect of *Scoparia dulcis*:effect on lipid peroxidation in streptozotocin diabetes. *Gen Physiol Biophys*., **24(1):** 13–26

Pari L and Latha M. 2004. Protective role of *Scoparia dulcis* plant extract on brain antioxidant status and lipid peroxidation in STZ diabetic male Wistar rats. *BMC Complement Alternative Med.*, **4**:16.

Querfurth HW and LaFerla FM. 2006. Alzheimer's disease. *N Engl J Med*. ,**2010**; 362:329-344.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG and Hoekstra WG. 1973. Selenium: biochemical role as a component of glutathione peroxidase. *Science*,**179**:588-590.

Sanches JR, França LM, Chagas VT, Gaspar RS, dos Santos KA, Gonçalves LM, Sloboda DM, Holloway AC, Dutra RP, Carneiro EM, Cappelli APG and Paes AM. 2016. Polyphenol-rich extract of *Syzygium cumini* leaf dually improves peripheral insulin sensitivity and pancreatic islet function in monosodium l-glutamate-induced obese rats. *Frontiers in Pharmacol*., **2016**; 7:48

Schneider LS. 2001. Treatment of Alzheimer's disease with cholinesterase inhibitors. *Clin Geriatr Med*., **17**:337-358

Sharma J, Chawla R, Kumar R, Sharma A, Sharma RK and Arora R. 2013. *Camellia sinensis* as a Safe Neuroprotective Radiation Counter Measure Agent. *Inter J Pharm Sci Inven*., **2**: 26-33

Sinha AK. 1972. Colorimetric assay of catalase. *Anal Biochem*.,**47**:389-394.

Soon YY and Tan BKH. 2002. Evaluation of the hypoglycemic and anti-oxidant activities of *Morinda ofcinalis* in streptozotocin-induced diabetic rats. *Singapore Med J*.,**43(2):** 77–85.

Taylor P. 1996. Anticholinesterase agents. In**: Goodman & Gilman's the Pharmacological Basis of Therapeutics. (9th Ed).** Hardman JG, Gilman AGand Limbird LE (Eds.). New York: McGraw-Hill Companies, pp 161-176.

Uddin MS, Al Mamun1 A, Hossain AS, Ashaduzzaman M, Noor MAS, Hossain MS, Uddin MJ, Sarker J and Asaduzzaman M.

2016. Neuroprotective effect of *Phyllanthus acidus* L. on learning and memory impairment in scopolamine-induced animal model of dementia and oxidative stress: Natural Wonder for Regulating the Development and Progression of Alzheimer's disease. *Advances in Alzheimer's Dis.*, **5**: 53-72.

Uttara B, Singh AV, Zamboni P and Mahajan RT. 2009. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacol*, **7**:65-74

Vincent AM, Russell JW, Low P and Feldman EL. 2004. Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocrine Reviews*, **25 (4):** 612–628.

Wacker P, Nunes PV and Forlenza OV. 2005. *Delirium* e demência no idoso: existem fatores de risco comuns? *Ver Psiq Clín*., **32**: 113–118.

Yu BP. 1994. Cellular defense against damage from reactive oxygen species. *Physiol. Rev.* **74**:139-162.

Zar JH. 1984. **Biostatistical Analysis**. Englewood Cliffs, NJ: Prentice Hall.