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Impacts of Dietary *Chrysophyllum albidum* Fruit Pulp on Brain Cholinesterase Function in High-Fat Diet/Streptozotocin-Induced Diabetic Rats

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Abstract

Epidemiologic studies have shown strong correlations between Alzheimer's disease and diabetes mellitus. The exact mechanism through which this happens remains unclear. However, the dependence on glucose for brain function has been proposed as one possible mechanism. Hence, this study investigated the neuroprotective potential of *Chrysophyllum albidum* fruit pulp (CAPP) with hypoglycaemic properties in diabetic rats induced with high-fat diet/streptozotocin (STZ). The animals were grouped into seven units as follows: control, STZ-induced, STZ + metformin (positive control), STZ + 5% CAPP, STZ + 10% CAPP, control + 5% CAPP and control + 10% CAPP and each group was made up of six rats. The animals were first placed on normal diet (non-diabetic groups) and high fat diet (diabetic groups) for a fortnight, respectively before induction with STZ and were treated with diets containing 5 and 10% CAPP for 14 days. After the experiment, the rat brain cholinesterase and antioxidant activities were determined. The results revealed that acetylcholinesterase (AChE), butylcholinesterase (BuChE), arginase, adenosine deaminase (ADA) and antioxidant activities were altered in STZ-diabetic group in comparison to the control. However, a significant decrease at $p < 0.05$ was found in the activities of AChE, BuChE, arginase and ADA. In addition, there was a concomitant rise in the levels of antioxidant in all the groups administered supplemented diets and the group treated with metformin in comparison to the STZ-diabetic group. Conclusively, we can suggest that the fruit pulp prevents neurological damage in diabetic rats via anticholinesterase activity and improvement of brain antioxidant status.

Keywords: *Chrysophyllum albidum;* Diabetes; Metformin; Cognitive function; Neuromodulation.

Introduction

There has been a constant increase in both neurodegenerative diseases such as Alzheimer's disease (AD) and diabetes mellitus since the last decade. These diseases have become global health challenges which affect a relatively large number of people. Although, diseases like these are often considered as autonomous conditions, growing evidence shows that a link exists

between these two disorders. However, the exact mechanism by which AD and diabetes are connected remains poorly understood. But, studies have shown that high blood sugar or insulin resistance can damaged the brain (Rivera et al. 2005, de la Monte 2017, Fiore et al. 2019).

The brain is susceptible to damages mainly because it has low antioxidant levels, uses large amount of oxygen, and largely depends on glucose and other chemicals, which are likely to be unstable due to high insulin resistance. Some of these changes may contribute towards the development of AD (Rivera et al. 2005, Lee et al. 2013, Talbot 2014).

Inhibition of key biochemical proteins such as monoamine oxidase (MAO), acetylcholinesterase (AChE), butyrylcholinesterase (BChE) in the brain, and the prevention of oxidative stress have been recommended as valuable therapeutic mechanisms in the management of neurodegenerative conditions (Youdim et al. 2006). AChE inhibitors such as galanthamine, rivastigmine, tacrine, and donepezil are well-known for treating AD, but these drugs may have possible side effects and therefore possess limitations for clinical uses (Sung et al. 2002, Zarotsky et al. 2003). Recently, researches are focused on discovering and developing natural AChE inhibitors. Interestingly, several fruits have shown promising potentials (Howes et al. 2003, Adsersen et al. 2006).

Several epidemiologic studies have strongly associated the consumption of vegetables and fruits with reduced risks of diabetes, AD, and other functional declines which are related to age (Liu 2013). Fruits are vital components of human's daily diet which have various bioactive compounds that promote health and prevent diseases. According to Paredes-López et al. (2010), flavonoids and hydrolysable tannins are the principal phenolic compounds present in most fruits. There are numerous health benefits of fruit polyphenol. For instance, it helps to prevent oxidative stress, inflammation, diabetes and diseases which include neurodegenerative and cardiovascular diseases (Hussain et al. 2018).

African star apple (*Chrysophyllum albidum*), a lowland rain forest tree species, is one of the over 800 species of the family Sapotaceae. At maturity, it grows up to about twenty-five to thirty-seven metres in height and has a girth which varies from 1.5 to 2 metres. It grows naturally in Nigeria, Uganda, and Niger Republic (Adebayo et al. 2011). The plant has diverse ethnomedicinal uses and different tribes in Nigeria attribute different local names to it (Amusa et al. 2003). For instance, the Yorubas call it "*agbalumo*" while the Igbos call it "*udara*". The plant is economically valuable in Nigeria (Oboh et al. 2009). The fleshy part of the fruit can be used as jam or eaten as a snack (Amusa et al. 2003). It is rich in ascorbic acid (about 1,000 mg to 3,300 mg per 100 g of edible fruit), 10 times more than the ascorbic acid content in guava and 100 times more than that of orange (Amusa et al. 2003). Oboh et al. (2018) confirmed that the fruit is rich in polyphenolic compounds. Some studies have shown that it possesses anti-inflammatory, antioxidant, and hypocholesterolemic properties (Ibrahim et al. 2017, Bobadoye et al. 2016). In addition, the fruit parts exhibited antioxidant and inhibitory properties on cholinesterase and monoamine oxidase activities *in vitro* (Oboh et al. 2018). Based on these findings, it is important to investigate their biological actions in various experimental animal models in order to justify their ethnopharmacological activities. Hence, the present study was designed to determine the hypoglycaemic and neuroprotective effects of *C. albidum* fruit pulp in type-2 diabetic rat model.

Materials and Methods

Materials

Africa star apple fruits which were verified at the Department of Plant Science and Biotechnology, Ekiti state University, Ado-Ekiti, Nigeria were originally sourced from a local market in the same town. The ripe fruits were collected around March, which is the regular season of the fruit. The

analysis started with thorough cleaning of the fruits followed by seed extraction and then the removal of fruit pulp. Then, the fruit pulp was frozen-parched and powdered. The fruit yield was 25.0 g dry sample/100 g fresh fruit pulp. Prior to the analysis, the sample was refrigerated at –10 °C. Also, in order to assess the nutritional content of the pulp, the sample was proximately examined. Voucher specimen of *C. albidum* was deposited in the Herbarium.

Experimental design

Following the method of Adefegha et al. (2014) with little modifications, we prepared the feed and carried out bioassays. For a period of two weeks, fifty male Wistar rats weighing 185–210 g were kept in cages which were well ventilated with light cycle which was well ordered. This was done to familiarize the animals. The animals were fed and given water as often as necessary during this period.

Animal ethics

This study strictly followed the criteria outlined by the EU Directive 2010/63/EU regarding the use and care for animals for experimental purposes. The ethic regulations in agreement with national and institutional guidelines for the protection of animals' welfare during experiments were trailed. The study was approved by the Ethics Committee for Animal Experimentation at the Ekiti State University, Ado-Ekiti, Nigeria with a bioethical allowance reference number of ORD/ETHICS/AP/028.

Induction and assessment of STZ-induced diabetic rats fed HFD (type 2 diabetic rat model)

After two weeks of acquaintance, we placed the rats on two different dietary manipulations for another two weeks. The first group was placed on a normal diet (NC) as the control group, while the second group (treated group) was placed on high fat diet (HFD). Table 1 shows how the feed was formulated. After a fortnight, following Srinivasan et al. (2005), the treated group was administered STZ for diabetes induction. This was administered intra-peritoneally (i.p.) at one dose of 35 mg kg^{-1} body weight. Also, the rats were not fed twelve hours prior to the examination/evaluation of their blood glucose level. Thus, the rats used for the research are those classified as diabetic based on a blood glucose level of 200 mg dL^{-1} or above. The experimental procedure commenced two days after the rats were diabetes-induced.

Table 1: Feed formulation for normal control (NC) and high-fat diet (HFD) fed rats

	NC diet (g kg^{-1})	HFD (g kg^{-1})
Skimmed milk	500	500
Lard	–	300
Rice bran	200	90
Corn starch	160	70
Premix	40	40
Groundnut oil	100	–

Skimmed milk contained 360 g kg^{-1} protein; mineral and vitamin premix (10 g kg^{-1}) contained 3200 i.u. vitamin A, 600 i.u. vitamin D3, 2.8 mg vitamin E, 0.6 mg vitamin K3, 0.8 mg vitamin B1, 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 iodine, 8.4 mg iron, 16 mg manganese, 0.08 mg selenium, 12.4 mg zinc, 0.5 mg antioxidant and lard contained 99% fat. African star apple fruit pulp contained 9.23% protein.

Experimental design

The rats were casually grouped into 7 categories of 6 rats each. These rats were placed on a different dietary treatment for 14 days. Table 2 presents the composition of the experimental diets on which the rats were placed. Non-diabetic animals received 1 mL of 0.1 mol L^{-1} citrate buffer i.p. The feed intake was monitored daily and their weight was checked on a regular basis within an interval of 3 days throughout the period of the experiment. The animal groups were as follows:

Group 1: Normal healthy rats given citrate buffer (pH 4.5) (1 mL/kg, intraperitoneally) and fed basal diet (skimmed milk 40 percent,

corn starch 46 percent, mineral and vitamin premix 4 percent and groundnut oil 10 percent).
Group 2: Untreated diabetic control rats retained on basal diet.
Group 3: Diabetic control rat retained on basal diet and oral standard drug (metformin) orally (25 mg/kg body weight).
Group 4: Diabetic control rats retained on basal diet augmented with 5% *C. albidum* fruit pulp powder.
Group 5: Diabetic control rats retained on basal diet augmented with 10% *C. albidum* fruit pulp powder.
Group 6: Non diabetic rats retained on basal diet augmented with 5% *C. albidum* fruit pulp powder.
Group 7: Non diabetic rats retained on basal diet augmented with 10% *C. albidum* fruit pulp powder.

Table 2: Diet formulation for basal and supplemented diets for control and test groups (g/100 g)

Ingredients	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Skimmed milk (g)	40	40	40	38.47	36.92	38.47	36.92
Oil (g)	10	10	10	9.84	9.68	9.84	9.68
Premix (g)	4	4	4	4	4	4	4
Corn starch (g)	46	46	46	43.69	49.40	43.69	49.40
Sample (g)	-	-	-	5%	10%	5%	10%
Total (g)	100	100	100	100	100	100	100

Note: Skimmed milk = 32% protein;

The vitamin premix (mg or IU/g) h had the following composition: 3200 IU vitamin A, 600 IU vitamin D3, 2.8 mg vitamin E, 0.6 mg vitamin K3, 0.8 mg vitamin B1, 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 iodine, 8.4 mg iron, 16 mg manganese, 0.08 mg selenium, 12.4 mg zinc, 0.5 mg antioxidant. Group 1: (Control) normal control rats fed basal diet; Group 2: diabetic control untreated rats placed on a basal diet; Group 3: diabetic rats placed on a basal diet and treated with metformin; Group 4: diabetic rats placed on a basal diet supplemented with 5% *C. albidum* fruit pulp powder; Group 5: diabetic rats placed on a basal diet supplemented with 10% *C. albidum* fruit pulp powder; Group 6: normal rats placed on a basal diet supplemented with 5% *C. albidum* fruit pulp powder; Group 7: normal rats placed on a basal diet supplemented with 10% *C. albidum* fruit pulp powder.

With an auto analyser (Fine test Auto-coding TM), we monitored the blood glucose level of each group. After 2 weeks of treatment, the rats were not fed (fasted) overnight and sacrificed by decapitation the next day. The doses were used to explore the likely biological effects relative to increased consumption of the materials. In addition, the sixth and seventh groups were included to examine the likely biological effects of the fruit's supplementation under normal and healthy condition. The percentage inclusions were used to investigate the possible biological effects in relation to increase in consumption of these materials.

Tissue homogenate and biochemical analysis preparation

The animals were sacrificed after 2 weeks. After isolating their brains, we placed the isolated brains on ice. This was followed by weighing and rinsing in cold (0.9 percent) normal saline (1:3, w/v). Afterward, they were regulated in sodium phosphate buffer (pH 6.9) and the homogenates were centrifuged at 5000×g. The biochemical assays were conducted using the obtained clear supernatant. Also, cholinesterases (AChE and BuChE) (Akinyemi et al. 2017), arginase (Zhang et al. 2001), and adenosine deaminaseactivities (ADA) were determined

(Guisti and Galanti 1984). Following the approach of Akinyemi et al. (2017), in vivo antioxidant enzymes and non-enzyme assays which included glutathione-S-transferase (GST) activity, lipid peroxidation, reduced glutathione (GSH) and non proteinthiol (NPSH) contents were also carried out.

Data analysis

Descriptive statistics such as mean ± standard error of mean (S.E.M.) were used in analysing the data. Using Graph Pad Prism 6.0 Software, a one-way analysis of variance (ANOVA) was adopted to examine the differences among the groups. Then, a post hoc Tukey's test was conducted to verify if the differences between the groups were significant or not. The level of significance was set at 5 percent.

Results

Effects of dietary *C. albidum* fruit pulp powder (CAPP) on blood glucose level in type-2 diabetic rats

The results as presented in Figure 1 showed a significant rise in blood glucose of the STZ (treated) group in comparison to the control group. For the treatment period, the blood glucose of the rats in treated groups was between 250.50 and 268.50 mg/dL. However, the blood glucose level of the STZ + metformin group was reduced from 248.00 to 191.00 mg/dL. Interestingly, as compared to the STZ untreated groups, there was a significant reduction in the blood glucose level of the STZ groups which was treated with diets supplemented of 5 and 10% CAPP. The results also revealed that the blood glucose levels of the rats treated with 10% CAPP diet (STZ + 10% CAPP) significantly reduced from 246.25 to 141.00 mg/dL compared to 5% CAPP (STZ + 5%) group (259.00 to 170.50 mg/dL), whereas a non-significant reduction in blood glucose level was also recorded in normal rats which were treated with both percentages of CAPP in comparison to the normal control group.

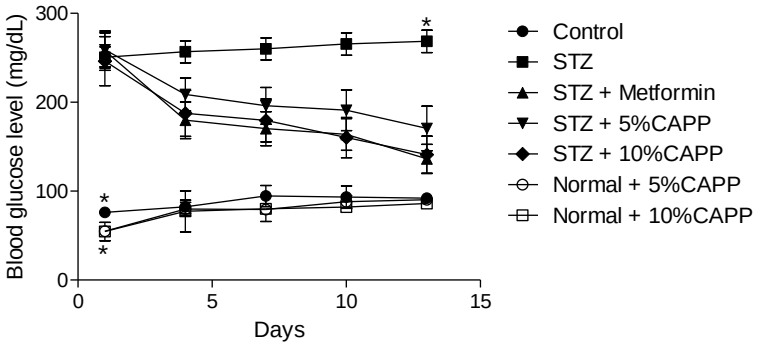

Figure 1: Effects of dietary *C. albidum* fruit pulp powder (CAPP) on blood glucose level in type-2 diabetic rats. Data are presented as mean ± SEM (n = 6). *Mean values are significantly different ($p < 0.05$) from other groups.

Effects of dietary *C. albidum* fruit pulp powder (CAPP) on arginase, acetylcholinesterase (AChE), butrylcholinesterase (BuChE), and adenosine deaminase activities in the brain of type-2 diabetic rats

The results of arginase activity in the brain homogenate of the rats presented in Figure 2a show that, in comparison with the control group, arginase activity in the STZ group significantly increased. However, after treatment with metformin and CAPP-supplemented diet, arginase activity was significantly reduced. Also, a significant difference was not found between the activity of arginase in the STZ-groups treated with 10% CAPP inclusive diet and the metformin-treated groups.

Also, it was observed that AChE activity significantly increased in the brain homogenate of the STZ-treated rats in comparison to the control group (Figure 2b).

A significant decrease in AChE activities was detected in the rat groups which were treated with metformin and CAPP diet as compared to the STZ group. However, STZ + metformin group had a significant ($P < 0.05$) decrease in AChE activity in comparison to STZ treated with CAPP-supplemented diet. Figure 2c shows that as compared to the control group, an increase was noted in BChE activity in the brain homogenate of the group treated with STZ-treated, but the treated group whose diet was supplemented with CAPP showed significant reduction ($P < 0.05$) in BChE activity in comparison to the STZ-untreated groups.

As shown in Figure 2d, in comparison to the control group, we observed a significant increase in ADA activity in the brain homogenate of the STZ-induced group. Conversely, compared to STZ-induced group, ADA activity in the brain of metformin-treated group significantly decreased. Similar results were obtained for the 5% and 10% CAPP diets treated groups.

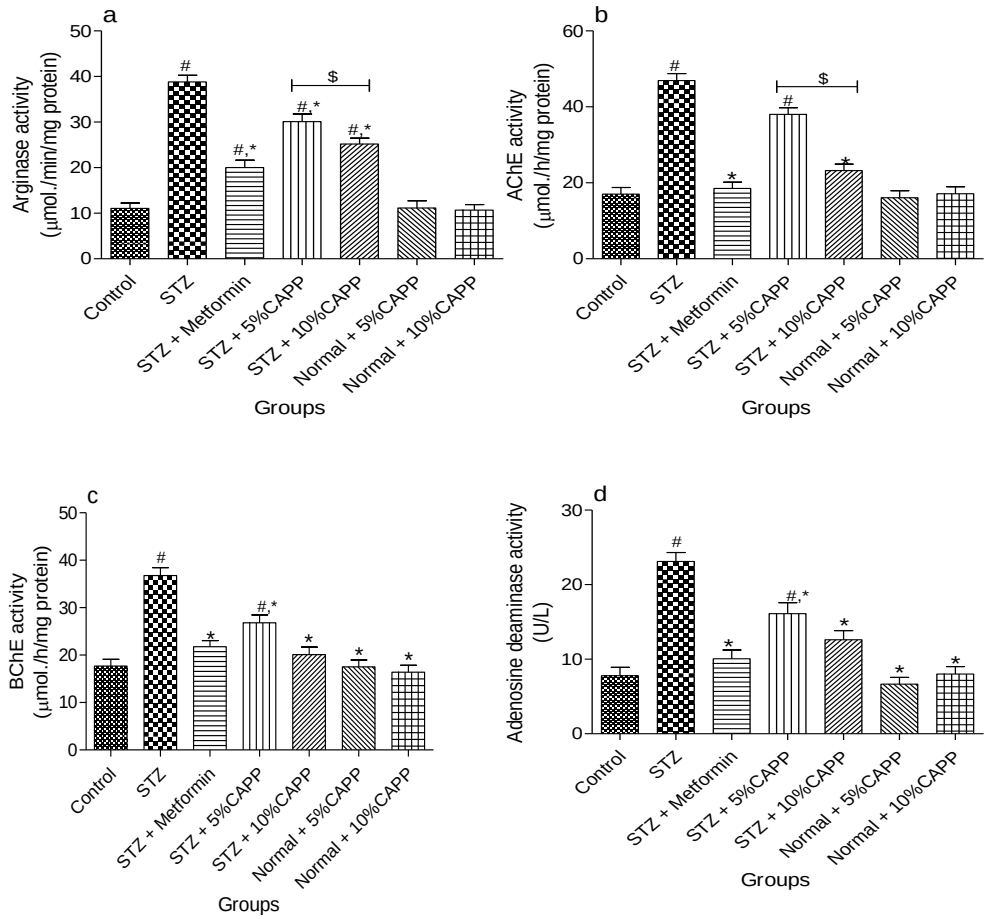

Figure 2: a = Arginase, b = Acetylcholinesterase (AChE), c = Butrylcholinesterase (BuChE), and d = Adenosine deaminase activities in the brain of type-2 diabetic rats treated with metformin and CAPP-dietary supplementation. Data are presented as mean ± SEM (n = 6). #Mean values are significantly different ($p < 0.05$) compared to control group.*Mean values are significantly different ($p < 0.05$) compared to STZ treated group. $Mean values are significantly different ($p < 0.05$) compared to STZ + metformin treated group.

Effects of dietary *C. albidum* fruit pulp powder (CAPP) on MDA levels, enzymatic (GST) and non-enzymatic TSH and NPSH antioxidant status in the brain of STZ-induced diabetic rats

Also, STZ induction led to a significant increase ($p < 0.05$) in the levels of MDA in brain homogenates of the rats in the STZ group in comparison to the control group (Figure 3a). Metformin and CAPP supplemented diet fed rats significantly decreased in the levels of MDA. Also, as compared to the control group, there was significant decrease in GST activity in the brain of the rats in the STZ group (Figure 3b). However, the activity significantly increased in metformin and CAPP supplemented diet fed rats when compared with STZ untreated group. Furthermore, comparing with the control group, there was a marked decrease in TSH and NPSH levels in the STZ group (Figure 3c and d). However, we observed a significant increase in all the groups treated with diets supplemented with CAPP and metformin when compared with STZ untreated group.

Discussion

Diabetes mellitus (DM) and its associated complications such as neuropathy and cardiovascular diseases continue to be global health concerns (ADA 2011). In recent time, the relevance and study of plants in treating diabetes has become important as a result of their therapeutic potentials. In the present study, rats were induced with DM through a high-fat diet with a low dose of streptozotocin (STZ). The model used in this study was chosen because it has the ability to reflect the clinical symptoms detected in people living with the disease (Skovsø 2014). In this study, it was observed that induction with high-fat diet/low dose STZ led to a significant rise in the levels of the blood glucose of the animals (Figure 1). This corroborates the findings of Srinivasan et al. (2005), Saliu et al. (2016), and Guex et al. (2019). This rise in blood glucose levels represents the first feature of patients which is an indication of insulin resistance (Wu et al. 2002). However, it is worth-noting that treatment with supplemented-diet and metformin group, respectively were able to lower the blood glucose levels.

Insulin-resistance plays a vital role in AD pathogenesis (Watson and Craft 2003). There are emerging evidences suggesting that the prevalence of insulin resistance and abnormalities in AD are likely to contribute to the clinical symptoms and disease pathophysiology. It has also been established that the transporters of insulin-sensitive glucose are localized to the same regions which support memory and insulin is important for memory functions, thereby suggesting that insulin may contribute to the functioning of normal cognitive and insulin abnormalities may intensify cognitive impairments, like those linked with AD. Insulin has also been implicated in regulating the acetylcholine, neurotransmitter responsible for learning and memory, but the main mechanism is still unclear (Watson and Craft 2003).

In this study, we demonstrated that high-fat diet coupled with small dose of STZ leads to the development of brain-specific insulin resistance with alterations in key biochemical enzymes responsible for the functionality of neurotransmitter in the brain (Figure 2a–d) and enhancing of oxidative stress (Figure 3a–d). It was observed that the activities of cholinesterases (AChE and BuChE), arginase and adenosine deaminase in STZ-diabetes rats were significantly increased in the brain. These results are consistent with the findings of Schmatz et al. (2009), Saravanan and Ponmurugan (2013), and Ademiluyi et al. (2015). Acetylcholine (ACh) is directly involved in motor processes, cognitive and memory and therefore, the main neurotransmitter of nervous stimuli from one neuron to another. Interestingly, it has been reported that an increased AChE activation will lead to degradation of ACh and a subsequent down stimulation of acetylcholine receptors which causes unpalatable effects on cognitive functions (Soreq and Seidman 2001).

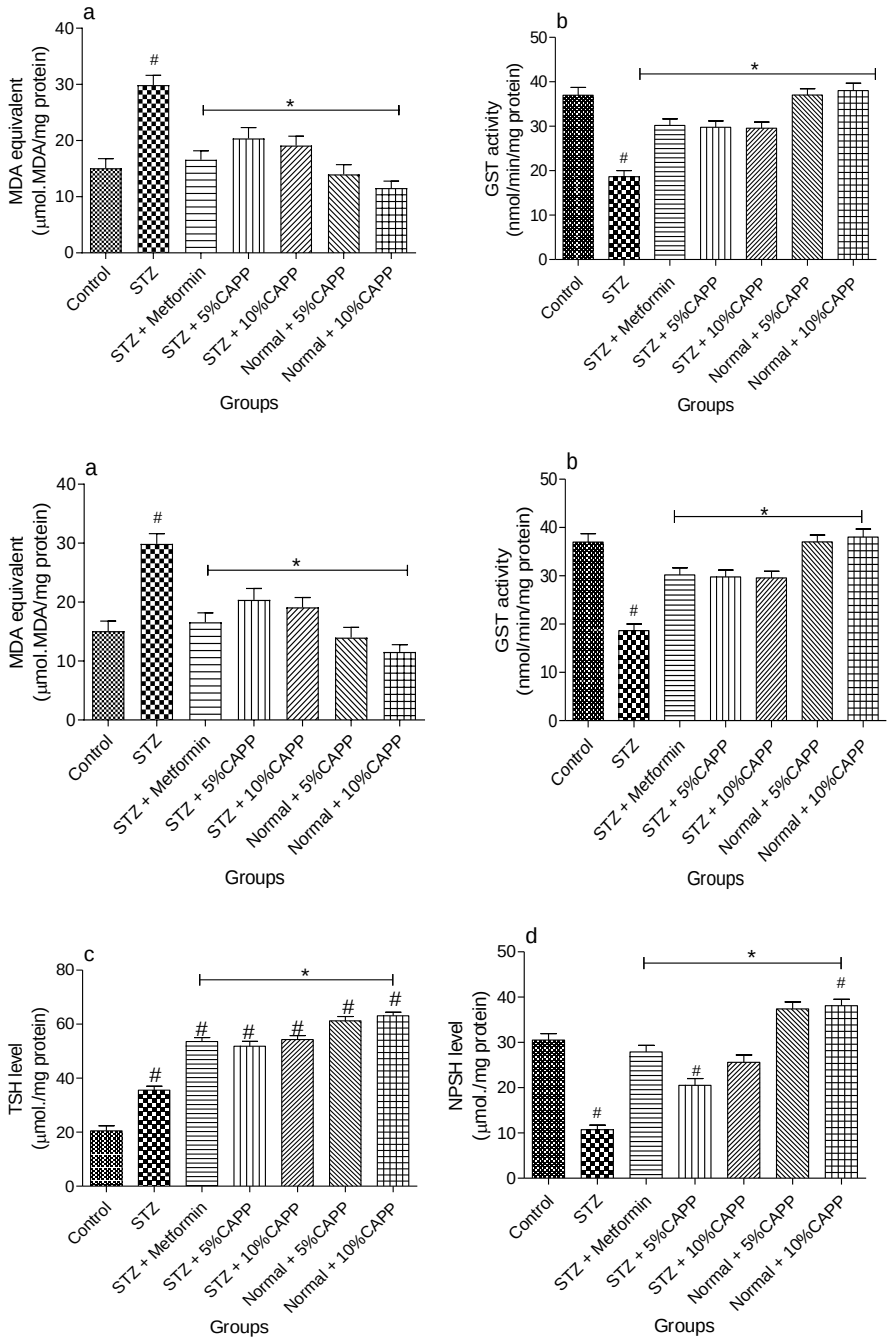

Figure 3: Effects of metformin and CAPP-supplementary diet on a. MDA levels, b. enzymatic (GST) and non-enzymatic, c. TSH, and, d. NPSH antioxidant status in the brain of STZ-induced diabetic rats.[#]Mean values are significantly different ($p < 0.05$) compared to control group.*Mean values are significantly different ($p < 0.05$) compared to STZ treated group. [$]Mean values are significantly different ($p < 0.05$) compared to STZ + metformin treated group.

Our findings therefore suggest that the increase in AChE activity caused by diabetes will affect the efficiency of cholinergic neurotransmission resulting from declining levels of acetylcholine in the synaptic cleft, therefore contributing to advanced cognitive impairment and other neurological dysfunctions exhibited by diabetic patients. However, treatment with supplemented-diet prevented alterations in the activities of cholinesterases (AChE and BuChE), arginase and adenosine deaminase when compared with the STZ-diabetic group via inhibition of these key biochemical enzymes (Figure 2a–d). This is an indication that African star apple exerts anticholinesterase properties in diabetic condition and this action is linked to their phenolic content as stated by Oboh et al. (2018). The activation of AChE in diabetes mellitus may be arbitrated by the production of free radicals and resultant oxidative stress in the brain.

According to Burnstock et al. (2011), ADA is essential for regulating the effects of adenosine in a number of systems such as the central nervous system (CNS). Adenosine is an endogenous anticonvulsant and antihypoxic. Also, it is a modulator of lipolysis, platelet aggregation, neurotransmission, blood flow, and glycogenolysis (Mcilwain 1983, Stone 1989). In mammals, it plays an important neuromodulatory role in the CNS and the brain (Burnstock 2006, Burnstock et al. 2011). Our findings revealed that high-fat diet coupled with a single small dose of STZ led to increase in the activity of ADA, which may mediate consequent decrease in the levels of adenosine. It has been reported that extracellular adenosine (EA) is an important neuromodulator in the establishment of long-term depression (LTD), long-term potentiation (LTP), and in synaptic plasticity. Thus, its depletion can lead to memory formation disruption (Burnstock 2006, Gutierres et al. 2012). However, the prevention in the alteration of activity of ADA caused by treatment with supplemented-diet as observed in this study may suggest that African star apple fruit as a promising functional food for neurological disorder involving impairment of the adenosinergic system in diabetic condition.

Also, it has been established that oxidative stress played a central role in neuronal and brain damage in both experimental and clinical diabetes (Vantyghem et al. 2000, Martín-Gallán et al. 2007). The neurotoxic effects of hyperglycaemia are arbitrated through the production of an increased reactive oxygen species (ROS) which is produced during glucose autooxidation (Bonnefont-Rousselot 2002). High production of ROS leads to DNA damage, lipid peroxidation, and oxidation of proteins which subsequently contribute to the death of neurons (Kahya et al. 2017). We evaluated the activity of GST as well as MDA and thiol levels and observed significant changes between the groups (Figure 3a–d). Our results confirm antioxidant properties of African star apple in diabetic rats (Oboh et al. 2018).

Conclusion

The results suggest that African apple prevents neurological disorders in diabetic rats primarily due to their anticholinesterase activity and improvement of brain antioxidant status.

Compliance with ethical standards

All animal procedures were approved and prior permission from Ekiti State University Animal Ethical Committee was obtained as per the prescribed guidelines. The bioethical allowance reference number was ORD/ETHICS/AP/028. All efforts were made to minimize the number of animals and their sufferings.

Conflict of interest: No conflict of interest.

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