Full Length Research Paper

Effect of methanolic extract of Dennettia tripetala (pepper fruit) on biomarkers of oxidative stress and lipid peroxidation in type 2 diabetic male Wistar rats

Nwaeme Ogochukwu1, Ude Victor Chibueze2, Augustine Ndubuisi3, Ugwudike Patrick2, Agu Francis1, Celestine Ani4*, Emeka Ugwuishi4, Sunday Njoba1, 5, Lilian Ndubuisi1, 3, Hector Obianyido3, Joachin Ezeokeke3 and Nwachukwu Daniel1

1Department of Human Physiology, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria.
2Department of Medical Biochemistry, College of Medicine, Enugu State University of Science & Technology, Parklane Enugu Nigeria.
3Department of Medical Biochemistry, College of Medicine University of Nigeria, Enugu Campus, Enugu State, Nigeria.
4Department of Human Physiology, Faculty of Basic Medical Sciences, Enugu State University of Science & Technology, Parklane Enugu Nigeria.
5Department of Human Physiology, Faculty of Medical Sciences, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

Received 15 December, 2019; Accepted 15 January, 2020

Oxidative stress resulting from chronic hyperglycemia in diabetes is due to high production of reactive oxygen species and/or a decrease in the antioxidant defense system activity. The study was designed to investigate the ameliorative effect of methanolic extract of Dennettia tripetala (DT) on oxidative stress and blood glucose level in type II diabetic male Wistar rats. Type II diabetes was induced by a single intraperitoneal injection of streptozotocin (40 mg/kg) after 2 weeks of 10% fructose diet. Twenty-five (25) rats were randomly divided into five groups, namely: 1 (normal control), 2 (negative control), 3 (10 mg/kg of glibenclamide), 4 (100 mg/kg of DT extract) and 5 (200 mg/kg of DT extract). The administration of the extract caused a significant decrease in blood glucose levels in all treatment groups compared to diabetic control. A significant (P<0.01) decrease in malonaldehyde (MDA) activity was observed in the group treated with 100 and 200 mg/kg of DT extract compared to diabetic control. Catalase (CAT) activity showed a significant (P<0.05) increase in the group treated with 200 mg/kg of DT extract compared to the normal control. However, the extract did not affect GSH-Px, SOD and CAT activities. The findings suggest ameliorative effect of DT extract in diabetic Wistar rats.

Key words: Dennettia tripetala, diabetes mellitus, oxidative stress, lipid peroxidation, Wistar rats.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease in which there is a high blood sugar level over a prolonged period (WHO, 2014), due to the inability of one’s body to properly use the energy from the food they eat. This is caused by inherited and/or acquired deficiency in production of insulin by the pancreas or by the
ineffectiveness of the insulin produced. Nigeria has a population of 186 million people and according to International Diabetic Foundation (IDF); 2 million of this population is believed to be known diabetics while a greater percentage are still undiagnosed. According to the World Health Organization (2014), the number of people with diabetes in Africa has jumped from 4 million in 1980 to 25 million in 2014. The disease was responsible for more than 320,000 deaths in 2015.

Oxidative stress is the outcome of an imbalance between the production and neutralization of reactive oxygen and nitrogen species (RONS) such that the antioxidant capacity of the cell is overwhelmed (Chikezie et al., 2018). Oxidative stress is a common mediator in pathogenicity of established risk factors, diabetes mellitus being one of them (Ho et al., 2013). The primary causative factor of oxidative stress in diabetes is the hyperglycemia (Chikezie et al., 2018).

Dennettia tripetala hereafter referred to as DT, is also known as pepper fruit, a member of Annonaceae family (Okwu, 2004). It is widely grown in the rain forest zones of Nigeria and some parts of West Africa. It is known in Nigeria by the following names: Ako (Edo), Mmimi (Igbo), and Ata Igbere (Yoruba). The fruits are green when developing but start to turn red with ripening. The fruits possess a very strong characteristic smell while the fruits and seeds are edible and are consumed because of the spicy nature. This highly nutritious fruit is rich in fatty acids, carbohydrates, proteins, calcium, potassium, magnesium, phosphorus, niacin, riboflavin, thiamine and vitamin A, C and E (Isegholi, 2015). This plant possesses phytochemical that have been shown to elicit antimicrobial, insecticidal, analgesic, and anti-inflammatory properties (Enwere, 1998). Diabetes is a serious medical problem worldwide, and due to the fact that most of the common menu is being restricted because of the carbohydrate content, a lot of diabetic patients are in search of healthy and cost-effective diets and better treatment strategy (WHO, 2014).

D. tripetala is used in traditional medicine as a remedy for cough, fever, toothache, diarrhea, diabetes, and nausea in pregnant women (Ejechi and Akpomedaye, 2005). However, there is paucity of information on the antidiabetic effect and specifically its effect on biomarkers of oxidative stress in diabetes. There is need to investigate the anti-hyperglycemic effect of D. tripetala in order to explore the possibility of using it as a non-pharmacological means of treating diabetes by low income earners of the tropical areas, especially in the rural and under-developed countries. This present study is also designed to investigate the effects of D. tripetala on biomarkers of oxidative stress in order to ascertain its ability to reduce the complications of diabetes, which are the major causes of mortality of the disease. This study was aimed at assessing the effect of methanolic extract of D. tripetala on biomarkers of oxidative stress and lipid peroxidation in type 2 diabetic male Wistar rats.

MATERIALS AND METHODS

Plant purchase, identification and authentication

D. tripetala fruits were purchased from New Market in Enugu metropolis, Enugu State. A sample of the fruit was identified and authenticated at the herbarium section of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. A voucher specimen was deposited in the herbarium for further reference with the number: UNH no 8c.

Extraction process

The method of Onyeso et al. (2016) was adopted and modified. D. tripetala fruits were properly de-stoned and pulverized using a mechanical milling machine. 1000 g of pulverized seeds was macerated in 5000 ml of a mixture of 80% methanol and 20% distilled water. This was followed by vigorous shaking at 1 h intervals for 48 h. After which, it was filtered using a clean sieve with tiny holes and finally filtered using No. 1 Whatman filter paper and measuring cylinders. The filtrates were poured into clean beakers and the solvents evaporated using moist air oven by Drawell Scientific Limited China (Model: DGT-G25). The extracts after evaporation were weighed and a total of 36 g of the pure extract was kept in a refrigerator at -2°C till ready for use.

Experimental animals

A total of forty (40) Wistar rats were purchased from the Animal House Unit of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State. The rats weighed between 110 and 160 g and were housed in stainless steel and well ventilated cages under controlled environmental conditions (12 h light/dark cycle) at the animal house unit of the Department of Anatomy, Faculty of Basic Medical Science, University of Nigeria, Enugu campus, Enugu.

Ethical clearance

Ethical clearance for this study was obtained from the Research Ethical Committee, College of Medicine, University of Nigeria, Enugu with protocol number 037/12/2017.

Preparation and administration of streptozotocin solution for the induction of diabetes

The solution of streptozotocin was prepared according to the method of Anioke et al. (2017). A total of 250 mg of powdered Streptozotocin purchased from Bridge Biotech, Ilorin (Santa Cruz Biotechnology, USA) was weighed using an electronic weighing balance and dissolved in 100 ml mixture of 0.1 M sodium citrate buffer (a mixture of 46.5 ml citric acid and 3.5 ml of sodium citrate solution) which served as the vehicle to get the appropriate stock concentration of 30 mg/ml. This was administered to the rats after overnight fast (8-12 h). The rats were induced with diabetes individually by single intraperitoneally administration of the dissolved streptozotocin powder at a dose of 40 mg/kg body weight. Using the formula stated as follows:

\[
Volume = \frac{dose \times weight \times stock\ concentration}{stock\ concentration} 
\]
Preparation of 0.1 M citric acid and sodium citrate solution

Approximately 2.87 g of powdered citric acid (C6H8O7·H2O) with molecular weight of 287 g/mol was weighed and dissolved in 100 ml of distilled water. Also 100 ml of 0.1 M solution of sodium citrate (C6H7O6·2H2O) was prepared following the same method. Then 44.5 ml of citric acid was mixed with 55.5 ml of sodium citrate and the mixture standardized with citric acid to a pH of 4.5. The preparation of 0.1 M solutions was done using the method of Opekuku (2002). The streptozotocin dissolved in citrate buffer (PH 4.5) after an overnight fast was to induce partial pancreatic β-cell destruction. Two days (48 h) after induction, diabetes was confirmed with a fasting blood glucose level of ≥200 mg/dl, using the ACCU-ANSWER Glucometer (ZH-G01) produced by Guangxi-China (Mainland) using a whole blood obtained from the caudal vein of the animals after a little incision with scissors.

Experimental design

Group 1 = Normal control rats; received feed and water ad libitum
Group 2 = Diabetic untreated group (negative control)
Group 3 = Diabetes + treatment with 10 mg/kg Glibenclamide orally only (positive control)
Group 4 = Diabetes + treatment with 100 mg/kg methanolic extract of D. tripetala only
Group 5 = Diabetes + treatment with 200 mg/kg methanolic extract of D. tripetala only

Acute toxicity studies

The median lethal dose (LD50) was determined by method of Lorke (1983) using twelve rats weighing between 100 and 190 g. In the first phase, four rats were divided into two groups of two rats each and they were treated with the extract of the fruit at 1600 and 2000 mg/kg body weight intraperitoneally, and then observed for 24 h for sign of toxicity. In the second phase eight rats were then divided into four groups of two rats each and also treated with extract of the fruit at doses 3000, 4000, 5000 and 6000 mg/kg of body weight intraperitoneally. The median lethal doses were then calculated as the square root of the highest dose that gave no mortality (\(\sqrt{D_{100}}\)) by multiplying the lowest dose that gave mortality (\(D_{100}\), LD50= \(\sqrt{D_{100}} \times D_{100}\)).

Administration of extract

\(D.\) tripetala extract was administered orally to the ten rats in groups 4 and 5 for 4 weeks giving a total of twenty-eight (28) days, with the use of an oral gastric gavage and 5 ml syringe. Administration was done in the evenings after which the rats were fed. Group 3, the positive control group, was given the standard diabetes drug for the same duration. \(D.\) tripetala extract was administered orally to the ten rats in groups 4 and 5 for 4 weeks giving a total of twenty-eight (28) days, with the use of an oral gastric gavage and two 5 ml syringes.

Preparation of urethane for anaesthesia

At the end of the treatment period, 1.25 g of granulated urethane purchased from Sigma Aldrich (Steinhen, Switzerland) was weighed using an electronic weighing balance and dissolved in approximately 5 ml of distilled water and administered intraperitoneally at a dose range of 500 mg/kg. Blood was collected via cardiac puncture using a sterile hypodermic syringe and introduced into a sterile sample and the blood allowed to clot followed by centrifugation and serum was collected using an automatic pipette for the analysis of the biomarkers of oxidative stress as stated.

Sample biochemical analysis

Malondialdehyde (MDA) activity, a measure of lipid peroxidation, was analyzed using the method described previously (Oikawa et al., 1979). The supernatant (0.2 ml) of homogenate was pipetted out in a test tube, followed by addition of 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 30% acetic acid (pH 3.5), 1.5 ml of 0.8% of thiobarbituric acid and the volume was made up to 4 ml with distilled water. The test tubes were incubated for 1 h at 95°C, then cooled and 1 ml of distilled water was added followed by addition of 5 ml of n-butanol-pyridine mixture (15:1 v/v). The tubes were centrifuged at 4000 g for 10 min. The absorbance of developed pink color was measured spectrophotometrically (DU 640B spectrophotometer, Beckman Coulter inc., CA, USA) at 532 nm. MDA was expressed as mmol/ml.

Glutathione peroxidase (GSH-Px) erythrocyte activity was measured according to Paglia and Valentine (1967). In the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted into the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm is measured. GSH-Px was expressed in unit per milligram protein (U/mg protein).

Superoxide dismutase (SOD) activity was measured according to the method described by Fridovich (1984). This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with p-iodonitrotetrazolium violet (INT) to form a red formazan dye which was measured at 505 nm. The assay medium consisted of the 0.01 M phosphate buffer: CAPS (3-cyclohexilamino-1-propanesulfonic acid) buffer solution (50 mM CAPS, 0.94 mM EDTA, saturated NaOH) with pH 10.2; solution of substrate (0.05 mM xanthine, 0.025 mM INT) and 80 U/L xanthine oxidase. SOD activity was expressed as unit per milligram protein (U/mg protein).

Catalase (CAT) activity was measured following the method described previously (Beutler, 1984). The activity was determined by measuring the decrease in absorbance at 240 nm of a reaction mixture consisting of hydrogen peroxide (H2O2) in phosphate buffer, pH 7.0 and requisite volume of serum sample. The molar coefficient of extinction (MCE) of 43.6 (mM)-1 cm-1 was used to determine the catalase activity. The assay medium consisted of 1 M Tris–HCl–5 mM Na2 EDTA buffer solution (pH 8.0), 1.0 M phosphate buffer solution (pH 7.0) and 10 mM H2O2. CAT activity was expressed as unit per min per milligram protein (U/min/mg protein).

Statistical analysis

The results obtained from this study were analyzed using statistical package for social science (SPSS version 21.0 for windows IBM Corporation, Armonk NY). Analysis of variance (ANOVA) was used to compare means and subjected to Tukey HSD for post Hoc for multiple comparisons, and values were considered statistically significant at p<0.05 or P<0.01. All results are presented as mean ± standard error of mean (SEM).

RESULTS

The LD50 for \(D.\) tripetala seed extract was 5785 mg/kg and this evoked paralysis in rats for 4 days coupled with discharge from the eyes and eventual death.

Table 1 shows that in the treatment group 3, there was a significantly decreased (P≤0.05) blood glucose level.
after the first week although groups 4 and 5 had a decreased blood glucose that was not significantly different (P>0.05). There was a significant decrease in blood glucose levels in group 3 in respect to group 2 in week 1. In the second week of this experiment, there was further significant decrease in groups 3, 4 and 5 still in respect to group 2. Table 2 shows the percentage changes in blood glucose levels between initial and weeks 2 and 4, respectively. Percentage reduction in blood glucose level was the highest in group 3 at both weeks 2 and 4, there was still a significant decrease in groups 4 and 5 for both weeks and in group 2 at week 4.

The malondialdehyde (MDA) levels of the experimental animals were only significantly different (P≤0.05) in group 3, which were lower than that of group 1. The difference in the groups was only significant at Ps≤0.01; there was a decrease in group 2 as compared to group 1, in group 4 as compared to groups 1 and 2 and also in group 5 as compared to group 2. There was not any significant difference (P>0.05) in the levels of GSH-Px, although there was a decrease in groups 2, 3 and 4 when compared with group 1 and an increase in group 5 as compared to group 1. The superoxide dismutase levels (SOD) levels were significantly increased (P<0.05) in group 3 as compared to group 2, although there was an increase in groups 3 and 4 when compared with group 2. The differences in the catalase activity (CAT) levels of the experimental animals were only significant (Ps≤0.05) in group 5, which was significantly increased as compared to group 1. Although there was an increase in the other groups (2, 3 and 4) when compared with group 1.

In Table 3, the diabetic negative control rats showed a significantly (Ps≤0.01) elevated levels of MDA compared with normal control rats. The diabetic positive control group that received glibenclamide showed a significant (P≤0.05) decrease in MDA levels compared to diabetic control group. The extract treated diabetic groups showed a significantly (Ps≤0.01) decreased lipid peroxidation as compared to the normal and diabetic control groups, 100 mg/kg of the extract had the lowest value for MDA as opposed to the 200mg/kg of D. tripetala extract which had a more increased MDA level. The changes in the GSH-Px levels were not significant (P>0.05), although the glibenclamide treated group had a decreased GSH-Px levels as compared to the diabetic negative and normal control group. The diabetic negative control group had a lower GSH-Px level as compared to normal control. The extract groups had increased level of GSH-Px as compared to diabetic controls and this increase was dose dependent with the values of 200 mg/kg higher than 100 mg/kg.

Table 4 shows a gradual increase in weight of the experimental animals in the various groups with the progression of the work, although this gradual weight gain was not significant. Group 1 had the highest increase in body weight by week 2 and 4 and with group 2 having the lowest at week 2 and group 4 the lowest at week 4. There was a significant increase in weight of animals in group 1 as compared to 2 in week 2, and a significant increase in percentage change in body weight of animals in groups 3 and 4. Overall, a significant (P<0.05) decrease in weight gain was observed in groups 2, 4 and 5 compared to group 1.
Table 3. Biomarkers of oxidative stress and lipid peroxidation in experimental animals.

| Group | MDA (10⁻⁷) mmol/mg protein | GSH-Px U/mg protein | SOD U/mg protein | CAT U/min/mg protein |
|-------|---------------------------|--------------------|-----------------|---------------------|
| 1     | 8.5±1.01                  | 3.61±0.53          | 4.17±0.42       | 0.81±0.14           |
| 2     | 12.8±0.5**                | 3.07±0.28          | 2.50±0.09       | 0.94±0.19           |
| 3     | 6.4±0.65**                | 2.81±0.38          | 5.05±1.12β      | 0.87±0.03           |
| 4     | 3.3±1.35* &β             | 3.28±0.46          | 3.87±0.36       | 1.07±0.03           |
| 5     | 8.38±0.79*β              | 3.72±0.48          | 4.14±0.48       | 1.46±0.42*          |

Values are expressed as Mean ± SEM, *P≤0.05 were considered significant to group 1, **P ≤ 0.01 compared to group 1.
&P≤0.05 compared to group 2, *P≤0.05 compared to group 2.

Table 4. The basal and weekly body weights (Grams) of experimental animals.

| Group | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 | Weight gain (Week4-Week1) |
|-------|--------|--------|--------|--------|--------|---------------------------|
| 1     | 124.5±2.43 | 149.8±7.67 | 174.8±3.52 | 183.6±8.51 | 188.1±4.49 | 63.6±2.06 |
| 2     | 121.7±2.48 | 124.3±8.44 | 127.0±2.83 | 138.7±3.50 | 140.8±7.46 | 19.1±5.16* |
| 3     | 145.9±2.88 | 163.2±5.88 | 169.6±6.91 | 188.2±5.87 | 202.0±6.81 | 56.1±3.93 |
| 4     | 153.6±2.17 | 164.2±8.15 | 165.6±4.97 | 173.7±5.26 | 175.7±6.67 | 22.1±1.50* |
| 5     | 147.8±2.41 | 162.3±2.14 | 161.2±2.08 | 166.3±1.77* | 177.2±2.69 | 29.4±0.28* |

Values are expressed as Mean± SEM; *P≤0.05 were considered significant to 1, **P ≤ 0.01 compared to 1

DISCUSSION

Diabetes is associated with a number of metabolic alterations and principal among these is hyperglycemia. Known secondary consequences of hyperglycemia such as cellular damage, increased extra cellular matrix production and vascular dysfunction have all been implicated in the pathogenesis of vascular disease type II diabetes (Dalle-Donne et al., 2006). Free radicals and oxidative stress may act as a common pathway to diabetes itself, as well as to its complications (Wolff, 1993). This study was conducted to evaluate the effect of pepper fruit on blood glucose level, MDA, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) in type two diabetes. From the results obtained, the blood glucose level which was used as an index of metabolic control was significantly increased in the diabetic groups after induction, which is one of the causes for increased production of free radicals by direct Amadori reaction (Wolff, 1993). Treatment with medium-dose of D. tripetala extract (100mg/kg body weight) and high-dose of DT extract (200 mg/kg body weight) produced an increased percentage reduction in blood glucose level comparable to glibenclamide. The phytochemical of DT seed revealed the presence of flavonoids, saponins, resins, alkaloids, and so forth. Studies in the recent past (Oyedemi et al., 2012; Unnikrishnan et al., 2013) reported that dietary flavonoids, alkaloids, saponins, tannins, and glycosides have antidiabetic potentials. These bioactive phytochemicals, as reported in previous studies (Ivorra et al., 1989; Kameswara et al., 2003) may either singly or in synergy with one another be responsible for the significant glucose-lowering activity reported in the current study. The hypoglycemic effect of D. tripetala extract found in this study agrees with previous reports (Isegholi, 2015; Anioke et al., 2017).

In Table 3, the diabetic negative control rats showed a significantly (P≤0.01) elevated levels of MDA compared with normal control rats. The diabetic positive control group that received glibenclamide showed a significant (P≤0.05) decrease in MDA levels compared to diabetic control group. This could be due to an effective glycemic control of the drug thereby decreasing hyperglycemic induced lipid peroxidation; this finding is in support of the work done by Ahmed et al. (2006). The extract treated diabetic groups showed a significantly (P≤0.01) decreased lipid peroxidation as compared to the normal and diabetic control groups, 100 mg/kg of the extract had the lowest value for MDA as opposed to the 200 mg/kg of D. tripetala extract which had a more increased MDA level. This shows that the ability of the extract to attenuate lipid peroxidation is not dose dependent. This decrease can be due to the high antioxidant capacity of the extract (Moussa, 2008; Anioke et al., 2017). The increased levels of lipid peroxidation in group 5 can be associated with the presence of toxic pyrrolizidine alkaloids (Ahmed et al., 2006; Anaga et al., 2006). Hypoinsulinaemia in diabetes increases the activity of the enzyme fatty acyl coenzyme A oxidase, which initiates β-oxidation of fatty acids,
resulting in lipid peroxidation (Horie et al., 1981). The products of lipid peroxidation are harmful to most cells in the body and are associated with a variety of diseases, such as atherosclerosis and brain damage. The changes in the GSH-Px levels were not significant (P>0.05), although the glibenclamide treated group had a decreased GSH-Px levels as compared to the diabetic negative and normal control group, this is in support of the work done by Lu et al. (2011). The diabetic negative control group had a lower GSH-Px level as compared to normal control. The extract groups had increased level of GSH-Px as compared to diabetic controls and this increase was dose dependent with the values of 200 mg/kg higher than 100 mg/kg. In diabetic patients, the auto-oxidation of glucose results in the formation of hydrogen peroxide which inactivates SOD and this accumulated hydrogen peroxide may be one of the explanations for decreased activity of SOD in type 2 diabetic patients (Nobar et al., 1999). The SOD activities were significantly increased in the glibenclamide group as compared to the diabetic negative control group. This could be due to effect of the drug on the attenuation of oxidative stress (Shinde et al., 2011). The SOD activities of the extract groups were increased in a dose dependent manner as compared to the diabetic negative control, which further shows the presence of phytochemical and antioxidant capacity of D. tripetala. The diabetic control groups had a mildly increased catalase activity as compared to the normal control. There was a significant increase in the extract treatment groups (200 mg/kg at P<0.05). The CAT activities of the extract groups were increased in a dose dependent manner as compared to the normal control which further shows the presence of phytochemical and antioxidant capacity of D. tripetala. This finding is in support of the previous works done by Acworth et al. (1997). In untreated diabetic rats, percentage increase in body weight decreased significantly compared to the normal control, which may be attributed to increased degradation of structural protein due to damage to the intracellular signaling pathways implicated in maintaining the balance between protein synthesis and degradation (Newsholine et al., 2011; Hulmi et al., 2012). Interestingly, there was a significant increase in the body weight when compared with the untreated (negative control) group after treatment. This suggests that D. tripetala may have a bioactive potency like IGF-1, which increases protein synthesis in diabetes to restore muscle wasting through the activation of Akt/mTOR (protein kinase B/mammalian target of rapamycin) pathways (Glass, 2005; Zhang et al., 2014).

**Conclusion**

The present experimental study demonstrated anti-hyperglycemic potency of D. tripetala (pepper fruit) which is comparable with glibenclamide in the treatment of diabetes. The potential antidiabetic properties linked with *D. tripetala* need to be therapeutically maximized to ameliorate the burden of diabetes and its complications in the society. Furthermore, the study shows that *D. tripetala* should be taken at an optimal dose of 100 mg/kg to prevent an increase in lipid peroxidation as seen at 200 mg/kg dose. There is a need for further investigation to elucidate the precise mechanism leading to the hypoglycemic effect present in *D. tripetala* fruits with the antidiabetic property observed in the study; also the lipid peroxidation present at higher doses should be investigated. Furthermore, glibenclamide which is the standard diabetic drug used in this study should be researched further to validate its glutathione peroxidase lowering effect and weight gain, and if consistent with this study, there is need for a review.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**REFERENCES**

Acworth IN, Mccabe T, Maher TJ (1997). The Analysis of Free Radicals, Their Reaction Products, and Antioxidants, In: S.I. Baskin, H. Salem (Eds.), Oxidants, Antioxidants and Free Radicals. Taylor and Francis, Washington, DC; Chapter 2.

Ahmed FN, Nagui FN, Shafiq F (2006). Lipid peroxidation and serum antioxidant enzymes in patients with type 2 diabetes mellitus. Annal of New York Academy of Science 1084:481-489.

Anaga AO, Shoyinka SVO, Asuzu IU (2006). Toxic Effects of *Dennettia tripetala* Root Extract. Pharmacology and Biology 44:451-461.

Anioko I, Okwuosa C, Uchendu I, Chijioke O, Dozie-Nwakile O, Ikewwuonu I, Kalu P, Maryann Okafor (2017). Investigation into Hypoglycemic, Antihyperlipidemic and Renoprotective Potentials of *Dennettia tripetala* (Pepper Fruit) seed in a Rat model of Diabetes. Hindawi Biomed Research International 6923629.1.

Beutler E (1984). Red Cell Metabolism. Grune and Stratton Inc., New York.

Chikezie PC, Ojiako OA, Nwufo KC (2018). Free Radicals and Free Radicals, In: S.I. Baskin, H. Salem (Eds.), Oxidants, Antioxidants and Free Radicals. Taylor and Francis, Washington, DC; Chapter 2.

Enwere NJ (1998). Peroxidase Activity and Peroxidase Enzymes in the Body. Ann. N.Y Acad. Sci. 852:116-128.

Ejichi DE, Akpomedaye BO (2005). Activity of Essential Oil and Phenolic acid Extracts of Pepper Fruit *Dennettia tripetala* G. Barker; Annonaceae against Some Food-Borne Microorganisms. African Journal of Biotechnology 4(2):258-261.

Enwere NJ (1998). Foods of Plant Origin. Afro-Orbis Publications Limited; University of Nigeria: Nsukka, Nigeria 169-180.

Fridovich I (1983). Superoxide Radical: An Endogenous Toxicant. Annual Review of Pharmacology and Toxicology 23:239-325.

Glass DJ (2005). Glass DJ (2005). Skeletal Muscle Hypertrophy and Atrophy Signaling Pathways. International Journal of Biochemistry and Cell Biology 37(10):1974–1984.

Ho E, Galongah I, Bhindi R, Figtree GA (2013). Biological markers of oxidative stress: Applications to cardiovascular research and practice. *Redox Biology* 1(1):483-491.

Horie S, Ishii H, Suga T (1981). Changes in Peroxosomal Fatty Acid Oxidation in Diabetic Rat Liver. Journal of Biochemistry 90:1691-1696.

Hulmi JJ, Silvennoinen M, Lehti M, Kivel"ar AR, Kainulainen H (2012)
Altered REDD1, Myostatin, and Akt/Mtor/Foxo/ MAPK Signaling in Streptozotocin-Induced Diabetic Muscle Atrophy. The American Journal of Physiology, Endocrinology Metabolism 302(3):E307-E315.

Isegholi SO (2015). A Review of The Uses and Medicinal Properties of Dennettia tripetala. Medical Science 3:104-111.

Ivorra MD, Paya M, Viller A (1989). A review of natural products and plants as potential antidiabetic drugs. Journal of Ethnopharmacology 27(3):243-275.

Kameswara B, Renuka R, Sudarshan P, Rajasekhar MD, Nagaraju N, Appa RC (2003). Antidiabetic Activity of Terminalia Pallida Fruit in Alloxan Induced Diabetic Rats. Journal of Ethnopharmacology 85(1):169-172.

Lorke D (1983). A new approach to practical acute toxicity testing. Toxicology Letter 20:15-26.

Lu HT, Chin S, Ya WC (2011). Arsenic induces pancreatic cell apoptosis via the oxidative stress regulated mitochondria dependent and endoplasmic reticulum stress triggered signaling pathways. Toxicology Letter 20:15-26.

Moussa SA (2008). Oxidative Stress in Diabetes Mellitus. Romanian Journal of Biophysics 18(3):225-236.

Newsholine P, Abdulkader F, Rebelato E (2011). Amino acids and diabetes. Implications for endocrine, metabolic and immune function. Frontiers in Bioscience 16(1):315-339.

Nobar MRE, Pour AR, Nobar MR, Beig FA, Mirhashemi SM (1999). Total antioxidant capacity, superoxide dismutase and glutathione peroxidase in diabetic patients. Medical Journal of Islamic Academy of Science 12(4):109-114.

Ohkawa H, Ohishi N, Yagi K (1979). Assay for Lipid Peroxidation in Animal Tissues by Thiobarbituric Acid Reaction. Analytical Biochemistry 95(2):351-358.

Ojokuku GO (2002). Practical chemistry for schools and colleges. Gbabeks Publishers Limited, New Edition P 166.

Okeke DE (2004). Morah FNI. Mineral and nutritive value of Dennettia tripetala fruits. Fruits 59:437-442.

Onyeso GI, Nkpaa KW, Onokohwomo JN, NwaemO VQA (2016). Co-administration of Caffeine and Hydromethanolic. Journal of Advanced Medicine and Pharmacy 5(2):1-7.

Oyedemi S, Bradley G, Afolayan A (2012). Antidiabetic Activities of Aqueous Stem Bark Extract of Strychnos Henningsii Gilg. In Streptozotocin-Nicotinamide Type 2 Diabetic Rats. Iranian Journal of Pharmaceutical Research 11(1):221-228.

Pagia DE, Valentine WN (1967). Studies on the Quantitative and Qualitative Characterization of Erythrocyte Glutathione Peroxidase. Journal of Laboratory and Clinical Medicine 70(1):158-169.

Shinde SN, Dhadke VN, Suryakar NA (2011). Evaluation of Oxidative Stress in Type 2 Diabetes Mellitus. Indian Journal of Clinical Biochemistry 26(1):74-77.

Unnikrishnan M, Veerapur V, Nayak YY, Mudgal P, Mathew G (2013). Antidiabetic, antihyperlipidemic and antioxidant effects of the flavonoids, in Polyphenols in Human Health and Disease. Elsevier Inc. 1:143-161.

Wolff SP (1993). Diabetes mellitus and Free Radicals. British Medical Bulletin 49(3):642-652.

World Health Organization (2014). About Diabetes. March 31, https://web.archive.org/web/20140331094533/http://www.who.int/diabetes/action_online/basics/en/ (accessed April 24, 2018).

Zhang J, Zhuang P, Wang Y (2014). Reversal of Muscle Atrophy by Zhimu-Huangbai Herb-Pair Via Akt/Mtor/Foxo3 Signal Pathway in Streptozotocin-Induced Diabetic Mice. Plos ONE 9(6):Article E100918.