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Effect of *Ficus exasperata* Leaf-Based Diet on Antioxidant Parameters of Type 2 diabetic Rats

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Abstract

The effect of *Ficus exasperata* leaf-based diet on antioxidant parameters of type 2 diabetic rats was investigated in this study. Forty-eight (48) male albino rats were randomly selected into 8 groups, namely A to H. Various proportion (10, 20, 30, 40 and 50%) of *Ficus exasperata* powdered leaf was incorporated into locally sourced ingredients and formulated into feed. The feed was administered *ad libitum* to animals in group D to H, after induction of diabetes using 10% fructose solution *ad libitum* and 40 mg/kg b.wt. streptozotocin injected intraperitoneally. Group A (positive control), B (negative control) and C (metformin-treated group) received feed without *F. exasperata* leaf. Biochemical parameters studied are; Fasting Blood Glucose (FBG), Superoxide dismutase, Catalase, Reduced glutathione and Malondialdehyde (MDA). Data analysis was done with SPSS version 16.0 and level of significance set at $p < 0.05$. Results showed alteration in the endogenous antioxidant parameters of diabetic animals when compared to the positive control. Treatment of the experimental animals with test diets resulted in a significant ($p < 0.05$) increase in the activity of superoxide dismutase, catalase and reduced glutathione level when compared to the negative control and no significant difference ($p > 0.05$) compared to the positive control group. MDA concentration was significantly reduced ($p < 0.05$) for all the treatment groups as well, compared to the negative control. In conclusion, Feeding of *Ficus exasperata* leaf-based diet to Type 2 diabetic rats for 16 days ameliorated the alteration in the endogenous antioxidant parameters studied. There was no significant difference in the ameliorative effect of the various proportion of the *Ficus exasperata* leaf in the feed.

Keywords: *Ficus exasperata* Leaf, Antioxidant parameters, Type 2 diabetes

Introduction

Mammalian cells are constantly exposed to a variety of oxidizing agents. These oxidizing agents may be produced by metabolic activity within the cells, however, it is important to maintain a balance between oxidants and antioxidants to be able to sustain optimal physiological conditions. Overproduction of oxidants can cause an imbalance, leading to oxidative stress (Ames *et al.*, 1993; Adom *et al.*, 2003). Oxidative stress can cause oxidative

damage to macromolecules such as lipids, proteins and DNA and consequently lead to increased risk for developing chronic diseases (Eleazu *et al.*, 2013). There is considerable evidence that hyperglycemia, a hallmark of diabetes mellitus, results in the generation of reactive oxygen species (ROS), ultimately leading to increased oxidative stress in a variety of tissues, and playing an important role in diabetic complications (Yfjord and Bodvarsdottir, 2005; Chandie *et al.*, 2006).

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Prolonged exposure to elevated plasma glucose levels leads to toxic effects in a variety of cell types (glucose toxicity). In humans, there is a strong relationship between chronic hyperglycemia and impaired function of vascular endothelium, with particular damage observed in retinal capillary endothelial cells and mesangial cells in the renal glomerulus (Coutinho *et al.*, 1999; Coutinho *et al.*, 2003). Diabetes mellitus (including type 1 diabetes mellitus [T1DM] and type 2 diabetes [T2DM]) is the leading cause of all new cases of blindness in adults and accounts for approximately 44% of all new cases of renal failure in the United States. In addition, injury to the microvasculature of the small vessels that supply the nerves contributes to neuropathy, which develops in 60% to 70% of all patients with DM (ADA, 2011). Managing chronically elevated glucose levels is crucial to reduce the risk of DM-related complications. Several large scale studies have shown that the risk of developing these adverse outcomes can be significantly reduced if tight glycemic control is maintained (Brown, *et al.*, 2010). Postprandial hyperglycemia induces an overproduction of superoxide that, after reacting with nitric oxide, produces a subsequent nitrosative stress with generation of such metabolic derivatives as peroxynitrite and nitrotyrosine. The toxicity of these substances can lead to endothelial damage and, furthermore, to microvascular and macrovascular complications (Ceriello *et al.*, 2002).

In order to prevent or reduce the oxidative stress induced by free radicals, sufficient amounts of antioxidants need to be consumed and fruits and vegetables are known to contain a variety of antioxidant compounds such as phenolics and carotenoids which may help protect cellular systems from oxidative damage and reduce the risk of developing chronic diseases (Wang *et al.*, 1996; Vinson *et al.*, 2001; Adom *et al.*, 2003).

Ficus exasperata Vahl (Family: *Moraceae*) also known as Sandpaper leaf (English), *Ewe Ipin* or *Eepin* (Yoruba-Western Nigeria), *Baure* (Hausa Northern Nigeria) and *Asefa* (Igbo-Eastern Nigeria) is a deciduous tree which grows up to 18- 20 m tall in evergreen forest or semi evergreen forests of Nigeria, India, East Africa, Middle East and Sri Lanka. Fresh leaves of *F. exasperata* are regularly included during the milling or pounding stage by the natives of Nigeria to improve the quality and stability of palm oil which could be attributed to its antioxidant potential (Umerie *et al.*, 2004).

The whole plant has been claimed to have several medicinal uses in African traditional medicine. For instance, the leaf has been used to treat diabetes mellitus, hypertension, rheumatism, arthritis, intestinal pains, colics, epilepsy, bleeding and wounds while the roots have been claimed to be used in managing asthma, dyspnoea and venereal diseases (Chhabra *et al.*, 1990). Studies have implicated *F. exasperata* to have pharmacological activities such as anti hyperlipidaemia, antihypercholesterolaemia and antihyperketonaemia (Nimenibo-Uadia 2003; Yakubu *et al.*, 2014). Antiarthritic and antioxidant effects of the leaf extract of *Ficus exasperata* was also reported by Wonder *et al.* (2010).

Although, there have been several reports on the therapeutic activities of different preparations/extracts of *F. exasperata* leaf, there is paucity of information on the antioxidant activity of *F. exasperata* leaf-based diet on Fructose and Streptozotocin-induced diabetic rats, which this study sought to achieve.

Materials and Methods

Plant material

Ficus exasperata leaf was collected within Ilorin community. It was identified and authenticated (Voucher number UILH/001/883) at the herbarium of

Department of plant Biology, University of Ilorin, Ilorin, Nigeria.

Ethical Clearance

Ethical clearance for this study was obtained from the University of Ilorin ethical committee.

Experimental animals

Male wistar albino rats (100 – 150g) of *Norvegicus* strain were obtained from the Small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were housed in well ventilated cages and allowed to acclimatize to Animal House conditions (i.e. temperature: 28 °C– 31 °C; photoperiod: 12 h natural light and 12 h dark; humidity: 50 %–55 %) for 7 days. They were fed with normal rat pellet and tap water.

Chemical and assay kits

Streptozotocin (Sigma-Aldrich, St. Louis, MO, USA) was purchased from Local Pharmacy store in Nigeria. Accu check active Glucometer, strips and fructose (Nature's Choice™ Whole food specialists, Meyerton, South Africa) were also purchased from a local pharmacy.

Metformin (Austell Laboratories Pvt. Ltd., Johannesburg, South Africa) was purchased from a local pharmaceutical company in Ilorin, Nigeria.

Assay kits for Glycosylated hemoglobin and Plasma Insulin was a product of Centronic GmbH Am Kleinfeld 11, 85456 Wartenberg/Germany. All other chemicals were of analytical grades and prepared in all glass distilled water.

Feed material/Preparation

Corn starch was prepared using yellow corn obtained from Ojatuntun market in Ilorin, Nigeria. It was rinsed and soaked for 48 hours, ground and sieved to remove shaft. The filtrate was drained and oven dried at 40°C to constant weight. Maize husk was purchased from Olufadi market in Ilorin, sun dried and pulverized using commercial grinder. Soy bean, Sucrose and Soy bean oil were purchased from the market as well. Soy bean was prepared by soaking in water for 6 hours and seed coat was removed. It was thereafter sun dried and ground to smooth texture. Vitamin, mineral mix, D-Methionine and L-Lysine were purchased from a local pharmacy store within Ilorin, Nigeria. Feed was compounded using the percentage nutrient composition of Mathew (2012) as reference.

Plant Preparation

Fresh leaf of *F. exasperata* was collected and air dried to a constant weight and pulverized into fine powder using an electronic blender. It was stored in air tight container kept in refrigerator prior to analyses.

Table 1: Feed Formulation Using *F. exasperata* Powdered Leaf

Ingredients g/kg	+ve Control	-ve Control	Reference drug	10%	20%	30%	40%	50%
Corn starch	512	512	512	412	312	212	112	12
Cellulose	40	40	40	40	40	40	40	40
Sucrose	100	100	100	100	100	100	100	100
Soybean	250	250	250	250	250	250	250	250
Soybean Oil	40	40	40	40	40	40	40	40
Vitamin/mineral mix	50	50	50	50	50	50	50	50
D- Methionine	4	4	4	4	4	4	4	4
L-lysine	4	4	4	4	4	4	4	4
<i>F.exasperata</i> leaf	-	-	-	100	200	300	400	500

Source: Matthew, (2012)

The ingredients were thoroughly mixed together, oil added at interval and water until a paste was formed. The paste was then grated on a wire mesh to form a pellet and oven dried at 40°C to a constant weight.

Diabetes induction and determination of blood glucose

All experimental animals apart from animals in the positive control group were administered orally with 10% fructose solution *ad libitum* for 2 weeks, while those in the positive control group were given distilled water. Streptozotocin (STZ) was then dissolved in 0.1 M citrate buffer (pH 4.5). At the end of the 2 weeks administration of Fructose solution, animals were fasted overnight and each of the fructose-fed animals were injected (ip) with a low dose STZ (40 mg/kg b.w.) (Wilson and Islam, 2012). Seven (7) days after the STZ injection, fasting blood glucose level of the animals was evaluated using AccuChek active glucometer and

compatible strips by withdrawing blood from the caudal vein of the rats' tail. Rats showing Fasting blood glucose concentration above 200 mg/dL were considered diabetic.

Animal grouping and feed administration

Forty-eight (48) male albino rats were randomly selected into 8 groups of 6 animals each. Group A (positive Control) – Non diabetic animals fed with diet without *F. exasperata* leaf; Group B (Negative Control) – Diabetic animals fed with diet without *F. exasperata* leaf; Group C (Reference drug) – Diabetic animals treated with metformin and fed with diet without *F. exasperata* leaf; Group D (Test Diet 1) – Diabetic animals fed with diet compounded with 10% *F. exasperata* leaf; Group E (Test Diet 2) - Diabetic animals fed with diet compounded with 20% *F. exasperata* leaf; Group F (Test Diet 3) - Diabetic animals fed with diet compounded with 30% *F. exasperata* leaf;

Group G (Test Diet 4) - Diabetic animals fed with diet compounded with 40% *F. exasperata* leaf; Group H (Test Diet 5) - Diabetic animals fed with diet compounded with 50% *F. exasperata* leaf.

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Compounded diet was administered to experimental animals *ad libitum* for a period of 16 days.

Animal Sacrifice and Tissue Collection

Animals were sacrificed 24 hrs after the last day of treatment. They were anaesthetized with diethyl ether and sacrificed by simply incising the jugular vein. Blood samples were collected into plain sample bottles for biochemical serum analysis. After sacrifice, the rats were dissected in order to isolate the liver. The isolated tissue was cleansed with cotton wool to remove blood stains, weighed and immediately stored in ice cold 0.25 M sucrose solution. One (1g) of the liver cut with a clean scalpel was homogenized in ice-cold

0.25 M sucrose solution (1:5^{w/v}). The homogenate was then stored in the freezer (-5°C) until required for further analysis. Serum was collected by allowing blood sample to stand at room temperature for 30 mins to form clot. The supernatant which is the serum was then collected using a Pasteur’s pipette.

Biochemical Assay

Catalase activity – Decomposition of H₂O₂ in the presence of catalase was followed at 620nm (Sinha, 1972). Procedure: 1ml of 0.01M phosphate buffer (pH 7.0) was added to 0.4ml of H₂O₂ (0.2M) followed by 0.1ml of sample and gently swirled at room temperature. The reaction mixture was stopped by the addition of 2ml of dichromate acetic acid reagent (5% of dichromate prepared in acetic acid). The changes in the absorbance was measured at 620nm and recorded after 3minutes interval.

$$\text{Percentage inhibition} = \frac{\text{Abs of blank} - \text{Abs of sample}}{\text{Abs of sample}} \times 100$$

$$\text{Enzyme activity (nmol/mL)} = \frac{\% \text{ inhibition} \times 1000 \times \text{df}}{50 \times 0.1}$$

Reduced glutathione level - Glutathione (GSH) concentration in the supernatant was determined according to the procedure described by Sedlak and Lindsay (1958).

Procedure

Appropriately diluted supernatant (5.0 mL) was mixed with 4.0 ml of distilled water. Aliquot (1.0 ml) of 20% trichloroacetic acid was added to mixture from step 1 and

centrifuged at 3000 x g for 15 minutes. Then 2 ml of the supernatant was mixed with 4ml of 0.4 M Tris buffer, pH 8.9.

Ellman reagent (DTNB) (0.1 ml) was added to the reaction mixture and vortexed for 5 minutes. The absorbance was read at 412 nm.

The concentration of reduced glutathione (µmol/mg) calculated with molar absorption coefficient of 13,600M was expressed as:

$$\text{Concentration (umol/mg protein)} = \frac{A \text{ sample} - A \text{ blank}}{13,600 \times \text{protein concentration}}$$

A sample = Absorbance of sample, A blank = Absorbance of blank

Determination of Malondialdehyde Concentration (MDA)

The concentration of MDA was quantified according to the method of Ohkawa *et al.* (1979) as outlined below:

A portion of TBA reagent (2ml of 0.7% and 1ml of TCA) were added to 2ml of the

sample. The mixture was thoroughly heated in a water bath at 100°C for 20minutes. It was then cooled and centrifuged at 78g (4000rpm) for 10minutes. The absorbance of the supernatant was read at a wavelength of 540nm against a reference blank of distilled water after centrifuging for another 10 minutes.

$$\text{Concentration of MDA} = \frac{\text{Absorbance} \times \text{dilution factor}}{\text{Extinction coefficient} \times \text{total protein}}$$

$$\text{Extinction coefficient of MDA} = 1.56 \times 10^5 \text{ nm}^{-1} \text{ cm}^{-1}$$

TBA: 0.7% i.e 0.7g in 100ml.

TCA: 20% i.e. 20g in 100ml

Superoxide Dismutase - Superoxide dismutase activity was determined by the method of Misra and Fridovich, (1972)

Procedure: An aliquot (0.2 ml) of diluted supernatant was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in a

spectrophotometric cuvette and the reaction was started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenalin. Blank cuvette contained 2.5 ml of carbonate buffer, 0.3 ml of adrenalin and 0.2 ml of distilled water. The increase in absorbance at 480 nm was read every 30 seconds for 150 seconds.

$$\text{SOD activity} = \frac{\text{Percentage inhibition} \times 1000 \times \text{df}}{50 \times 0.1}$$

df = dilution factor

Result

The blood glucose level of all the diabetic animals increased significantly ($p < 0.05$) compared to the positive control group (C) by

day 1 of the experiment confirming diabetes induction. Day 0 of this experiment was the Fasting Blood Glucose level of experimental animals before diabetes induction (Figure 1).

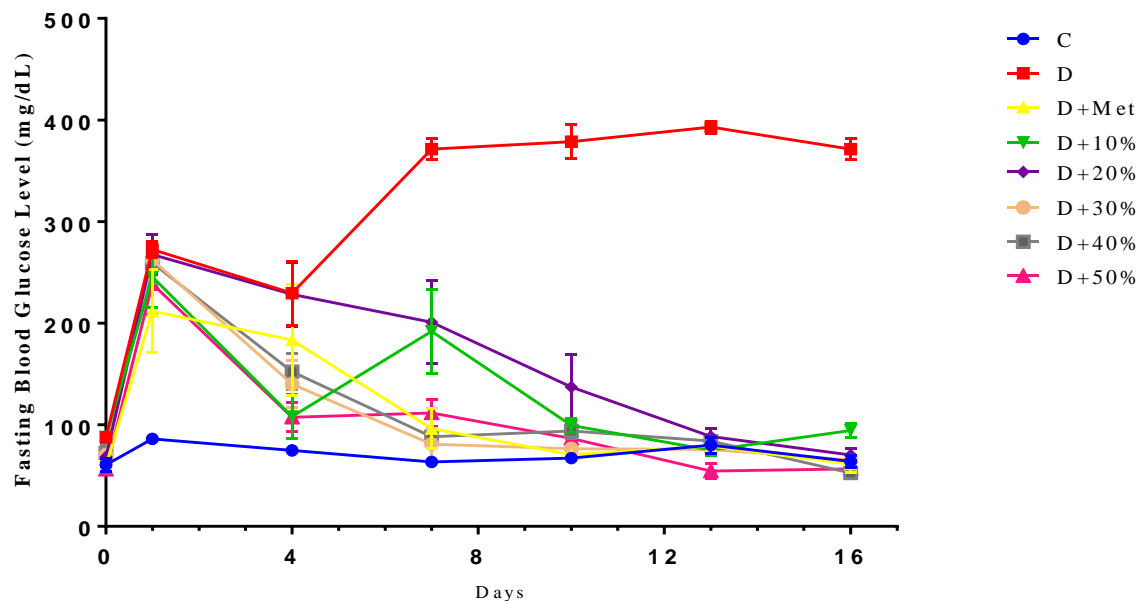


Figure 1: Fasting Blood Glucose concentration of Fructose and Streptozotocin-induced diabetic rats treated with *F. exasperata* leaf-based diet

Values are expressed as mean of 6 replicates \pm S.E.M.

C – Positive control (non diabetic animals fed with diet without *F. exasperata* leaf)

D- Negative control (Diabetic animals fed with diet without *F. exasperata* leaf)

D+Met - Diabetic animals fed with diet without *F. exasperata* leaf and treated with metformin

D+10% - Diabetic animals fed with diet containing 10% *F. exasperata* leaf

D+20% - Diabetic animals fed with diet containing 20% *F. exasperata* leaf

D+30% - Diabetic animals fed with diet containing 30% *F. exasperata* leaf

D+40% - Diabetic animals fed with diet containing 40% *F. exasperata* leaf

D+50% - Diabetic animals fed with diet containing 50% *F. exasperata* leaf

Table 2 showed the result of superoxide dismutase and catalase activity (mU/mg/dL) of fructose and streptozotocin-induced diabetic rats treated with *F. exasperata* leaf-based Diet. There was a significant reduction ($p < 0.05$) in the enzyme activity of the negative control group compared to the

positive control group. For all the treatment groups however, there was a significant ($p < 0.05$) increase in the enzymes activity when compared to the negative control and no significant difference ($p > 0.05$) when compared to the positive control group.

Table 2: Superoxide dismutase and Catalase Activities of Fructose and Streptozotocin-induced Diabetic Rats Treated with *F. exasperata* leaf-based Diet

Group	Superoxide dismutase activity(mU/mg/dL)	Catalase activity (nmol/ml)
C	2147.40 ± 44.22 ^a	6324.0 ± 118.01 ^a
D	569.58 ± 13.93 ^b	4692.3 ± 53.82 ^b
D+Met	2088.80 ± 14.98 ^a	6183.8 ± 33.31 ^a
D+10%	2133.20 ± 30.14 ^a	61.82 ± 137.70 ^a
D+20%	2114.00 ± 15.25 ^a	6055.7 ± 74.64 ^a
D+30%	2135.50 ± 74.99 ^a	6134.3 ± 74.82 ^a
D+40%	2144.60 ± 53.88 ^a	6184.4 ± 91.70 ^a
D+50%	2131.10 ± 49.15 ^a	6264.7 ± 113.09 ^a

Values are expressed as mean of 6 replicates ± S.E.M. and those with different superscripts along a column are statistically different ($p < 0.05$)

C – control (non diabetic animals fed with diet without *F. exasperata* leaf)

D- Diabetic animals fed with diet without *F. exasperata* leaf

D+Met - Diabetic animals fed with diet without *F. exasperata* leaf and treated with metformin

D+10% - Diabetic animals fed with diet containing 10% *F. exasperata* leaf

D+20% - Diabetic animals fed with diet containing 20% *F. exasperata* leaf

D+30% - Diabetic animals fed with diet containing 30% *F. exasperata* leaf

D+40% - Diabetic animals fed with diet containing 40% *F. exasperata* leaf

D+50% - Diabetic animals fed with diet containing 50% *F. exasperata* leaf

There was a significant reduction ($p < 0.05$) of reduced glutathione concentration compared to positive control. The various treatment groups on the other hand increased

significantly ($p < 0.05$) when compared to the negative control group. This increase was however not as high as the positive control (Figure 2).

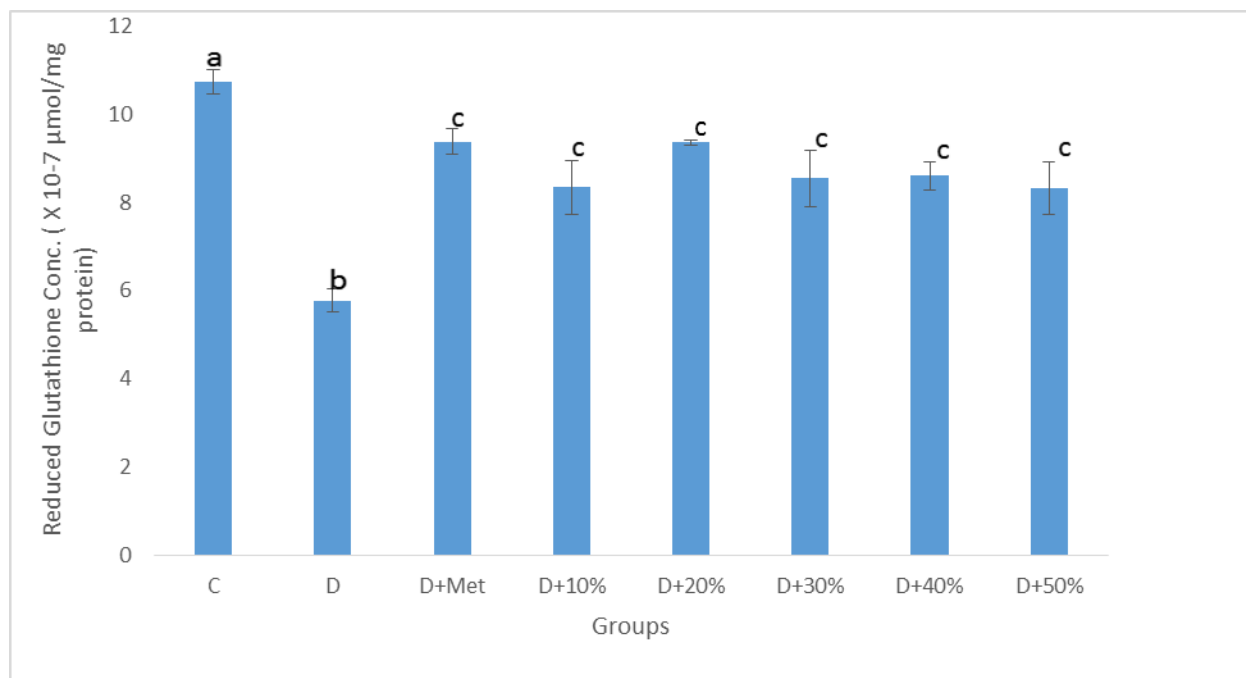


Figure 2: Reduced Glutathione Concentration of Fructose and Streptozotocin-induced diabetic rats treated with *F. exasperata* leaf-based diet

Values are expressed as mean of 6 replicates \pm S.E.M. and those with different superscripts are statistically different ($p < 0.05$)

C – control (non diabetic animals fed with diet without *F. exasperata* leaf)

D- Diabetic animals fed with diet without *F. exasperata* leaf

D+Met - Diabetic animals fed with diet without *F. exasperata* leaf and treated with metformin

D+10% - Diabetic animals fed with diet containing 10% *F. exasperata* leaf

D+20% - Diabetic animals fed with diet containing 20% *F. exasperata* leaf

D+30% - Diabetic animals fed with diet containing 30% *F. exasperata* leaf

D+40% - Diabetic animals fed with diet containing 40% *F. exasperata* leaf

D+50% - Diabetic animals fed with diet containing 50% *F. exasperata* leaf

The malondialdehyde activity was significantly increased ($p < 0.05$) in the negative control group compared to the positive control and the various treatment groups.

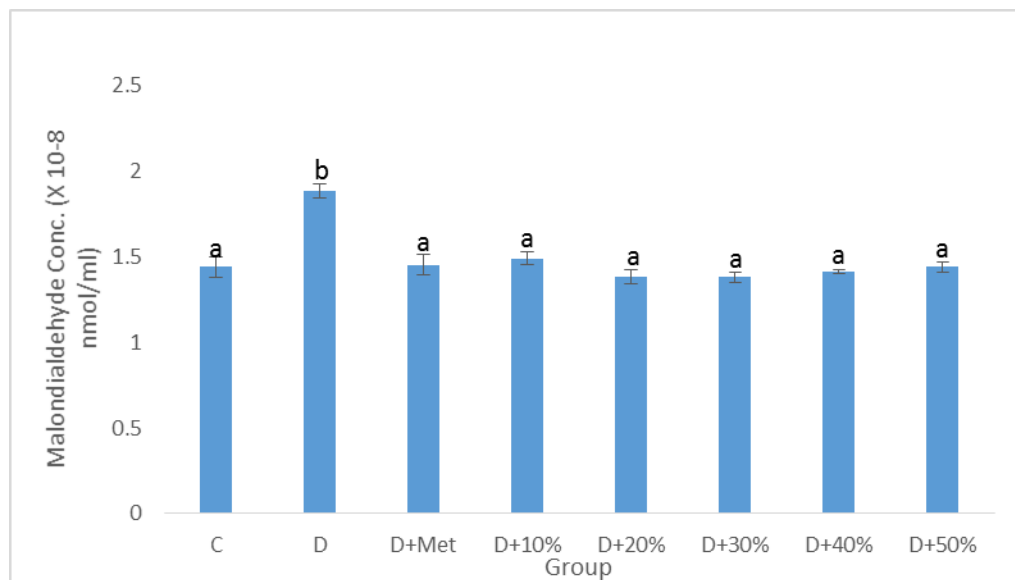


Figure 3: Malondialdehyde Concentration of Fructose and Streptozotocin-induced diabetic rats treated with *F. exasperata* leaf-based diet

Values are expressed as mean of 6 replicates \pm S.E.M. and those with different superscripts are statistically different ($p < 0.05$)

C – control (non diabetic animals fed with diet without *F. exasperata* leaf)

D- Diabetic animals fed with diet without *F. exasperata* leaf

D+Met - Diabetic animals fed with diet without *F. exasperata* leaf and treated with metformin

D+10% - Diabetic animals fed with diet containing 10% *F. exasperata* leaf

D+20% - Diabetic animals fed with diet containing 20% *F. exasperata* leaf

D+30% - Diabetic animals fed with diet containing 30% *F. exasperata* leaf

D+40% - Diabetic animals fed with diet containing 40% *F. exasperata* leaf

D+50% - Diabetic animals fed with diet containing 50% *F. exasperata* leaf

Discussion

The results of this study showed that there was alteration in the endogenous antioxidants parameters of fructose and streptozotocin-induced diabetic rats resulting from oxidative stress due to persistent hyperglycemia (figure 1). This effect was however ameliorated by the administration of the various proportions of *F. exasperata* leaf-based diet in the experimental animals. Previous studies had shown that extracts of *F. exasperata* leaf possess antioxidant effect (Abotsi *et al.*, 2010;

Faiyaz *et al.*, 2012) and this effect has been attributed to some of the phytoconstituents (flavonoids, saponins, tannins, phlobatanins, phenols, glycolyside) present in the plant. Several researches on the phenolic constituent and antioxidant activities in various plants have been conducted by Siddiq *et al.* (2005), Aher *et al.* (2009) and Ullah *et al.* (2009). It is however, imperative to isolate the bioactives of *F. exasperata* leaf eliciting the observed antioxidant effect.

The blood glucose level of all the diabetic animals increased significantly ($p < 0.05$) compared to the positive control group (C) by day 1 of the experiment confirming diabetic induction. Day 0 of this experiment was the Fasting Blood Glucose level of experimental animals before diabetes induction (Figure 1). In order to study the antioxidant effect of the test diet on the experimental animals, confirming diabetes induction is imperative. There is considerable evidence that hyperglycemia, a major hallmark of diabetes mellitus results in the generation of reactive oxygen species (ROS), ultimately leading to increased oxidative stress in a variety of tissues, and playing an important role in diabetes complications (Yfjord and Bodvarsdottir, 2005; Chandie *et al.*, 2006).

In the present study, the result of superoxide dismutase and catalase activities of fructose and streptozotocin-induced diabetic rats treated with *F. exasperata* leaf-based diet showed a significant reduction ($p < 0.05$) in the enzyme activity of the negative control group compared to the positive control group. For all the treatment groups however, there was a significant ($p < 0.05$) increase in the enzymes activity when compared to the negative control and no significant difference ($p > 0.05$) when compared to the positive control group. Upon entry of glucose into the cell, glucose is metabolized and generates electron donors for transfer to the mitochondria, where they are used to make energy in the form of adenosine triphosphate. If more glucose is being oxidized, more electron donors are generated. However, when the mitochondrial capacity is exceeded, excess electron donors may form superoxide, an ROS.

There was a significant reduction ($p < 0.05$) of reduced glutathione concentration compared to positive control. The various treatment groups on the other hand increased significantly ($p < 0.05$) when compared to the negative control group. This increase was

however not as high as the positive control (Figure 2). An important antioxidant in the cell is glutathione. Glutathione reductase, the enzyme that converts glutathione to its reduced form, which is critical for its antioxidant activity, requires the cofactor nicotinamide adenine dinucleotide phosphate (NADPH). NADPH becomes depleted when intracellular glucose levels increase excessively due to increased flux of glucose through the NADPH-dependent polyol pathway. When NADPH is depleted, generation of reduced glutathione is limited, leaving the cell vulnerable to the effects of ROS (Brownlee, 2005; Di Mario and Pugliese, 2009).

The malondialdehyde concentration was significantly increased ($p < 0.05$) in the negative control group compared to the positive control and the various treatment groups. Oxygen derived free radicals and reactive oxygen species interact with the lipid bilayer of the cell membrane resulting in lipid peroxidation. Malondialdehyde (MDA) is a stable end product of lipid peroxidation. Elevated MDA levels alter the structural integrity of the cell membranes. Inactivation of membrane bound enzymes and surface receptor molecules leads to cell-regulating errors (Mahreen *et al.*, 2010).

Conclusion

Feeding of *F. exasperata* leaf-based diet to Type 2 diabetic rats for 16 days ameliorated the alteration in endogenous antioxidant parameters of the experimental animals.

Declaration of interest

Authors declare that there is no conflict of interests.

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