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Antioxidant Activity of Hydro-Ethanolic Extract of Blighia sapida Stem Bark in the Pancreas of Alloxan-Induced Diabetic Rats

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Abstract
Blighia sapida is a plant belonging to the family of sapindaceae. In this study we aimed at investigating the possible antioxidant activities of hydro-ethanolic extract of Blighia sapida stem bark in the pancreas of alloxan-induced diabetic rats. Administration of the extract at 100mg/kg body weight significantly ($P<0.05$) increased the activities of antioxidant enzymes catalase, glutathione peroxidase and superoxide dismutase in the pancreas of diabetic rats. Also the concentration of reduced glutathione increased in the pancreatic tissues of the diabetic rats while the levels of malondialdehyde and protein carbonyl generally decreased in the pancreas of alloxan-induced diabetic rats during the course of the experiment. These are indications of antioxidant properties of the stem bark of Blighia sapida with 100mg/kg body weight of the hydro-ethanolic extract showing good antioxidant activities by comparing favourably well with metformin, a standard antidiabetic drug.
Keywords: *Blighia sapida*, diabetes mellitus, antioxidants, antioxidant enzymes
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1. Introduction

Diabetes mellitus is a group of metabolic disease caused by a defect in insulin production, insulin action or both. Type 1 diabetes is caused by a lack of insulin due to the destruction of insulin-producing β – cells in the pancreas. It is widely accepted that type 1 diabetes is an autoimmune disease involving T-cell mediated destruction of pancreatic β – cells. Type 2 diabetes, the most common form of diabetes, is caused by a combination of factors, including insulin resistance, a condition in which the body's muscle, fat and liver cells do not use insulin effectively.

Nigeria probably accounts for a quarter of all diabetes mellitus cases in Africa by deduction. Almost all the cases of diabetes mellitus in Nigeria are caused by the inability of the human body to properly metabolize glucose either due to insufficient endogenous supply of insulin or body's insulin resistance.

The World Health Organization (WHO) in its 2014 release said that the prevalence of diabetes has reached epidemic proportions. In 2014 the global prevalence of diabetes was estimated to be 9% among adults aged 18+ years. In 2012, an estimated 1.5 million deaths were directly caused by diabetes. More than 80% of diabetes deaths occur in low- and middle-income countries (WHO 2014).

Diabetes mellitus is associated with an increase in reactive oxygen species (ROS) generation by mononuclear cells and an increased oxidative load resulting in oxidative damage to lipids, proteins and DNA. Chronic hyperglycemia and subsequent augmentation of reactive oxygen species (ROS) deteriorate β-cell functions and increase insulin resistance which leads to the aggravation of type 2 diabetes. In addition, chronic hyperglycemia and ROS are also involved in the development of atherosclerosis which is often observed under diabetic conditions (Kaneto *et al.* 2010). NADPH oxidase-derived ROS plays a physiological role in the regulation of endothelial function and vascular tone and a pathophysiological role in endothelial dysfunction, inflammation, hypertrophy, apoptosis, migration, fibrosis, angiogenesis and rarefaction; important processes underlying cardiovascular and renal remodeling in hypertension and diabetes. These findings have evoked considerable interest because of the possibilities that therapies against nonphagocytic NADPH oxidase to decrease ROS generation and/or strategies to increase nitric oxide (NO) availability and antioxidants may be useful in minimizing vascular injury and renal dysfunction and thereby prevent or regress target organ damage associated with hypertension and diabetes (Paravicini & Touyz 2008).

Chronic hyperglycemia is a cause of impairment of insulin biosynthesis and secretion. This process is called β-cell glucose toxicity which is often observed under diabetic conditions. β-cells are rather vulnerable to ROS due to the relatively low expression of antioxidant enzymes such as catalase and glutathione peroxidase. Therefore it is likely that ROS are involved in β–cell deterioration found in diabetes (Evans *et al*. 2003). The potential mechanism of oxidative stress includes the reduction of antioxidant defense.

Blighia sapida is a plant belonging to the family of Sapindaceae. It is native to Western Tropical Africa and was introduced into Jamaica in the late 18th century. It has spread to other parts of tropical America but it is still more widely known in Jamaica than elsewhere. It is commonly known as ackee. In Nigeria, it is called Gwanja Kusa (Hausa), Isin (Yoruba) and Okpu (Igbo). The seed of the fruit is not edible, whereas the fleshy aril is edible. The fruit is known to contain saponins, which are hemolytic (Aderinola *et al.* 2007).

Most of the earlier studies on *Blighia sapida* have been on the nutritional qualities of the root (Abolaji *et al*. 2007) and the leaves as a dry season feed resource for West African dwarf goats in the Northern savanna zone of Nigeria (Aderinola *et al*. 2007). More recently, the physicochemical properties of the oil from the fruit of the species and toxicological evaluation of the oil – based diet in Wister rats have been investigated (Oladiji *et al*. 2009). However, the scanty information on the antioxidant activity of extract of *Blighia sapida* stem bark prompted this study. Tree bark is an important component of African traditional medicine as herbal medicine is still the main source of health care for the majority of Africans and in particular, Nigerians.

There has been increasing demand for the use of plant products with anti-diabetic activity. The high cost, availability, uncertainty of use during pregnancy and undesirable side effects of synthetic drugs or drugs from other animal sources are some of the factors leading to a strong preference for hypoglycemic drugs of plants origin.

This study is thus aimed at investigating the antioxidant activity of hydro-ethanolic extract of readily available *Blighia sapida* stem bark.

2. Materials and Methods

2.1 Chemicals

All chemicals used were of analytical grade and items are products of BDH and Sigma Chemical Ltd., UK and Accu-chek ® Advantage, Roche Diagnostic, Germany.

2.2 Animals

Male albino rats (Ratus norvegicus) weighing between 100g and 120g were used for the experiment. The rats were bred in the animal holding of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, were maintained on standard rat pellets (Ladokun feeds, Ibadan, Nigeria), and were given water ad libitum.

2.3 Sourcing for the Tree Bark of Blighia sapida

A sizeable quantity of the tree bark of Blighia sapida was obtained from the compound of the Federal Polytechnic, Ado Ekiti, Nigeria.

2.4 Identification of Plant:

The fruits and leaves of Blighia sapida plant were obtained from the compound of the Federal polytechnic, Ado Ekiti, Ekiti State, Nigeria and were used for the purpose of authentication of the identity of the plant at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. The voucher number of identification is UIH624.

2.5 Processing of sample and preparation of extract:

The sample obtained was air-dried at room temperature for fifty-six (56) days until a constant weight was obtained. The air-dried tree bark of Blighia sapida was pulverized. 100g of the pulverized sample was extracted with 800ml of 15% ethanol (hydro-ethanol) solution for seventy-two (72) hours in an extractor. The hydro-ethanolic extract was obtained by filtering with Whatman filter paper and subsequently freeze-dried in Armfield freeze-drier for ten (10) days.

2.6 Induction of experimental diabetes mellitus:

After an overnight fasting, rats were induced by intraperitoneal administration of alloxan monohydrate at a dose of 120mg/kg body weight. Alloxan monohydrate was freshly dissolved in distilled water and maintained on ice prior to use. Four days after the administration, the animals were fasted for 16 hours and blood glucose levels were determined in mg/dl using a digital glucometer (Accu-chek ®, advantage, Roche, Diagnostic, Germany). On the seventh day of administration, animals which had basal glycemia levels of 125mg/dl were used in the experiment. Animals had free access to food and water after the alloxan injection.

2.7 Experimental Design:

Randomized Complete Block Design (RCBD) method was used.

Eighty male albino rats were grouped as follows:

Group 1**:** Control group administered with distilled water orally.

Group 2**:** The alloxan-induced diabetic group left untreated

Group3**:** The alloxan-induced diabetic group treated with oral administration of hydro-ethanolic extract of Blighia sapida at 100mg/1000g body weight

Group 4**:** The alloxan-induced diabetic group treated with oral administration of Metformin hydrochloride at 21.4mg/1000g body weight.

All the animals were fed with vital finisher made up of maize and soya bean mainly. The administration of the extracts as written above will be carried out every 24 hours for 21 days.

Analysis of the various parameters as stated were carried out after eight days of diabetes induction and then at intervals of seven days for twenty-one days.

2.8. Repeated administration of the hydro-ethanolic extract of Blighia sapida stem bark in control and diabetic groups:

The fasting blood glucose levels of all groups were measured and then the extract dissolved in distilled water. The solution of the extract was administered to one of the diabetic groups orally at 100mg/kg body weight once a day for twenty-one (21) days.The diabetic control and untreated (without alloxan induction). Five animals each were sacrificed from each of the four groups by diethyl ether anaesthesia and the pancreas obtained from them. The pancreas so obtained were stored in phosphate buffer (0.1M, pH = 7.0) maintained below -20^{0}C until required for analysis.

2.9. In vivo Antioxidant Assay

Pancreas was homogenized with cold 1.5% KCl to make a 10% homogenate.

2.9.1. Determination of the activity of Catalase (CAT): Catalase activity was determined in the lysate using Aebi's method (Aebi, 1984).

2.9.2. Determination of the activity of Superoxide dismutase (SOD): This method is well described by Mccord and Fridovich (1969) and can be applied for determination of antioxidant activity of a sample.

2.9.3. Determination of the activity of Glutathione Peroxidase (GPx): Glutathione peroxidase (GPx) was measured by the method described by Rotruck et al. (1973).

2.9.4. Determination of reduced glutathione (GSH): Reduced glutathione (GSH) was measured by the method of Beatler et al. (1963)

2.9.5. Determination of Malondialdehyde (MDA): Total amount of lipid peroxidation products present in the samples was estimated by the thiobarbituric acid (TBA) method which measures the malondialdehyde (MDA) reactive products according to the method of Ohkawa et al. (1979).

2.9.6. Determination of Protein Carbonyl Content: The protein carbonyl content was assayed according to a previous method of Levine et al (1990).

2.9.7. Determination of Protein: Protein determination was carried out according to the method of Lowry et al., (1951) as described by Holme & Peck (1998).

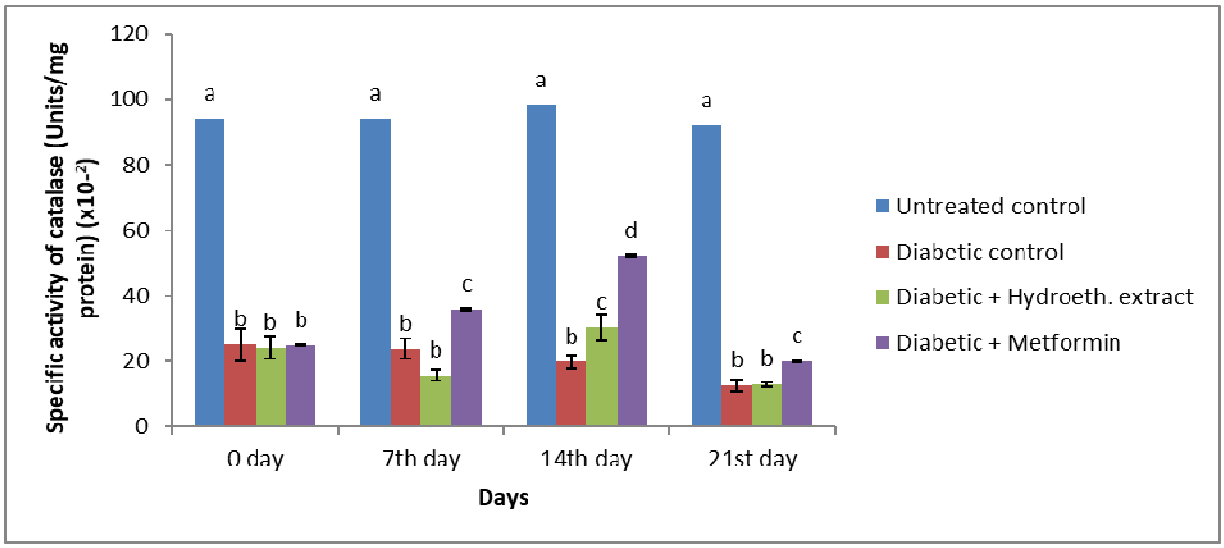
2.10. Statistical Analysis: Data were expressed as mean $\pm$ S.E.M. of five replicates and subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to determine significant differences in all the parameters. Values were considered statistically significant at $P<0.05$.

3.0 Results

3.1 Catalase activity**:** Specific activity of catalase was found to increase ($P<0.05$) in pancreas following administration of hydro-ethanolic extract of *Blighia sapida* stem bark while the administration of metformin, a standard antidiabetic drug increased the specific activity of catalase in pancreas till the fourteenth day of the experiment (Fig.1). The specific activity of catalase was found to reduce in the pancreas of untreated, diabetic animals.

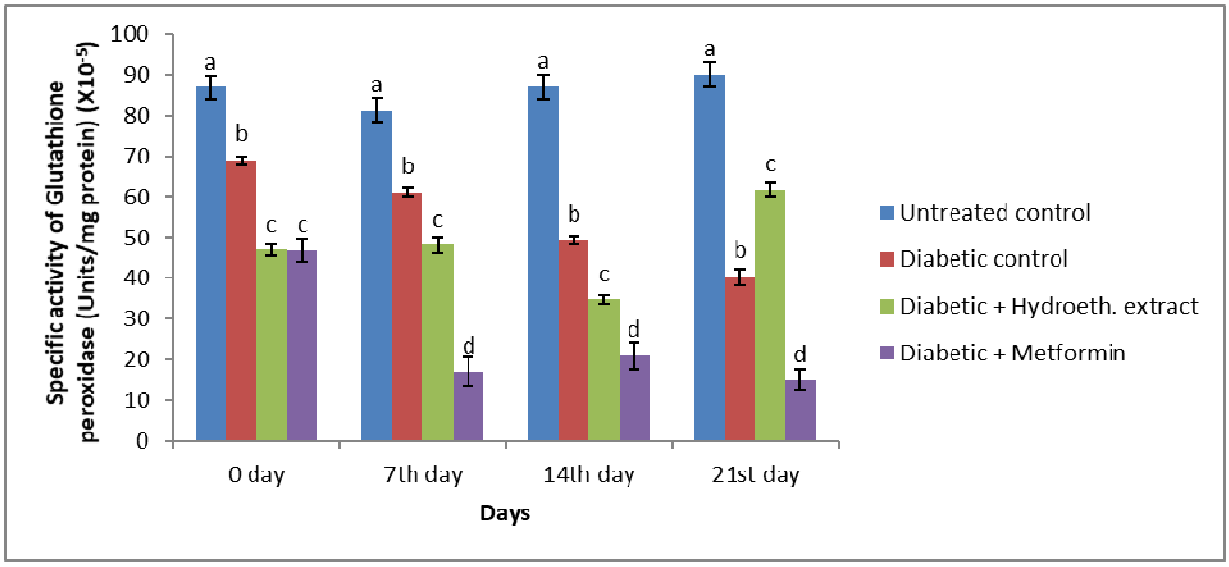
3.2 Glutathione peroxidase (GPx) activity**:** A significant increase ($P< 0.05$) was observed in the specific activity of glutathione peroxidase in the pancreas of the diabetic rats after an initial reduction, following administration of hydro-ethanolic extract of *Blighia sapida* stem bark (Fig.2).

3.3 Superoxide dismutase (SOD) activity: Fig 3 shows that the specific activity of superoxide dismutase in the pancreas of the diabetic rats significantly increased ($P < 0.05$) during the course of the experiment. A significant increase ($P < 0.05$) in the specific activity of superoxide dismutase was also observed in pancreas of diabetic rats following administration of metformin, a standard antidiabetic drug.



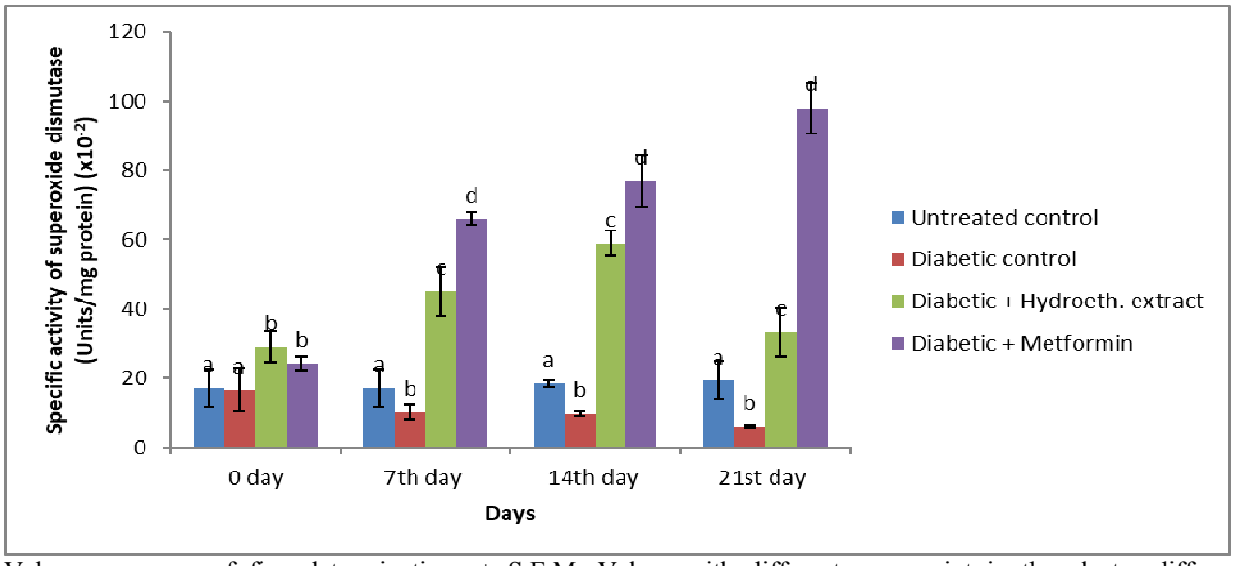
Values are mean of five determinations ± S.E.M. Values with different superscript in the cluster differ significantly ($p<0.05$)

Fig. 1: Specific activity of catalase in pancreas of diabetic albino rats following administration of hydro-ethanolic extract of *Blighia sapida* stem bark



Values are mean of five determinations ± S.E.M. Values with different superscript in the cluster differ significantly ($p<0.05$)

Fig. 2: Specific activity of Glutathione peroxidase (GPx) in pancreas of diabetic albino rats following administration of hydro-ethanolic extract of *Blighia sapida* stem bark



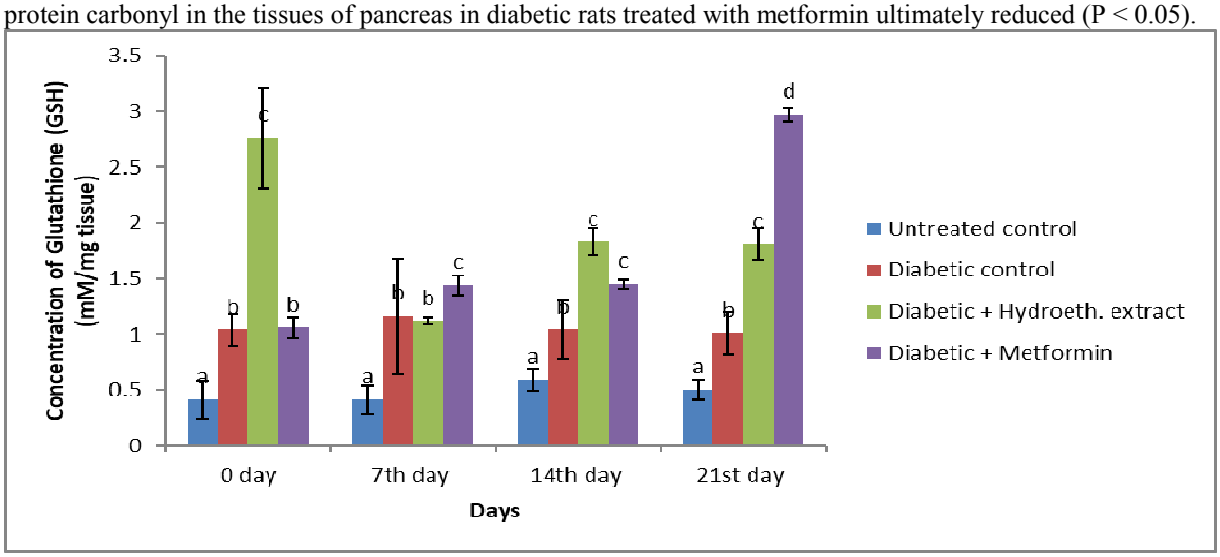
Values are mean of five determinations ± S.E.M. Values with different superscript in the cluster differ significantly ($p<0.05$)

Fig. 3: Specific activity of superoxide dismutase (SOD) in pancreas of diabetic albino rats following administration of hydro-ethanolic extract of *Blighia sapida* stem bark

3.4 Reduced glutathione: Fig. 4 shows the effect of administration of hydro-ethanolic extract of *Blighia sapida* stem bark on concentration of reduced glutathione (GSH) in pancreas of diabetic rats. There was a significant ($P < 0.05$) increase in the level of reduced glutathione, a potent antioxidant, in the pancreas of diabetic rats after an initial reduction, following the administration of hydro-ethanolic extract of *Blighia sapida* stem bark.

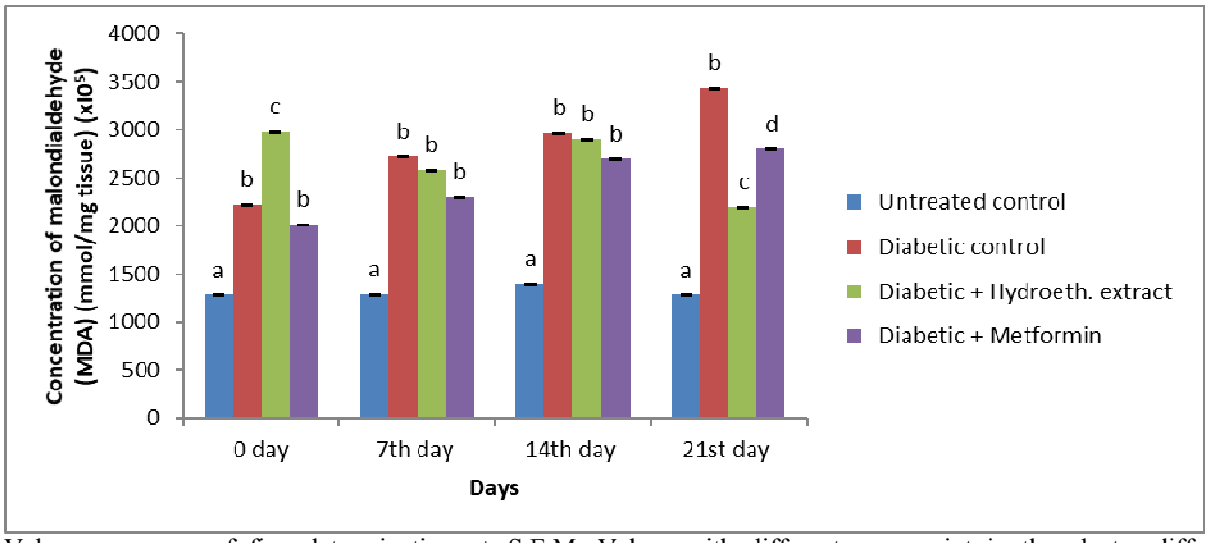
3.5 Malondialdehyde**:** A significant reduction ($P < 0.05$) in the level of malondialdehyde (MDA) was noticed in the pancreas of diabetic rats following the administration of hydro-ethanolic extract of *Blighia sapida* stem bark (Fig. 5). On the other hand, the administration of metformin, a standard antidiabetic drug, did not reduce the concentration of malondialdehyde, Instead the level of malondialdehyde increased throughout the course of the experiment in the pancreatic tissues of the diabetic rats treated with metformin, a similar result obtained in the group of untreated diabetic rats.

3.6 Protein carbonyl: The level of protein carbonyl in the tissue of the pancreas of diabetic rats treated with hydro-ethanolic extract of *Blighia sapida* stem bark did not follow any particular pattern. Also, the level of protein carbonyl in the tissues of pancreas in diabetic rats treated with metformin ultimately reduced ($P < 0.05$).



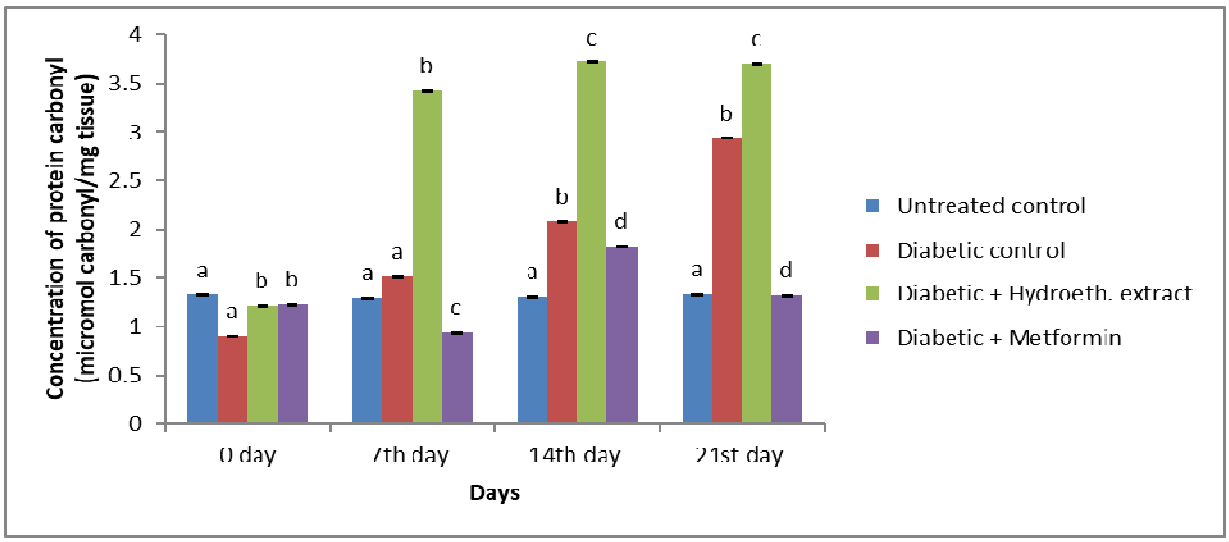
Values are mean of five determinations ± S.E.M. Values with different superscript in the cluster differ significantly ($p<0.05$)

Fig. 4: Concentration of reduced glutathione (GSH) in pancreas of diabetic albino rats following administration of hydro-ethanolic extract of *Blighia sapida* stem bark



Values are mean of five determinations ± S.E.M. Values with different superscript in the cluster differ significantly ($p<0.05$)

Fig. 5: Concentration of malondialdehyde (MDA) in pancreas of diabetic albino rats following administration of hydro-ethanolic extract of *Blighia sapida* stem bark



Values are mean of five determinations ± S.E.M. Values with different superscript in the cluster differ significantly ($p<0.05$)

Fig. 6: Concentration of protein carbonyl in pancreas of diabetic albino rats following administration of hydro-ethanolic extract of *Blighia sapida* stem bark

4.Discussion

Diabetes mellitus is associated with an increase in reactive oxygen species (ROS) generation by mononuclear cells and an increased oxidative load resulting in oxidative damage to lipids, proteins and DNA. Acute hyperglycemia has been shown to result in an increase in blood pressure, which is prevented by antioxidants, this suggests that acute hyperglycemia probably causes increased generation of ROS. Chronic hyperglycemia and subsequent augmentation of reactive oxygen species (ROS) deteriorate β-cell functions and increase insulin resistance which leads to the aggravation of type 2 diabetes (Kaneto *et al*. 2010).

It has been shown that ROS are produced in various tissues under diabetic conditions (Baynes & Thorpe 1999). There are several sources of ROS in cell such as nonenzymatic glycosylation reaction, the electron transport chain in mitochondria, and membrane-bound NADPH oxidase (Mohazzab *et al*. 1994; Browlee 2001; Harrison *et al*. 2003). Chronic hyperglycemia is a cause of impairment of insulin biosynthesis and secretion. This process is called β-cell glucose toxicity which is often observed under diabetic conditions. In diabetic state, hyperglycemia and subsequent production of ROS decrease insulin gene expression and finally bring about apoptosis. In addition, ROS are induced and involved in the β-cell glucose toxicity. B-cells are rather vulnerable

to ROS due to the relatively low expression of antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase. Therefore it is likely that ROS are involved in β-cell deterioration found in diabetes (Evans *et al*. 2003). The potential mechanism of oxidative stress includes the reduction of antioxidant defense. In this study, the levels of catalase, glutathione peroxidase and superoxide dismutase activities in the tissues of pancreas of diabetic group were significantly reduced and treatment with *Blighia sapida* stem bark hydro-ethanolic extract improved the catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities not only on acute experiments but also after 21 days of treatment. Decreased levels of CAT, GPx and SOD in the diabetic state may be due to the inactivation caused by reactive oxygen species. In treated groups, the increased CAT specific activity could be due to higher production of H_2O_2. It is possible that CAT activity which in turn would protect SOD inactivation H_2O_2 causes an increase in SOD activity. Increase in SOD activity would protect GPx and CAT against inactivation by superoxide anion (Blum and Fridovich 1985). An increase in the level of reduced glutathione could be due to it been spared as a result of the protection offered by superoxide dismutase to glutathione peroxidase.

The increase in free radicals in diabetic condition is suggested to be due to the increased lipid peroxidation and the damage to antioxidant defense systems. Protein glycation and glucose autoxidation can generate free radicals that catalyze the lipid peroxidation (Altan *et al*. 2006).

In particular O_2^- and $^{\cdot}OH$ induce various injuries to the surrounding organs and play a vital role in some clinical disorders. Therefore, removal of O_2^- and $^{\cdot}OH$ is the most effective defense of the living body against disease (Lin *et al*. 1995). Any compound, natural or synthetic, with antioxidant activity might totally or partially alleviate this damage. In this study, direct effects of hydro-ethanolic extract of *Blighia sapida* stem bark on malondialdehyde (MDA) levels in diabetic group were found to be higher ($P < 0.05$) than those in control group, indicating increased free radical generation. Treatment of diabetes with the hydro-ethanolic extract of *Blighia sapida* stem bark caused a general reduction in the MDA levels in pancreas after 21 days of treatment.

Direct effects on protein carbonyl levels in diabetic group were found to be higher than those in control group ($P < 0.05$), indicating increased free radical generation via production of various kinds of glycated proteins such as glycosylated hemoglobin, albumin and lens. Treatment of diabetes with the hydro-ethanolic extract of *c* caused a reduction in the level of protein carbonyl in pancreas within 21 days of administration.

The result obtained in this study is in agreement with an earlier report by Amira and Oloyede (2017) where they evaluated the antioxidant activity of aqueous extract of *Blighia sapida* stem bark in alloxan-induced diabetic rats.

5.Conclusion

It was observed that *Blighia sapida* stem bark hydro-ethanolic extract generally caused a significant increase in the activities of catalase, glutathione peroxidase and superoxide dismutase in the pancreas of diabetic rats during 21 days of treatment. It was also noticed that hydro-ethanolic extract of *Blighia sapida* stem bark extract possess the capability of inhibiting or reducing both lipid and protein peroxidation in diabetes.

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