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Antioxidant Activity of Aqueous Extract of Blighia sapida Stem Bark in Alloxan-induced Diabetic Rats

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Abstract

Blighiasapida is a plant belonging to the family of sapindaceae. In this study we aimed to evaluate thein vivo antioxidant activitiesof aqueous extract of Blighiasapida stem bark in 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 kidney and pancreas tissues of diabetic rats. Also the concentration of reduced glutathione increased in the kidney and pancreas tissues of the diabetic rats while the levels of malondialdehyde and protein carbonyl generally decreased in the kidney and pancreas tissues of alloxan-induced diabetic rats during the course of the experiment. These are indications of antioxidant properties of the stem bark of Blighiasapida with 100mg/kg body weight of the aqueous extract showing good antioxidant activities by comparing favourably well with metformin, a standard antidiabetic drug.

Index terms— blighia sapida, diabetes, antioxidant enzymes, biomolecules.

1 I. Introduction

iabetes 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 ? -cells in the pancreas. 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.

Diabetes mellitus is a multifactorial disease, which is characterized by hyperglycemia (Ugochukwu et al., 2003), lipoprotein abnormalities **??**Scoppola et al., 2001), raised basal metabolic rates (Okwu et al., 2006), defect in reactive oxygen species scavenging enzymes and altered intermediary metabolism of major food substances (Unwin et al., 2001).

Hyperglycemia causes many of the health problem associated with diabetes, including eye, kidney, heart disease and nerve conditions. Hypoglycemic agents have been used in the management of diabetes mellitus (DM).

The World Health Organization (WHO) in its 2014 release reported 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 middleincome 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 (Marfella et al., 1995;Giugliano et al., 1997; **??**aoliso and Giugliano, 1996).

Chronic hyperglycemia and subsequent augmentation of reactive oxygen species (ROS) deteriorate ?-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 and Thorpe, 1999).

Chronic hyperglycemia is a cause of impairment of insulin biosynthesis and secretion. This process is called ?-cell glucose toxicity which is often observed under diabetic conditions. ?-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 ?-cell deterioration found in diabetes (Evans et al., 2003). It is also known that lipotoxicity is also involved in the deterioration of ?-cell function found in type 2 diabetes (Kaneto et al., 2010).

Blighia sapida is a plant belonging to the family of Sapindaceae. It is commonly known as ackee. In Nigeria, it is called Gwanja Kusa (Hausa), Isin (Yoruba) and Okpu (Igbo) (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). The repellant potential of the fruit part components against stored-product insect pests (Khan and Gumbs, 2003) as well as neutropenia and thrombocytopenia effects of the aqueous and lipid extracts of the unripe fruit have been investigated in mice (Gardiner et al, 1996). 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. Stem 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. 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) and animals which had basal glycemia levels of 125mg/dl were used in the experiment. 7. Experimental Design: Randomized Complete Block Design (RCBD) method was used. Eighty male albino rats were grouped as follows:

2 II. Materials and Methods

Group 1: Control group administered with distilled water orally.

Group 2: The alloxan-induced diabetic group left untreated Group 3: The alloxan-induced diabetic group treated with oral administration of distilled water 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 was carried out every 24 hours for 21 days.

Analysis of the various parameters as stated was carried out weekly after diabetes detection for three weeks.

3 Repeated administration of the aqueous 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 chloroform anaesthesia and the pancreas and kidney obtained from them. The pancreas and kidney so obtained were stored in phosphate buffer (0.1M, pH = 7.0) maintained below -20 0 C until required for analysis.

4 9.

In vivo Antioxidant Assay: Pancreas and kidney tissues were homogenized with cold 1.5% KCl to make a 10% homogenate.

5 Determination of the activity of Catalase (CAT):

Catalase activity was determined in the lysate using Aebi's method (Aebi, 1984).

6 Determination of the activity of Superoxide dismutase (SOD):

This method is well described by Mccord and Fridovich (1969).

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

10. Statistical Analysis: Data were expressed as mean + 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 $P<0.05$.

7 III. Results

1. Catalase activity: Specific activity of catalase was found to increase ($P<0.05$) in kidney and pancreas following administration of aqueous extract of Blighia sapida stem bark while the administration of metformin, a standard antidiabetic drug increased the specific activity of catalase in kidney and pancreas till the fourteenth day of the experiment (Table 4). The specific activity of catalase was found to reduce in the kidney and pancreas

of untreated, diabetic animals. 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 aqueous extract of Blighia sapida stem bark. On the other hand, the specific activity of glutathione peroxidase in the kidney of diabetic rats did not increase but significantly reduced (P < 0.05) during the course of the experiment, a result similar to the one obtained for the untreated diabetic rats (Table 5).

Superoxide dismutase (SOD) activity: Table 6 shows an initial significant increase (P < 0.05) in the specific activity of superoxide dismutase followed by a slight reduction toward the end of the experiment in the kidney of the diabetic rats following administration of aqueous extract of Blighia sapida stem bark. However, 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 observed in both the kidney and pancreas of diabetic rats following administration of metformin, a standard antidiabetic drug. 4. Reduced glutathione: Table 7 shows the effect of administration of aqueous extract of Blighia sapida stem bark on concentration of reduced glutathione (GSH) in kidney and pancreas of diabetic rats. There was a significant (P < 0.05) increase in the level of reduced glutathione, a potent antioxidant, in the kidney and pancreas of diabetic rats after an initial reduction, following the administration of aqueous extract of Blighia sapida stem bark.

8 5.

Malondialdehyde: A significant reduction (P < 0.05) in the level of malondialdehyde (MDA) was noticed in the kidney and pancreas of diabetic rats following the administration of aqueous extract of Blighia sapida stem bark (Table 8). 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 kidney and pancreas tissues of the diabetic rats treated with metformin, a similar result obtained in the group of untreated diabetic rats. 9 shows a significant reduction (P < 0.05) towards the end of the experiment after an initial increase, in the level of protein carbonyl in the kidney tissues of diabetic rats following the administration of aqueous extract of Blighia sapida stem bark. On the other hand, the level of protein carbonyl in the tissue of the pancreas of diabetic rats treated with aqueous extract of Blighia sapida stem bark did not follow any particular pattern. Also, while the level of protein carbonyl in the tissues of pancreas in diabetic rats treated with metformin ultimately reduced (P < 0.05) those in the tissues of kidney of diabetic rats treated with metformin did not follow a definite pattern.

9 Protein carbonyl: Table

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10 IV. 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 ?-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 and Thorpe, 1999). There are several sources of ROS in cell such as nonenzymatic glucosylation reaction, the electron transport chain in mitochondria, and membrane-bound NADPH oxidase (Browlee, 2001; Harrison et al, 2003, Mohazzab et al, 1994). Chronic hyperglycemia is a cause of impairment of insulin biosynthesis and secretion. This process is called ?-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 ?-cell glucose toxicity. ? -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 ?-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 kidney and pancreas of diabetic group were significantly reduced and treatment with Blighia sapida stem bark aqueous 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 2 O 2 . It is possible that CAT activity which in turn would protect SOD inactivation by H 2 O 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 . OH induce various injuries to the surrounding organs and play a vital role in some clinical disorders. Therefore, removal of O 2 and . 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 aqueous 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 aqueous extract of Blighia sapida stem bark caused a general reduction in the MDA levels in kidney and 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 aqueous extract of Blighia sapida stem bark caused a reduction in the level of protein carbonyl in kidney and pancreas within 21 days of administration.

11 V. Conclusion

A major finding of this study is that Blighia sapida stem bark aqueous extract generally caused a significant increase in the activities of catalase, glutathione peroxidase and superoxide dismutase in the kidney and pancreas of diabetic rats during 21 days of treatment. It is also noticed that aqueous extract of Blighia sapida stem bark extract possess the capability of inhibiting or reducing both lipid and protein peroxidation in diabetes. [1] [2] [3]

4

Tissue	Group of animal	Specific activity of catalase (Units/mg protein) (x10 -2)			
		0 day	7th day	14th day	2st d
Kidney	Untreated control	148.44+ 2.41 a	149.35+ 2.10 a	148.52+ 3.01 a	148.24+ 2.52 a
	Diabetic control	128.20+ 13.60 b	113.85+ 14.00 b	103.98+ 13.20 b	82.65+ 13.40 b
	Diabetic + Aqueous extract	128.20+ 13.60 b	104.71+ 2.88 c	230.22+ 1.86 c	279.55+ 0.01 c
	Diabetic + Metformin	123.50+ 0.08 b	71.40+ 0.08 d	112.00+ 0.23 b	86.00+ 0 b
Pancreas	Untreated control	94.13+ 2.53 a	94.13+ 2.53 a	98.32+ 3.12 a	92.01+ 2.51 a
	Diabetic control	25.06+ 4.90 b	23.76+ 3.10 b	19.85+ 2.00 b	12.58+ 1.80 b
	Diabetic + Aqueous extract	25.06+ 4.90 b	11.51+ 2.30 c	21.08+ 2.90 b	120.83+ 3.32 a
	Diabetic + Metformin	24.90+ 0.28 b	35.80+ 0.31 d	52.20+	0 c

[Note: cValues are mean of five determinations + S.E.M. Values with different superscript in the row and column differ significantly (p<0.05)]

Figure 1: Table 4 :

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Figure 2: Table 5 :

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Tissue	Group of animal	Specific activity of superoxide dismutase (SOD (Units/mg protein) (x10? 3) 0			
Kidney	Untreated control	19.35+ 10.06 a	20.16+ 1. 26 a	19.56+ 1.01 a	19.35+ 1.16 a
	Diabetic control	28.05+ 1.23 b	26.52+ 0.96 a	28.95+ 1.24 a	19.98+ 1.02 a
	Diabetic + Aqueous extract	28.05+ 1.23 b	193.36+ 3.04 b	116.07+ 2.34 b	109.22+ 1.81 b
	Diabetic + Metformin	32.00+ 1.40 b	84.00+ 1.31 c	123.00+ 1.32 b	382.00+ 0.50 c
Pancreas	Untreated control	17.32+ 5.48 a	17.32+ 5.48 a	18.51+ 1.05 a	19.32+ 5.46 a
	Diabetic control	16.72+ 6.28 a	10.27+ 2.15 b	9.81+ 0.53 b	6.01+ 0.23 b
	Diabetic + Aqueous extract	16.72+ 6.28 a	166.91+ 12.21 c	116.53+ 16.28 c	245.27+ 52.55 c
	Diabetic + Metformin	24.23+ 1.90 b	66.00+ 1.72 d	77.00+ 7.50 d	98.00+ 7.40

[Note: d Values are mean of five determinations + S.E.M. Values with different superscript in the row and column differ significantly (p<0.05)]

Figure 3: Table 6 :

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[Note: dValues are mean of five determinations + S.E.M. Values with different superscript in the row and column differ significantly (p<0.05)]

Figure 4: Table 7 :

8

Tissue Group of animal	0 day	7 th day	14 th day	21 st day
Kidney Untreated control	.1186.68+ 0.14 a	1193.00+ 0.10 a	1I86.88+ 0.11 a	1099.80+ 0.10 a
Diabetic control,	1426.50+ 0.12 b	1522.20+ 0.10 b	1700.90+ 0.12 b	lS92.30+ 0.12 b
Diabetic + Aqueous extract	1426.50+ 0.12 b	1364.38+ 0.99 b	1351.30+ 0.03 a	1052.30+ 0.08 a
Diabetic + Metformin	1390.00+ 0.06 b	2570.00+ 0.51 c	3570.00+ 0.25 c	9750.00+ 0.22 c
Pancreas Untreated control	1278.59+ 0.22 a	1288.60+ 0.10 a	1392.60+ 0.20 a	1278.59+ 0.22 a
Diabetic control	2227.10+ 0.08 b	2723.50+ 0.09 b	2965.20+ 0.09 b	3435.20+ 0.09 b
Diabetic + Aqueous extract	2227.10+ 0.08 b	1625.82+ 0.07 c	1227.10+ 0.28 a	1106.20+ 0.06 a
Diabetic + Metformin	2010.00+ 0.17 b	2300.00+ 0.17 b	27000.07 + b	2800.00 + 0.31

[Note: cValues are mean of five determinations + S.E.M. Values with different superscript in the row and column differ significantly (p<0.05)]

Figure 5: Table 8 :

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[Note: cValues are mean of five determinations + S.E.M. Values with different superscript in the row and column differ significantly (p<0.05)]

Figure 6: Table 9 :

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