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Anti-hyperglycemic and in vivo Antioxidant Activities of Aqueous Extract of *Blighiasapida* Stem Bark in Alloxan-Induced Diabetic Rats

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Anti-hyperglycemic and *in vivo* Antioxidant Activities of Aqueous Extract of *Blighiasapida* Stem Bark in Alloxan-Induced Diabetic Rats

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Abstract- ***Blighiasapida*** **is a plant belonging to the family of Sapindaceae. This study is aimed at evaluating the hypoglycemic and possible *in vivo* antioxidant activities of the aqueous extract of the stem bark of the plant for 21 days in alloxan-induced diabetic rats. Administration of the extract at 100mg/kg body weight significantly (P<0.05) decreased blood glucose levels, increased body weight as well as increased the activities of antioxidant enzymes catalase, glutathione peroxidase and superoxide dismutase in the plasma and liver tissues of diabetic rats. Also, the concentration of reduced glutathione increased in the plasma and liver tissues of the diabetic rats while the levels of malondialdehyde and protein carbonyl significantly decreased in the plasma and liver tissues of alloxan-induced diabetic rats during the course of the experiment. These are indications of antihyperglycemic and antioxidant properties of the stem bark of *Blighiasapida* with 100mg/kg body weight of the extract showing good hypoglycemic and antioxidant activities by comparing favourably well with metformin, a standard hypoglycemic drug.**

Keywords: blighiasapida, diabetes, antioxidant enzymes, epidemic, biomolecules.

I. Introduction

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). Diabetes being a major degenerative disease is found in all parts of the world and it is becoming the third most lethal disease of mankind and rapidly increasing. It is affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders (Saidu *et al.*, 2012).

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. 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.

Too much glucose circulating in the blood results in hyperglycemia, one of the major symptoms of diabetes. Hyperglycemia causes many of the health problem associated with diabetes, including eye, kidney, heart disease and nerve conditions.

The World Health Organization (WHO) in its 2014 release repoeted 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 (Marfella *et al.*, 1995; Giugliano *et al.*, 1997; Paoliso and Giugliano, 1996).

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).

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 cells such as the nonenzymatic glycosylation reaction, the electron transport chain in mitochondria, and membrane-bound NADPH oxidase (Brownlee, 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 β-cell glucose toxicity which is often observed under diabetic conditions (Evans *et al*, 2003).It is also known that lipotoxicity is also involved in the deterioration of β-cell function found in type 2 diabetes (Kaneto *et al*, 2010).

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Blighiasapida 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). It is an evergreen tree of about 33 to 40ft (10-12m) with a dense crown of spreading branches. The leaves are compound with three to five pairs of oblong, ovate-oblong, or elliptical leaflets 1.5-3.0cm long. 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 *Blighiasapida* 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 *Blighiasapida* stem bark and its anti-hyperglycemic effect 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 prohibitively high cost, unavailability, uncertainty of use of the common anti-diabetic agents during pregnancy and undesirable side effects of these drugs have been some of the factors limiting their use and leading to a preference for anti-diabetic drugs of plants origin. This study is thus aimed at isolation of hypoglycemic agents from readily available *Blighiasapida*.

II. Materials and Methods

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.

Animals: Male albino rats (*Ratusnorvegicus*) 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 and were maintained on standard rat pellets (Ladokun feeds, Ibadan, Nigeria), and were given water *ad libitum*.

Sourcing for the Tree Bark of Blighiasapida: A sizeable quantity of the tree bark of *Blighiasapida* was obtained from the compound of the Federal Polytechnic, Ado Ekiti, Nigeria.

Identification of Plant: The fruits and leaves of *Blighiasapida* 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.

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 *Blighiasapida* was pulverized. 100g of the pulverized sample was extracted with 800ml of distilled water for seventy-two (72) hours in an extractor. The aqueous extract was obtained by filtering with What man filter paper and subsequently freeze-dried in Armfield freeze-drier for ten (10) days.

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) and animals which had basal glycemia levels of 125mg/dl were used in the experiment.

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

Group 3: The alloxan-induced diabetic group treated with oral administration of distilled water extract of *Blighiasapida* 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 stated was carried out weekly after diabetes detection, for three weeks.

Repeated administration of the aqueous extract of Blighiasapidastem 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). Body weight and blood glucose levels of the groups were monitored daily, blood sample was obtained from the tail vein of the animals and their fasting blood glucose level was determined in mg/dl using a digital glucometer (Accu-chek ® Advantage, Roche Diagnostic, Germany). Five animals each were sacrificed from each of the four groups by chloroform anaesthesia and the blood and liver obtained from them. The plasma was obtained from the blood by using centrifuge at 3000g for 15 minutes. The plasma and liver so obtained were stored in phosphate buffer (0.1M, pH = 7.0) maintained below -20^{0}C until required for analysis.

In vivoantioxidant assay: Liver tissues were homogenized with cold 1.5% KCl to make a 10% homogenate.

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

Determination of the activity of Superoxide dismutase (SOD): This method is well described by Mccord and Fridovich (1969).

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

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

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).

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

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

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 $P<0.05$.

III. Results

Blood Glucose Level: The administration of aqueous extract of *Blighiasapida* stem bark was found to significantly ($P<0.05$) reduce the blood glucose in diabetic albino rats at the end of the experiment (Table 1). The effect was more rapid in the first week of administration and compared favourably well with metformin-treated diabetic rats.

Weight gain or loss: There was a significant reduction ($P<0.05$) in the weight gained by the untreated diabetic rats when compared with the metformin-treated and extract-treated groups. Generally, the effect of treatment with 100mg/kg body weight of aqueous extract of *B. sapida* stem bark compared favourably well with that of metformin hydrochloride which is a known standard drug for diabetes.

Catalase activity: Specific activity of the antioxidant enzyme catalase was found to be increased ($P<0.05$) plasma and liver following administration of aqueous extract of *B. sapida* stem bark while the administration of metformin, a standard antidiabetic drug did not seem to have any ameliorative effect on the reduced specific activity of catalase in the plasma and liver of diabetic rats when compared with the untreated diabetic rats (Table 3).

Glutathione peroxidase (GPx) activities: A significant increase ($P<0.05$) was noticed in the specific activity of glutathione peroxide in the plasma of diabetic rat following administration of aqueous extract of *B. sapida* stem bark at the later stage of the experiment. On the other hand, the specific activity of glutathione peroxidase in the liver 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 4).

Table 1: Blood Glucose level of alloxan-induced diabetic rats following administration of Aqueous extract of *Blighiasapida* stem bark.

Groups	Serum Glucose Level (mg/dl)			
	0 day	7th day	14th day	21st day
Untreated control	91.50 ± 1.94^a	90.41 ± 2.50^a	90.21± 1.61^a	88.10 ± 2.02^a
Diabetic control	154.80 ± 14.00^b	172.41± 17.32^b	203.50 ±11.20^b	253.00 ± 13.20^b
Diabetic + Aqueous extract	154.80 ± 14.00^b	86.40±5.43^a	74.00±4.48^c	58.00 ± 6.04^c
Diabetic + Metformin	153.84 ±10.26^b	113.75 ±5.41^c	70.75 ± 6.50^c	57.00 ± 9.60^c

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

Table 2: Body weight of alloxan-induced diabetic albino rats following administration of Aqueous extract of *Blighiasapida* stem bark.

Groups	Average body weight of animals (g)			
	0 day	7th day	14th day	21st day
Untreated control	129.20 ±2.30[a]	133.70 ±1.09[a]	146.20± 1.12[a]	157.00± 1.16[a]
Diabetic control	132.01 ± 1.09[a]	125.20 ±2.01[b]	112.00 ±0.98[b]	98.20 ±2.02[b]
Diabetic + Aqueous extract	131.00 ±6.06[a]	149.00 ±7.12[c]	157. 00 ±6.57[c]	124.00 ±6.06[c]
Diabetic + Metformin	132.00 ± 2.96[a]	116.25 ± 5.41[b]	127.50 ± 6.50[d]	147.50 ±9.60[d]

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

Table 3: Specific activity of catalase in plasma and liver of alloxan-induced diabetic albino rats following administration of Aqueous extract of *Blighiasapida* stem bark

Tissue	Group of animal	Specific activity of catalase (Units/mg protein) (x10^{-2})			
		0 day	7th day	14th day	21st day
Plasma	Untreated control	5.86± 0.14[a]	5.91± 0.20[a]	5.86± 0.16[a]	5.82± 0.16[a]
	Diabetic control	3.86± 0.70[b]	3.52± 0.20[b]	2.14±0.10[b]	1.27± 0.80[b]
	Diabetic + Aqueous extract	3.86± 0.70[b]	4.25± 0.90[c]	7.81± 1.80[a]	15.66± 3.20[c]
	Diabetic + Metformin	5.30± 0.28[a,a]	6.10± 0.10[a]	2.10± 0.07[b]	1.90± 0.62[b]
Liver	Untreated control	178.48± 4.90[a]	179.32± 3.20[a]	178.52± 5.20[a]	176.05± 5.10[a]
	Diabetic control	92.26± 6.30[b]	92.52± 2. 10[b]	79.16± 5.20[b]	49.56± 6.20[b]
	Diabetic + Aqueous extract	97.26± 6.30[b]	195.02± 3.93[c]	289.36± 8.30[c]	190.85± 3.20[a]
	Diabetic + Metformin	97.30± 0.28[b]	84.30± 0.28[b]	87.00± 0.31[b]	65.80± 0.63[c]

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

Table 4: Specific activity of Glutathione peroxidase (GPx) in plasma and liver of alloxan-induced diabetic albino rats following administration of Aqueous extract of *Blighiasapida* stem bark

Tissue	Group of animal	Specific activity of Glutathione peroxidase (Units/mg protein) (XI0·^{5})			
		0 day	7th day	14th day	21st day
Plasma	Untreated control	13.68± 0.48[a]	13.54± 0.39[a]	13.67± 0.46[a]	13.83± 0.49[a]
	Diabetic control	13.61± 0.18[a]	11.3S± 0.15[a]	10.23± 0.18[b]	8.26± 0.16[b]
	Diabetic + Aqueous extract	13.61± 0.18[a]	7.96± 0.61[b]	6.10± 0.08[c]	8.85± 0.85[b]
	Diabetic + Metformin	I3.50± 0.6I[a]	4.94± 0.53[c]	4.79± 0.59[c]	6.10± 0.4I[c]
Liver	Untreated control	47.00± 1.02[a]	47.00±1.I2[a]	48.00± 1. 02[a]	46.00± 1.01[a]
	Diabetic control	30.40± 0.72[b]	25.10± 0.69[b]	I7.30± 0.59[b]	10.20± 0.30[b]
	Diabetic + Aqueous extract	30.40± 0.72[b]	44.I0± 0.99[a]	32.70± 0.40[c]	18.00± 0.31[c]
	Diabetic + Metformin	3I.60± 0.53[b]	49.00± 0.53[a]	4780± 0.40[a]	60.00± 0.35[a]

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

Superoxide dismutase (SOD) activity: A significant increase ($P<0.05$) in the specific activity of superoxide dismutase was observed in the plasma and liver diabetic rats administered with aqueous extract of *B. sapida* stem bark similar to what was observed in those treated with metformin, a standard antidiabetic drug. However, the specific activity of superoxide dismutase in the plasma and liver of untreated diabetic rats was found to reduce significantly ($P<0.05$) during the course of the experiment (Table 5).

Reduced glutathione: Table 6 shows the effect of administration of aqueous extract of *B. sapida* stem bark on concentration of reduced glutathione (GSH) in plasma and liver of diabetic rats. A significant increase ($P<0.05$) in the oxidant was noticed in the plasma and

liver of diabetic rats following the administration of aqueous extract of *B. sapida* stem bark.

Malondialdehyde: A significant reduction (P<0.05) in the level of malondialdehyde (MDA) was observed in the plasma and liver of diabetic rats following the administration of aqueous extract of *B. sapida* stem bark (Table 7). On the other hand changes in the concentration of malondialdehyde in plasma and liver tissues of diabetic rats did not follow a particular pattern following the treatment of diabetic rats with metformin hydrochloride, a standard antidiabetic drug.

Protein carbonyl: A significant reduction (P<0.05) was noticed towards the end of the experiment after an initial increase in the concentration of protein carbonyl in the plasma and liver tissues of diabetic rats following the administration of aqueous extracts of *B. sapida* stem bark (Table 8). A similar result was obtained for the diabetic rats treated with standard antidiabetic drug, metformin.

Table 5: Specific activity of superoxide dismutase (SOD) in plasma and liver of alloxan-induced diabetic albino rats following administration of Aqueous extract of *Blighiasapida*stem bark

Tissue	Group of animal	Specific activity of superoxide dismutase (SOD (Units/mg protein) ($x10^{-3}$)			
		0 day	7thday	14th day	21stday
Plasma	Untreated control	10.88± 1.23[a]	19.53± 1.06[a]	22.11± 1.00[a]	19.93± 1.23[a]
	Diabetic control	7.91± 1.40[b]	5.25± 1.30[b]	3.28± 1.0l[b]	1.96± 0.91[b]
	Diabetic + Aqueous extract	7.91± 1.40[b]	27.12± 4.05[c]	24.03± 3.56[a]	28.64± 7: 12[c]
	Diabetic + Metformin	8.10± 1.10[b]	12.90± 0.70[d]	17.20± 0.30[c]	28.70± 0.30[c],
Liver	Untreated control	55.87± 9.65[a]	55.87± 9.65[a]	66.01± 7.51[a]	51. 78± 1.20[a]
	Diabetic control	35.40± 5.98[b]	19.87± 4.43[b]	11.35± 3.25[b]	9.36± 1.54[b]
	Diabetic + Aqueous extract	35.40± 5.98[b]	150.52± 1.93[c]	161.80± 2.27[c]	1153.95± 12.11[c]
	Diabetic + Metformin	58.70± 1.10[b]	12.90± 0.70[b]	17.20± 0.30[d]	28.70± 0.30[d].

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

Table 6: Concentration of reduced glutathione (GSH) in plasma and liver of alloxan-induced diabetic albino rats following administration of Aqueous extract of *Blighiasapida* stem bark.

Tissue	Group of animal	Concentration of Glutathione (GSH) (mM/mg tissue)			
		0 day	7th day	14th day	21st day
Plasma	Untreated control	3.63± 0.56[a]	3.45± 0.32[a]	3.63± 0.56[a]	3.65± 0.56[a]
	Diabetic control	3.21± 0.43[a]	2.51± 0.55[b]	2.11± 0.30[b]	1.82± 0.25[b]
	Diabetic + Aqueous extract	3.21± 0.43[a]	2.83± 0.34[b]	2.63± 0.41[c]	3.15± 0.25[c]
	Diabetic + Metformin	3.64± 0.15[a]	4.71± 0.l5[c]	l.86± 0.18[b]	4.27± 0.99[a]
Liver	Untreated control	1.21± 0.19[a]	1.21± 0,.19[a]	1.56± 0.15[a]	1.35± 0.20[a]
	Diabetic control	2.54± 0.53[b]	1.83± 0.32[b]	1.24± 0.22[b]	1.03± 0.16[b]
	Diabetic + Aqueous extract	2.54± 0.53[b]	1.24± 01.14[a]	1.46± 0.10[b]	4.06± 0.27[c]
	Diabetic + Metformin	2.35± 0.03[b]	1.18± 0.03[a]	1.92± 0.18[c]	1.93± 0.07[a]

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

Table 7: Concentration of malondialdehyde (MDA) in plasma and liver of alloxan-induced diabetic albino rats following administration of Aqueous extract of *Blighiasapida stem* bark

		Concentration of malondialdehyde (MDA) (mmol/mg tissue) (xl0·^{5})			
Tissue	Group of animal	0 day	7thday	14thday	21stday
Plasma	Untreated control	1316.99± 0.16^{a}	1428.60± 0.13^{a}	1316.90± 0.16^{a}	1439.80± 0.12^{a}
	Diabetic control	1619.69± 0.08^{a}	2002.10± 0.07^{b}	4698.70± 0.09^{b}	7023.60± 0.06^{b}
	Diabetic + Aqueous extract	1619.69± 0.08^{a}	1372.55± 0.05^{a}	1065.36± 0.06^{a}	1125.82± 0.04^{a}
	Diabetic + Metformin	280.00±0.11^{b}	420.00± 0.11^{c}	380.00± 0.51^{d}	500.00± 0.17^{c}
Liver	Untreated control	1286.70± 0.01^{a}	l286.70± 0.01^{a}	1096.40± 0.01^{a}	1193.00± 0.01^{a}
	Diabetic control	1513.10± 0.08^{b}	1735.20± 0.08^{b}	2012.30± 0.08^{b}	2523.20± 0.06^{b}
	Diabetic + Aqueous extract	1513. 10± 0.08^{b}	1388.89± 0.06^{a}	1197.71± 0.06^{a}	1040.S5± 0.05^{a}
	Diabetic + Metformin	1720.00± 0.08^{b}	2000.00± 0.07^{b}	1900.00± 0.08^{b}	3200.00± 0.07^{d}

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

Table 8: Concentration of protein carbonyl in plasma and liver of alloxan-induced diabetic albino rats administration of Aqueous extract of *Blighiasapida* stem bark

		Concentration of protein carbonyl (micromol carbonyl/mg tissue)			
Tissue	Group of animal	0 day	7th day	14th day	21st day
Plasma	Untreated control	0.56± 1.05E-05^{a}	0.52± 1.12 E-05^{a}	0.59± 1.05E-05^{a}	0.60± 1.03E-05^{a}
	Diabetic control	0.46± 6.66E-07^{b}	0.68± 8.63 E.07^{b}	1.26± 7.65E-07^{b}	1.80± 8.20E-07^{b}
	Diabetic + Aqueous extract	0.46± 6.66E-07^{b}	1. 09± 1. 11 E-06^{c}	0.98± 1.15E-06^{c}	0.32± 9.09E-07^{c}
	Diabetic + Metformin	0.53± 1.36E-05^{a}	0.55± 1.36 E-05^{a}	0.74± 1.81E-05^{a}	0.53± 1.39E-05^{a}
Liver	Untreated control	2.53± 1.08E-05^{a}	2.53± 1.09 E-05^{a}	2.47± 1.11E-05^{a}	2.51± 1.08E-05^{a}
	Diabetic control	0.78± 7.80E-07^{b}	1.86± 8.22 E-07^{b}	2.60± 6.11E-07^{b}	3.82± 8.52E-07^{b}
	Diabetic + Aqueous extract	0.78± 7.80E-07^{b}	2.08± 1.05E-06^{c}	0.94± 1.05E-06^{c}	0.66± 7.42E-07^{c}
	Diabetic + Metformin	1.27± 6.22E-06^{c}	2.06± 6.22E-06^{c}	1.97± 2.71E-05^{a}	2.08± 5.I2E-06^{d}

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

IV. Discussion

The increase in blood glucose concentration is an important characteristic feature of diabetes. *Blighiasapida* extract produced significant hypoglycemic effect on diabetic rats, and by day 14, the glucose levels tended towards normalcy as found in the control rats. Phytochemical screening of the aqueous extract of the root bark of *B. sapida* had indicated the presence of saponins (Saidu*et al.*, 2012), which have been reported to possess hypoglycemic activity in diabetic rabbits (Abdel-Hassan *et al.*, 2000).

The marked increase in the body weight in the *B. sapida* stem bark extract-treated rats could be attributed to the increase in the metabolic activity of their body systems. This clearly indicates that the plant extract increase glucose metabolism which enhanced body weight gain in rats. This observation was reported by Sunmonu and Afolayan (2013). According to these authors, *Artemisia afra* leaves and stem increased the body weight of diabetic rats. It is interesting to note that the effect of *B. sapida* stem bark aqueous extract at the dose of 100mg/kg body weight compared favorably well with metformin.

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 and Thorpe, 1999). There are several sources of ROS in cells

such as the nonenzymatic glycosylation reaction, the electron transport chain in mitochondria, and membrane-bound NADPH oxidase (Brownlee, 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 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. β – cells are rather vulnerable to ROS due to the relatively low expression of antioxidant enzymes such as catalase, glutathione peroxide 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 general, antioxidants such as phenolic compounds (tocopherols, flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), carotenoids and ascorbic acid (Hall and Cuppett, 1997; Larson, 1988) compounds inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reaction. In this study, the levels of catalase, glutathione peroxidase and superoxide dismutase activities in plasma and liver tissues of diabetic group were significantly reduced and treatment with *B. sapida* stem bark aqueous extract generated the catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities not only on acute experiments but after 21 days of treatment. Decreased levels of CAT, GPx and SOD in the diabetic state may be due to 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 by 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 thus be due to it been spared as a result of the protection offered by superoxide dismutase to glutathione peroxidase.

It is known that lipotoxicity is also involved in the deterioration of β – cell function found in diabetes. The increase in free radicals in diabetic condition is suggested to be due to the increased lipid peroxidation and the damage to antioxidant defense system. Protein glycation and glucose autoxidation can generate free radicals that catalyze the lipid peroxidation (Altan*et al*., 2006). Any compound, natural or synthetic, with antioxidant activity might totally or partially alleviate this damage. In this study, direct effects of aqueous extract of *B. sapida* stem bark on malondialdehyde (MDA) levels in diabetes group were found to be higher than those in control group ($P<0.05$), indicating free radical generation via lipid peroxidation. Treatment of diabetes with the aqueous extract of *B. sapida* stem bark caused an eventual reduction in the MDA levels in plasma and liver after 21 days of treatment. Furthermore, direct effects of aqueous extract of *B. sapida*extract on protein carbonyl levels in diabetes 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 *B. sapida* stem bark caused a reduction in the levels of protein carbonyl in plasma and liver after 21 days of administration.

V. Conclusion

One of the major findings of this study is that oral administration of aqueous extract of *B. sapida* stem bark caused anti-hyperglycemic activity in alloxan-induced diabetes in experimental albino rats. The results also revealed that *B. sapida* stem bark aqueous extract caused a significant increase in the activities of catalase, glutathione peroxidase and superoxide dismutase in the plasma and liver of diabetic rats after 21 days of treatment. It is also observed that aqueous extract of *B. sapida* stem bark extract possess the capability of inhibiting both lipid and protein peroxidation in diabetes.

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