Glucose utilization and anti-oxidative mechanisms of the aqueous *hunteria umbellata* seed extract in alloxan-induced diabetic rats

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Summary: In South-west Nigeria, water decoctions of *Hunteria umbellata* seeds are highly valued by traditional healers in the local management of diabetes mellitus, obesity and hyperlipidemia. Previous studies hypothesized one of the antihyperglycemic mechanisms of the aqueous seed extract of *Hunteria umbellata* (*HU*) to be mediated probably via increased peripheral glucose utilization. The present study, therefore, was designed at evaluating the peripheral glucose utilization and anti-oxidative mechanisms of 50 mg/kg, 100 mg/kg and 200 mg/kg of *HU* in alloxan-induced diabetic rats in Groups IV-VI rats as well as in the control groups (Groups I-III). Experimental type 1 DM was induced in male Wistar rats through intraperitoneal injection of 150 mg/kg of alloxan monohydrate in cold 0.9% normal saline after which the diabetic rats were orally treated with 50-200 mg/kg of *HU* for 14 days. Effects of *HU* on the rat body weight, percentage body weight changes and fasting blood glucose (FBG) were determined on days 1 and 15 of the experiment. Also, on day 15 of the experiment, *HU* effect on serum insulin, liver enzyme markers, proteins, albumin, triglyceride, total cholesterol and lactate dehydrogenase as well as on hepatic tissue oxidative stress markers, liver glycogen and glucose-6-phosphatase were determined after sacrificing the rats under diethyl ether anesthesia. Results showed that oral treatments with 50-200 mg/kg of *HU* caused significant ($p<0.0001$) improvements in the weight loss caused by alloxan-induced diabetes, while causing significant ($p<0.05$, $p<0.001$ and $p<0.0001$) dose-related reductions in the FBG levels despite causing non-significant ($p>0.05$) alterations in the serum INS levels in the treated rats. Also, repeated oral treatment with *HU* caused significant ($p<0.0001$) reversal in the decrease and increase in the hepatic glycogen levels and glucose-6-phosphatase activity, respectively, caused by alloxan-induced diabetes. Similar significant ($p<0.0001$) and complete reversal effects were recorded in the serum hepatic enzyme markers, total protein, albumin, triglyceride, total cholesterol and lactate dehydrogenase as well as on hepatic tissue oxidative stress markers such as superoxidase dismutase (SOD), catalase (CAT), malonialdehyde (MDA) and reduced glutathione (GSH) of *HU*-treated rats when compared to that of untreated alloxan-induced diabetic rats. In conclusion, results of this study showed *HU* treatment to significantly ameliorate the hyperglycemia and oxidative stress in alloxan-induced diabetic rats which was mediated via increased hepatic glycogen deposit, decreased hepatic glucose-6-phosphatase activity and improvement in antioxidant/free radicals scavenging activities.

Keywords*: Hunteria umbellata*, Alloxan-induced diabetes, Fasting blood glucose, Liver glycogen, Glucose-6-phosphatase, Oxidative stress markers

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INTRODUCTION

Diabetes mellitus (DM) is a state of carbohydrate, protein and lipid metabolic disequilibrium characterized by sustained hyperglycemia and other metabolic derangements, and resulting from pancreatic insulin insufficiency and/or due to defects in tissue insulin receptors (Frier and Fisher, 2010). Recent World Health Organization (WHO) data suggests that the current world's population affected by DM stands at 171 million and this figure is estimated to reach 366 million by the year 2030 (Wild *et al.*, 2004) with the estimated global cost of $1 trillion incurred yearly for treating it and its associated complications (Rahul *et al.*, 2006). Basically, DM is classified into two major common types, namely: insulin-dependent (type 1) DM and non-insulin dependent (type 2) DM (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2002). Other forms of DM include pregnancy-related (gestational) DM, maturity-onset diabetes of the young (MODY), surgical diabetes,

drug-induced DM, *etc*. (Frier and Fisher, 2010). In all of these DM types, chronic hyperglycemia and oxidative stress have been identified as common features in both their etiopathology and pathophysiology (Robertson, 2004; Kaneto *et al.*, 2007).

Oxidative stress has been implicated in the etiology of DM since reactive oxygen species (ROS) radicals generated have been established to play a pivotal role in the development of DM complications such as nephropathy, retinopathy, vasculopathy, neuropathy and cardiovascular disease (Kaneto *et al.*, 2007). Oxidative stress promotes the onset and development of DM either by directly decreasing insulin sensitivity and/or causing direct cytotoxicity to the pancreatic insulin-producing β-cells (Maiese *et al.*, 2007). Documented ROS-induced cytotoxic mechanisms include lipid peroxidation as well as disruption of mitochondrial respiratory machinery (mitochondrial electron transport) which is regulated by NADPH ubiquinone oxidoreductase and ubiquinone-cytochrome *c* reductase systems (Maiese *et al.*, 2007). Thus, disruptions of mitochondrial *oxidoreductase* and *citrate synthase* activities result in significant reductions in mitochondrial oxidative and phosphorylation activities as well as reduction of the levels of mitochondrial proteins and mitochondrial DNA in adipocytes, particularly in type 2 DM (Petersen *et al.*, 2003). Oxidative stress has also been shown to trigger the opening of the mitochondrial membrane permeability transition pore which results in a significant depletion of mitochondrial NAD^+ stores and subsequently apoptotic cell injury (Maiese *et al.*, 2007). In the pancreatic tissues, these cellular events result in depletion of the β-cells population and insulin deficiency while in the skeletal muscle, it manifests as insulin resistance (Robertson *et al.*, 2003).

Hunteria umbellata (K. Schum.) Hallier f., belonging to Apocynaceae family, is a tropical rainforest tree which is locally known as "Abeere" among the Yorubas and the Binis tribes in Nigeria while in French it is known as "Demouain" (Adeneye and Adeyemi, 2009a). Ethnomedical uses of the plants include treatment of yaws and sexually transmitted infections, stomach ache and ulcers, diabetes mellitus and dysmenorrhoea (Falodun *et al.*, 2006). Previous studies have reported the antihyperglycemic (Adeneye and Adeyemi, 2009a; Adeneye and Adeyemi, 2009b; Igbe *et al.*, 2009) anti-obesity and antihyperlipidemic (Adeneye *et al.*, 2010) effects of the crude aqueous seed extract of *Hunteria umbellata* (*HU*) in rats. Recent studies have reported the isolation of a new bisindole alkaloid, erinidine, from the crude alkaloid extract of *HU* (Adeneye *et al.*, 2012) which mediated an *in vivo* antihyperglycemic activity via intestinal glucose uptake inhibition (Adeneye *et al.*, 2013). Also, we have reported the *in vivo* anti-inflammatory and in *vitro* anti-oxidant activities of *HU* and its fractions (Adeneye *et al.*, 2011). In a previous study, HU was hypothesized to regulate glucose homeostasis via enhanced peripheral glucose utilization in experimental models of DM (Adeneye and Adeyemi, 2009a; Adeneye and Adeyemi, 2009b). Unfortunately, till date there has not been any study further investigating the exact mechanism by which *HU* regulates glucose through enhanced peripheral glucose metabolism and its possible anti-oxidative role in *in vivo* hyperglycemic models. In view of the above, the present study is designed at investigating the exact peripheral glucose utilization mechanism and the possible role of *HU* in attenuating the oxidative stress in alloxan-induced hyperglycemic rats (being a prototype of type 1 DM animal model). In doing this, 50-200 mg/kg/day of *HU* were administered to alloxan-induced diabetic rats for 14 days after which its antidiabetic and anti-oxidative profile and mechanisms were investigated in the treated diabetic rats.

MATERIALS AND METHODS

Plant Materials
Fresh matured fruits of *Hunteria umbellata* were collected from the deciduous forests of Imoroko Village, Atan-Ijebu in Ijebu East Local Government Area of Ogun State, in the month of December, 2012 and plant authentication was done as previously reported by Adeneye and Adeyemi (2009a). Fresh seeds from the fruits were separated and rinsed in tap water after which it was continuously and completely dried in an aerated oven preset at the temperature of 35 ℃ and protected from direct sunlight for 4 week.

Preparation of the cold aqueous seed extract of Hunteria umbellata
Aqueous extract of *Hunteria umbellata* seed was prepared by soaking 50 g of powdered seed in 500 ml of distilled water and kept in the refrigerator for 72 hours. After 72 hours, the solution was continuously stirred using a magnetic stirrer for 2 hours after which the solution was filtered using a piece of clean white cotton cloth. The filtrate was then completely dried-off using an aerated oven preset at 40 ℃ until a solid residue of constant weight was obtained.

Experimental Animals
Young adult male Wistar rats weighing between 110-130 g were procured from Bayo Animal Farm, Sango-Otta, Ogun State, Nigeria, after institutional ethical approval obtained. The rats were acclimatized and maintained on standard rat chow, potable drinking water and standard laboratory conditions (temperature: 28-30 ℃ and humidity: 55-65%,) under natural 12 hour daylight/ night periodicity for 14 days before being used for the experiment. All rats were processed under same sham-handling using standard principles guiding the care and use of laboratory

animals as contained in the NIH publication No. 85-23 (1985).

Induction of experimental diabetes mellitus
Experimental type 1 diabetes was induced in rats using the method described by Venugopal *et al.* (1998) and as modified by Iwalewa *et al.* (2008). Rats were injected with freshly prepared 150 mg/kg body weight of alloxan monohydrates dissolved in sterile cold normal saline, given via the intraperitoneal route. The rats were then kept for the next 24 hours on 5% glucose solution bottles in their cages to prevent hypoglycemia which is often associated from alloxan-related hyperinsulinemia (Gupta *et al.*, 1984). Fasting blood glucose levels in rats were measured on the 3rd and 5th day post-alloxan injection and treated rats with fasting blood glucose levels equal to or above 250 mg/dl were considered diabetic and used for study.

Body weight measurement
Body weights of all rats were measured on the 1st and 15th day of the experiment, respectively, using digital mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland). The weight difference on the 1st and 15th day in reference to the initial weight per group was calculated.

Experimental design and oral treatment of alloxan-induced hyperglycemic rats
Oral treatments of alloxan-induced hyperglycemic rats for 14 days are as follows:
Group I: normal control rats received 10 ml/kg and 1 ml/kg of distilled water via the oral and intraperitoneal routes, respectively, intraperitoneal alloxan, having being injected to other groups of rats
Group II: Alloxan-induced diabetic rats received 10 ml/kg of distilled water
Group III: Alloxan-induced diabetic rats orally received 5 mg/kg of glibenclamide in distilled water
Group IV: Alloxan-hyperglycemic rats orally treated with 50 mg/kg of *HU* in distilled water
Group V: Alloxan-hyperglycemic rats orally treated with 100 mg/kg of *HU* in distilled water
Group VI: Alloxan-hyperglycemic rats orally treated with 200 mg/kg of *HU* in distilled water.

Blood collection
On day 15, after an overnight fast, the final fasting blood glucose was determined before the treated rats were sacrificed after light diethyl anesthesia. After anesthesia, blood samples were collected directly from the heart chamber into 10 ml plain bottles. The blood samples obtained were immediately frozen at -70 ℃ and centrifuged at 3000 rpm for 20 min to separate out the serum that was then analyzed for the biochemical assays such as serum insulin, liver enzyme markers, proteins, albumin, triglyceride, total cholesterol and lactate dehydrogenase.

Blood glucose measurement
Whole blood fasting blood glucose (FBG) of treated rats was collected by tail tipping method and determined by the glucose oxidase method of Trinder (1969) using a One Touch Basic Blood Glucose Monitoring System® (Life Scan Inc., Milpitas, California, U.S.A.). The blood glucose monitor was calibrated and validated at the beginning of, midway into and at the end of the experiment.

Measurement of serum insulin levels
Serum insulin was assayed by the modified method of Herbert *et al.* (1965) using insulin radioimmunoassay kits (BARC, Mumbai, India). Dextran coated charcoal in 0.2 M glycine buffer was used to separate bound and free insulin.

Measurement of serum liver enzyme markers, proteins, albumin, triglyceride, total cholesterol and lactate dehydrogenase
Serum activities of aspartate transaminase (AST) and alanine transaminase (ALT) were assayed by the method of Moss and Henderson (1999). Serum total protein (TP) and albumin (ALB) levels were estimated using the methods of Kingsley and Frankel (1939), and Doumas *et al.* (1971) while that of TG and TC were assayed using standard diagnostic test kits (Randox Laboratories, Crumlin, U.K.) on Automated Clinical System (Sychron Clinical System®, model: CX5 PRO) (Beckman Coulter Inc., Galway, Ireland). Serum lactic dehydrogenase activity (LDH) was measured by the method of Wroblewski and LaDue (1955).

Collection of liver tissues and determination of liver tissue SOD, CAT, MDA and GSH
Immediately the blood samples were collected, the liver was identified and removed and briskly rinsed in ice-cold 1.14% KCl solution in order to preserve activities of the oxidative stress markers before it was homogenized in 0.1 M tris-HCl buffer of pH 7.4 to give a 10% homogenate. This homogenate was used for the appropriate oxidative stress markers estimation. Superoxide dismutase (SOD) activity in the liver tissue was determined by the method of Kakkar *et al.* (1984) while that of liver MDA, catalase (CAT) and reduced glutathione (GSH) were determined by the methods of Kumar *et al.* (2010), Sinha (Sinha, 1972) and Kaur *et al.* (2006), respectively.

Determination of liver glycogen and glucose utilization
Liver glycogen content was measured by methods of Carroll *et al.* (1956) and Chattopadhyay *et al.* (1992), while the hepatic glucose-6-phosphatase concentration (being the rate limiting enzyme for glucose release from glycogen storage into the blood) was quantified by the method of Baginsky *et al.* (1992).

Statistical analysis

The values for the average body weights and percentage were expressed as mean ± S.D. while the biochemical values were expressed as mean ± standard error of mean (SEM) of six rats for each treatment group. Data were analyzed using one-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test on GraphPad Prism (version 5.00, 2007; La Jolla, California, U.S.A.) statistical software. Significant values were considered at $p < 0.05$, $p < 0.001$ and $p < 0.0001$.

RESULTS

Aqueous extraction of pulverized *Hunteria umbellata* seed

Extraction of pulverized *Hunteria umbellata* seed with distilled water yielded a deep brown, sweet-smelling solid residue weighing 7 g and giving a yield of 14%.

Effect of 50-200 mg/kg of HU on body weight in alloxan-induced diabetic rats

Table 1 shows the effect of repeated daily oral treatment with 50-200 mg/kg of *HU* on the average body weight and %weight change of treated alloxanized rats. There was a significant (p<0.0001) weight loss in the untreated alloxanized diabetic (Group II) rats during the treatment period when compared to that of untreated normal (Group I) rats (Table 1). However, oral treatments of the alloxan-induced diabetic rats with 50, 100 and 200 mg/kg of *HU* significantly (p<0.0001) improved body weight in Groups IV-V rats relative to that of untreated alloxanized diabetic (Group II) rats although these improvements were significantly (p<0.05) lower than that caused by glibenclamide treatment (Table 1).

Effect of 50-200 mg/kg of HU on FBG, %ΔFBG and serum INS in alloxan-induced diabetic rats

Effects of repeated single daily dosing of alloxan-induced diabetic rats on the 1st and 15th day FBG and serum INS concentrations with 50-200 mg/kg of *HU* are shown in Table 2. Treatment with alloxan resulted in sustained significant (p<0.0001) reductions in the circulating INS with concomitant significant (p<0.0001) rise in the FBG levels in Groups II rats when compared to untreated normal (Group I) rats (Table 2). Treatment with 50-200 mg/kg of *HU* for 14 days, however, did not significantly (p>0.05) alter the serum INS levels despite significant (p<0.05, p<0.001 and p<0.0001) dose-related reductions in the FBG levels induced by *HU* treatment relative to the values obtained for untreated alloxan-induced diabetic (Group II) rats (Table 2).

Effect of 50-200 mg/kg of HU on liver glycogen and glucose-6-phosphatase levels in alloxan-induced diabetic rats

Alloxan treatment was observed to have caused significant (p<0.0001) reductions in the hepatic glycogen while causing significant elevation in glucose-6-phosphatase activity when compared to untreated normal (Group I) rats (Table 3). With repeated oral treatment with *HU*, there were significant (p<0.05, p<0.001 and p<0.0001) dose-related increases in the hepatic glycogen and concomitant reduction in glucose-6-phosphatase activity with the highest levels recorded in rats treated with 200 mg/kg/day of *HU* and with the values for both relatively comparable to that of glibenclamide (Table 3).

Table 1. Effect of 14 days of oral treatment with 50-200 mg/kg *HU* on average body weights and percentage weight changes (%ΔWt) in alloxan-induced diabetic rats

Group	Day 1 wt. (g)	Day 15 wt. (g)	%ΔWt
I	135.20 ± 1.60	147.20 ± 2.27	8.91 ± 1.57
II	138.20 ± 1.70	112.00 ± 4.41	-19.06 ± 2.28[f]
III	137.50 ± 2.49	157.80 ± 2.69	14.86 ± 1.72[c]
IV	137.70 ± 2.06	144.80 ± 2.66	5.38 ± 0.59[a]
V	137.30 ± 1.80	144.70 ± 2.22	5.36 ± 1.16[a]
VI	136.80±2.61	141.00 ± 3.22	3.02 ± 0.61[a]

[f] $p < 0.0001$ Vs Group I, [a] $p < 0.05$ vs Group II, [c] $p < 0.0001$ vs Group II values. Group I: normal, Group II: Diabetic untreated, Group III: Diabetic+glibenclamide, Group IV: Diabetic+50 mg/kg *HU*, Group V: Diabetic+100 mg/kg *HU*, Group VI: Diabetic+200 mg/kg *HU*

Table 2. Effect of 14 days of oral treatment with 50-200 mg/kg of *HU* on the 1st and 15th day FBG, %FBG changes (%ΔFBG) and serum insulin in alloxan-induced diabetic rats

Group	Day 1 FBG (g)	Day 15 FBG (g)	%ΔFBG	serum insulin (ng/dl)
I	56.33±1.26	57.17±2.69	1.36 ± 3.60	0.96 ± 0.04
II	255.20±1.89[c+]	302.50±5.17[c]	18.52 ± 1.30[c]	0.35 ± 0.03[f]
III	255.50±1.34[c+]	149.20±4.	-41.64± 1.49[f]	0.42 ± 0.04[f]
IV	255.20±2.18[c+]	207.00± 3.33[e]	-18.89± 0.79[e]	0.42 ± 0.02[f]
V	257.30 ± 2.49[c+]	191.70± 1.59[e]	-25.03± 0.60[e]	0.38 ± 0.03[f]
VI	256.20 ± 3.21[c+]	159.00± 7.24[f]	-38.05± 2.06[f]	0.34 ± 0.03[f]

[c+] $p < 0.0001$ Vs Group I, [f] $p < 0.0001$ Vs Group I, [e] $p < 0.001$ Vs Group II, and [f] $p < 0.0001$ Vs Group II. Group I: normal, Group II: Diabetic untreated, Group III: Diabetic+glibenclamide, Group IV: Diabetic+50 mg/kg *HU*, Group V: Diabetic+100 mg/kg *HU*, Group VI: Diabetic+200 mg/kg *HU*

Table 3. Effect of 50-200 mg/kg of *HU* on liver glycogen and glucose-6-phosphatase levels in alloxan-induced diabetic rats

Group	liver glycogen (mg/g)	glucose-6-Phosphatase (U/mg of protein)
I	5.78 ± 0.14	3.03 ± 0.25
II	3.34 ± 0.18[c]	4.53 ± 0.08[c+]
III	6.73 ± 0.17[c]	2.48 ± 0.06[e]
IV	4.07 ± 0.15[a]	2.89 ± 0.08[d]
V	4.80 ± 0.22[b]	2.26 ± 0.08[e]
VI	7.15 ± 0.19[c]	2.04 ± 0.10[f]

[c+] and [c-] represent significant increases and decreases at $p < 0.0001$ Vs Group I. [d] $p < 0.05$, [e] $p < 0.001$, and [f] $p < 0.0001$ vs Group II. Group I: normal, Group II: Diabetic untreated, Group III: Diabetic+glibenclamide, Group IV: Diabetic+50 mg/kg *HU* , Group V: Diabetic+100 mg/kg *HU* ,Group VI: Diabetic+200 mg/kg *HU*

Table 4. Effect of 50-200 mg/kg of *HU* treatment on serum TP, ALB, TG and TC in alloxan-induced diabetic rats

Group	TP (mg/dl)	ALB(mg/dl)	TG (mg/dl)	TC (mg/dl)
I	6.08 ± 0.15	3.70± 1.45	141.00±7.17	116.50±3.92
II	2.38±0.10[c-]	1.43±0.07[c-]	272.30±2.70[c+]	253.00±3.76[c-]
III	5.03±1.80[c]	3.00±0.14[c]	223.50±5.07[e]	208.70±3.82[e]
IV	3.08±0.09[a]	1.82±0.06[a]	233.20±7.10[d]	221.70±6.33[d]
V	3.86±0.12[b]	2.60±0.10[b]	206.30±3.07[e]	186.70±4.18[e]
VI	5.00±0.09[c]	3.10±0.06[c]	174.50± 4.19[f]	156.50± 2.84[f]

[c-] and [c+] represent significant decrease and increase at $p<0.0001$, respectively, relative to untreated normal (Group I) rats. [a,] [b] and [c] represent significant increases at $p<0.05$, $p<0.001$ and $p<0.0001$, respectively, while [d, e] and [f] represent significant decrease at $p<0.05$, $p<0.001$ and $p<0.0001$, respectively, relative to untreated diabetic (Group II) values Group I: normal, Group II: Diabetic untreated, Group III: Diabetic+glibenclamide, Group IV: Diabetic+50 mg/kg *HU* , Group V: Diabetic+100 mg/kg *HU* , Group VI: Diabetic+200 mg/kg *HU*

Table 5. Effect of 50-200 mg/kg of *HU* treatment on the serum levels of AST, ALT, ALP and LDH in alloxan-induced diabetic rats

Group	AST(U/mg protein)	ALT(U/mg protein)	ALP(U/mg protein)	LDH(U/mg protein)
I	37.00 ± 1.75	58.50 ± 1.23	37.67 ± 4.35	288.30 ± 3.18
II	156.70 ± 4.65[c+]	142.00 ± 5.87[c+]	149.70 ± 5.57[c+]	560.20± 7.44[c]
III	67.00 ± 7.53[e]	53.00 ± 5.02[e]	57.00 ± 5.15[e]	296.60 ± 6.83[f]
IV	90.83 ± 2.59[e]	57.33 ± 2.46[e]	79.17 ± 2.71[d]	351.50 ± 2.95[d]
V	83.17 ± 2.75[e]	53.33 ± 2.97[e]	66.50 ± 4.40[e]	336.90±10.30[e]
VI	55.14 ± 2.66[f]	33.50 ± 1.93[f]	41.40 ± 2.79[f]	255.80 ± 7.60[f]

[c+] represents a significant increase at $p<0.0001$ relative to untreated normal (Group I) rats while [d, e] and [f] represent significant decreases at $p<0.05$, $p<0.001$ and $p<0.0001$, respectively, relative to untreated diabetic (Group II) values Group I: normal, Group II: Diabetic untreated, Group III: Diabetic+glibenclamide, Group IV: Diabetic+50 mg/kg *HU* , Group V: Diabetic+100 mg/kg *HU* ,Group VI: Diabetic+200 mg/kg *HU*

Table 6. Effect of 50-200 mg/kg of *HU* treatment on hepatic tissue SOD, CAT, GSH and MDA in alloxan-induced diabetic rats

Group	SOD(U/mg protein)	CAT(U/mg protein)	GSH(U/mg protein)	MDA(nM/mg protein)
I	14.57 ± 1.10	7.68 ± 0.34	8.83 ± 0.47	0.67 ± 0.05
II	04.55 ± 0.36[c]	03.63 ± 0.41[c-]	01.55 ± 0.24[c-]	02.13 ± 0.13[c+]
III	24.70 ± 1.02[c]	09.02 ± 1.17[c]	12.73 ± 0.63[c]	0.61 ± 0.10[e]
IV	07.12 ± 0.38[a]	05.30 ± 0.18[a]	02.45 ± 0.15	02.10 ± 0.23
V	13.53 ± 0.89[b]	07.48 ± 0.39[b]	07.73 ± 0.61[a]	00.59 ± 0.04[e]
VI	20.50 ± 0.78[c]	08.73 ± 0.27[c]	11.50 ± 0.31[b]	0.41 ± 0.04[f]

[c-] and [c+] represent significant decrease and increase at $p<0.0001$, respectively, relative to untreated normal (Group I) rats. [a,] [b] and [c] represent significant increases at $p<0.05$, $p<0.001$ and $p<0.0001$, respectively, while [e] and [f] represent significant decrease at $p<0.001$ and $p<0.0001$, respectively, relative to untreated diabetic (Group II) values Group I: normal, Group II: Diabetic untreated, Group III: Diabetic+glibenclamide, Group IV: Diabetic+50 mg/kg *HU* , Group V: Diabetic+100 mg/kg *HU* ,Group VI: Diabetic+200 mg/kg *HU*.

Effect of HU treatment on serum proteins (TP and ALB) and lipids (TG and TC) in alloxan-induced diabetic rats

Alloxan treatment resulted in significant ($p<0.0001$) reductions in the serum TP and ALB levels and concomitant significant ($p<0.0001$) elevations in the serum TG and TC when compared to the untreated normal (Group I) rats (Table 4). However, oral treatments with 50-200 mg/kg of *HU* significantly ($p<0.05$, $p<0.001$, $p<0.0001$) reversed the effect of alloxan in the treated rats in a dose-related fashion with the most significant effect recorded for the group treated with the highest dose of *HU* (Table 4).

Effect of HU treatment on serum AST, ALT, ALP and LDH in alloxan-induced diabetic rats

Table 5 represents the effect of 50-200 mg/kg of *HU* on the serum levels of AST, ALT and ALP in alloxan-induced diabetic rats following oral treatment with the extract for 14 days. Alloxan treatment resulted in significant ($p<0.0001$) elevations in the serum AST, ALT and ALP levels and these were significantly ($p<0.05$, $p<0.001$ and $p<0.0001$) reversed by repeated oral treatment with 50-200 mg/kg of *HU* in a dose-related fashion (Table 5).

Effect of HU treatment on liver SOD, CAT, GSH and MDA levels in alloxan-induced diabetic rats

Treatment of rats with 150 mg/kg of alloxan given intraperitoneally resulted in significant ($p<0.0001$) reductions in the hepatic SOD, CAT activities and GHS levels while at the same time significantly ($p<0.0001$) enhancing the activity of MDA relative to untreated normal (Group I) control (Table 6). These effects were significantly ($p<0.05$, $p<0.001$ and $p<0.0001$) reversed by oral treatment with 50-200

mg/kg *HU* dose dependently with the most significant improvement recorded for the group treated with the highest dose (200 mg/kg) of *HU* (Table 6).

DISCUSSION

Recent and accumulating reports from both preclinical and clinical studies showed that oxidative stress plays a central and important role in the onset and course of DM as well as in the development of its associated vascular and neurological complications (Hunt *et al.*, 1988; Niedowicz and Daleke, 2005), with diversion of glycolytic intermediates into pathological pathways (Turk, 2010). Also, in DM, there is increased oxygen free radicals generation and disproportionately increased decline in antioxidant defense mechanism resulting in glucose oxidation, non-enzymatic glycation of proteins and subsequent oxidative degradation of cellular organelles and enzymes, increased lipid peroxidation, and rapid decline in pancreatic β-cells population (Domínguez *et al.*, 1998; Ceriollo, 2006) with consequent progression and development of secondary diabetic complications such as retinopathy, nephropathy, neuropathy, and accelerated coronary artery disease (McGrowder, 2013).

In this study, type 1 DM was induced through the intraperitoneal injection of cold alloxan monohydrate in normal saline into Wistar rats. Alloxan is known to cause diabetes and oxidative/nitrosative stress after its intracellular accumulation in the pancreatic β-cells via the GLUT2 glucose transporters through ROS mechanism which results in pancreatic β-cell destruction (Lenzen, 2008a). In addition, pancreatic β-cells are known to be highly sensitive and susceptible to oxidative stress as the intrinsic antioxidative defense mechanisms of pancreatic β-cells are weak and are easily overwhelmed by redox imbalance from reactive oxygen and nitrogen species with attendant deleterious consequences such as lipid peroxidation, protein oxidation, DNA damage (Lenzen, 2008b). Interference of reactive species [such as superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), toxic hydroxyl radicals ($\cdot OH$), singlet oxygen, nitric oxide, and peroxynitrite] with signal transduction pathways contributes significantly to β-cell dysfunction and death (Lenzen, 2008b; Djordjevic *et al.*, 2004). In *in vivo* experimental models, tissue oxidative stress markers such as SOD, CAT and GSH are useful and reliable markers of antioxidant status while MDA is a sensitive and reliable marker for lipid peroxidation (Kumar *et al.*, 2010; Feillet-Coudray *et al.*, 1999). Similarly, the integrity of hepatocytes is reliably assessed by levels of the serum liver enzyme markers such as AST, ALT and ALP (Moss and Henderson, 1999) and often these marker enzymes are elevated in DM (Rao *et al.*, 1989). In the current study, oxidative stress were

reliably induced with alloxan in the treated rats as indicated by marked reductions in the hepatic tissue SOD, CAT, GSH and marked elevation in hepatic tissue MDA levels and concomitant elevations in the serum AST, ALT and ALP levels which are in complete agreement with results of other studies (Golberg *et al.*, 1977; Stanely and Menon, 2001; Ezekwesili, 2012; Rajaram, 2013). Reversal in the hepatic tissue levels of these oxidative markers and hepatic enzyme markers following repeated oral treatment with 50-200 mg/kg/day of *HU* strongly indicate the effective therapeutic role of *HU* in attenuating oxidative stress associated with type 1 DM which was probably mediated via free radical scavenging activities and improving glutathione status in the liver tissues. Consequently, this result is in consonance with that of an earlier study which reported the *in vitro* anti-oxidant activity of *HU* (Adeneye *et al.*, 2011). Similarly, significant reductions in the serum liver enzyme markers, total cholesterol, triglyceride, and concomitant significant elevations in the serum total protein and albumin levels are suggestive of the hepatoprotective potential of *HU* against oxidative stress induced by alloxan since these biochemical parameters were normalized by *HU* treatments.

Alloxan, as a thiol reagent, selectively inhibits glucose-induced insulin secretion through its ability to inhibit β-cell glucose sensor, glucokinase, an essential rate-limiting step glucose metabolic enzyme (Lenzen, 2008b). The alloxan treated animals exhibited a decrease in hepatic glycogen content which may be due to enhancements in the glucose-6-phosphatase activity and deactivation/inhibition of glucokinase activity (Shirwaikar *et al.*, 2004). Glucose-6-phosphatase is known to catalyze the final step of glucose production by liver and kidney and its activity is often elevated in DM (Liu *et al.*, 1994; Clore *et al.*, 2000). Similarly, in type 1 DM, LDH activity is often significantly enhanced, resulting in lactic acidosis as a metabolic complication of type 1 DM (Zappacosta *et al.*, 1995; Raju *et al.*, 2001). The fact that glucose-6-phosphatase activity was significantly enhanced in the rats treated with alloxan in this study shows that our result is in accord with report of Liu *et al.* (1994) and Clore *et al.* (2000). Thus, the profound control of hyperglycemia coupled with increased hepatic glycogen content, decreased glucose-6-phosphatase and reduced lactate dehydrogenase activities in the *HU*-treated rats as recorded in this study suggest that the glycemic control achieved by *HU* was probably mediated via decreased release of glucose from hepatic tissue glycogen and increased hepatic glycogen deposition/storage due to decreased glucose-6-phosphatase activity through inhibition of glycolysis. Also, literature has it that an increase in hepatic

glycogen is often mediated through the activation of glycogen synthase for which the substrate glucose-6-phosphate could have been readily provided by increased hexokinase activity (Shirwaikar *et al.*, 2004; Bouche *et al.*, 2004; Lawrence and Roach, 1997). Again, significant reduction in glucose-6-phosphatase activity following treatment with *HU* coupled with insignificant alterations in the serum insulin levels lends support to the earlier report that *HU* achieves glycemic control via increased peripheral glucose utilization (Adeneye and Adeyemi, 2009b). Thus, the current study has further provided an insight into the enhanced peripheral glucose utilization mechanism of *HU* in alloxan-induced diabetic rats.

Another significant finding of this study is the improvement in the average body weight and percentage weight changes following repeated oral treatment with 50-200 mg/kg/day of *HU*. Results of the current study showed that untreated alloxan-induced diabetic rats manifested significant weight loss, hypercholesterolemia and hypertriglyceridemia which are in concordance with earlier studies that reported alloxan to cause significant weight loss with metabolic derangements such as hyperglycemia, dyslipidemia, hyperketonemia, lactic acidosis, *etc.* (Abdulrahman *et al.*, 2013). The fact that these metabolic alterations were restored to near normal levels following *HU* treatment also suggest that these metabolic re-arrangements by *HU* treatment could probably have accounted for the improvement in the body weight changes seen in the *HU*-treated rats.

Overall, results of this study show a positive correlation between chronic hyperglycemia and oxidative stress in alloxan-induced diabetic rats and both the hyperglycemia and oxidative stress were profoundly ameliorated with *HU* treatment via enhanced hepatic glycogen deposition mediated via decreased hepatic glucose-6-phosphatase activity and improvement in antioxidant/free radicals scavenging activities, respectively. Thus, this study provides further insight into the antidiabetic and antioxidant mechanisms of *HU* in experimental type 1 DM.

REFERENCES

Abdulrahman, M.M., El-Hefnawy, M.H., Aly, R.H., *et al*. (2013). Metabolic effects of honey in type 1 diabetes mellitus: a randomized crossover pilot study. *Journal of Medicinal Food* 16(1): 66-72.

Adeneye, A.A. and Adeyemi, O.O. (2009a). Hypoglycemic effects of the aqueous seed extract of *Hunteria umbellata* in normal and glucose- and nicotine-inducedhyperglycemic rats. *International Journal of Applied Research and Natural Products* 2(1): 9-18.

Adeneye, A.A. and Adeyemi, O.O. (2009b). Further evaluation of the antihyperglycemic effect of *Hunteria umbellata* (K. Schum.) Hallier f. seed extract in experimental diabetes. *Journal of Ethnopharmacology* 126(2): 238-243.

Adeneye, A.A., Adeyemi, O.O., Agbaje, E.O. (2010). Anti-obesity and antihyperlipidemic effect of *Hunteria umbellata* seed extract in experimental hyperlipidemia. *Journal of Ethnopharmacology* 130(2): 307-314.

Adeneye, A.A., Crooks, P.A., Fadhel-Albayati, Z., *et al*. (2013). Antihyperglycemic profile of erinidine isolated from *Hunteria umbellata* seed. *African Journal of Traditional, Complementary and Alternative Medicine* 10(2): 189-202.

Adeneye, A.A., Crooks, P.A., Miller, A-F., *et al*. (2012). Isolation and structure elucidation of a new indole alkaloid, erinidine, from *Hunteria umbellata* seeds. *Pharmacologia* 3(7): 204-214.

Adeneye, A.A., Sofidiya, M.O., Adenekan, O.S. (2011). Anti-inflammatory and antioxidant activities of *Hunteria umbellata* seed fractions. *Pharmacologia* 2(6): 165-171.

Baginsky, E.S., Foa, P.P., Zak, B. (1992). In: Bergmeyer, H.U. and Gawehn, K. (eds). *Methods of enzymatic analysis*, 2nd edition. Academic Press, New York, pp. 876-880.

Bouche, C., Serdy, S., Kahn, C.R., *et al*. (2004). The cellular fate of glucose and its relevance in type 2 diabetes. *Endocrine Reviews* 25: 807-830.

Carroll, N.V., Longley, R.W., Roe, J.H. (1956). The determination of glycogen in liver and muscle by use of anthron reagent. *Journal of Biological Chemistry* 220: 583-593.

Ceriollo, A. (2006). Oxidative stress and diabetes-associated complications. *Endocrine Practice* 12(Suppl1): 60-62.

Chattopadhyay, R.R., Sakar, S.K., Ganguly, S., *et al*. (1992). Effect of leaves of *Vinca rosea* Linn. on glucose utilization and glucose deposition by isolated rat hemi-diaphragm. *Indian Journal of Physiology and Pharmacology* 36: 137-138.

Clore, J.N., Stillman, J., Sugerman, H. (2000). Glucose-6-phosphatase flux *in vitro* is increased in type 2 diabetes. *Diabetes* 49(6): 967-974.

Djordjevic, A., Spasic, S., Jovanovic-Galovic, A., *et al*. (2004). Oxidative stress in diabetic pregnancy: SOD, CAT and GSH-Px activity and lipid peroxidation products. *Journal of Maternal, Fetal and Neonatal Medicine* 16: 367-372.

Domínguez, C., Ruiz, E., Gussinye, M., *et al*. (1998). Oxidative stress at onset and in early stages of type 1 diabetes in children and adolescents. *Diabetes Care* 21(10): 1736-1742.

Doumas, B.T., Watson, W.A., Biggs, H.G. (1971). Albumin standard and the measurement of serum

albumin and bromocresol green. *Clinica Chimica Acta* 31: 131-137.

Ezekwesili, C.N., Ogbunugafor, H.A., Ezekwesili-Ofili, J.O. (2012). Antidiabetic activity of aqueous extract of *Vitex dodiana* leaves and *Cinchona calisaya* bark in alloxan-induced diabetic rats. *International Journal of Tropical Disease and Health* 2(4): 290-300.

Falodun, A., Nworgu, Z.A.M., Ikponmwonsa, M.O. (2006). Phytochemical components of *Hunteria umbellata* (K. Schum.) and its effect on isolated non-pregnant rat uterus in oestrus. *Pakistani Journal of Pharmaceutical Sciences* 19(3): 256-258.

Feillet-Coudray, C., Rock, E., Coudray, C. (1999). Lipid peroxidation and antioxidant status in experimental diabetes. *Clinica Chimica Acta* 284: 31-43.

Frier, B.M. and Fisher, M. (2010). Diabetes mellitus. In: Colledge, N.R., Walker, B.R., Ralston, S.H. (eds.). *Davidson's Principles and Practice of Medicine*, 21st edition. Churchill Livingstone Elsevier, Edinburg, pp. 795-834.

Goldberg, D.M., Martin, J.V., Knight, A.H. (1977). Elevation of serum alkaline phosphate activity and related enzymes in diabetes mellitus, *Clinical Biochemistry* 10(1): 8-11.

Gupta, M.P., Solis, N.G., Avella, M.E., *et al.* (1984). Hypoglycemic activity of *Neurolaena lobata. Journal of Ethnopharmacology* 10: 323-327.

Herbert, V., Lau, K.S., Gottlieb, C.W., *et al.* (1965). Coated charcoal immunoassay of insulin. *Journal of Clinical Endocrinology and Metabolism* 25: 375-384.

Hunt, J., Dean, R.T., Wolff, S.P. (1988). Hydroxyl radical production and autoxidative glycosylation. Glucose autoxidation as the cause of protein damage in the experimental glycation model of diabetes and ageing. *Biochemical Journal* 256: 205-212.

Igbe, I., Omogbai, E.K.I., Ozolua, R.I. (2009). Hypoglycemic activity of aqueous seed extract of *Hunteria umbellata* in normal and streptozotocin-induced diabetic rats. *Pharmaceutical Biology* 47(10): 1011-1016.

Iwalewa, E.O., Adewale, I.O., Taiwo, B.J., *et al.* (2008). Effects of *Harungana madagascariensis* stem bark extract on the antioxidant markers in alloxan-induced diabetic and carrageenan-induced inflammatory disorders in rats. *Journal of Complementary and Integrative Medicine* 5(1): 1-18.

Kakkar, P., Dos, B., Viswanathan, P.N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics* 21: 130-132.

Kaneto, H., Katakami, N., Kawamori, D., *et al.* (2007). Involvement of oxidative stress in the pathogenesis of diabetes. *Antioxidant and Redox Signaling* 9: 355-366.

Kaur, G., Jabbar, Z., Anthar, M., *et al.* (2006). *Punica granatum* (pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice. *Food and Chemical Toxicology* 44: 984-993.

Kingsley, S.R. and Frankel, S.J. (1939). The determination of serum total protein, albumin and globulin by the biuret reaction. *Journal of Biological Chemistry* 128: 131-137

Kumar, R.P., Sujatha, D., Saleem, T.S.M., *et al.* (2010). Potential antidiabetic and antioxidant activities of *Morus indica* and *Asystasia gangetica* in alloxan-induced diabetes mellitus. *Journal of Experimental Pharmacology* 2: 29-36.

Lawrence, J.C. and Roach, P.J. (1997). New insights into the role and mechanism of glycogen synthase activation by insulin. *Diabetes* 46: 541-547.

Lenzen, S. (2008a). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* 51(2): 216-226.

Lenzen, S. (2008b). Oxidative stress: vulnerable beta-cell. *Biochemical Society Transactions* 36(Pt 3): 343-347.

Liu, Z., Barrett, E.J., Dalkin, A.C., *et al.* (1994). Effect of acute diabetes on rat hepatic glucose-6-phosphatase activity and its messenger RNA level. *Biochemical and Biophysical Research Communications* 205(1): 680-686.

Maiese, K., Chong, Z.Z., Shang, Y.C. (2007). Mechanistic insights into diabetes mellitus and oxidative stress. *Current Medicinal Chemistry* 16(16): 1729-1738.

McGrowder, D.A., Anderson-Jackson, L., Crawford, T.V. (2013). Biochemical evaluation of oxidative stress in type 1 diabetes. In: Escher, A.P. and Li, A. (eds). *Diabetes type 1.* InTech Publishing Co., Crotia, pp. 223-248. DOI: 10.5772/52836.

Moss, D.W. and Henderson, A.R. (1999). Clinical enzymology. In: Burtis, C.A. and Ashwood, E.R. (eds). *Tietz's Textbook of Clinical Chemistry*, 3rd edition. WB Saunders, Philadelphia, pp. 617-721.

Niedowicz, D. and Daleke, D. (2005). The role of oxidative stress in diabetic complications. *Cell Biochemistry and Biophysics* 43: 289-330.

Petersen, K.F., Befroy, D., Dufour, S., *et al.* (2003). Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 300(5622): 1140-1142.

Rajaram, K. (2013). Antioxidant and antidiabetic activity of *Tectona grandis* Linn. in alloxan induced albino rats. *Asian Journal of Pharmaceutical and Clinical Research* 6(Suppl 3): 174-177.

Rahul, S., Sanjay, K., Abhay, K.S. (2006). Antidiabetic potential of *Butea monosperma* in rats. *Fitoterapia* 77: 86-90.

Raju, J., Gupta, D., Rao, A.R., *et al*. (2001). *Trigonella foenum graecum* (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. *Molecular and Cellular Biochemistry* 224(1-2): 45-51.

Rao, G.M., Morghom, L.O., Kabur, M.N., *et al*. (1989). Serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels in diabetes mellitus. *Indian Journal of Medical Sciences* 43(5): 118-121.

Robertson, R.P. (2004). Chronic oxidative stress as central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *Journal of Biological Chemistry* 279(41): 42351-42354.

Robertson, R.P., Harmon, J., Tran, P.O., *et al*. (2003). Glucose toxicity in β-cells: Type 2 diabetes, good radicals gone bad, and glutathione connection. *Diabetes* 52(3), 581-587.

Shirwaikar, A., Rajendran, K., Kumar, C.D., *et al*. (2004). Antidiabetic activities of aqueous leaf extract of *Annona squamosa* in streptozotocin-nicotinamide type 2 diabetic rats. *Journal of Ethnopharmacology* 91: 171-175.

Sinha, A.K. (1972). Colorimetric assay of catalase. *Analytical Biochemistry* 47: 389-394.

Stanely, M.P.P. and Menon, V.P. (2001). Antioxidant action of *Tinospora cordifolia* root extract in alloxan diabetic rats. *Phytotherapy Research* 15: 213-218.

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. (2002). Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 25(Suppl.l): S5-S20.

Trinder, P. (1969). Determination of blood glucose using 4-aminophenzone as oxygen acceptor. *Journal of Clinical Pathology* 22: 246-248.

Turk Z. (2010). Glycotoxines, carbonyl stress and relevance to diabetes and its complications. *Physiological Research* 59: 147-156.

United States National Institutes for Health. (1985). Publication no. 85-23.

Venogopal, P.M., Prince, P.S.M., Pari, L. (1998). Hypoglycemic activity of *Syzigium cumini* seeds: effect on lipid peroxidation in alloxan diabetic rats. *Journal of Ethnopharmacology* 61: 1-7.

Wild, S., Roglic, G., Green, A., *et al.* (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 27: 1047-1053.

Wroblewski, F. and LaDue, J.S. (1955). Lactic dehydrogenase activity in blood. *Proceedings of Society for Experimental Biology and Medicine* 90: 210-213.

Zappacosta, B., De Sole, P., Rossi, C., *et al*. (1995). Lactate dehydrogenase activity of platelets in diabetes mellitus. *European Journal of Clinical Chemistry and Clinical Biochemistry* 33(8): 487-489.