GLUCOSE UTILIZATION AND ANTI-OXIDATIVE MECHANISMS OF THE AQUEOUS SEED EXTRACT OF *HUNTERIA UMBELLATA* IN ALLOXAN-INDUCED HYPERGLYCEMIC RATS

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PRESENTATION OUTLINE

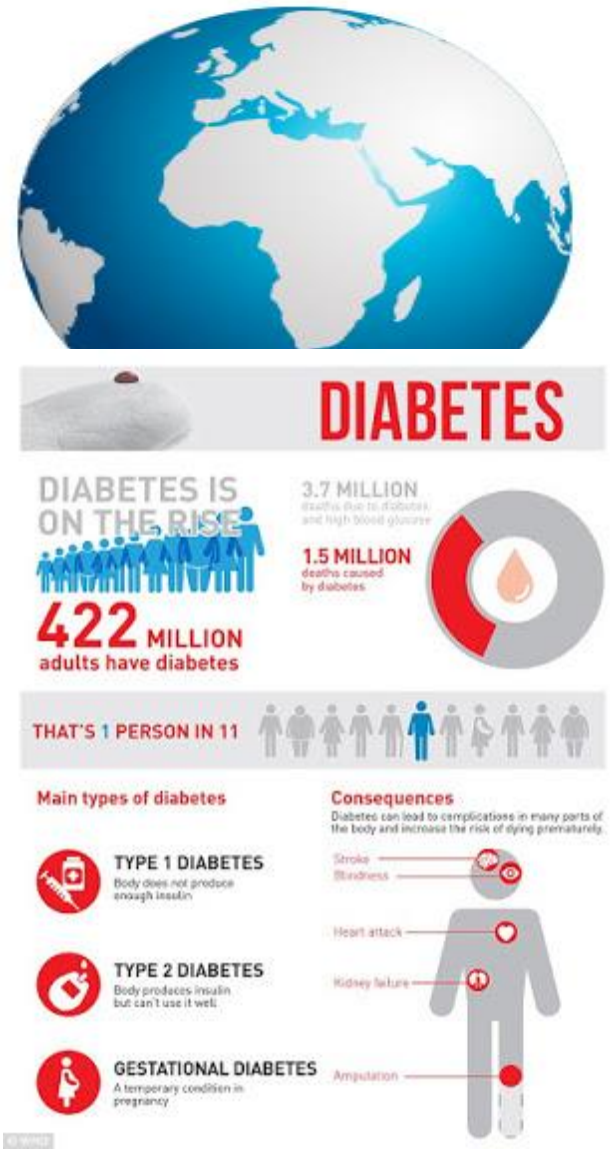
- Introduction
- Aims & Objectives of study
- Methodology
- Results
- Summary of findings
- References

INTRODUCTION

- Diabetes mellitus (DM) : 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 & Fisher, 2010).

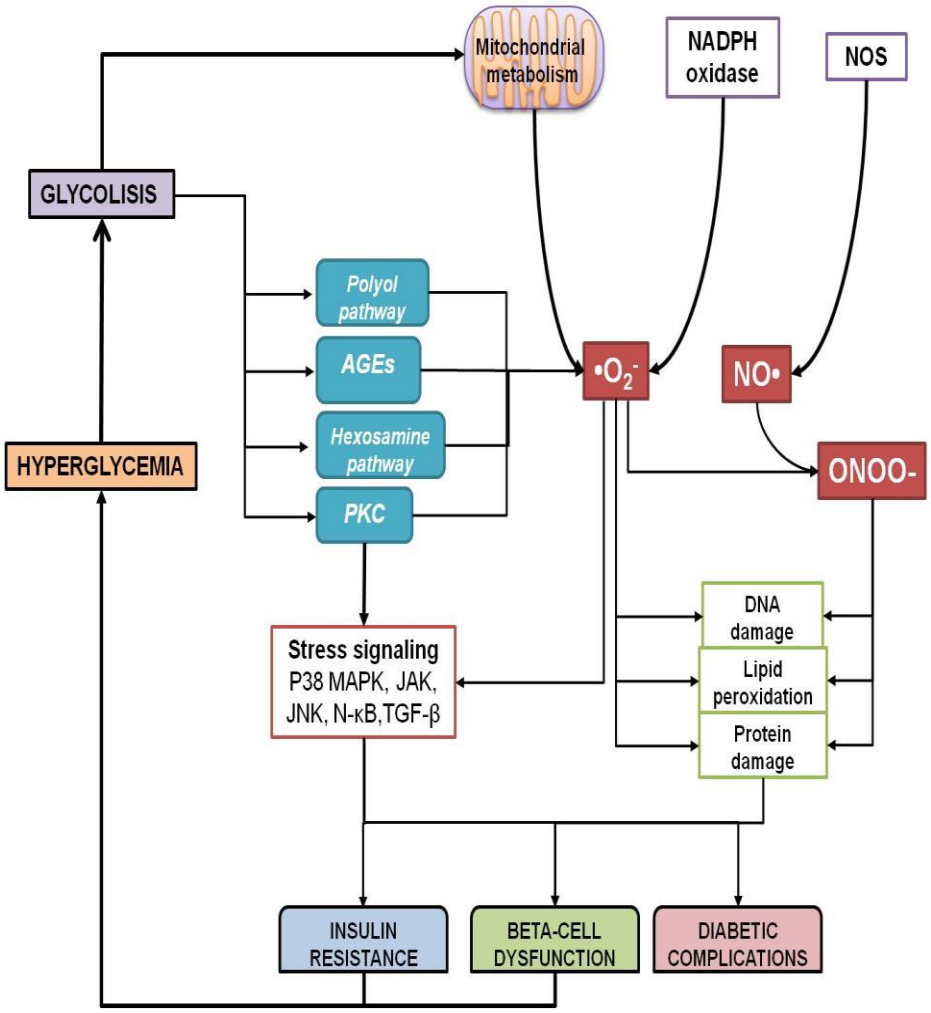
- DM is a leading pan-systemic endocrine disorder with attendant high morbidity and mortality owing to its deleterious effects on vital body organs such as the kidneys, eyes, liver, brain, *etc*., caused by untreated chronic hyperglycemia, attendant oxidative stress and glycation processes (WHO, 2016).

Estimated Global DM prevalence



Y2014 → an estimated 422 million adults (8.5% adult population) suffer DM, and yearly global cost of US$ 827 billion (WHO, 2016)

Y2030 → expected to rise to 552 million and yearly global GDP loss of US$ 1.7 trillion (Whiting *et al.*, 2011)



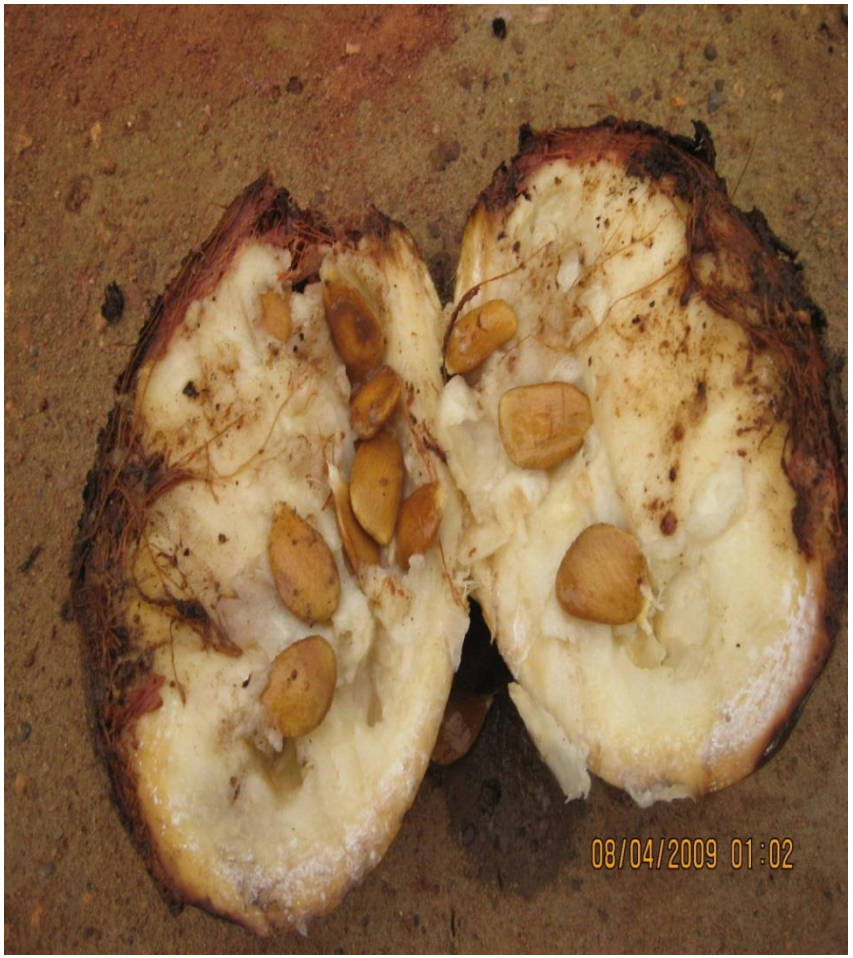
- Oxidative stress: implicated in the etiopathophysiology and complications of DM such as nephropathy, retinopathy, vasculopathy, neuropathy and cardiovascular disease (Lazo-de-la-Vega-Monroy & Fernández-Mejía , 2013).

- Oxidative stress promotes the onset and development of DM either by directly:

- ↓decreasing insulin sensitivity

-↑ INS-producing β-cells cytotoxicity (Maiese *et al.*, 2007)

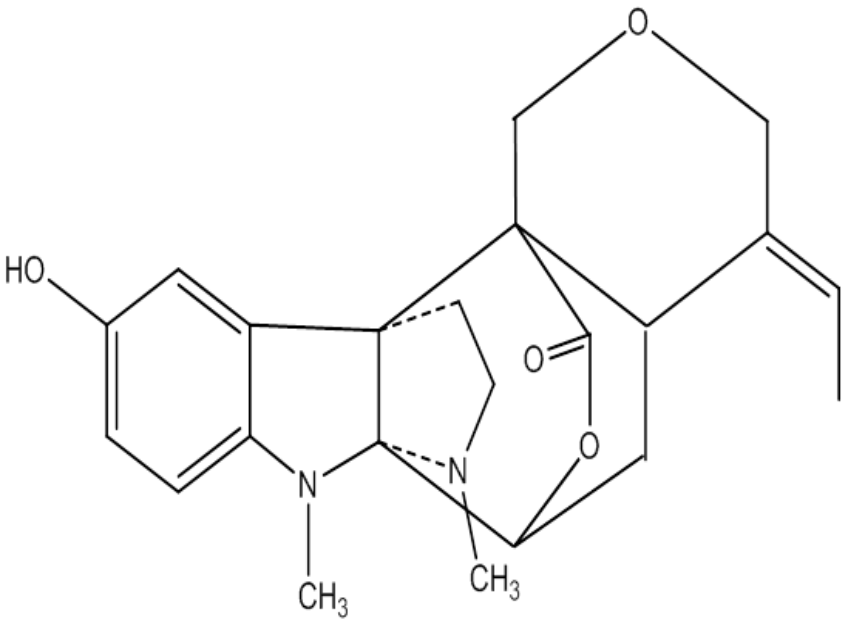
- *Hunteria umbellata* (K. Schum.) Hallier f., (family: Apocynaceae) is a tropical West African rainforest tree, locally known as “Abeere” (among the Yorubas and the Binis tribes in Nigeria) and “Demouain” in French (Boone, 2006)



- Its ethnomedical uses include Rx of STIs, stomach ulcers, diabetes mellitus & dysmenorrhea (Falodun *et al.*, 2006).

- Previous studies have reported its antihyperglycemic (Igbe *et al.*, 2009) anti-obesity and antihyperlipidemic (Adeneye *et al.*, 2010)

- erinidine



- A new bisindole alkaloid, erinidine, isolated from its crude alkaloid extract mediated an *in vivo* antihyperglycemic activity via intestinal glucose uptake inhibition (Adeneye *et al.*, 2012; 2013).
- Also, an *in vivo* anti-inflammatory and *in vitro* anti-oxidant activities of *HU* and its fractions have been reported (Adeneye *et al.*, 2011)

STUDY OBJECTIVES

- Previous studies have hypothesized *HU* was hypothesized to regulate glucose homeostasis via enhanced peripheral glucose utilization in experimental DM (Adeneye and Adeyemi, 2009a; 2009b).

- Unfortunately, no further studies have investigated the exact mechanism by which *HU* regulates glucose through enhanced peripheral glucose metabolism.

- Thus, the present study investigates the exact peripheral glucose utilization mechanism and the possible role of 50-200 mg/kg/day of *HU* in attenuating the oxidative stress in alloxan-induced hyperglycemic rats

METHODOLOGY

A. Plant Material Collection

- Fresh matured fruits of *HU* collected from Imoroko Village, Atan-Ijebu in Ijebu East L.G.A. of Ogun State, in December, 2012;

- Plant authentication & voucher specimen done as previously reported (Adeneye & Adeyemi, 2009a).

- Fresh seeds separated from fruits, rinsed in tap water & completely dried in an aerated oven preset at 25 ºC and protected from direct sunlight for 4 week.

B. Extraction Process

- 50 g of pulverized seeds dissolved in 500 ml of dH2O, stored in the refrigerator for 72 hours after which it was rigorously stirred with a magnetic stirrer for 2 hours before being filtered.

- The filtrate was completely air dried in an aerated oven preset at 40 ºC until a solid residue of constant weight was obtained.

- The crude extract obtained was stored in the air- and water-tight container and stored in the refrigerator

C. Experimental Animals

- Young adult male Wistar rats (wt. 110-130 g, 6-8 wks old) were procured from Bayo Animal Farm, Sango-Otta, Ogun State, Nigeria, after ethical approval was obtained.

- Acclimatized, maintained on standard rat chow, potable drinking water using Standard Principles Guiding the Care and Use of Laboratory Animals as contained in the NIH Publication No. 85-23 (1985).

D. Induction of Alloxan-Induced hyperglycemia

- I.P. Injection with freshly prepared 150 mg/kg alloxan monohydrates in sterile cold normal saline (Iwalewa *et al.*, 2008)
- Oral exposure to 5% glucose solution for 24 hr to prevent hypoglycemia (Gupta *et al.*, 1984)
- FBG levels checked on the 3rd & 5th day post-induction for sustained hyperglycemia (250 mg/dl)

E. Oral Treatment

- **Group I**: normoglycemic control - 10 ml/kg and 1 ml/kg of d H2O *p.o.* and *i.p.*, respectively
- **Group II**: alloxan-induced hyperglycemia + 10 ml/kg of dH2O *p.o.*
- **Group III**: alloxan-induced hyperglycemia + 5 mg/kg GLIB in dH2O *p.o.*
- **Group IV**: alloxan-hyperglycemia + 50 mg/kg of *HU* in dH2O *p.o.*
- **Group V**: alloxan-hyperglycemia + 100 mg/kg of *HU* in dH2O *p.o.*
- **Group VI**: alloxan-hyperglycemia + 200 mg/kg of *HU* in dH2O *p.o.*

F. Bioassays

- FBG: measured using tail tipping method & measured using glucose oxidase method (Trinder, 1969)

- Serum INS: measured using insulin RIA kits (Herbert *et al.*,1965)

- Serum AST and ALT, TP, ALB, TG and TC: 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).

F. Bioassays (cont'd)

- Serum lactic dehydrogenase activity (LDH) was measured by the method of Wroblewski & LaDue (1955).

- Hepatic tissue SOD, MDA, CAT & GSH activities: determined using commercial test kits

- Hepatic glycogen content: measured by method of Chattopadhyay *et al*. (1992)

- Hepatic glucose-6-phosphatase concentration (the rate limiting enzyme for glucose release from glycogen storage into the blood): quantified by the method of Baginsky *et al*. (1992).

G. Data Analysis

- 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) statistical software.

- Significant values were considered at $p<0.05$, $p<0.001$ and $p<0.0001$.

RESULTS

Table 1. Effect of 50-200 mg/kg of *HU* on body weight in alloxan-induced hyperglycemic rats

Group	1st day Wt. (g)	15th day 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]$p<0.001$ *vs* Group I values, [a]$p<0.05$ and [c] $p<0.001$ *vs* Group II values

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 hyperglycemic rats

Groups	1st DFBG (mg/dl)	15th DFBG (mg/dl)	%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 ± 4.21^{f}	-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, $^{e}p<0.001$ and $^{f}p<0.0001$ *vs* Group II

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

Groups	liver glycogen (mg/g)	G-6-P (U/mg 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}

- $^{a}p<0.05$, $^{b}p<0.001$, $^{c}p<0.0001$, $^{c-}p<0.0001$, $^{c+}p<0.0001$ *vs* Group I; $^{d}p<0.05$, $^{e}p<0.001$, $^{f}p<0.0001$ *vs* Group II values.

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

Groups	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}

$^{a}p<0.05$, $^{b}p<0.001$, $^{c}p<0.0001$, $^{c-}p<0.0001$, $^{c+}p<0.0001$ *vs* Group I; $^{d}p<0.05$, $^{e}p<0.001$, $^{f}p<0.0001$ *vs* Group II values

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

Groups	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}	36.90±10.30^{e}
VI	55.14 ± 2.66^{f}	33.50 ± 1.93^{f}	41.40 ± 2.79^{f}	255.80±7.60^{f}

$^{c+}p<0.0001$ *vs* Group I; $^{d}p<0.05$, $^{e}p<0.001$, $^{f}p<0.0001$ *vs* Group II values.

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

Groups	SOD (U/mg prot.)	CAT (U/mg prot.)	GSH (U/mg prot.)	MDA (nM/mg prot.)
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 ± 0.1276^{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}

$^{a}p<0.05$, $^{b}p<0.001$, $^{c+}p<0.0001$, $^{c-}p<0.0001$ *vs* Group I; $^{d}p<0.05$, $^{e}p<0.001$, $^{f}p<0.0001$ *vs* Group II values

SUMMARY OF FINDINGS

- → Recent preclinical and clinical evidences have shown that oxidative stress plays a central role in the onset and course of DM & its complications (Turk, 2010).

- → Type 1 DM characterized by sustained hyperglycemia was successfully induced through the intraperitoneal injection of cold alloxan monohydrate in normal saline into Wistar rats btw 3^{rd} - 5^{th} day post induction

- → This sustained hyperglycemia was associated with progressive weight loss which was attenuated by repeated oral Rx with 50-200 mg/kg *HU* in a dose related pattern

- → Repeated oral Rx with 50-200 mg/kg *HU* profoundly & progressively lowered whole blood FBG and increased serum INS levels 15th day post-Rx, dose dependently in the alloxan-induced hyperglycemic rats.

- These findings are in support of earlier reports on its antihyperglycemic effects (Adeneye *et al*., 2009a; 2009b, Igbe *et al*., 2009)

- → This Rx was also associated with dose-dependent hepatic glycogen deposit mediated via decreased hepatic glucose-6-phosphatase activities

- → Oral 50-200 mg/kg *HU* Rx was also associated with profound dose related increased TP & ALB and decreased TG & TC biosynthesis.

- These findings are in consonance with previous report on the anti-obesity and antihyperlipidemic effect of HU in experimental *in vivo* models (Adeneye *et al.*, 2010)

- → Similarly, oral 50-200 mg/kg HU Rx induced significant dose related reductions in the serum ALT, AST, ALP and LDH indicating its protection on the liver function

- → On oxidative stress markers, oral Rx with 50-200 mg/kg HU profoundly attenuated reduced hepatic SOD, CAT activities, GSH & MDA levels associated with alloxan-induced hyperglycemia

- → Overall, results of this study show a positive correlation between chronic hyperglycemia and oxidative stress in alloxan-induced hyperglycemia

- → 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.

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