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Antidiabetic and Plasma Endogenous Antioxidant Activity of *alstonia boonei* in Alloxan-Induced Male Diabetic Rabbits

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ABSTRACT: Alstonia boonei is a well-known plant of medicinal value but its effect on endogenous plasma antioxidant in diabetes remains unknown. Thus, need to investigate the effects of the methanolic extract of the plant on plasma bilirubin and uric acid level in alloxan induced diabetes rabbits. Twenty five rabbits divided into five groups of four rabbits each were used. There was a significant change in the levels of total bilirubin and uric acid in the plasma of treated groups as compared with both non-diabetes and untreated diabetes group, while conjugated bilirubin level was relatively unchanged in the treated group. This work clearly indicates that methanolic extract of *Alstonia boonei* stem bark is effective in the management of diabetes as well as restoration of lost endogenous plasma antioxidants experienced in diabetes mellitus. © JASEM

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Introduction

Diabetes mellitus is a metabolic disease that is as old as mankind; it is a major endocrine disorder and also a growing health problem globally (Pickup and Williams, 1997). Development of diabetes mellitus, course and its complications are closely associated with imbalance of pro- and antioxidative cell impairments and change of redox potentials (colak, *et al*., 2007), with concomitant increased production of free radicals and reduced capacity of antioxidant defence (Aronson and Rayfield, 2002). Diabetes mellitus accounts for over 4.8million annual deaths (9% of global total) are attributed to either diabetes or its complications (Sunmonu and Afolayan, 2013).

Uric acid and bilirubin are part of the endogenous antioxidant defence mechanism present in the human system (Nag, *et al*., 2009, Ames, *et al*., 1981). Uric acid is the end product of purine metabolism, where purines by enzymatic hydrolytic deamination form xanthine and hypoxanthine compounds which are than oxidized to uric acid (White, *et al*., 2004). It is an important physiological antioxidant by providing a primary defence against oxidants based upon its capacity to scavenge singlet oxygen and its ability to inhibit lipid peroxidation (Bhargava, *et al*., 2015). Bilirubin, on the other hand is an end product of heme catabolism in mammals. It can effectively scavenge peroxyl radicals generated in a reaction (Stocker, *et al*., 1987). Serum uric acid levels had been shown to have an important role in the pathogenesis and progression of long term complications associated with diabetes mellitus (Ashakiran, *et al*., 2010). Likewise, circulating bilirubin is associated with decreased risk of type 2 diabetes, but the nature of the relationship remain unknown (Abbasi, *et al*., 2015).

Throughout the world, many cultures still rely on indigenous medicinal plants for their primary health care needs (Farnsworth, *et al*., 1985). The use of natural remedies for diabetes treatment is strengthened due to the belief that herbs can provide some benefits over allopathic medicine and allows users to feel that they have some control in their choice of medication. However, their general acceptability has been limited by lack of dose regimen and adequate data on their toxicity (Joshi and Kaul, 2004).

Alstonia boonei (De Wild) a genus of to the family Apocynaceae with about 40-60 species is a large deciduous evergreen tree, usually up to 45m tall and 1.2m in diameter (Owolabi, *et al*., 2014, Akinloye, *et al*., 2013). The plant is commonly being sold in the local markets of West and Central Africa and used for ameliorating some disease conditions (Bello, *et al*., 2009) however, to the best of our knowledge, there is limited information on the effect of this plant on diabetes mellitus. In an attempt to carry out

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further scientific scrutiny on this plant, this study aims at investigating possible hypoglyceamic and the corresponding effects of the methanolic extracts of *A.boonei* on plasma uric acid and bilirubin levels of alloxan induced rabbits.

MATERIALS AND MEHODS

*Animals***:** Adult rabbits weighing between 1-2kg were obtained from the Animal House of the Faculty of life Sciences, University of Benin, Benin City. The animals were acclimatised for fourteen (14) days in well ventilated animal cages under good hygienic and standard environmental conditions, with 12 hours of light/dark cycle and were maintained on a regular feed (vital feed) and water *ad libitum*.

Collection and preparation of methanolic extracts: The stem bark, leaves and root of *A. Boonei* were collected around the premises of the University of Benin, Benin city, between June and July 2014. The plant was authenticated by Dr. Akinibosun, A.O. of the Department of Plant Biology and Biotechnology, University of Benin, Benin city, Edo State, Nigeria.

Plant parts of *A. boonei* were ensured to be free of debris by washing and air-dried at room temperature for 7 days. The plant parts were then pulverized into powder, using commercial blender and the powdered plant parts were stored in different air-tight containers prior to extraction.

2kg each of the pulverized plant parts were weighed and transferred into different containers. 4.0 litres of methanol was added to each container containing the stem bark, leaf and root powder. They were thoroughly mixed and allowed to stand for 24 hours. They were shaken at intervals to ensure thorough extraction. The macerated extract was filtered with a cheese cloth and the filtrates concentrated to dryness in a water bath set at 40 degree centigrade. The extracts were reconstituted prior to administration using normal saline. 100mg per kilogram of body weight of extracts (stem bark, leaf and root) was administered orally to the rabbits once daily (in the morning) for a period of seven (7) days.

Induction of experimental diabetes Diabetes mellitus was induced in sixteen rabbits according to the method of Katsumata, *et al*., (1993). The animals were allowed to fast overnight. The alloxan-momohydrate was injected intravenously (through the marginal ear vein) in a 12 hrs fasted rabbits at a dose of 100mg/kg body weight. Each 100mg of alloxan-momohydrate was diluted in 1ml of physiological saline. 10ml of 20% glucose D solution was injected to each rabbit immediately after induction of alloxan monohydrate in order to overcome sudden decrease in the animal blood glucose level. Diabetes was confirmed ten (10) days later, glucose level greater than 200mg/dl were considered as diabetic (Enechi, *et.al.,* 2014). Baseline fasting blood glucose levels and diabetes status determination was monitored on blood obtained from the marginal ear vein puncture using an automated glucose sensor machine Glucometer Analyser (Accu Chek Active).

Experimental protocol/design: A total of thirty twenty five (25) rabbits were divided into five (5) groups of four (4) rabbits each. The animals were grouped as follows: Group 1: (Reference/Normal control): Rabbits were fed with normal diet and water *ad libitum*, neither alloxan nor plant extract was administered. Group 2: This is the positive control group. Alloxan was administered to the animals in this group. No plant extract was administered. Group 3: Rabbits received root extract along feeds and water for seven days, alloxan was also administered Group 4: Rabbits received leaf extract along feeds and water for seven days, alloxan was also administered Group 5: Rabbits received stem bark extracts along feeds and water for seven days, alloxan was also administered

*Collection and processing of blood and tissue samples***:** On the day of sacrifice, food and water were withdrawn 6 hours prior to the time of sacrifice. This was done to minimize the glycogen stored in the body. The animals were sacrificed under chloroform anaesthesia, blood samples were collected using 5ml syringes from the abdominal aorta and left ventricle of the heart. The blood samples were introduced into lithium heparinized bottles and then mixed properly by gentle inversion. The blood samples were centrifuged at 4000 revolution per minute for 10minutes. The resulting plasma were collected into plain sterile bottles and stored at -20^0C until analyses. Uric acid was estimated by phosphotungstate technique while total and conjugated bilirubin in plasma was estimated by the methods of Jendrasik and Grof (Sood, *et al*., 2006).

Statistical analysis: Data generated were evaluated using Statistical Package for Social Sciences program (SPSS) version 16.0. Values were expressed as mean (±SD). The difference between the mean were analysed statistically with one way analysis of variance (ANOVA; 95% confidence interval), followed by Duncan Multiple Range Test. Values of $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

It was observed that all the rabbits that were not induced with alloxan-monohydrate moved freely throughout the period of experimentation while, the diabetic rabbits both treated and untreated appeared sluggish and highly lethargic. The blood glucose level of diabetic rabbits was significantly ($p<0.05$) higher than that of other groups and was also accompanied by significant reduced body weight. The mean blood glucose concentrations of all the rabbits in each experimental group are shown in table 1. There is also a non-significant ($p>0.05$) high concentration of plasma uric acid in untreated diabetes rabbits when compared to non-diabetes rabbits. This uric acid concentration is reversed in treated diabetes rabbits with stem bark extract exerting the most significant ($p<0.05$) effect. However, total bilirubin from our study revealed a significant ($p<0.05$) increase in untreated diabetes rabbits when compared with non-diabetes rabbits, with stem bark extract exerting the most significant ($p<0.05$) effect while, conjugated bilirubin did not show any significant ($p>0.05$) effect.

Table 1: Concentrations of blood glucose, plasma uric acid and bilirubin in controls and experimental Rabbits

Groups					
Parameters	1	2	3	4	5
Gluc	$65.5\pm5.6_a$	$316.9\pm28.4_b$	$244.4\pm29.6_c$	$295.9\pm12.7_d$	$199.6\pm32.8_e$
UA	$2.07 \pm 0.56_a$	$1.8 \pm 0.13_b$	$2.3 \pm 0.40_c$	$2.53 \pm 0.34_d$	$3.18 \pm 0.45_a$
TB	$0.35 \pm 0.10_a$	$2.10 \pm 0.13_b$	$0.55 \pm 0.06_c$	$1.83 \pm 0.17_d$	$0.11 \pm 0.01_e$
CB	$0.05 \pm 0.10_a$	$0.06 \pm 0.02_a$	$0.12 \pm 0.04_a$	$0.09 \pm 0.02_a$	$0.06 \pm 0.01_a$

Values with different subscript along the same row are significantly different at $p<0.05$.
Gluc = Glucose, UA = Uric acid, TB = Total Bilirubin CB = Conjugated Bilirubin

Hyperglycemia, a major feature of diabetes mellitus generates oxidative stress, which is exacerbated by metabolic stress (Dave, *et al*., 2015). Our present study therefore, investigated the antidiabetic activities of methanolic extracts of *A. boonei* and its effects on plasma endogenous antioxidants of bilirubin and uric acid in diabetes induced rabbits.

The methanol extracts of *A.boonei* from our study lowered the rabbit blood glucose level effectively, with the stem bark extract producing the most significant effects. A result that support the earlier work on *A.boonei* by Owolabi, *et al*., (2014) and Akinloye, *et al*., (2013), This antidiabetic effect though un-established may have produced hypoglycaemic action by being insulinomimetic (Owolabi, *et al*., 2014) or by stimulating glucose catabolising enzymes and inhibiting gluconeogenic enzymes (Akinloye, *et al*., 2013).

Bilirubin, a strong antioxidant and anti-inflammatory compound exerts its action on vasculature, thereby acting as a cytoprotectant to the vasculature (Stocker, *et al*., 1987, Cho, 2011). It also scavenges lipid peroxides and other products of physiological oxidation (Ramesh, *et al*., 2015). Serum bilirubin levels have been proven to be associated with microalbuminuria and sub clinical atherosclerosis in patients with type 2 diabetes (Fukui, *et al*., 2008), this present study however, showed that only total bilirubin and not conjugated bilirubin has a significant increase in the plasma of untreated diabetes rabbits when compared to non-diabetes rabbits, suggesting a response of the animal body system to oxidative stress caused by diabetes by way of producing of more bilirubin to mop-up the oxidants in the animal system as bilirubin may compensate the oxidative stress which might be an important factor in the pathophysiology of diabetes (). Increased level of bilirubin had been shown by Fu, *et al*., (2010) and Dong, *et al*., (2014) to reduced streptozotocin-induced pancreatic beta-cells damage in mice by attenuating oxidative stress and increasing insulin sensitivity respectively. An investigation on the effects of the extracts on plasma bilirubin in diabetes induced rabbits also revealed that all the extracts have potentials of reversing the hyperbilirubineamia with stem bark exerting the most significant effects.

Uric acid is involved in a complex reaction with several oxidants and may have some protective effects under certain conditions (Sautin and Johnson 2008). It is an antioxidant in the extracellular environment, reacting with superoxide (to make allantoin) and with peroxynitrite (to make triuret). These antioxidant properties of uric acid were proposed to be beneficial by protecting against ageing and associated oxidative stress (Ames, *et al*., 1981). The uric acid concentration in the body of our diabetes induced animals shows a non significant low level when compared to non-diabetes animals, a

feature that is different from earlier reported work of Cooke, *et al*., (1986) and Safi, et al., that reported hyperuricaemia in body of diabetes human. It was also observed that stem bark extract produced the most significant increased level of uric acid in the body of our treated diabetes animals. We therefore proposed that the reduced level may be due to oxidative stress experienced in diabetes condition.

*Conclusion***:** This study has clearly revealed that methanolic stem bark extract of *Alstonia boonei* is an effective antidiabetic agent that can be of help in the treatment of diabetes mellitus and restoration of the lost endogenous antioxidant activities of both plasma uric acid and bilirubin concentrations. More work needs to be done on the effect of the plant extract on both enzymatic and non enzymatic antioxidant mechanism in the management of diabetes using large pool of animals.

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