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Effect of Supplementation with a Locally Prepared Nutriceutical on Renal Function Profile in Alloxan-Induced Diabetic Rats

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ABSTRACT: Diabetes mellitus is characterized by increased levels of marker of oxidative stress which play a role in the development of diabetes complications. Antioxidants are thought to be beneficial in curtailing the lipid peroxidation. In the current work, antioxidant- rich nutriceutic was formulated from onions, garlic, lemon, palm oil and crayfish (in ratio 6:6:2:1:5 respectively), which are known sources of vitamins A, C and E and Cu, Cr, Mn and Zn. The nutriceutic was administered to alloxan-induced diabetic rats for 3 weeks and the effect of the supplementation on renal function profile was studied. The results shows that supplementation significantly ($P<0.05$) reduced blood glucose, urea, creatinine and potassium. It also not significantly ($P>0.05$) reduced serum sodium and bicarbonate. The findings suggest that supplementation with naturally occurring antioxidant nutriceutic may reduce the risk of oxidative stress and complications associated with diabetes mellitus and might be beneficial in the routine treatment of diabetes mellitus patients.
Key Words: Diabetes, nutriceutic, glucose, and renal function profile**.**

INTRODCTION

Diabetes mellitus is a state of sustained hyperglycaemia due to absolute or relative insulin deficiency or inactivity (Nsirim, 2000). There is progressive increase in the global prevalence of diabetes probably due to life style changes (Shaw *et al.*, 2010). The current estimate shows that more than 285 million people worldwide are affected by the disease representing 6.4% of the world population (Shaw *et al.*, 2010). It has been predicted that the worldwide estimate of diabetes will reach 7.7% by the year 2030 (Shaw *et al.*, 2010). Nigerian diabetic population is put at 3.9% as at 2010 (Shaw *et al.*, 2010).

Although diabetes mellitus is defined in terms of high blood glucose level, it is associated with multiple metabolic, endocrine and haematological derangements which are important in the pathogenesis of the disease and its complications (Carl and Burtis, 2001). Micro and macro vascular complications associated with diabetes mellitus are responsible for significant morbidity and mortality (WHO 1994; Edemeka *et al.*, 1999). The diabetic population is characterized by higher rate of blindness, kidney disease, gangrene and coronary heart disease several times more than non-diabetics (Nsirim, 2000).

Antioxidants participate in the protection of human body against free radical pathology and its complications (Nsirim, 2000). Antioxidant micronutrients have been indicated to boost the antioxidant defences and curtail the deleterious effects of reactive oxygen species (Armstrong, 1996). Deficiencies of micronutrients may increase susceptibility of diabetic mellitus and the associated complications (Aliyu *et al.*, 2005). Complex antioxidant mechanism including antioxidant vitamins and trace elements exists to limit the effects of these reactions (Packer, 2002).It is expected that this study will stimulate interests, discussion and further studies on the role of antioxidant vitamins and trace elements vis-à-vis complications of diabetes mellitus.

In this study an antioxidant rich nutriceutical (containing Vitamins A, C and E, Cu, Cr, Mn and Zn) was formulated and administered to alloxan-induced diabetic rats. The effect of the supplementation on blood glucose, and renal function profile of the diabetic rats were assessed.

MATERIALS AND METHODS

Chemicals

All the reagents used for the study were of analytical grade. Alloxan monohydrate and Metformin were purchased from Lab. Tech. Chemicals, India. Kits for the assay of glucose, urea, cretinine, sodium, potassium and bicarbonate were obtained from Randox Laboratories, Switzerland.

Preparation of the Nutriceutical

The nutriceutic was prepared from onions, garlic, lemon, palm oil and crayfish in ratio 6:6:2:1:5 respectively and administered to alloxan-induced diabetic rats for three weeks with and without treatment with metformin according to Leslie *et al.*,(1998). The amounts of antioxidants in the nutriceutic are shown in Table 1.

Table 1: Amount of Antioxidants Administered per kg Body Weight

Antioxidant	Amount (µg)
Vit.A	310.28
Vit.C	15,000
Vit.E	813.75
Cu	250.90
Cr	4.65
Mn	598.60
Zn	730.50

After the last day, the animals were fasted overnight and anaesthetised by dropping each in a transparent plastic jar saturated with chloroform vapour. Blood specimens were collected through cardiac puncture and placed in labelled centrifuged tube allowed to clot. The blood specimens were centrifuged in a bench top centrifuge at 4000 rpm for 5 min. The sera were collected from the centrifuged blood samples using Pasteur pipettes into labelled specimen bottle and stored at – 20^{o}C until required. Serum glucose was determined within 6 h from blood collection.

Experimental Animals

Thirty nine (39) apparently healthy mature albino rats (weighing between 130 and 220g) of both sexes purchased from the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria were used for the study. They were allowed to acclimatized at the Animal House of the Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, for a week before commencement of the experiment. During the period, they were fed on rat chow product of GCOM, Bukuru Jos, Nigeria, and allowed access to clean water *ad libitum*.

Induction of diabetes

Experimental rats were made diabetic by intraperitoneal injection of 80mg/kg body weight alloxan monohydrate for three consecutive days (kato and Miura, 1994; Pari and Maheswari, 1999; Stanley and Menon, 2000). A week after the last dose, the animals were observed for polydipsia, polyuria and polyphagia as well as general reduction on body weight. The animals were fasted overnight and the fasting blood glucose was estimated using a commercial glucose kit. Only rats that had fasting blood glucose level of $\geq$ 7.0 mmol/l (126mg/dl) and partial destruction of pancreas tested with positive response to metformin as described by Dulin and Soret, (1978), were included in the study.

Experimental design

The rats were divided into 6 groups as follows:

Group A: normal, non-diabetic rats. They received neither the antidiabetic drug nor were supplemented with the nutriceutic;

Group B: normal, non-diabetic rats. Supplemented with 200mg/kg body weight nutriceutic but not treated with antidiabetic drug;

Group C: alloxan-induced diabetic rats which were neither treated with antidiabetic drug nor were supplemented with nutriceutic;

Group D: alloxan-induced diabetic rats supplemented with 200mg/kg body weight nutriceutic but not treated with antidiabetic drug;

Group E: alloxan-induced diabetic rats treated with 250mg/kg body weight metformin but not supplemented with nutriceutic;

Group F: alloxan-induced diabetic rats treated with 250mg/kg body weight metformin and 200mg/kg body weight nutriceutic.

Measurement of Biochemical Parameters

Serum glucose concentration was estimated using glucose oxidase method (Trinder, 1969). Urea

concentration was estimated using Diacetyl-monoxime method (Ranjna, 1999). Creatinine concentration was estimated using Jaffe's alkaline picrate method (Ranjna, 1999). Sodium and Potassium concentrations were estimated using Flame photometry method (Ranja, 1999). Bicarbonate concentration was estimated using titrimetric method (Ranjna, 1999).

Statistical Analysis: All data were expressed as mean ± standard deviation (S.D). Data was analyzed by analysis of variance (ANOVA) using Instat 3 software (San Diego, USA). Difference in mean (±SD) were considered to be significant at $P<0.05$.

RESULTS

The fasting blood glucose levels of experimental albino rats are presented in Table 2.

Table 3 shows renal function parameters of diabetic rats supplemented with combination of metformin and the nutriceutical. Supplementation appears to reduce the renal function parameters levels to the levels of non-diabetic rats.

Table 2: Serum Glucose Levels (mmol/l) of Experimental Rats.

Group	Fasting Blood Sugar	
	Before treatment	After treatment
A	4.96 ± 1.21	5.18 ± 0.99[a]
B	4.96 ± 0.67	4.25 ± 060[a]
C	7.27 ± 0.20	7.80 ± 0.36[b]
D	7.20 ± 0.23	7.03 ± 0.23[b]
E	7.52 ± 0.34	6.26 ± 0.39[a]
F	7.37 ± 0.16	5.67 ± 0.27[a]

Values are mean ± SD. Values bearing different superscripts on the same column differ significantly ($P<0.05$). Using analysis of variance, instat 3 software (San Diego, USA)

Key: A: Non-diabetic-non-supplemented (n=7);
B: Non-diabetic supplemented (n=7);
C: Diabetic non-treated non-supplemented (n=7);
D: Diabetic non-treated supplemented (n=6);
E: Diabetic treated non-supplemented (n=6);
F: Diabetic treated and supplemented (n=6).

Supplementation with the nutriceutical returned the serum glucose level to the level of the non-diabetic rats.

Table 3: Renal Function Parameters of Diabetic Rats Supplemented with Locally Prepared Nutriceutical.

Group	Urea (mmol/l)	Creatinine (mg/dl)	Na^+ (mmol/l)	K^+ (mmol/l)	HCO_3^-(mmol/l)
A	5.48 ± 0.51[a]	1.18 ± 0.21[a]	142.00 ± 3.34[a]	4.80 ± 0.25[a]	26.00 ± 1.93[a]
B	4.60 ± 0.61$_a$	0.90 ± 0.19[a]	140.25 ± 1.26[a]	3.95 ± 0.28[a]	25.13 ± 099[a]
C	10.47 ± 0.18[b]	1.65 ± 0.27[b]	128.50 ± 1.31[b]	5.27 ± 0.35[b]	17.33 ± 0.82[b]
D	8.05 ± 0.85[c]	1.27 ± 0.14[c]	132.83 ± 1.45[b]	4.35 ± 0.54[b]	21.00 ± 2.61[c]
E	7.18 ± 0.57[d]	1.06 ± 0.17[a]	133.40 ± 2.46[a]	5.00 ± 0.14[b]	29.40 ± 1.34[d]
F	5.86 ± 0.34[a]	1.12 ± 0.18[a]	131.20 ± 1.50[a]	4.30 ± 0.44[a]	24.80 ± 0.84[a]

Values are mean ± SD. Values bearing different superscripts on the same column differ significantly ($P<0.05$). Using analysis of variance, instat 3 software (San Diego, USA)
Key: A: Non-diabetic-non-supplemented(n=7); **B:** Non-diabetic supplemented (n=7); **C:** Diabetic non-treated non-supplemented (n=7); **D:** Diabetic non-treated supplemented (n=6); **E:** Diabetic treated non-supplemented (n=6); **F:** Diabetic treated and supplemented (n=6).

Supplementation appears to reduce the renal function parameters levels to the levels of non-diabetic rats. Treatment with anti-diabetic drug also improve renal function parameters levels to the levels of non-diabetic rats

DISCUSSION

Increased oxidative stress has been proposed to be one of the major causes of hyperglycaemia-induced diabetic complication, & hyperglycaemia in an organism stimulates reactive oxygen species (ROS) formation from variety of sources (Volko *et al.*, 2007). Supplementation with antioxidants are thought to be effective in increasing the activities of antioxidant defence enzymes, scavenging free radicals, preventing oxidative damage and thereby sparing lipid components of the cells against lipid peroxidation (Zingg *et al.*, 2000).

The decrease concentration of fasting blood sugar (FBS) in diabetic treated and supplemented group

compared with diabetic treated non-supplemented group ($P<0.05$). This might be connected with increased availability of antioxidants that are important components and co-factors of the antioxidant enzymes (Fridovich, 1995).

The increase concentrations of urea, creatinine, potassium and dereased sodium and bicarbonate in diabetic patients might be due to excessive lipolysis in severe diabetic mellitus leading to ketosis and later acidosis (Mayne, 1993). Osmotic diuresis induced by glycosuria in poorly controlled diabetics may lead to urinary phosphate loses with negative phosphorus balance (Carl and Burtis, 2001). Diabetes affects the kidneys by causing them to become glucosuric, polyuric, and nocturic, which are caused by the heavy demands made on the kidneys to diurese hyperosmotic urine (Micheal *et al.,* 2005). Early treatment of diabetics that focuses on tight control of blood glucose and prevention of high blood pressure may prolong the onset of chronic renal failure (Micheal *et al.,* 2005). There were also decrease concentrations of urea, potassium, creatinine, sodium and bicarbonate in diabetic treated and supplemented rats compared with diabetic treated and not supplemented onces. Numerous studies (Micheal et al., 2005) have found alterations in micronutrient status of patients with diabetes mellitus. In some studies deficiency of certain minerals and vitamins has been correlated with presence of diabetics complications such as nephropathy, neuropathy, atherosclerosis etc. Quite a number of antioxidants participate in the protection of human body against free radical pathology and its consequences (Kahler *et al.*, 1993). Diabetic is one of the leading causes of death and disability due to complications such as coronary heart disease, stroke, end-stage renal disease and peripheral vascular disease (Khosh and Khosh, 2001; WHO, 1994). Oxidative stress contributes to impairment of islet cell function (Ceriello and Motz, 1999) insulin resistance, micro and macro vascular disease (Rema and Alttabori, 1994) and may increase their requirement for micronutrients with antioxidant effects (Attah, 2000).

CONCLUSION

Based on the results of the current study therefore, it can be concluded that supplementation with the nutriceutical rich antioxidant vitamins and minerals may reduced the risk of renal related complications that may be associated with diabetes mellitus.

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