Journal of Medicine, Physiology and Biophysics
ISSN 2422-8427 An International Peer-reviewed Journal
Vol.33, 2017

Haematological and Biochemical Changes in Alloxan-Induced Diabetic Dogs Treated with Aqueous Extract of *Moringa oleifera* Leaves

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Abstract

Moringa oleifera is a medicinal plant that has been used traditionally for the treatment of a number of ailments. The haematological and biochemical changes following treatment of alloxan-induced diabetic dogs with aqueous extract of *M. oleifera* leaves was investigated in this study. Fifteen dogs with mean weight of 10 ± 1.3 kg) were divided into three equal groups. Group A were neither induced nor treated. Group B were induced but, untreated. Groups C were induced and treated with Moringa extract 5.2% (300mg/kg) orally in water, once daily, for 42 days post induction. Blood was collected from all dogs pre-induction, post-induction and post-treatment for haematological and biochemical analysis. Values obtained were expressed as mean ± standard error of means and data were analysed by one way Analysis of Variance (ANOVA) and Bonferroni's Multiple Comparison Test using Graph Pad. Fasting blood glucose (FBG) of group C decreased significantly ($P < 0.05$) post treatment compared to group B. The Packed Cell Volume, red blood cell count and haemoglobin concentration insignificantly ($P < 0.05$) decreased in diabetic groups post-induction and post-treatment, while white blood cells count increased. Platelet count increased post-induction and post-treatment. There was a significant increase ($P < 0.05$) in creatinine, urea, alanine aminotransferase and aspartate aminotransferase levels in diabetic groups compared to non-diabetic group post-induction and then decreased in group C post-treatment with moringa extract. Albumin and total protein concentrations increased significantly ($P < 0.05$) post-induction in diabetic groups but, decreased in group C post-treatment. In conclusion, diabetes caused decrease in haematological parameters, and increased platelet count and some biochemical parameters, while moringa extract decreased biochemical parameters in dogs.

Key words: Haematobiochemical changes, *M. oleifera*, Diabetes, dogs

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose concentration caused by insulin deficiency, often combined with insulin resistance (Jelodar *et al*., 2007). It is an endocrine disease in origin, but its major manifestations are those of a

metabolic disturbance. Diabetes is caused by the impairment of insulin signalling pathways, and the defect usually results from pancreatic β-cell deficiency and/or a deficiency of insulin (Kahn, 1994). It is associated with an increased risk of thrombotic, atherosclerotic and cardiovascular disorders (DeFronzo and Ferrannini, 1991). Diabetes is characterized by increased thirst, polyuria, dehydration, pruritus, and unexplained weight loss (Birchard and Sherding, 2006). Hyperglycemia, the primary clinical manifestation of diabetes, is thought to contribute to diabetic complications by altering vascular cellular metabolism, vascular matrix molecules and circulating lipoproteins, (Chattopadhyay *et al.*, 2005).

Some conditions like breed (Cocker Spaniel, Beagle, Poodles, Keeshounds, Cairn Terrier, Dachshund, Schnauzer), female gender, obesity and age of five to seven years predispose dogs to hyperglycemia. The incidence of diabetes in the different breeds of dogs differs widely, suggesting that there may be some important genetic factors in its aetiology (Fall *et al.*, 2007). In human also, the major risk factors are overweight, age more than 45 years, higher High Density Lipoprotein(HDL) level, high blood pressure, history of gestational diabetes and impaired glucose tolerance, race/ethnicity (Susman, 2010). Diabetes due to insulin deficiency is considered to be caused by the autoimmune destruction of insulin-producing β-cells, pancreatitis or islet hypoplasia (Cook *et al*., 1993). Treatment of the cause of insulin resistance in the dog may result in the remission of the condition. However, untreated cases lead to insulin deficiency because chronic hyperglycaemia induces permanent β-cell dysfunction (Catchpole *et al.*, 2005). Despite the presence of different types of anti-diabetic drugs in the pharmaceutical market, diabetes with related complications continues to be a major medical problem all over the world (Hussein, *et al*., 2006).

Interest in medicinal plants as a re-emerging health aid in the maintenance of personal health and well-being has been fuelled by the bio-prospecting of new plant-derived drugs (Ayo, 2010). *Moringa oleifera* is one of such medicinal plants and has been used traditionally for the treatment of a number of ailments including fomentation to relieve spasm, diarrhoea, as diuretic and stimulant in paralytic affliction, epilepsy and hysteria (Anwar *et al*., 2007; Paliwal *et al*., 2011). It has also been used in the treatment of ascites, rheumatism (Anwar *et al*., 2007) and as cardiac and circulatory stimulants (Limaye *et al*., 1995). The extract of the leaves of *Moringa oleifera* has been found to lower fasting blood glucose of streptozocin-induced diabetic wistar rats (Tende *et al.*, 2011).). Chemical constituents of the crude aqueous extract of the fresh leaves of *Moringa oleifera* were found to be tannins, saponins, carbohydrates, flavonoids, cardiac glycosides, alkaloids, steroids and terpenes while the crude aqueous extract of the dried leaves contain the same chemical components except for the absence of steroids and terpenes (Kwaghe and Ambali, 2009). This study investigated the haematological and biochemical changes in alloxan-induced diabetic dogs treated with aqueous extract of moringa leaves.

2. MATERIALS AND METHODS

2.1 Experimental animals

Fifteen Nigerian indigenous dogs of both sexes with mean body weight of 10 ± 1.30kg were used for this work. They were kept in wooden cages and allowed to acclimatize for three weeks during which they were treated against some haemoparasites, using 5% oxytetracycline (Tetranor 5.5^{R}, Jubaili Agrotec, China) intra-venously at the dose of 10mg/kg. They were also treated for endo- and ecto-parasites using albendazole (Zolat^{R}, Emzor Pharmaceutical Ind. Ltd., Lagos, Nigeria) per os at the dose of 7mg/kg and Fipronil (Frontline-plus®, Merial) spray, respectively. The dogs were fed twice daily with cooked rice and fish, and water was provided *ad libitum*.

2.2 Aqueous extraction of *Moringa oleifera*

The *Moringa oleifera* leaves were air-dried indoor and then ground into fine powder. Eight hundred grammes (800g) of the powder was soaked in 1000ml of distilled water at room temperature for 24 h. It was then filtered using Wartmann filter paper (size no. 1). The filtrate was evaporated to dryness in a water bath at 60^0C within seven hours. A brownish residue weighing 51.6 g was obtained and then kept in an air-tight bottle in a refrigerator (4^0C) until used.

2.3 Experimental design

An open, randomised controlled design was used for this study. The design was approved by the Ethical Committee of College of Veterinary Medicine, Federal University of Agriculture, Abeokuta. The dogs were randomly divided into three equal groups. Group A comprised of five dogs in which diabetes was neither induced nor treated but were given normal saline. Group B comprised of five dogs in which diabetes was induced but were not treated. Group C comprised of five dogs in which diabetes was induced and thereafter treated with *Moringa oleifera* leaves extract (300 mg/kg).

2.4 Acute toxicity testing

Three dogs (A, B and C) were subjected to acute toxicity testing by respectively giving 1000mg, 3000mg and 5000mg of moringa aqueous extract orally to the dogs. The dogs were observed for signs of toxicity.

2.5 Determination of blood glucose level and induction of diabetes mellitus

Blood was obtained from the animals before fasting through the cephalic vein to determine their non-fasting blood glucose levels in mg/dl using Accu-Chek Glucometer (Roche, Germany). Food was withdrawn from the dogs overnight, and 5% solution of alloxan monohydrate (Sigma, USA) was administered intravenously to groups B and C dogs through the cephalic vein at the dose of 80 mg/kg body weight. The fasting blood glucose of each dog was then determined at 0, 8, 16, 24 and 48 hours after the administration of alloxan monohydrate.

2.6 Administration of *Moringa oleifera* extract

The 51.6g of aqueous extract of *Moringa oleifera* was made up to 1000ml with distilled water to make a 5.2% solution. This was administered orally once daily to group C dogs at the dose of 300mg/kg for a period of 42 days. The FBG was measured at 0h, 2h, 4h, 8h, 16h, and 24h.

2.7 Determination of haematological and biochemical parameters

Blood was collected from the dogs through cephalic vein before and after induction of diabetes, and then day 42 post treatment with aqueous extract of moringa leaves for haematology as described by Jain, 1986. Part of the blood was centrifuged for 10 minutes at 2000 rpm and plasma collected for biochemistry using spectrometric and enzymatic colorimetric methods.

3. Statistical analysis

Data generated from the study were expressed as mean ± standard error of means. Differences between the groups were compared by analysis of variance (ANOVA) and Bonferroni's Multiple Comparison Test using Graph pad software.

4. Results

4.1 Fasting blood glucose post induction and post treatment

There was a decrease in the FBG levels of groups B and C below the pre-induction level at 8 hours post administration of alloxan monohydrate without significant difference ($P < 0.05$) compared to group A. The FBG after falling below the pre-induction level progressively rose up to 48 hours when diabetes was achieved with a significant difference ($P < 0.05$) between the induced and non-induced groups. The clinical signs observed after the administration of alloxan monohydrate were nausea, vomiting, diarrhoea and dehydration at 3-8 hours post administration of alloxan monohydrate. The elevated levels of the FBG of groups C decreased significantly ($P < 0.05$) at 2h and 8h post treatment with *Moringa oleifera* extract compared to untreated group B dogs. The fasting blood glucose of Moringa extract treated group started rising again at 8h. The clinical signs of polyuria and polydipsia persisted in both treated and untreated groups post treatment but less severe in treated group C.

4.2 Acute toxicity determination

There were no apparent clinical signs of acute toxicity in the 3 dogs at any level of the administered doses.

4.3 Haematological changes

Pack Cell Volume (PCV), red blood cells and haemoglobin concentration (Hb) decreased significantly ($p < 0.05$) in the diabetic groups B and C post induction compared to group A. The white blood cells (WBC) insignificantly increased in diabetic groups post induction and post treatment. The platelet count increased in diabetic groups B and C post induction without significant difference ($P > 0.05$) between non-diabetic and diabetic groups. After treatment, the platelet count further increased with significant difference ($p < 0.05$) compared to both non-diabetic and diabetic-untreated groups (Table 1).

4.4 Biochemical changes

The creatinine, urea, alanine aminotransferase and aspartate aminotransferase levels in diabetic groups B and C increased significantly ($P < 0.05$) compared to non-diabetic group A post-induction and then decreased in group C post-treatment with moringa extract. Total protein concentration increased significantly ($P < 0.05$) post-induction in diabetic groups but, decreased in group C post-treatment with aqueous moringa extract. Albumin decreased significantly post induction and post treatment in both diabetic groups B and C. Alkaline phosphatase increased slightly in diabetic groups B and C post induction and post treatment (Table 2).

Table 1: Some haematological parameters of dogs before and after induction, and after administration of *Moringa oleifera* leaves extract

	Parameters	A	B	C	Reference range
X	PCV (%)	35 ± 1.3	36.0 ± 1.1	35 ± 1.0	35 – 55
Y	PCV (%)	35 ± 0.3[a]	27.0 ± 2.9[b]	31.0 ± 1.4[b]	35 – 55
Z	PCV (%)	32 ± 2.9 [a]	25.0 ± 2.1[b]	27.0 ± 0.6[b]	35 – 55
X	HB (g/dl)	12.76 ± 0.3	12.8 ± 0.6	13.2 ± 0.4	12 – 18
Y	HB (g/dl)	10.77 ± 0.2 [a]	9.0 ± 1.0[b]	9.0 ± 1.0[b]	12 – 18
Z	HB (g/dl)	10.67± 0.9 [a]	8.7 ± 0.6[b]	9.0 ± 0.2[b]	12 – 18
X	WBC X $10^3(Cm^3)$	18.23 ± 3.2	18.3 ± 1.4	18.2 ± 2.0	6 – 17
Y	WBC X $10^3(Cm^3)$	16.21 ± 3.1	20.2 ± 2.6	16.0 ± 1.2	6 – 17
Z	WBC X $10^3(Cm^3)$	16.70± 2.4	20.4 ± 0.7	18.5 ± 2.6	6 – 17
X	RBC X $10^{12/L}$	5.52 ± 0.2	5.6 ± 0.1	5.6 ± 0.1	5.5 - 8.5
Y	RBC X $10^{12/L}$	5.27 ± 0.1 [a]	4.2 ± 0.5[b]	4.9 ± 0.2[b]	5.5 - 8.5
Z	RBC X $10^{12/L}$	5.36 ± 0.4 [a]	4.0 ± 0.3[b]	4.2 ± 0.1[b]	5.5 - 8.5
X	PLAT X $10^{11/L}$	6.75 ± 0.2	5.2 ± 0.2	6.4 ± 0.2	2 – 9
Y	PLAT X $10^{11/L}$	6.50 ± 0.3	5.9 ± 0.4	6.4 ± 0.2	2 – 9
Z	PLAT X $10^{11/L}$	6.51 ± 0.3[a]	6.0 ± 0.3[a]	7.0 ± 0.2[b]	2 – 9

Key: A= Non-diabetic, B = Diabetic-Untreated, C = *Moringa oleifera* treated group, PCV = packed cell volume, HB = haemoglobin, WBC = white blood cells, RBC = red blood cells, PLAT = platelet, X = basal value, Y = post induction of diabetes, Z = post treatment with moringa leaves extract, [a, b] Values in the same rows with different superscripts are significantly different ($P < 0.05$).

Table 2: Some biochemical parameters of dogs before and after induction, and after administration of *Moringa oleifera* leaves extract

	Parameters	A	B	C	Reference Value
X	T. Pro (g/L)	50.0 ± 1.4	58.1 ± 2.7	53.2 ± 2. 2	55-75
Y	T.Pro (g/L)	47.0 ± 2.5[a]	59.3 ± 4.0[b]	70.4 ± 2.2[b]	
Z	T.Pro (g/L)	46.6 ± 1.6[a]	63.2 ± 1.6[b]	62.2 ± 2.9[b]	
X	Albumin (g/L)	29.1 ± 3.6	31.0 ± 0.3	31.7 ± 1.9	26-40
Y	Albumin (g/L)	28.8 ± 2.9[a]	35.1 ± 1.7[b]	37.5 ± 1.7[b]	
Z	Albumin (g/L)	27.8 ± 1.0[a]	34.1 ± 1.7[b]	33.8 ± 0.6[b]	
X	Urea mg/dl	10.0 ± 1.4	11.5 ± 0.3	12.4 ± 1.9	8.8-26
Y	Urea mg/dl	9.6 ± 1.4[a]	23.8 ± 6.6[b]	22.1 ± 1.2[b]	
Z	Urea mg/dl	10.1 ± 1.6[a]	35.8 ± 3.4 [b]	16.4 ± 1.6[c]	
X	Creat (mg/dl)	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.1	0.5-1.6
Y	Creat (mg/dl)	0.7 ± 0.1[a]	1.4 ± 0.2[b]	1.4 ± 0.2[b]	
Z	Creat (mg/dl)	1.2 ± 0.2[a]	1.6 ± 0.1[b]	0.9 ± 0.1[c]	
X	AST (IU/L)	38.6 ± 5.5	37.5 ± 2.4	37.1 ± 4.2	8.9-49
Y	AST. IU/L	36.9 ± 1.9[a]	104.6 ± 8.5[b]	99.3 ± 3.6[b]	
Z	AST. IU/L	38.4 ± 4.1[a]	97.5 ± 3.2[b]	55.9 ± 3.3[c]	
X	ALT IU/L	28.4 ± 3.4	20.8 ± 6.9	20.1 ± 3.0	8.2-57
Y	ALT IU/L	30.1 ± 3.7[a]	73.9 ±12.6[b]	59.6 ± 4.6[b]	
Z	ALT IU/L	30.1 ± 3.7 [a]	88.8 ± 5.4[b]	53.0 ± 3.5[c]	
X	ALP IU/L	35.3 ± 9.8	35.4 ± 5.5	35.0 ± 2.2	10.6-101
Y	ALP IU/L	34.8 ± 8.7	94.7 ± 5.9	85.8 ± 2.3	
Z	ALP IU/L	35.6 ± 6.8	92.8 ± 6.7	72.2 ± 6.6	

Key: A= Non-diabetic, B = Diabetic-Untreated, C = *Moringa oleifera*-Treated, T. Pro = total protein, Creat = creatinine, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP =

alkaline phosphatise, X = baseline value, Y = post induction of diabetes, Z = post treatment with moringa leaf extract

5. Discussion

The clinical signs of nausea, vomiting, diarrhoea and dehydration observed between 3 and 8 hours post administration of alloxan monohydrate were due to the adverse effect of the chemical. Hypoglycaemia was observed at 8 hours after the intravenous administration of alloxan monohydrate. The destruction of beta-cells by alloxan monohydrate trigger the release of excess insulin into the vascular system resulting in over utilization of blood glucose. This observation was in consonance with that of Kim *et al*., (2006), was reported hypoglycaemia in dogs after induction of diabetes with alloxan monohydrate. The moringa extract decreased the fasting blood glucose after 2 hours of administration after which it started rising at 8 hours. This showed that it has hypoglycemic effect which can only last for 8 hours. It can be suggested that it needs 8 hourly dosing to be able to maintain plasma concentration. Alkaloids have been reported to produce hypoglycaemic effect in mice, (Kubo, *et al*., 2002). Li *et al*., (2000), reported the hypoglycaemic effect of saponins in mice, while, Ojo *et al.*, (2010), reported that flavonoids produced hypoglycaemic effect in rats. In this study, the hypoglycaemic activity of the leaves of *Moringa oleifera* may probably be due to the presence of the biological principles such as flavonoids, alkaloids, saponins, glycosides (niazirin and niaziminin), which are involved in the stimulation of the B-cells and subsequent secretion of preformed insulin.

The decrease in PCV, RBC and haemoglobin concentration might be due to the inflammatory process seen in diabetic condition. The activities of proinflammatory cytokines especially, interleukin-6 and tumor necrosis factor alpha have been reported to be contributors of anaemia of inflammatory diseases. Tumor necrosis factor (NF-α) contributes to anaemia of inflammatory diseases by inhibiting erythropoietin secretion and /or response along with direct toxicity on the erythroid precursor cells (Chikazawa and Dunning, 2016), while interleukin-6 induces production of hepcidin which binds to ferroportin thereby blocking the intestinal absorption of iron (Ganz and Nemeth, 2011). *Diabetes mellitus* is characterized by enhanced platelets activation and coagulation proteins and reduced fibrinolytic activity (Carr, 2001). In this study, there was increased platelet count in the diabetic groups which significantly increased in the moringa treated group. This observation is consistent with the report of Oru *et al*., (2016), who observed significant increase of platelet count in streptozotocin-induced diabetic rats, and Hisham *et al*., (2012) who reported significant increase in platelet count in rats and rabbits fed daily with M. oleifera for 21 days. The significant increase in platelet count in the moringa treated group portended risk of macro and micro cardiovascular complications. Many authors have reported that increased morbidity and mortality in type 2 *Diabetes mellitus* are associated with macro vascular (cardiovascular diseases, stroke, and peripheral arterial disease) and micro vascular (nephropathy, neuropathy and retinopathy) complications due to platelet dysfunction (Vinik *et al*, 2001; Ferroni *et al*., 2004).

The increase in levels of both creatinine and urea seen in this study is suggestive of kidney damage which could be due to the cytotoxic effect of alloxan monohydrate as reported by Valilou *et al.*, (2007) as well as low kidney perfusion as a result of anaemia. The decrease in albumin concentration might be due to degenerative changes in the liver. The decrease seen

in total protein concentration could have resulted from dehydration. The activities of ALT and AST were high and it indicates hepatocytes damage which must have resulted from the inflammatory process in hyperglycaemic conditions. The cytotoxic effect of alloxan chemical is another possible means of hepatic damage, and it has been reported by Valilou *et al.*, (2007). The decrease in the biochemical parameters in diabetic group C treated with aqueous extract of moringa is an indication that apart from having a hypoglycaemic effect, aqueous extract of moringa leaves also has an anti-infammatory effect.

Conclusion

Moringa oleifera has a hypoglycaemic effect and so can be useful in the management of diabetes mellitus in dogs. Plasma creatinine and urea concentrations, Alanine aminotransferase and Aspartate aminotransferase activities were elevated in alloxan-induced diabetic dogs and it was decreased by aqueous extract of moringa leaves. Platelet count was increased in alloxan-induced diabetic dogs treated and remained high after treatment with aqueous extract moringa leaves.

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