Antidiabetic and haematinic effects of *Parquetina nigrescens* on alloxan induced type-1 diabetes and normocytic normochromic anaemia in Wistar rats

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Abstract

Background: The plant, *Parquetina nigrescens* is used in folklore medicine to treat diabetes mellitus and its complications in several parts of West Africa.

Objective: To determine the effect of *Parquetina nigrescens* extract on fasting blood glucose in alloxan-induced diabetic rats. **Methods:** The blood glucose levels, complete blood count, erythrocyte indices and osmotic fragility, body and organ weights were evaluated.

Results: Diabetic rats treated with the extract showed significant (P < 0.01) reduction of the blood glucose to levels comparable to that of the non-diabetic control and those treated with chlorpropamide (standard drug). Similarly, there was significant (P < 0.01) reduction in the complete blood count in the diabetic rats.

Discussion: The anaemia, leucopenia and thrombocytopenia associated with the diabetes were corrected in the animals treated with the extract and chlorpropamide. The extract also reduced the erythrocyte osmotic fragility, body and organ weights. *Parquetina nigrescens* demonstrated antidiabetic property by reducing the elevated blood glucose in alloxan treated rats which is comparable to animals that received the standard drug.

Conclusion: Paraquetina nigrescens stabilized the erythrocyte membrane, decreased the body weight probably by lowering lipogenesis. However, the mechanism underlying the antidiabetic and haematinic properties of *Parquetina nigrescens* remains to be elucidated.

Keywords: Parquetina nigrescens, alloxan, blood glucose, haematology, osmotic fragility African Health Sciences 2010; 10(3): 276 - 283

Introduction

The use of medicinal plants as food supplements and in the treatment of specific diseases dates back to antiquities. In actual facts, herbal preparations and their therapeutic uses can be traced to the origin of man himself. Although several synthetic drugs are available, attention is currently being focused on the use of plants and plant products in prevention or correction of various metabolic disorders or in the treatment of specific diseases because of several side effects associated with the use of synthetic drugs.^{1,2} Several plants are now known to have medicinal effects across the different regions of the world. Some of these have been demonstrated to be of significant value in the treatment of diabetes and its complications. Different laboratories have reported

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Dr A. Bernard Saba Department of Veterinary Physiology Biochemistry and Pharmacology University of Ibadan, Ibadan, Nigeria Phone numbers: +2348033963853, +2348054138127 E mail: sabadee2000@yahoo.com the lipid lowering effects of *Murraya koenigii* (curry leaf) on alloxan-induced diabetics in male Wistar rats and *Artemisia sphaerocephala* (Krasch seed) polysaccharide have also been shown to have hypoglycaemic as well as hypocholesterolaemic effects in diabetic rats.^{3,2} Similarly, *Eruca sativa* seed oil has also been shown to ameliorate biochemical alterations in alloxan-induced diabetes in rats.⁴

In the present study, the hypoglycaemic effect of *Parquetina nigrescens* on alloxan induced diabetes was evaluated in rats. Its ameliorative effect on anaemia and erythrocyte osmotic fragility was also investigated. *Parquetina nigrescens* is an herbaceous, perennial twine belonging to the family *Asclepiadaceae*. It is usually woody at the base and measures between 7-8 m in length. The plant is commonly found growing on ant-hills across the African regions, from Senegal to Nigeria, and over the Congo basin down to south tropical Africa.⁵ *Parquetina nigrescens* has been found to contain cardenoides, glycosides and alkaloids.^{5,6} It has been shown to possess haematopoietic activities, increasing erythrocytes indices in anaemic rats on dose basis.^{7,8} It also

stimulates increased uterine contraction as a result of mobilization of extracellular calcium in a manner that is similar to the effects of oxytocin.⁹

Methods

Experimental animals

Twenty five (25) male Wistar rats weighing 70-140g were obtained from the Experimental animal unit of the Faculty of Veterinary Medicine, University of Ibadan for the experiment. They were divided into five groups A-E with each group containing five rats. The rats were maintained on pelletized rats grower feed (Vital Feed Ltd, Nigeria) while water was provided *ad libitum*. Diabetes was induced in the animals in groups C-E. However rats in group A which serve as the control were given normal saline while those in group B were given only aqueous extract of *Parquetina nigrescens*.

Induction of diabetes

Diabetes was induced in animals in groups C-E using Alloxan monohydrate (5% W/V) (BDH Laboratory Reagents and Chemical Limited, Poole, England). The alloxan was administered intraperitonealy at a dosage of 100mg/kg body weight. Fasting glucose level was determined in all the five groups 72 hours after induction of diabetes and continually measured weekly afterwards for four weeks with glucometer test strips (Accucheck Advantage II).

Extract preparation

Freshly harvested leaves of Parquetina nigrescens were identified at the Forest Research Institute (FRIN) Ibadan, Nigeria with voucher number FHI 107128. The leaves were air-dried, grinded into powder using electric blender/mill grate and was then soaked in 300 ml of distilled water for 24 h. The resultant mixture was filtered with cheesecloth and the filtrate concentrated under reduced pressure at 40°C for 20 min using a rotary evaporator (Gallenkamp UK). The resulting residue, the aqueous extract, was stored at 4°C10. 1000mg/ kg of the extract was prepared by dissolving 20g of the concentrated extract in 100ml of distilled water and was administered to rats in groups B (1000mg/kg of Parquetina nigrescens alone) and C (1000mg/kg of Parquetina nigrescens plus Alloxan), animals in group D received (Alloxan alone) while animals in group E were administered with 1000mg/kg of Parquetina nigrescens and (Diabenes[®], Pfizer Plc) at a dosage of 25mg/kg body weight through oral cannula. All treatments were administered orally on a daily basis for a period of 4 weeks following the induction of diabetes by alloxan. Animals in group A however were given sterile normal saline throughout the period of the experiment.

Body weight

Each animal was weighed daily using mouse balance (Adventurer SL, Switzerland), for the period of four weeks which the experiment lasted.

Blood sample collection and determination of haematological parameters

After four weeks of experiment, 5 ml of blood each was collected from all the rats through the retroorbital venous sinus into heparinized bottles, and was analyzed immediately. The packed cell volume (PCV) was determined by microhaematocrit method; concentration haemoglobin (Hb) bv Cyanmethaemoglobin method, red blood cell (RBC) and white blood cell (WBC) counts were obtained by the haemocytometer methods. From the haematological parameters obtained, the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated.¹¹ The platelet count was determined by manual counting on improved Neubauer slide.

Erythrocyte osmotic fragility

The osmotic fragility of erythrocytes was determined as described previously by using 1% phosphatebuffered sodium chloride (NaCl) solution diluted to concentrations ranging from 0.0% to 0.9% NaCl.¹² The percentage haemolysis in each NaCl concentration was determined using the tube with distilled water (0.0% NaCl) as having maximum haemolysis (i.e. 100% haemolysis).

Statistical analysis

The results were analyzed by one way analysis of variance (ANOVA) and Tukey comparison of individual groups, in Graphpad Prism 4 (2003) statistical software. Difference of means were considered significant at P < 0.05.

Results

Blood Glucose

As shown in Table 1, at the first week of treatment, the fasting blood glucose increased immediately after induction of diabetes with alloxan in groups C and D, being significantly higher (P<0.001) than that of either group A or B. Animals in group E that were

treated with chlorpropamide on the other hand showed normal blood glucose level that is comparable to those of the animals in groups A and B in which diabetes was not induced. It was significantly lower (P<0.01) than the blood glucose obtained in groups C and D. As from the second week of treatment, there was a drastic reduction in the blood glucose of the diabetic animals in group C that were placed on 1000mg/kg of *Parquetina nigrescens* extract to values comparable to those of the animals in the control group (group A) and animals placed on chlorpropamide. On the contrary, blood glucose levels of the diabetic rats in group D (untreated group) remained persistently high. It was significantly higher (P<0.001) than those of animals in the other four groups (A, B, C and E).

Table 1: The fasting blood glucose (mean \pm SD) of alloxan-induced diabetes (mmol/L) in Wistar rats during four weeks of treatment with crude extract of *Parquetina nigrescens* and chlorpropamide.

Group Week	Group A Control(5)	Group B Extract alone (1,000mg/kg) (5)	Group C Alloxan + Extract (1,000mg/kg)(5)	Alloxan alone (5)	Group E Alloxan + chlorpropamide (5)
Week 1	4.42 ± 0.49	4.88 ± 0.43	$8.89 \pm 0.22^{\mathrm{ab^{**}e^{**}}}$	$8.96 \pm 2.41^{ab^{**}e^{**}}$	5.66 ± 1.42
Week 2	3.90 ± 0.29	4.28 ± 1.54	$5.12 \pm 0.08^{a^{***}}$	$8.60 \pm 0.98^{\mathrm{abce}^{***}}$	$5.42 \pm 0.63^{a^{***}}$
Week 3	4.24 ± 0.27	4.88 ± 0.45	5.28 ± 0.45	$11.68 \pm 0.98^{\text{abce}^*}$	$5.70 \pm 0.63^{a^{**}}$
Week 4	5.20 ± 0.43	5.52 ± 0.55	5.30 ± 0.26	$16.46 \pm 2.70^{\text{abce}^{*}}$	** $6.90 \pm 0.41^{\text{ac}^{\text{***b}^{\text{**}}}}$

Superscript alphabets are significantly different from their corresponding group. Asterisks indicate level of significance. * = P < 0.05, ** = P < 0.01, *** = P < 0.001

Haematology

Table 2 shows the effects of aqueous extract of Parquetina nigrescens and chlorpropamide on the haematological parameters in alloxan-induced diabetes in the Wistar rats. The PCV and RBC count of the untreated diabetic animals (group D) were significantly lower (P<0.05) than those of either the control (group A) or those given Parquetina nigrescens extract only (group B). They were also lower (P < 0.01) than those of the diabetic rats (group C) treated with aqueous extract of Parquetina nigrescens. The PCV of the untreated diabetic rats was also significantly lower (P<0.001) than that of the diabetic rats treated with chlorpropamide (group D). The Haemoglobin concentration of the untreated diabetic rats was also significantly lower (P < 0.05) than that of either group A or B. It was also lower than that of the diabetic animals treated with the extract (P<0.05) and those treated with chlorpropamide (P<0.01).

In like manner, the MCHC of the untreated diabetic rats was significantly lower (P<0.001) than that of either the control or that of diabetic rats treated with the extract as well as that of the diabetic rats treated with chlorpropamide. It was also lower (P<0.01) than the value obtained in animals that received alloxan alone (group B). There was however no significant difference in the MCV and MCH among all the groups. The platelet count of the rats in groups A, B, C and E were significantly higher (P<0.001) than that of the untreated diabetic rats (group D) while platelet count obtained in diabetic rats treated with the extract was higher (P<0.001) than that of either the control group or that of group B which received alloxan alone. Similarly, platelets count in the animals treated with chlorpropamide was higher than that of either the control or the rats placed on the extract alone.

Table 2: Haematological parameters of alloxan-induced diabetic rats after four weeks of treatment with aqueous extract of *Parquetina nigrescens* and chlorpropamide. Values are expressed as mean \pm SD.

Group Haemat-	Group A Control(5)	Group B Extract alone	Group C Alloxan +	Group D Alloxan alone	Group E Alloxan +
ological		(5)	Extract (5)	(5)	chlorpropamide (5)
parameters					
PCV %	41.00 ± 6.04	41.80 ± 3.35	42.60 ± 2.51	$28.8 \pm 10.08^{\text{ab*c**e***}}$	46.61 ± 2.88
RBCx 10 ⁶ /µL)	6.88 ± 1.31	6.72 ± 0.58	7.32 ± 0.56	$5.05 \pm 1.69^{\mathrm{ab*c^{**}}}$	7.66 ± 0.21
Hb g/dl	13.00 ± 0.52	12.54 ± 0.95	12.60 ± 0.52	$9.54 \pm 3.37^{abc*e^{**}}$	13.86 ± 0.58

Continuation of table 2							
Group	Group A	Group B	Group C	Group D	Group E		
Haemat-	Control(5)	Extract alone	Alloxan +	Alloxan alone	Alloxan +		
ological		(5)	Extract (5)	(5)	chlorpropamide (5)		
parameters							
MCV fl	60.40 ± 4.34	62.40 ± 3.85	58.40 ± 2.97	57.00 ± 2.86	60.80 ± 2.68		
MCH pg	20.00 ± 4.47	18.60 ± 1.14	17.20 ± 0.84	19.10 ± 1.46	18.00 ± 0.45		
MCHC g/dl	32.60 ± 5.27	29.80 ± 1.48	29.60 ± 1.14	$16.00 \pm 0.25^{\text{ace}^{***b^{**}}}$	30.00 ± 0.71		
Platelets x $10^3/\mu$ L)	41.18 ± 0.35	43.20 ± 1.53	$58.22 \pm 3.77^{abd^{***}}$	$1.09 \pm 0.25^{abc^{***}e^{**}}$	$64.78 \pm 9.95^{abd^{**}}$		
WBC x10 ³ / μ L) 8	$8.22 \pm 0.92^{\text{bd}^{***}}$	5.48 ± 0.77	$18.26 \pm 10.6^{\rm abde^{*}}$	5.76 ± 0.72	$8.70 \pm 2.80^{\mathrm{bd}^{**}}$		

Superscript alphabets are significantly different from their corresponding group. Asterisks indicate level of significance. * = P<0.05, ** = P<0.01, *** = P<0.001

Contrary to the observed trend in the other parameters, the total white blood cell (WBC) count was highest in the diabetic rats treated with Parquetina *nigrescens* extract. It was significantly higher (P < 0.05) than that of the control or that of the non-diabetic rats placed on the extract. It was also higher (P < 0.05)than WBC values obtained in the untreated diabetic

animals and those treated with chlorpropamide. WBC count in the control group was however higher (P < 0.001) than that of either group B or D. Similarly, WBC count obtained in the animals treated with chlorpropamide was higher (P < 0.01) than those of group A or B.

Table 3: Variations in the organ weights (g) of alloxan-induced diabetic rats after treatment with *Parquetina nigrescens* extract and chlorpropamide. Values are mean \pm SD.

	Group A	Group B	Group C	Group D	Group E
	Control(5)	Extract alone (5)	Alloxan +	Alloxan	Alloxan +
			Extract (5)	alone (5)	chlorpropamide (5)
Liver	4.32 ± 0.37	$3.70 \pm 0.96^{d^*}$	$3.36 \pm 0.44^{\mathrm{ae}^{**}d^{***}}$	4.88 ± 0.55	4.26 ± 0.14
Kidney	0.90 ± 0.12	$0.72 \pm 0.08^{a^*d^{**}}$	$0.65 \pm 0.19^{\rm ad^*}$	0.91 ± 0.11	0.83 ± 0.18
Spleen	0.66 ± 0.17	$0.48 \pm 0.09^{a^*e^{**}}$	$0.52 \pm 0.21^{e^*}$	0.61 ± 0.13	0.73 ± 0.14
Heart	0.48 ± 0.07	$0.43 \pm 0.02^{d^*}$	0.52 ± 0.20	0.46 ± 0.03	0.52 ± 0.12

Superscript alphabets are significantly different from their corresponding group. Asterisks indicate level of significance. * = P<0.05, ** = P<0.01, *** = P<0.001

Erythrocyte osmotic fragility

As shown in Figure 1 and 2, at 0% NaCl (distilled water) the erythrocyte osmotic fragility of the normal non-diabetic rats (control) was significantly higher (P < 0.01) than that of the diabetic rats treated with Parquetina nigrescens. It was also significantly higher (P < 0.05) than that of the diabetic rats treated with chlorpropamide. At 0.1% NaCl concentration, the fragility of erythrocytes of the untreated diabetic rats (Grp D) was significantly higher (P < 0.05) than that of the rats treated with chlorpropamide (Grp E). Finally, at 0.7% NaCl the erythrocytes of the nondiabetic rats treated with the extract has lower osmotic fragility than those of the diabetic rats treated with the extract.

Organ weight

Following treatment of the alloxan-induced diabetic rats with Parquetina nigrescens extract and Chlorpropamide, the mean weight of the liver of the diabetic rats treated with the extract (Group C) was significantly lower (P<0.01) than those of either the control or those treated with chlorpropamide (Table 4). It was also lower (P < 0.001) than those of the untreated rats (Grp D). Similarly, it was lower (P < 0.05) than that of the non-diabetic rats given the extract (Grp B).

	Group A	Group B	Group C	Group D	Group E
	Control(5)	Extract alone (5)	Alloxan	Alloxan	Alloxan +
			+ Extract (5)	alone (5)	chlorpropamide (5)
Initial body weight (g)	122	199	125	117	133
Final body weight (g)	170	132	105	112	162
Percentage weight gain/loss (%)	+39.3	-33.7	-16	-4.3	+22.5

Table 4: percentage weight gain of alloxan-induced diabetic rats treated with aqueous extract of *Parquetina nigrescens* and chlorpropamide.

The mean weight of the kidneys of the diabetic rats that were given the extract was also lower (P < 0.05) than those of either the control or the untreated diabetic rats, while the weight of the kidneys of the rats in group B were also lower (P<0.05) than those of the control, as well as those of the untreated group (P<0.01). In like manner, the spleen of the rats in group B were significantly lower in weight (P<0.05) than that of the control. It was also lower (P < 0.01) than that of the diabetic rats treated with chlorpropamide. Mean weight the kidney of the rats treated with chlorpropamide was also higher (P < 0.05) than that of the rats in group C (alloxan + extract) only the heart of the normal non-diabetic rats administered with the extract showed any significant variation. It was lower (P<0.05) than that of the untreated diabetic rats (Group D).

Average weight gain or loss by the animals

There was a considerable weight loss in all the rats that were given the aqueous extract of *Parquetina nigrescens* (Groups B-D) while those rats in the control group as well as those treated with chlorpropamide (Table 4) showed increased weight. Animals in group B lost 33.7% of their body weight while those in groups C and D lost 16% and 4% body weight respectively. Animals in group A (control) had 39.3% weight increase while those in group E added 22% to their weight.

Discussion

The use of *Parquetina nigrescens*, in this study has demonstrated its antidiabetic properties, significantly lowering blood glucose in the alloxan-induced diabetes to values that are comparable to those of the non-diabetic control consistently for a period of 4 weeks. The glucose lowering effects was even more pronounced than the effects of chlorpropamide (Table 1). This is the first time the antidiabetic property of *Parquetina nigrescens* is being reported, although it had been shown to have uterotonic and cardiotonic activities.9,13 The antidiabetic properties of Parquetina nigrescens is comparable to those of several plants which have been demonstrated to possess hypoglycaemic and antidiabetic actions, for example, significant antidiabetic and antihyperlipidaemic effect was reported with neem seed (Azadirachta indica) on alloxan induced diabetes in rats,14 while the leaves extract was found to antagonize the glycogenolytic effects and increased peripheral utilization of glucose by epinephrine in alloxan and streptozocin induced diabetic and normal rats.^{2,15} Similarly, chronic administration of crude aqueous extracts of Momordica charantia and Swertia chirayita also showed hypoglycemic effect in streptozocin treated rats and mice. Although S. chirayata was more effective.¹⁶ Aqueous leaves extract of Murraya koenigii (curry leaf) has also been demonstrated to lower the lipid profile of alloxan induced diabetic rats³ thereby reducing the risk of cardiovascular complications in diabetes as a result of its antioxidant properties. Eruca sativa seed oil also ameliorates the oxidative damage induced by alloxan diabetic rats as a result of its antioxidant properties.4

After being taken up by the pancreatic **β** cells via GLUT 2 glucose transporters, alloxan generates reactive oxygen species (ROS) in a cyclic redox reaction with its reduction product, dialuric acid, autoxidation of which generates superoxide radicals, hydrogen peroxide and, in a final iron-catalyzed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the beta cells.¹⁷ It may then follow that the extract acts as an antioxidant blocking the formation of the reactive oxygen species; mechanism of which remains to be elucidated. Following treatment of the alloxan-induced diabetes, apart from its ameliorative effects on the blood glucose of the diabetic rats, the normocytic hypochromic anaemia

observed in the diabetic rat as a result of reduction in haemoglobin concentration, haematocrit, mean corpuscular haemoglobin as well as mean corpuscular haemoglobin concentration was also corrected by the extract.¹⁸ This is similar to earlier reports that observed significant increase in the red blood cell count, haemoglobin concentration, haematocrit and reticulocytes count in rats that have suffered from acute blood loss after treatment with Parquetina *nigrescens* extract.^{7,8} In the current study, there was also severe thrombocytopenia in the diabetic rats which was corrected in those rats treated with Parquetina *nigrescens* in a manner that is similar to the effect of chlorpropamide (a standard antidiabetic drug). Apart from the ameliorative effects of the extract on the erythrocyte parameters, the diabetic rats treated with the extract also showed leucocytosis whereas normal rats given the extract as well as the untreated groups had leucopenia. This is an indication of increased cell mediated immunity in the diabetic rats that were treated with the extract. The activity of the extract on the total leucocyte count might not be unconnected with the stimulating effects of Parquetina *nigrescens* on haemopoiesis generally, (characterized by increased PCV, RBC and WBC values) which had previously been reported in experimentally induced acute haemorrhagic anaemia in rats.^{7,8} Since the extract did not significantly increase the total WBC in the normal rats, but only in the diabetic rats, its effects may probably be on stimulation of erythropoietin, thrombopoietin and granulopoietin production in response to anaemia, thrombocytopenia and leucopenia associated with alloxan induced diabetes observed in the present study.

The effects of alloxan induced diabetes has been demonstrated to be due to oxidative damage following the generation of superoxide radicals as a result of increased xanthine oxidase production, an enzyme involved in the generation of superoxide radicals.^{17,19} Glucose auto-oxidation, protein glycation, and overproduction of superoxide radicals in mitochondria and via NAD(P)H oxidase have also been incriminated in the pathogenesis of oxidative damage in diabetes mellitus.²⁰ These may be responsible for the observed enlargement of the liver, kidney and the heart in the untreated diabetic group in this study while those treated with the extract and chlorpropamide showed relatively normal weight of these organs. Similarly, hyperglycaemia associated with diabetes has been shown to increase the erythrocyte osmotic fragility and membrane lipid peroxidation in human erythrocytes.²¹ Recently,

oxidative damage associated with diabetes mellitus in the dog was reported lead to significant increase in RBC thiobarbituric acid reacting substance (TBARS).²² Increased TBARS level on the other hand has been known to be associated with increased fragility of erythrocytes during oxidative stress.²³ In the current study, the erythrocytes of the diabetic rats showed increased fragility at 0.1% NaCl concentration and at 0.7% while the extract conferred some degree of stability to the erythrocytes of the normal non-diabetic rats that were given the extract (group B) as well as the diabetic rats treated with the extract. This is in agreement with the observations who reported higher resistance to osmotic lysis in anaemic rats that were treated with Parquetina *nigrescens* extract.⁸ This was attributed to elevated level of reticulocytes which are more resistant to osmotic lysis in the blood; an indication of regeneration and increased erythropoiesis in the rats treated with Parquetina nigrescens extract, which may be true of the current study. The increased osmotic resistance observed in the current study may also be due to the ameliorative effects of the extract on the oxidative damage associated with diabetes.

However, the mechanism by which the extract ameliorates the oxidative damage, which may either, be by scavenging the superoxide radicals or by increasing the levels of endogenous antioxidants such as glutathione are subjects for further investigation. Finally, Parquetina nigrescens extract also reduced the body weight in all the groups that received the extract. This may be due to lipid or cholesterol lowering effects of the extract, but this also requires further investigation. From the foregoing, it can be deduced that Parquetina nigrescens reduced hyperglycaemia and corrected the normocytic hypochromic anaemia associated with diabetes. It also increased the erythrocytes resistance to osmotic lysis as well as reduced oxidative damage to the liver, kidney and the heart whilst also reducing the body weight. The molecular mechanism(s) underlying with the antidiabetic properties of Parquetina nigrescens will be investigated in our laboratory. Also, the isolation and quantification of the plant materials by Thin layer chromatography (TLC), High performance liquid chromatography (HPLC) and Revised high performance liquid chromatography (RHPLC) of the various active principles with the aim of identifying the isolate (s) that confer the antidiabetic properties on Parquetina nigrescens will be investigated.

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