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Effects of Oral Administration of *Garcinia kola* Seeds on Haematological and Defence Parameters of Diabetic Rats

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ABSTRACT

The effects of oral administration of *Garcinia kola* seeds on haematological and defence parameters of diabetic rats were investigated. Thirty acclimatized wistar rats weighing between 240-250 g were divided into six groups of five animals per group ($n = 5$). The first two groups of rats; non-diabetic control and non-diabetic treated groups orally received normal saline and 600 mg kg^{-1} b.wt. of *Garcinia kola* seed powder (GKP), respectively. The last four groups which were made diabetic by intra-peritoneal injection of alloxan monohydrate had one diabetic control that orally received normal saline and three diabetic groups that got 300, 600, 900 mg kg^{-1} b.wt. of GKP. The GKP was then given twice daily for 21 days after which the animals were sacrificed, blood collected by cardiac puncture for glucose and haematological analysis. Results showed that GKP significantly reduced blood glucose, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and lymphocyte of diabetic rats. It significantly increased RBC, WBC, PCV, neutrophil and monocyte of diabetic treated rats. This study therefore depicts GKP as a hypoglycaemic agent with potential to normalize aberrant haematological and defence parameters associated with diabetes.

Key words: *Garcinia kola*, haematological, diabetic, hypoglycaemic, defence parameters

INTRODUCTION

Diabetes mellitus is the commonest endocrine disorder known to man. WHO (1999) estimated that there were 135 million people in the world with diabetes and that this figure would rise to 380 million by 2025. This report also pointed out that low and middle income countries will bear the brunt of the increase with Africa contributing significantly to this rise (King *et al.*, 1998). Diabetes primary defect in fuel metabolism and this culminates in widespread multi-organ complications that ultimately affect every system of the body including the hematopoietic system.

The laboratory determination of blood products and parameters for the purpose of disease diagnosis is highly accurate, sensitive and reliable and has remained the bedrock of ethical and rational research, disease diagnosis, prevention and treatment (Okokon *et al.*, 2004). Reactive Oxygen Species (ROS) have been implicated in the mechanism of damage of red blood cells in diabetic patients (Vives Corrons *et al.*, 1995). As a consequence, complications develop which consist of mainly abnormalities in function, morphology and metabolism of erythrocyte, leucocyte and platelets (Comazzi *et al.*, 2004). It is an established fact that that RBC and WBC decrease in diabetic than non-diabetic patients (Palmieri *et al.*, 2001). Anaemia has also been identified succinctly as a common complication of diabetes mellitus (Thomas *et al.*, 2005).

The use of herbs has a long drawn history in health care delivery in Africa. Several plants are now being used in parts or whole to treat and manage many diseases (Adedeji *et al.*, 2006). The plant *Garcinia kola* is one of such plants. *Garcinia kola*, commonly called bitter kola is found mainly in tropical rain forest region of Central and Western Africa (Uko *et al.*, 2001). It is used for traditional medicine purposes for the treatment of cirrhosis and hepatitis (Okwu, 2005), treatment of chest cold (Iwu, 1986), hepato-protective effects (Braide, 1991), anti-cancer (Farombi *et al.*, 2005), anti-diarrhoea, antifungal (Okwu and Morah, 2007) and antibacterial (Adegboye *et al.*, 2008). Its hypoglycaemic effects have also been reported (Nwangwa, 2012; Udenze *et al.*, 2012a, b).

Conventional treatment of diabetes is based on oral hypoglycaemic agents and use of insulin. Unfortunately these agents do not restore normal glycaemic state and even fail after some time; these are outside the numerous complications or side effects they present on prolonged usage. The need to discover an oral hypoglycaemic agent that will not only restore normoglycaemia but also ameliorate the gamut of complications associated with diabetes becomes apparent. One of such pragmatic efforts is the use of *Garcinia kola* seeds as an oral hypoglycaemic agent. The study therefore sets out to determine the effects of oral administration of *Garcinia kola* seed on haematological and defence parameters of alloxan-induced diabetes rats.

MATERIALS AND METHODS

Plant material: Fresh *Garcinia kola* seeds were purchased from Watt Market, Calabar, Nigeria in September 2010. They were identified and authenticated by Mr. Frank Apejaye, a botanist at the Botany Department of the University of Calabar. Voucher samples (No. 176) were kept in the herbarium of Botany Department for record purposes.

Preparation of seed samples: The outer testa of each *Garcinia kola* seed was removed, washed and air dried for about 24 h. Each seed was cut into small pellets with kitchen knife and the resulting pellets were subsequently dried in an electric oven for 12 h at 40°C. The dry seed pellets were ground to fine powder using manual grinder and then sieved with 10 µm sieve. The resulting powder aliquots were used for phytochemical analysis and the remaining reconstituted with normal saline to obtain suspensions of appropriate concentration for oral administration.

Phytochemical screening: A portion of the powder was subjected to phytochemical analysis using (Trease and Evans, 1983; Harborne, 1983) methods to test for alkaloids, tannins, flavonoids, saponins and cardiac glycosides. The intensity of the colouration determines the abundance of the compound.

Acute toxicity (LD₅₀) studies: Acute toxicity studies was done using the probit method and was determined to be 6741.43 mg kg⁻¹ showing that it is relatively non-toxic and doses up to 900 mg kg⁻¹ b.wt. were found to be safe. All doses used in this study were carefully chosen to exclude the lethal dose.

Laboratory animals: Thirty albino wistar rats weighing about 240-250 g were purchased from the animal unit of the department of pharmacology, University of Calabar. The animals were kept in cages to acclimatize with conditions of the animal housing facility with ambient temperature 26-28°C and adequate ventilation for two weeks and fed with standard growers mash (Vita Feeds

Nig. LTD) and clean water *ad libitum*. They were handled in accordance with National Institute of Health (NIH) guide for the care and use of laboratory animals (NRC., 1996).

Induction of diabetes mellitus: A single dose of freshly prepared alloxan monohydrate (Sigma, St Lois MO, USA) in normal saline at a dose 150 mg kg⁻¹ b.wt. (Ebong *et al.*, 2008) was injected intra-peritoneally into twenty rats. Blood samples collected by tail vein tapping were monitored for glucose levels, using a glucometer. After 72 h, rats that had blood glucose level above 200 mg dL⁻¹ were considered diabetic and selected for the study.

Experimental design: The thirty albino wistar rats were divided into six groups of five rats per group. Animals in all groups received orally different doses of GKP as follows:

- Non-diabetic control received normal saline (0.5 mL kg⁻¹)
- Non-diabetic treated group received 600 mg kg⁻¹ of *Garcinia kola* seed (GKP)
- Diabetic control received normal saline (0.5 mL kg⁻¹)
- Diabetic treated I received 300 mg kg⁻¹ of GKP
- Diabetic treated II received 600 mg kg⁻¹ of GKP
- Diabetic treated III received 900 mg kg⁻¹ of GKP

The blood glucose and body weight changes were also monitored every three days during the period. *Garcinia kola* suspended in normal saline was administered to all the test groups twice daily for every 12 h (6.00 am and 6.00 pm) for 21 days. The blood glucose and body weight changes were strictly monitored every three days during the period.

Collection and analysis of samples: At the end of the 21 day period, the animals were fasted for 12 h, anaesthetized with chloroform and sacrificed. Whole blood was collected by cardiac puncture first for glucose analysis and the rest which was emptied in EDTA bottles for haematological analysis. Blood glucose concentration was determined by GOD-PAP method based on Barham and Trinder (1972), Haematological analysis using Sysmex* Automated Heamatology Analyser KX-21N, Sysmex Corporation, Kobe-Japan (whole blood mode) and differential WBC was determined from areas under the WBC histogram as percent of entire WBC volume.

Statistical analysis: Data was collected and analyzed by ANOVA using Statistical Package for Social Science (SPSS) software for windows and *post hoc* testing was performed for inter-group comparison using the Least Significant Difference (LSD). All data was expressed as Mean±Standard error of the mean (SEM). The p<0.05, 0.01 and 0.001 were considered significant.

RESULTS

Phytochemical studies: Phytochemical analysis of *Garcinia kola* seeds revealed the presence of saponins, tannins, flavonoids and glycosides as shown in Table 1.

Effects of oral administration of *Garcinia kola* (GKP) on fasting blood glucose concentration of diabetic and non-diabetic rats: Table 2 presents the results of fasting blood glucose concentration of diabetic and non-diabetic rats. There was a significant (p<0.001) increase in blood glucose concentration of diabetic control animals compared to the non-diabetic controls.

Table 1: Phytochemical studies of *Garcinia kola* seed powder

Components	Presence
Alkaloids	-
Glycoside	++
Flavonoids	++
Tannins	+
Saponins	++

+: Trace amount present, ++: Abundant amount present, -: No amount present

Table 2: Effects of oral administration of *Garcinia kola* seeds on fasting blood glucose of diabetic and non-diabetic rats

Treatments	Fasting blood glucose concentration (mg dL ⁻¹)
Non-diabetic control (GKP, 0 mg kg ⁻¹)	67.50±3.02
Non-diabetic treated (GKP, 600 mg kg ⁻¹)	62.89±3.76
Diabetic control (GKP, 0 mg kg ⁻¹)	350.33±13.29***
Diabetic treated I (GKP, 300 mg kg ⁻¹)	171.12±4.70*** ^c
Diabetic treated II (GKP, 600 mg kg ⁻¹)	139.12±4.69 ^c
Diabetic treated III (GKP, 900 mg kg ⁻¹)	77.92±2.88

***Significantly different from non-diabetic controls (p<0.001), ^cSignificantly different from diabetic control (p<0.001)

Table 3: Effects of oral administration of *Garcinia kola* seed powder (GKP) on RBC, WBC, Hb and PCV of diabetic and non-diabetic rats

Treatment	RBC (×10 ⁶ μL ⁻¹)	WBC (×10 ³ μL ⁻¹)	Hb (g dL ⁻¹)	PCV (%)
Non-diabetic control (GKP, 0 mg kg ⁻¹)	6.31±0.16	16.25±0.44	12.27±0.44	45.00±0.57
Non-diabetic treated (GKP, 600 mg kg ⁻¹)	6.50±0.31	19.32±1.48*	12.38±0.23	44.90±0.64
Diabetic control (GKP, 0 mg kg ⁻¹)	3.25±0.24***	10.92±0.67***	12.15±0.24	38.84±2.46***
Diabetic treated I (GKP, 300 mg kg ⁻¹)	6.59±0.22 ^c	12.32±0.73**	12.00±0.22	40.82±0.39*
Diabetic treated II (GKP, 600 mg kg ⁻¹)	7.44±0.24 ^c	14.86±0.29 ^c	12.18±0.24	45.48±0.38 ^c
Diabetic treated III (GKP, 900 mg kg ⁻¹)	8.39±0.83*** ^c	17.89±0.47 ^c	14.72±0.21*** ^c	46.42±0.53 ^c

Values are Mean±SEM, *Significantly different from non-diabetic controls (p<0.05), **Significantly different from non-diabetic control (p<0.01), ***Significantly different from non-diabetic control (p<0.001), ^cSignificantly different from diabetic control (p<0.001)

Treatment with GKP significantly (p<0.001) attenuated the hitherto increased blood glucose in a dose related manner and almost brought it to normal at the maximum dose.

Effects of oral administration of *Garcinia kola* seed powder (GKP) on RBC, WBC, Hb and PCV of diabetic and non-diabetic rats: Table 3 shows the effect of graded doses of GKP on RBC, WBC, Hb and PCV of diabetic and non-diabetic experimental animals.

There was a significant (p<0.001) reduction in the levels of RBC and WBC of diabetic control animals as compared to the non-diabetic control ones. Treatment with the various doses of GKP, increased significantly (p<0.001) these hitherto reduced levels in a dose response manner compared to the diabetic control. Interestingly, the highest dose of GKP produced a significant (p<0.001) increase in RBC compared to the non-diabetic control. There was no significant difference in the level of haemoglobin across all groups, although the diabetic control recorded the lowest value. The highest dose of the treatment showed a significant increase at p<0.001 compared to the non-diabetic and diabetic control. Treatment with various doses of GKP produced a dose response significant (p<0.001) increase in PCV of diabetic treated groups compared to the diabetic control which was reduced significantly (p<0.001) compared to the non-diabetic control animal values.

Table 4: Effects of oral administration of *Garcinia kola* seed powder (GKP) on ESR, MCV, MCH and MCHC of diabetic and non-diabetic rats

Treatments	ESR (mm h ⁻¹)	MCV (fL)	MCH (pg)	MCHC (g dL ⁻¹)
Non diabetic control (GKP, 0 mg kg ⁻¹)	12.60±0.68	71.50±1.99	19.30±1.18	26.94±1.13
Non diabetic treated (GKP, 600 mg kg ⁻¹)	13.20±0.58	69.66±3.16	19.19±0.79	27.58±0.47
Diabetic control (GKP, 0 mg kg ⁻¹)	12.40±0.24	121.77±11.45***	38.23±2.90***	31.80±2.10**
Diabetic treated I (GKP, 300 mg kg ⁻¹)	12.80±0.37	62.22±2.16 ^c	18.31±0.74 ^c	29.40±0.29
Diabetic treated II (GKP, 600 mg kg ⁻¹)	13.00±0.32	61.41±1.94 ^c	16.31±0.65 ^c	26.65±1.27 ^b
Diabetic treated III (GKP, 900 mg kg ⁻¹)	14.20±0.37* ^a	57.02±4.35 ^c	18.13±1.55 ^c	31.72±0.41

Values are represent Mean±SEM, *, **, ***Significantly different from non-diabetic controls at p<0.05, p<0.01 and p<0.001 respectively.

^{a,b,c}Significantly different from diabetic control p<0.05, p<0.01 and p<0.001 level, respectively

Table 5: Effects of oral administration of *Garcinia kola* seed powder (GKP) on differential WBC count of diabetic and non-diabetic rats

Treatments	Percentage				
	Neutrophils	Lymphocytes	Basophils	Eosinophils	Monocytes
Non diabetic control (GKP, 0 mg kg ⁻¹)	39.00±1.22	51.00±1.00	1.00±0.00	2.40±0.24	5.80±0.37
Non diabetic treated (GKP, 600 mg kg ⁻¹)	51.00±0.71***	40.00±1.58***	1.00±0.00	2.40±0.51	5.60±0.51
Diabetic control (GKP, 0 mg kg ⁻¹)	30.20±0.86***	65.00±3.61***	0.94±0.04	2.60±0.24	4.00±0.32**
Diabetic treated 1 (GKP, 300 mg kg ⁻¹)	36.00±1.22 ^c	53.00±0.84 ^c	1.20±0.20	2.60±0.24	4.00±0.32**
Diabetic treated 2 (GKP, 600 mg kg ⁻¹)	40.00±1.14 ^c	50.00±0.71 ^c	1.20±0.20	2.80±0.20	5.20±0.37 ^a
Diabetic treated 3 (GKP, 900 mg kg ⁻¹)	46.00±0.71*** ^c	46.00±0.71 ^c	1.60±0.24 ^b	2.60±0.24	4.00±0.32**

Values are Means±SEM, **, ***Significantly different from non-diabetic control at p<0.01, p<0.001 ^{a,b,c}Significantly different from diabetic control p<0.05, p<0.01 and p<0.001 level, respectively

Effects of oral administration of *Garcinia kola* seed powder (GKP) on ESR, MCV, MCH and MCHC of diabetic and non-diabetic rats: Table 4 presents the result of the oral administration of GKP on ESR, MCV, MCH and MCHC of diabetic and non-diabetic animals. There was no significant difference in the levels of ESR, except that the highest dose showed a significant increase (p<0.05) compared to normal and diabetic control. Results of red cell indices, showed that MCV and MCH of diabetic animals increased significantly (p<0.001) and that MCHC increased significantly (p<0.01) compared to the non-diabetic controls. Treatment attenuated these increases significantly (p<0.001 and 0.01) compared to the diabetic control.

Effects of oral administration of *Garcinia kola* seed powder (GKP) on differential WBC count of diabetic and non-diabetic rats: Table 5 depicts the results obtained from differential WBC count. The neutrophil fraction in the diabetic control was significantly (p<0.001) reduced compared to the non-diabetic control. Treatment with various doses of GKP produced a significant (p<0.001) dose dependent increase in neutrophil fraction. The lymphocyte fraction of diabetic control increased significantly (p<0.001) as compared to the non-diabetic control animals. Treatment produced a dose related significant (p<0.001) reduction in the lymphocytes. The basophil and eosinophil fractions showed no significant difference across all groups. The monocyte fraction of WBC reduced significantly (p<0.01) in diabetic control compared to normal control. Treatment with GKP only produced significant (p<0.05) elevation in the group that received the 600 mg kg⁻¹ dose compared to the diabetic control.

DISCUSSION

The study evaluated the effects of oral administration of *Garcinia kola* seeds on haematological and defence parameters of alloxan-induced diabetic rats. Results of phytochemical analysis of *Garcinia kola* seeds demonstrated the presence of flavonoids, tannins, glycosides and saponins, as observed by Udenze *et al.* (2012a) and Adesuyi *et al.* (2012).

At the end of the treatment period, blood glucose concentrations of diabetic control rats were significantly higher as compared to non-diabetic controls and non-diabetic treated groups. This finding agrees with the reports of Nwangwa (2012) and Udenze *et al.* (2012b) that also used alloxan as their diabetogenic agent. Alloxan induces diabetes by damaging insulin secreting cells of the pancreas, decreasing or stopping insulin secretion and hence precipitating hyperglycaemia characteristics of diabetes mellitus. Oral administration of graded doses of GKP reduced the increased glucose concentrations in diabetic animals and almost brought the glycaemic state to normal at the highest dose (Nwangwa, 2012; Udenze *et al.*, 2012b). The mechanism for hypoglycaemic effect of GKP could be possibly by modulation of carbohydrate mechanism, restoration of β -cell integrity and function, insulin releasing activity, improvement in glucose uptake or utilization and antioxidant properties (Mansi and Lahham, 2008).

Alterations of haematological parameters and anaemia are common features of diabetes mellitus and these findings have variously been corroborated by researchers (Azeez *et al.*, 2010; Ekor *et al.*, 2010; Musa *et al.*, 2010). This was in line with the present study as alloxan-diabetic rats had significantly deranged blood parameters. It was observed that the RBC, HB, PCV, ESR of diabetic control animals were decreased when compared to non-diabetic control animals and non-diabetic treated animals; while MCV, MCH and MCHC of the diabetic control group were significantly increased compared to the non-diabetic control. Treatment with GKP reversed these reductions in RBC, PCV, HB and ESR bringing them to normal and it appears that these increases were dose related. The observed alteration can be attributed to both effects of alloxan on rapidly dividing haematopoietic cells and suppression of haematopoiesis as a result of insulin deficiency occasioned by the selective damage of β -cells of the islet of Langerhans of the pancreas by alloxan (Azeez *et al.*, 2010; Zhang *et al.*, 2004; Phillips *et al.*, 2004). Reactive Oxygen Species (ROS) have also been implicated in the mechanism of red blood cell destruction (Rao *et al.*, 2003). In diabetes, hyperglycaemia enhances the non-enzymatic glycosylation of haemoglobin and RBC membrane proteins, thus precipitating anaemia typical of alloxan-diabetes (Mohammed *et al.*, 2009). This could possibly be due to increased erythrocyte osmotic fragility that has been consistently observed in diabetic patients as a result of persistent hyperglycaemia (Chattopadhyay and Bandyopadhyay, 2005), peroxidation of membrane lipid and other oxidative damage to the erythrocyte membrane. It is also important to add, that increase in MCV, MCH and MCHC in diabetic rats, showed that alloxan diabetes in this study was associated with anaemia of the macrocytic type and in contrast to Azeez *et al.* (2010) who reported anaemia of the microcytic type.

The possible reason for the attenuation of anaemia by GKP, could be due to the presence of flavonoids and other phyto-constituents which have antioxidant effects, hence scavenging ROS (which damage RBC) and reducing lipid peroxidation of its membrane as well. The favourable effects of GKP on the haematological parameters of the non-diabetic treated group in this study also correlates well with its erythropoietic effects (Oluyemi *et al.*, 2007; Esomonu *et al.*, 2005).

GKP treatment also increased and almost brought to normal at the highest dose the reduced total WBC count caused by diabetes. Azeez *et al.* (2010) also corroborated this finding with another plant which contained copious phytochemicals akin to GKP. It has been suggested that the body's

defence mechanism against infections was usually altered due to diabetes mellitus (Mansi and Lahham, 2008; Musa *et al.*, 2010). This explains the reason why diabetic patients are usually prone to opportunistic infections. In this study, it was demonstrated that GKP treatment increased the diabetic-lowered neutrophil and monocyte count in diabetic treated groups. This therefore showed that GKP treatment might also increase the defence mechanism of the body against infections in the diabetic. Treatment with GKP also reduced significantly the increased lymphocyte count in the diabetic rats, showing that the body was recovering from diseased state. There was no significant difference in levels of the eosinophils and basophils except that the highest dose produced a significant increase in the later when compared to the diabetic control rats.

CONCLUSION

Restoration of normoglycaemia, improvement in haematological and defence parameters of diabetes from the study has demonstrated *Garcinia kola* seed as an anti-diabetic agent with strong potential to ameliorate anaemia and body defence abnormalities associated with diabetes mellitus.

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