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Research Article

Effects of *Strophanthus hispidus* DC. (Apocynaceae) aqueous root extract on antioxidant status in Streptozotocin-induced diabetic rats

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ABSTRACT: *Strophanthus hispidus* is a multipurpose medicinal plant which has been reported to have diverse medicinal uses in the treatment of sexual diseases, malaria, dysentery, stroke, heart failure, gonorrhoea, arthritis, diabetes, snake bites, constipation, inflammatory, rheumatism and ulcers. This study investigated the hypoglycemic and antioxidant effects of *Strophanthus hispidus* aqueous root extract, using the activities of superoxide dismutase, total peroxidases, gamma glutamyl transferase, glutathione - S- transferase, glutathione peroxidase, glutathione reductase, as well as the concentrations of glucose, glutathione, vitamin C, nitric oxide, total thiols and malondialdehyde as indices. Forty rats were divided into five (A, B, C, D and E) Groups. Group A served as control, Group B were streptozotocin – induced diabetes mellitus untreated rats, Group C were streptozotocin – induced diabetes mellitus rats treated with 600µg/ kg body weight of glibenclamide, Group D and E were streptozotocin – induced diabetes mellitus rats treated with 500mg/kg and 1000mg/kg body weight of the extract respectively for fourteen consecutive days. The concentrations of blood glucose, nitric oxide and malondialdehyde were significantly ($p < 0.05$) decreased in all the Groups that received the different doses of extract as compared with the negative control Group (Group B). In conclusion, *Strophanthus hispidus* aqueous root extract exhibited hypoglycemic and antioxidant functions.

KEYWORDS: *Strophanthus hispidus*, diabetes, rats, antioxidant.

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INTRODUCTION

The use of medicinal plant is a major African socio-cultural heritage which has been in existence for hundreds of years. These plants have been the focus for wider coverage of primary health care delivery in Africa and the rest of the world (Elujoba *et al.*, 2005; Okigbo and Mmekka, 2006). There is a general belief that medicinal plants are safer and less damaging to the human body than synthetic drugs. Scientific research should be conducted on safety, efficacy and quality of these medicinal plants (Akerlele, 1993; Okigbo and Mmekka, 2006) Therefore laboratories around the world are engaged in screening of plants for biological activities with therapeutic potential for the management of priority disease like malaria, HIV/AIDS, sickle-cell anemia, diabetes and hypertension (Elujoba *et al.*, 2005). Certain toxic plants are beneficial for health if small amounts are ingested infrequently and in a specific context of illness. A number of plants consumed as food are recognized as having pharmacological properties alongside their nutritional value and are purposely ingested as medicine in certain traditional human societies. Additionally, a large number of drugs used in modern medicine are, or have been, obtained from plants, or were discovered from their traditional use (Masi *et al.*, 2012).

Strophanthus hispidus DC belongs to the family of plants known as Apocynaceae. They are popularly known as poison arrow vine, brown strophanthus and hairy strophanthus in western part of Africa including Nigeria (Ayoola *et al.*, 2008; Ojiako and Igwe, 2009; Agbaje and Fageyinbo, 2012; 2014; Agyare *et al.*, 2013; Ezuruike and Prieto, 2014; Balde *et al.*, 2015). *Strophanthus hispidus* DC (Apocynaceae) is a climbing shrub of the open savanna woodland, reaching 16 m long, and occurs widely throughout West African countries (Ayoola *et al.* 2008; Agyare *et al.*, 2013; Ishola *et al.*, 2013). It is native mainly to West, Central and tropical Africa, extending to South Africa, with a few species in Asia, from southern India to the Philippines and southern China. Common names in Nigeria include “Sagere”, “Isagere”, “Isagira” (Yoruba tribe of southwest Nigeria), “Ota”, “Kwan-kwani”, “Osisi” (Igbo tribe of southeast Nigeria) and “Kaguru” (Hausa tribe of northern Nigeria) (Ishola *et al.*, 2013). *Strophanthus hispidus* is a multipurpose medicinal plant which has been reported to have diverse medicinal uses; for example, in the Savannah Zone of West Africa, the latex and seeds of *Strophanthus hispidus* are used as arrow poison, while decoctions of root, stem bark or leaf are used externally to treat skin diseases, leprosy. Also various parts of the plant are used as analgesic and in the treatment of sexual diseases, malaria, dysentery, stroke, heart failure, gonorrhoea, arthritis, diabetes, snake bites, constipation, inflammatory, rheumatism and ulcers (Ishola *et al.*, 2013; Ojiako and Igwe, 2009; Ezuruike and Prieto, 2014; Agbaje and Fageyinbo, 2012; 2014). In Nigeria and Ghana, the root decoction is ingested to treat rheumatic diseases, while in Togo; the root bark macerate is employed for treating oedema (Ishola *et al.*, 2013; Agbaje and Fageyinbo, 2012; 2014). While boiled stem and leaves with palm wine are used for the treatment of

swollen stomach and burning sensation in heart (Uzodimma, 2013). Preliminary phytochemical screening by Ayoola *et al.* (2008) showed that the methanol root/stem extracts tested positive for flavonoids, tannins, cardiac glycosides and terpenes and negative for saponins, phlobatannins and anthraquinones. Most of the respondents attested to the common use of *Strophanthus hispidus* as a “great medicine” used to strengthen others in medicinal preparations (Uzodimma, 2013).

This study was carried out to investigate the hypoglycemic and antioxidant effects of *Strophanthus hispidus* aqueous root extract using the activities of superoxide dismutase (SOD), total peroxidases (POD), gamma glutamyl transferase (GGT), glutathione-S-transferase (GST), glutathione peroxidase (GPX), glutathione reductase (GR), as well as the concentrations of glucose, glutathione (GSH), vitamin C (ASC), nitric oxide (NO), total thiols (T.SH) and malondialdehyde as indices

MATERIALS AND METHODS

Chemical and reagents: All chemical and reagents used were of pure quality and analytical grade. Chemicals used were purchased from Sigma Chemical Co., Saint Louis, MO, USA and British Drug House (BDH) Chemical Ltd., Poole, UK.

Preparation of Plant Extract: Fresh roots of *Strophanthus hispidus* were harvested from neighbouring bushes in Odeda, Odeda Local Government Area, Ogun State, Nigeria. Sample of the root with its leaves was identified and authenticated by a botanist (Prof. D.A. Agboola) from the Department of Botany, Federal University of Agriculture Abeokuta, Nigeria. The harvested fresh roots of *S hispidus* were cleaned, chopped into tiny bits and air dried at room temperature for some weeks to obtain a constant weight. A known weight was boiled in a measured volume of distilled water on a hot plate for 1 hour; and there after macerated for 72 hours before filtering through sterile cotton wool. The filtrate was thereafter evaporated to dryness in an oven at 40° C. Percentage yield (w/w) of 5.48 % was obtained (Agbaje and Fageyinbo, 2012).

Experimental animals, induction of diabetes and administration of the extracts: Forty eight (48) adult female albino rats were purchased from the College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Nigeria. They were kept in well ventilated animals cages and allowed to acclimatize for two weeks with free access to feed and water *ad libitum*. Out of the forty eight rats, eight were separated to serve as control (Group A), while others were induced diabetes with streptozotocin (STZ) injection intraperitoneally (60mg/kg body weight), which was dissolved in 0.1M citrate buffer, pH 4.5, after overnight fasting (12 hours). Control rats (Group A) were injected 0.1M citrate buffer. The other animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced

hypoglycemia. The blood glucose levels were analyzed three days (within 72 hours) after injection of streptozotocin using the blood from the tail end of each rat (after overnight fasting) with the aid of glucometer (Accu Chek Active). Animals demonstrating hyperglycemia (>250 mg/dL) were considered diabetic and were used. Thirty two hyperglycemic rats were divided into four (B, C, D and E) Groups of eight rats each. Group A served as control and were administered distilled water, Group B were untreated streptozotocin – induced diabetes mellitus rats that served as negative control and were given distilled water, Group C were streptozotocin – induced diabetes mellitus rats treated with 600µg/ kg body weight of glibenclamide (a standard drug for treatment of diabetes) which served as standard, Group D were streptozotocin – induced diabetes mellitus rats treated with 500mg/kg body weight of *S hispidus* and Group E were streptozotocin – induced diabetes mellitus rats treated with 1000mg/kg body weight of *S hispidus*. Oral treatment of the diabetic rats with specified doses of *S hispidus* aqueous root extract and glibenclamide was carried out daily for fourteen days. The blood glucose level was determined using the blood from the tail end of each rat (after overnight fasting) with the aid of glucometer at baseline (before the whole experiment started), day 0 (when the animals were confirmed diabetic), days 5 and 10 (during treatment with the extract and standard drug). On the fifteenth day, just before sacrificing the rats, the blood glucose level was determined using the blood from the tail end of each rat (after overnight fasting) with the aid of glucometer. Then the animals were anaesthetized under light diethyl ether and sacrificed. Blood was collected by cardiac puncture into heparinized tubes in order to estimate the biochemical parameters, while their livers and pancreas were excised for biochemical examinations. Approval of the Departmental ethical committee was obtained before commencement of the study.

Preparation of plasma and RBC

Whole blood in the heparinized tubes was centrifuged at 3,000 rpm for 10 minutes to separate the plasma for biochemical analysis. Buffy coat was also removed from the remaining layer and RBCs were washed with 10 mM phosphate buffered saline (PBS, pH 7.2). The washing of the RBCs was carried out by suspending them in PBS solution (2 times volumes of RBCs) and mixed gently by inversion of the tube. The supernatant was discarded after centrifugation at 2,000 rpm for 5 minutes; the washing of RBCs continued until the supernatant was clear, and the washed RBCs were further used for biochemical analysis (Akinloye *et al.*, 2014).

Preparation of organ (liver and pancreas) homogenate

Ten percent liver / pancreas homogenate was prepared by homogenizing 0.2 grams of excised washed (with 0.9% NaCl) liver / pancreas in PBS solution to make up a total suspension of 2 ml. The suspension was then centrifuged at 3,000 rpm for 10 minutes and the supernatant was used for biochemical analysis (Akinloye *et al.*, 2014).

Biochemical assays

Nitric oxide concentrations were determined indirectly using a spectrophotometer based on the principles of the Griess reaction, as described by Nathan and Matthew (2007). Total peroxidases (POD) activities in the plasma, red blood cells, pancreas and liver were assayed spectrophotometrically as described Zhang *et al.* (2005). Reduced glutathione (GSH) in the plasma, red blood cells, pancreas and liver was estimated by the method of Ellman (1959). Total thiols (T. SH) content was quantified in the pancreas and liver by the method of Sedlak and Lindsay (1968). The concentration of plasma ascorbic acid was estimated according to the method described by Rajarajeswari and Pari (2011). Malondialdehyde (MDA) concentration was estimated in the plasma, red blood cells, pancreas and liver according to the method of Fernandez *et al.* (1997). Total protein was estimated according to the method of Gornal *et al.* (1949). Superoxide Dismutase (SOD) activity was measured according to the method of Marklund and Marklund (1974). Glutathione peroxidase (GPX) activity was measured according to the method of Rotruck *et al.* (1973). GR activity was determined by method of Ramos-Martinez *et al.* (1983). Glutathione-S-Transferase (GST) activity was determined according to the method of Habig *et al.* (1974). Gamma glutamyl transferase (GGT) assay was carried according to the method of Persijn and Van der Slik (1978).

Statistical analysis

Data thus obtained were analyzed using the Statistical Package for Social Science (SPSS) 17.0 version and were expressed as Mean ± S.E.M (Standard Error of Mean). The level of homogeneity among the Groups was tested using Analysis of Variance (ANOVA). Where homogeneity occurred, the Groups were separated using the Duncan Multiple Range Test (DMRT).

RESULTS

The results on the effects of the extract on the blood glucose are shown in Figure 1. The results showed that on baseline before the administration of extract, the blood glucose levels were not significantly different in all the Groups treated with streptozotocin. On day zero, before the administration of the treatments (extracts and glibenclamide), the blood glucose levels were greatly increased significantly ($p < 0.05$) compared with the control. On days 5, 10 and 15 after the administration of extract, the blood glucose levels were significantly lower in all the Groups that received the different doses of extract compared with the negative control Group (Group B) with exception of Group E which had almost the same blood glucose level on day 15. A drastic decrease in blood glucose was observed in the Groups treated with the extract (Groups D and E) at day 5 compared with Groups B and C respectively. Blood glucose levels were significantly lower in the positive control Group (Group A) compared with all the other Groups and at no time did the blood glucose levels of other Groups reach Group A.

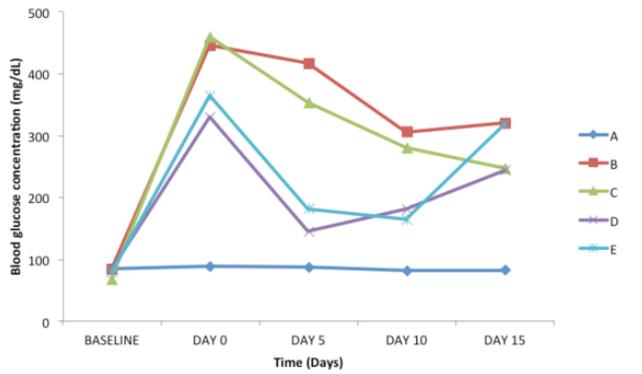


Figure 1: The effects of *Strophanthus hispidus* aqueous root extract on blood glucose level. Each point represent the mean value of blood glucose level of eight rats (n= 8). Values expressed as Mean \pm S.E.M, n= 8. Values along the same column with different superscripts are significantly different at $p < 0.05$.

Table 1: The effects of *Strophanthus hispidus* aqueous root extract on liver activities of antioxidant enzymes and on concentration of liver GSH, MDA and T.SH.

	A (Control)	B (Untreated diabetic rats)	C (Treated diabetic rats with glibenclamide)	D (Treated diabetic rats with 500 mg/kg.bwt of extract)	E (Treated diabetic rats with 1000 mg/kg b.wt of extracts)
SOD in liver (U/mg protein)	19.61 \pm 0.19 ^a	0.01 \pm 0.00 ^a	1.65 \pm 0.06 ^a	6.02 \pm 0.03 ^b	23.71 \pm 0.01 ^a
GPX in liver (U/mg protein)	24.71 \pm 0.27 ^a	25.61 \pm 0.04 ^a	25.00 \pm 0.04 ^a	24.81 \pm 0.12 ^a	24.97 \pm 0.03 ^a
POD in liver (U/mg protein)	0.09 \pm 0.01 ^a	0.12 \pm 0.01 ^b	0.08 \pm 0.01 ^a	0.10 \pm 0.01 ^a	0.08 \pm 0.01 ^a
GR in liver (U/mg protein)	9.31 \pm 0.09 ^a	14.82 \pm 0.09 ^c	10.32 \pm 0.09 ^b	8.03 \pm 0.07 ^a	12.37 \pm 0.11 ^{bc}
GGT in liver (U/mg protein)	1.90 \pm 0.01 ^a	2.29 \pm 0.02 ^a	2.28 \pm 0.03 ^a	3.39 \pm 0.03 ^b	1.92 \pm 0.01 ^a
GST in liver (U/mg protein)	1.01 \pm 0.02 ^a	0.78 \pm 0.03 ^a	0.51 \pm 0.02 ^a	0.88 \pm 0.02 ^a	0.89 \pm 0.02 ^a
GSH in liver (mmol/mg protein)	0.08 \pm 0.01 ^a	0.12 \pm 0.01 ^{ab}	0.15 \pm 0.03 ^b	0.14 \pm 0.02 ^b	0.12 \pm 0.02 ^{ab}
T.SH in liver (mmol /mg protein)	72.19 \pm 0.04 ^c	65.78 \pm 0.06 ^d	61.87 \pm 0.05 ^b	53.35 \pm 0.03 ^a	63.12 \pm 0.05 ^c

Values expressed as Mean \pm S.E.M, n=8. Values along the same role with different superscripts are significantly different at $p < 0.05$. Kg. bwt = Kilogram per body weight.

Table 2: The effects of *Strophanthus hispidus* aqueous root extract on pancreas activities of antioxidant enzymes and on concentration of pancreas GSH, MDA, T.SH and NO

	A (Control)	B (Untreated diabetic rats)	C (Treated diabetic rats with glibenclamide)	D (Treated diabetic rats with 500 mg/kg.bwt of extract)	E (Treated diabetic rats with 1000 mg/kg.bwt of extracts)
SOD in pancreas (U/mg protein)	17.86 \pm 0.94 ^a	19.07 \pm 1.18 ^a	19.11 \pm 1.15 ^a	14.47 \pm 0.86 ^a	15.98 \pm 1.19 ^{ab}
GPX in pancreas (U/mg protein)	29.78 \pm 1.18 ^a	30.54 \pm 0.24 ^a	29.57 \pm 0.23 ^a	29.94 \pm 0.10 ^a	29.83 \pm 0.39 ^a
POD in pancreas (U/mg protein)	0.04 \pm 0.01 ^a	0.05 \pm 0.01 ^b	0.03 \pm 0.00 ^a	0.03 \pm 0.00 ^a	0.03 \pm 0.00 ^a
GGT in pancreas (U/mg protein)	3.23 \pm 0.03 ^a	3.72 \pm 0.02 ^a	4.30 \pm 0.02 ^b	3.96 \pm 0.02 ^b	3.70 \pm 0.02 ^{ab}
GST in pancreas (U/mg protein)	1.09 \pm 0.02 ^b	0.74 \pm 0.02 ^{ab}	0.84 \pm 0.02 ^{ab}	0.64 \pm 0.02 ^{ab}	0.43 \pm 0.01 ^a
T.SH in pancreas (mmol /mg protein)	60.02 \pm 0.11 ^b	32.23 \pm 0.03 ^a	62.33 \pm 0.05 ^b	61.35 \pm 0.04 ^b	71.01 \pm 0.06 ^c
GSH in pancreas (mmol /mg protein)	0.87 \pm 0.09 ^a	1.18 \pm 0.12 ^{ab}	0.97 \pm 0.04 ^b	1.41 \pm 0.07 ^b	1.28 \pm 0.07 ^{ab}
NO in pancreas (mmol/L)	0.12 \pm 0.01 ^a	0.33 \pm 0.03 ^b	0.10 \pm 0.01 ^a	0.10 \pm 0.01 ^a	0.12 \pm 0.02 ^a

Values expressed as Mean \pm S.E.M, n=8. Values along the same role with different superscripts are significantly different at $p < 0.05$. Kg. bwt = Kilogram per body weight.

The effects of *Strophanthus hispidus* aqueous root extract on liver activities of antioxidant enzymes and on concentration of liver GSH, MDA and T.SH are shown in Table 1. GPX, POD and GST activities showed no significant difference ($p > 0.05$) in all the tested Groups as compared to control (Group A). There were slight changes in the concentrations of GSH and T.SH of all tested Groups as compared to Group A. Only Group D showed a significant increase ($p < 0.05$) in GGT activities as compared to Group A, while other tested Groups showed no significant difference. The SOD activities in Group B, C and D were significantly ($p < 0.05$) lower in comparison to Group A, with Group B showing the lowest value; while Group E showed a significant higher value than Group A. There was no significant difference in the level of GR activities in Groups C and D as compared to Group A, while Group B and E showed a significant increase in GR activities. MDA levels were gradually decreased significantly ($p < 0.05$) from Group C to Group E as compared to Group B but showed no significant difference as compared to Group A.

Table 3: The effects of *Strophanthus hispidus* aqueous root extract on RBC activities of antioxidant enzymes and on concentration of liver GSH and MDA.

	A (Control)	B (Untreated diabetic rats)	C (Treated diabetic rats with glibenclamide)	D (Treated diabetic rats with 500 mg/kg.bwt of extract)	E (Treated diabetic rats with 1000 mg/kg.bwt of extracts)
SOD in RBC (U/mg protein)	22.77 ± 0.50 ^a	15.38 ± 0.94 ^d	15.46 ± 0.63 ^a	16.35 ± 0.85 ^a	16.60 ± 0.84 ^a
GPX in RBC (U/mg protein)	27.51 ± 0.25 ^a	27.12 ± 0.18 ^a	27.64 ± 0.16 ^a	27.34 ± 0.03 ^a	27.11 ± 0.05 ^a
POD in RBC (U/mg protein)	0.06 ± 0.00 ^{bc}	0.07 ± 0.01 ^c	0.02 ± 0.00 ^a	0.05 ± 0.00 ^b	0.04 ± 0.00 ^b
GR in RBC (U/mg protein)	64.46 ± 0.13 ^c	54.46 ± 0.14 ^b	69.28 ± 0.15 ^c	71.81 ± 0.16 ^c	21.12 ± 0.06 ^a
GGT in RBC (U/mg protein)	1.95 ± 0.01 ^a	3.86 ± 0.02 ^f	2.28 ± 0.02 ^a	2.05 ± 0.02 ^a	3.2 ± 0.02 ^b
GST in RBC (U/mg protein)	0.64 ± 0.02 ^a	0.67 ± 0.01 ^a	1.07 ± 0.01 ^a	0.44 ± 0.01 ^a	0.22 ± 0.01 ^a
GSH in RBC (mmol/mg protein)	0.06 ± 0.01 ^a	0.07 ± 0.01 ^a	0.04 ± 0.01 ^a	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a
MDA in RBC (µmole/L)	1.67 ± 0.01 ^a	8.50 ± 0.03 ^b	1.08 ± 0.01 ^a	1.34 ± 0.01 ^a	1.25 ± 0.01 ^a

Values expressed as Mean ± S.E.M, n=8. Values along the same role with different superscripts are significantly different at p<0.05. Kg. bwt = Kilogram per body weight.

The effects of *Strophanthus hispidus* aqueous root extract on pancreas activities of antioxidant enzymes and on concentration of pancreas GSH, MDA, T.SH and NO are shown in Table 2. GPX activities showed no significant difference in all test Groups as compared to control (Group A); while there were slight changes in the activities of SOD, GGT and GST in all test Groups as compared Group A. POD activities were significantly (p<0.05) higher in Group B, while other treated Groups showed no significant different as compared to Group A.

Group B showed 54 % decrease in the level of T.SH as compared to Group A, while other Groups showed no significant (p>0.05) different (with exception of Group E) as compared to control (Group A). The level of NO was significantly higher in Group B, while other treated Groups showed no significant different as compared to Group A.

Table 4: The effects of *Strophanthus hispidus* aqueous root extract on plasma activities of antioxidant enzymes and on concentration of plasma GSH, MDA and ASC.

	A (Control)	B (Untreated diabetic rats)	C (Treated diabetic rats with glibenclamide)	D (Treated diabetic rats with 500 mg/kg.bwt of extract)	E (Treated diabetic rats with 1000 mg/kg.bwt of extracts)
SOD in plasma (U/mg protein)	1.69 ± 0.12 ^a	0.37 ± 0.01 ^a	1.62 ± 0.11 ^a	3.58 ± 0.47 ^a	1.16 ± 0.27 ^a
GPX in plasma (U/mg protein)	12.12 ± 0.46 ^b	12.07 ± 0.08 ^{ab}	11.40 ± 0.05 ^a	11.68 ± 0.07 ^{ab}	11.90 ± 0.09 ^{ab}
POD in plasma (U/mg protein)	0.22 ± 0.02 ^c	0.27 ± 0.01 ^d	0.17 ± 0.01 ^b	0.20 ± 0.02 ^c	0.14 ± 0.02 ^a
GSH in plasma (mmol/mg protein)	0.12 ± 0.02 ^a	14.28 ± 1.97 ^b	0.34 ± 0.05 ^a	0.09 ± 0.02 ^a	0.09 ± 0.02 ^a
ASC in plasma (mg/L)	0.22 ± 0.02 ^a	0.23 ± 0.01 ^a	0.24 ± 0.02 ^{ab}	0.28 ± 0.01 ^b	0.28 ± 0.02 ^b
MDA in plasma (µmole/L)	0.56 ± 0.01 ^a	1.59 ± 0.01 ^b	0.42 ± 0.01 ^a	0.17 ± 0.01 ^a	1.32 ± 0.01 ^b

Values expressed as Mean ± S.E.M, n=8. Values along the same role with different superscripts are significantly different at p<0.05. Kg. bwt = Kilogram per body weight.

The effects of *Strophanthus hispidus* aqueous root extract on RBC activities of antioxidant enzymes and on concentrations of RBC GSH and MDA are shown in Table 3. GPX and GST activities in RBC showed no significant difference in all tested Groups as compared to control. The level of RBC GSH also showed no significance different in all tested Groups as compared to control. There were slight changes observed in the levels of RBC SOD, GR and POD in all tested Groups as compared to control. The levels of MDA and GGT activities in RBC which were increased by diabetes (Group B) were significantly decreased (p<0.05) to normal value by both the extracts (Groups D and E) and glibenclamide (Group C) as compared to control (control).

The effects of *Strophanthus hispidus* aqueous root extract on plasma activities of antioxidant enzymes and on concentration of plasma GSH, MDA and ASC are shown in Table 4. Plasma GSH and MDA concentrations were

increased in streptozotocin - induced diabetic rats (Group B) as compared to control rats (Group A). Administration of *S hispidus* root extract (Groups D and E) and glibenclamide (Group C) reversed the concentrations of GSH and MDA to normal as compared to control (Group B). GPX, SOD, POD and ASC levels showed slight changes in all test Groups as compared to control (Group A).

DISCUSSION

Diabetes mellitus is a Group of complex metabolic disorders characterized by hyperglycemia, with disturbances of carbohydrate, lipid and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Maritim *et al.*, 2003; Marzouk *et al.*, 2013). Fasting blood sugar in diabetic rats represents an important indicator of diabetic status (Marzouk *et al.*, 2013). The significant reduction in fasting blood glucose produced by different doses of *S hispidus* extract in this study suggests the possibility that, the extract has hypoglycaemic effect on streptozotocin -induced diabetic rat; though at no time did the blood glucose reach the base line level. The present observation agrees with results from a previous study that reported on the hypoglycaemic effect of various part of *S hispidus* plant (Ojiako *et al.*, 2009). The exact mechanism of reduction in the blood glucose level is not well understood but the probable reason adduced was that it might be due to an increased uptake of glucose peripherally and increased sensitivity of insulin receptor (Mostofa *et al.*, 2007).

The measurement of various enzyme activities in tissues and body fluids plays a significant role in the toxicity of drugs including plant extracts (Yakubu *et al.*, 2003; Akinloye *et al.*, 2014). This study assayed for activities of SOD, POD, GGT, GST, GPX, GR, as well as the concentrations of GSH, ASC, NO, T.SH AND MDA as indices in various tissues (plasma, erythrocytes, pancreas and liver) of the rats. It has been established that the site of injury to the cell could be correlated and determined by assaying the level of activities of 'marker' enzymes in such cells (Ngaha, 1981; Akinloye *et al.*, 2014).

MDA, a highly reactive molecule which is an end product of membrane lipid peroxidation and one of the most frequently used indicators of lipid peroxidation (Nielsen *et al.*, 1997; Akinloye *et al.*, 2015). Increase in concentration of MDA can be interpreted as a result of cellular membrane damage initially caused by increased formation of radicals (Niedernhofer *et al.*, 2003; Akinloye *et al.*, 2015). There is increasing evidence that complications related to diabetes are associated with oxidative stress induced by the generation of free radicals (Kim *et al.*, 2006; Marzouk *et al.*, 2013). Hyperglycemia occurring in diabetes is the crucial factor that is responsible for the development of oxidative stress and reactive oxygen species (ROS) which are the main mediators of cellular damage in diabetes (Marzouk *et al.*, 2013). The increase in concentration of MDA in

streptozotocin-induced diabetic rats (Group B) as compared to control (Group A), support earlier reports of some researchers (Sathishsekar and Subramanian, 2005; Marzouk *et al.*, 2013) that oxidative stress plays an important role in chronic complications of diabetes and is postulated to be associated with increases in lipid peroxidation (MDA) and nitric oxide (NO) generation and decreases in antioxidant activities as well as pancreatic insulin contents when compared with the control animals in experiments. The level of MDA which was increased by diabetes (Group B) was reversed to normal level by different concentrations of the extract (Groups D and E) as compared to control (control). The possible mechanism by which the extract reversed MDA concentration to normal may be due to direct protective role of natural antioxidants that are present in the extract (Akinloye *et al.*, 2015). The extract may carry out these antioxidant activities by one or the following mechanisms; scavenging free radicals, interaction with oxidative cascade and preventing its outcome, reactive species quenching and inhibition of oxidative enzymes (Rukkumani *et al.* 2004; Akinloye *et al.*, 2015).

Nitric oxide (NO) is a free radical gas synthesized by a family of enzymes present in most of the cells of the body. Total NO concentration is commonly determined as a sum of nitrite and nitrate concentrations because it is unstable and undergoes various reactions in biological fluids resulting in the formation of nitrites, nitrates and peroxynitrites (Wioletta *et al.*, 2013). The ubiquitous localization of NO demonstrates its implication in a wide range of physiological process, but it can be harmful due to its reactivity, mainly with proteins, when involved in pathophysiological processes (Elfering *et al.*, 2002; Nikhil *et al.*, 2012.).

NO is produced by a Group of enzymes denominated nitric oxide synthases (NOS) (Elfering *et al.*, 2002; Guzik *et al.*, 2003). NO can also be produced by the xanthine oxidase pathway or by H₂O₂ and L-Arg in a non-enzymatic way (Alderton *et al.*, 2001.), or by the reduction of nitrites in acid and reducing conditions, as occurs in ischemic processes (Maiese and Boccone, 1995). When NO is produced in an excessive amount, NO changes from a physiological neuromodulator to a neurotoxic factor (Guix *et al.*, 2005). The high concentrations of total NO observed in our study before treatment could be secretion of NO by cells of the immune system (Jablonska *et al.* 2006). Similar observations by Alusik *et al.* 2008, was that increase in NO production by neutrophils and macrophages, as mediated by inducible nitric oxide synthase (iNOS) occur under pathological conditions and would be expected during inflammation. Decreased NO level after administration of the extract may be related to formation of the extract antioxidant constituent with NO; as it was observed by Atakisi *et al.* (2010) that administration of GSH reduced the level of NO concentration by binding to it.

Glutathione (L-γ-glutamyl-L-cysteinylglycine) is the main antioxidant in mammalian cells and is a ubiquitous thiol-containing tripeptide, which plays a central role in cell biology (Whitfield, 2001). It is implicated in the cellular defense

against xenobiotics and naturally occurring deleterious compounds, such as free radicals and hydro-peroxides. The key function of GSH molecule is the maintenance of protein structure and function, the regulation of protein synthesis and degradation, the maintenance of immune function, protection against oxidative damage, and detoxification of reactive chemicals (Atakisi *et al.*, 2010). Glutathione status is a high sensitive indicator of cell functionality and viability (Whitfield, 2001). Gamma-glutamyl transferase (gamma-glutamyl transpeptidase, GGT) is an enzyme present in serum and most cell surfaces (Meister *et al.*, 1976). It is involved in glutathione metabolism by transferring the glutamyl moiety to a variety of acceptor molecules including water, some of the L-amino acids and peptides. This reaction produces cysteinyl-glycine moieties, which are usually taken within the intracellular milieu by the action of membrane dipeptidases as precursors for glutathione re-synthesis (Whitfield, 2001). GGT is used as an oxidative stress marker (Lee *et al.*, 2004). The increased in the activities of RBC GGT and Plasma GSH concentration (component of blood) in streptozotocin - induced diabetic rats (Group B) which then reversed to normal by the administration of *S hispidus* root extract (Groups D and E) and glibenclamide (Group C) could be attributed to increased transport of glutathione into cells by increased GGT activity, to maintain high intracellular antioxidant glutathione levels in order to compensate for the oxidative stress (Lee *et al.*, 2004; Shankar and Li, 2007) that could have been caused by diabetes. The significant increase of SOD activity in rat liver (1000 mg/kg.bwt of extract) showed the possible effect that could be exerted by the extract polyphenolic compounds (Akinloye *et al.*, 2014). This result agreed with the report of Cui *et al.* (2000) that a high concentration of polyphenols, which are known to influence detoxification processes in liver, caused a favorable increase of SOD activity.

Peroxidases are a family of wide-spread enzymes (EC 1.11.1.X) which perform distinct tasks. They have the ability to catalyze the oxidation of a large variety of substrates through a reaction with hydrogen peroxide (Banci, 1997). They act as preventive antioxidants to detoxify damaging lipid peroxides or other peroxides from blood and organic substrates (Halliwell and Gutteridge, 1998). Total peroxidase system activity is known to play a key role in a number of human diseases (Hasan and Dawood, 2012). Enhanced production of oxygen free radicals are responsible for peroxidation of membrane lipids and the degree of peroxidative damage of cells is controlled by the potency of antioxidative peroxidase enzyme system (Velikova *et al.*, 2000). The presence of ROS, under normal conditions induces the expression of antioxidant enzymes as a defense mechanism (Habib and Samir, 2014). An increase in the peroxidase activity is a common response to oxidative stress (Fieldes and Gerhardt, 1998). Increased total peroxidases activities in streptozotocin-induced diabetic rats (Group B) as compared to control rats (Group A) confirm the pro-oxidant-antioxidant imbalance in diabetes (Palanisamy *et al.*, 2010). Hyperglycemia is known to stimulate specific processes for

oxidative stress and pro-inflammatory state, especially, an increase in the production of reactive oxygen species (ROS) in mitochondria's matrix (Pajunen *et al.*, 2000; Habib and Samir, 2014). The reverse in the activities of peroxidases to normal as compared to control (Group A) by administration of *S hispidus* root extract (Groups D and E) confirmed the anti-inflammatory potential of the extract (Agbaje and Fageyinbo, 2012).

Conclusion

The aqueous root extract of *Strophanthus hispidus* exhibited a hypoglycemic function and would be helpful in the prevention of diabetic complications through enhancing the antioxidant defense system. However, the precise mechanisms for its actions were not investigated in this work and further investigations in purifying the active compounds from the plant will be necessary to elucidate the precise mechanisms of the plant hypoglycemic and correction oxidative stress effects.

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