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J Vet Res 62, 371-377, 2018
DOI:10.2478/jvetres-2018-0053

Ameliorative effects of *Helianthus annuus* against nephrotoxic, cardiac, and haematological disorders in alloxan-induced hyperglycaemia in albino rats

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Received: April 27, 2018 *Accepted: September 17, 2018*

Abstract

Introduction: The study evaluated the ameliorative effects of *Helianthus annuus* leaf extract on nephrotoxicity, cardiac, and haematologic disorders in alloxan-induced hyperglycaemic rats. **Material and Methods:** The cold maceration method with 80% methanol was used in the preparation of *H. annuus* extract. Thirty alloxan-induced hyperglycaemic rats were randomly assigned to five equal groups (A–E). Groups A and B received 5% tween-20 solution in water (5 mL/kg) and glibenclamide (2 mg/kg), respectively; while groups C, D, and E received 150, 300, and 600 mg/kg of the extract, respectively, *per os* once daily for 21 consecutive days. The levels of serum urea, creatinine, haematological indices, and histopathological changes in the kidneys and heart were evaluated 24 h after the last treatment on day 21. **Results:** The extract and glibenclamide significantly ($P < 0.05$) reduced the levels of serum urea and urea : creatinine ratio in diabetic rats when compared with the vehicle treated group. The extract and glibenclamide also ameliorated haematological disorders and kidney and cardiac damage induced by alloxan. **Conclusion:** *H. annuus* extract produced nephroprotective, cardioprotective, and haematoprotective effects and might prevent the advancement of diabetic complications such as diabetic nephropathy and cardiovascular diseases in diabetic patients.

Keywords: rats, diabetes mellitus, *Helianthus annuus*, nephro-, cardio- and haematoprotective properties.

Introduction

The development and progression of diabetes and its complication are linked to chronic inflammatory conditions. Increased expressions of systemic inflammatory markers are associated with the pathogenesis of insulin resistance and type 2 diabetes (12). Macro- and microvascular complications of diabetes are mediated by inflammatory processes, thus prevention or treatment of inflammation will inhibit, retrogress, and/or ameliorate the complications of diabetes mellitus (DM) (14). Most antidiabetic drugs have been reported to possess anti-inflammatory properties (29).

One of the most frequent complications of DM in both humans and experimental animals is diabetic nephropathy (DN). DN develops as a result of non-enzymatic glycation of protein mediated by hyperglycaemia and reactive oxygen species (ROS) (28). Blood cell dyscrasias such as anaemia and neutrophilia usually co-occur with DN (1). Anaemia in DN and other diseases associated with inflammatory condition are presumed to be caused by reduced iron availability, low erythropoietin, and decrease in life span of red blood cells (RBC) (3). Inflammatory mediators such as cytokines and tumour necrosis factor are in abundance in DN and they accelerate the destruction of RBC precursors and lower the number of erythropoietin receptors in progenitor cells (21). Hypertension is also another cardiovascular complication of DM and often accompanies DN.

The ethnomedical management of DM is evolving rapidly. *Helianthus annuus* is among the herbs used in the folkloric care of diabetic patients. It is a perennial

plant with many pharmacological properties. The anti-inflammatory, analgesic, antihyperglycaemic, antioxidant, hepatoprotective, antifertility, and antimicrobial activities of *H. annuus* have been documented (8, 9, 26). The present study was designed to evaluate the ameliorative effects of hydromethanol leaf extract of *H. annuus* on nephrotoxicity, cardiac and haematologic disorders induced by alloxan in rats.

Material and Methods

Plant collection and extract preparation. The leaves of *H. annuus* were collected from the wild in Nsukka, Enugu State, Nigeria and identified by Mr. A.O. Ozioko, a plant taxonomist. The leaves were air dried at ambient temperature (25–27ºC). Hydromethanol extract of *H. annuus* was prepared using the cold maceration method as described by Onoja and Anaga (26) and was referred to as hydromethanol leaf extract of *H. annuus* (HLEHA). The extract was stored at 4ºC in a refrigerator while the experiment lasted.

Animals. A total of 40 male albino Wistar rats (100–120 g) were used for the study. They were housed in aluminum cages in a well-ventilated room at ambient temperature (25–27ºC) and with a natural light/darkness cycle. The rats were fed pelleted commercial grower feed (Vital Feed PLC, Nigeria) *ad libitum* except when fasting was required and had free access to clean drinking water. The rats were acclimatised for two weeks and the experimental protocol was approved by the institution's animal ethical committee.

Induction of experimental DM. DM was induced by a single intraperitoneal (i.p) alloxan injection at a dose of 160 mg/kg. Briefly, the rats were fasted for 16 h, after which their normal fasting blood glucose (FBG) levels were determined using an Accu-Chek Active blood glucose meter (Roche Diagnostics, Germany) and alloxan monohydrate (Sigma-Aldrich, Germany) was administered at dose of 160 mg/kg i.p. Seven days after alloxan injection the FBG was measured and rats with FBG > 126 mg/dL were used in the study.

Experimental design. A total of 30 diabetic rats were randomly assigned to five equal groups (A–E). The groups were treated as follows:
Group A received only the vehicle, 5% tween-20 (Sigma Aldrich, Germany) (5 mL/kg);
Group B received a reference drug, glibenclamide (GLB) (GNC, Nigeria) (2 mg/kg);
Groups C received HLEHA, 150 mg/kg;
Groups D received HLEHA, 300 mg/kg;
Groups E received HLEHA, 600 mg/kg.

All compounds were administered *per os* once daily for 21 consecutive days. The body weights were measured on days 7, 14, and 21. After the last treatment, the rats were fasted for 16 h, and blood samples were collected *via* the retro-orbital venous plexus into plain and EDTA-coated sample bottles. Thereafter, the rats were sacrificed by cervical dislocation, and evisceration and excision of the kidneys and heart were carried out. The kidneys and heart were fixed in 10% formal saline for processing and histopathological examination.

Haematological evaluation. The blood samples in EDTA-coated bottles were used for haematological evaluation. The total RBC and white blood cell (WBC) counts were measured using the improved Neubauer haemocytometer method. The cyanomethaemoglobin method was used to measure haemoglobin (Hb) concentration, while packed cell volume (PCV) was determined by the microhaematocrit method (4). Total and differential leukocyte counts were done as described in Thrall *et al*. (33). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae (33).

Biochemical evaluation. The serum urea and creatinine levels were measured using the urease-Berthelot method (6) and Jaffe's reaction method (31), respectively, using a commercially available reagent kit (Randox Laboratories, UK).

Histopathology. Histopathological sections of kidneys and heart were prepared as described by Ezeja *et al*. (13). Photomicrographs were captured at 400 × magnification with an Olympus photo microscope (Olympus Scientific Equipment, USA).

Statistical analysis. The obtained data were subjected to one-way ANOVA, followed by Least Significant Difference (LSD) Post-hoc analysis using SPSS software (SPSS Inc, USA). The mean values were considered significant at $P < 0.05$.

Results

Effects of HLEHA on body weight. The vehicle-treated group had significant ($P < 0.05$) sustained time-dependent weight loss, while the GLB- and HLEHA-treated groups had significant ($P < 0.05$) time-dependent weight gain over the period of the experiment. On day 21, the percentage body weight gains of vehicle-treated rats were significantly ($P < 0.05$) lower than the percentage body weight gains of GLB- and HLEHA-treated groups (Fig. 1).

Erythrocytic profile. There were no significant ($P > 0.05$) differences in PCV, RBC, HB, or MCV among various treatment groups. The MCH and MCHC of the group treated with HLEHA (150 mg/kg) were lower ($P < 0.05$) when compared with the vehicle-treated group, while the MCH and MCHC of the GLB- and HLEHA- (300 and 600 mg/kg) treated groups were not significantly different ($P > 0.05$) to those of the vehicle-treated group (Table 1).

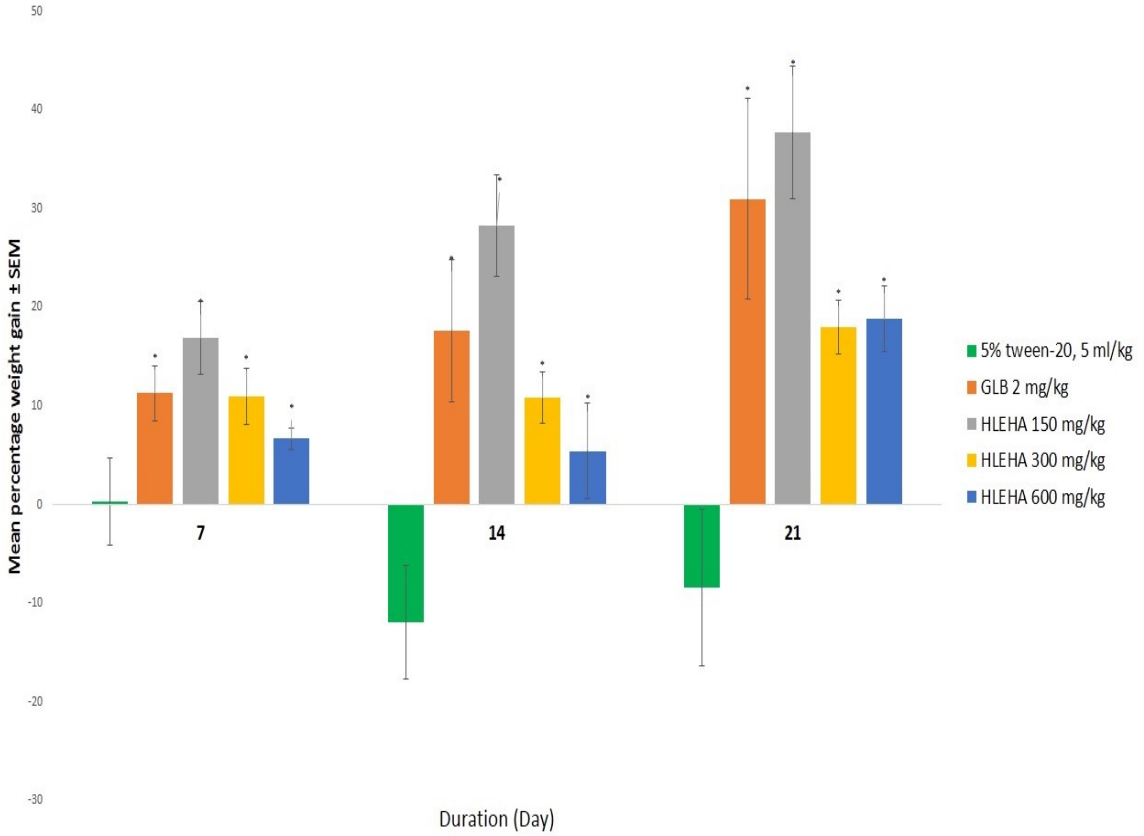

Fig. 1. Effects of HLEHA on body weight (mean ±SEM)
* P < 0.05 when compared with 5% tween-20–treated group, SEM – standard error of mean, GLB – glibenclamide, HLEHA – hydromethanol leaf extract of *H. annuus*

Table 1. Effect of HLEHA treatment on erythrocytic profile (mean ±SEM)

Treatment	PCV (%)	HB (g/dL)	RBC ($\times 10^6/\mu L$)	MCV (fL)	MCH (pg)	MCHC (g/dL)
5% tween-20, 5 mL/kg	43.8 ± 1.17	15.52 ± 0.56	7.81 ± 0.54	58.56 ± 3.1	20.69 ± 0.97	35.4 ± 0.59
GLB, 2 mg/kg	42.6 ± 0.87	14.18 ± 0.63	7.5 ± 0.25	57.31 ± 1.99	19.16 ± 1.03	33.18 ± 0.74
HLEHA, 150 mg/kg	45.0 ± 0.91	14.26 ± 0.57	8.21 ± 0.62	55.49 ± 3.12	17.51 ± 0.69*	31.48 ± 0.71*
HLEHA, 300 mg/kg	44.2 ± 1.11	14.88 ± 0.31	8.21 ± 0.35	54.22 ± 2.54	18.29 ± 1.06	33.75 ± 1.09
HLEHA, 600 mg/kg	44.13 ± 0.52	14.67 ± 0.48	7.8 ± 0.62	57.45 ± 3.68	19.03 ± 0.98	33.24 ± 0.84

* P < 0.05 when compared with 5% tween-20–treated group, SEM – standard error of mean, GLB – glibenclamide, HLEHA – hydromethanol leaf extract of *H. annuus*, PCV – packed cell volume, HB – haemoglobin, RBC – red blood cells, MCV – mean corpuscular volume, MCH – mean corpuscular haemoglobin, MCHC – mean corpuscular haemoglobin concentration

Leukocytic profile. The total WBC count of the vehicle-treated group was insignificantly (P > 0.05) higher than that of other treatment groups (Table 2). The neutrophil and basophil counts of the GLB- and HLEHA-treated groups were lower (P < 0.05) than the 5% tween-20–treated group. The lymphocyte and eosinophil counts of the GLB- and HLEHA-treated groups were higher (P < 0.05) than the 5% tween-20–treated group, except eosinophil in the HLEHA 150 mg/kg treated group where the increase was not significant. The neutrophil : lymphocyte ratio of GLB- and HLEHA-treated groups were reduced (P < 0.05) when compared with the 5% tween-20–treated group (Table 2).

Effect of HLEHA treatment on serum markers of kidney function. The serum urea level of the 5% tween-20–treated group was significantly higher (P < 0.05) when compared with the groups treated with

GLB and HLEHA (150, 300, and 600 mg/kg). The serum creatinine level of all the groups did not differ significantly (P > 0.05) (Table 3).

Histopathology. The kidney structure of the 5% tween-20–treated group showed areas of renal tubular degeneration, congested blood vessels, and fibroplasias of the Bowman's capsule and peritubular spaces. The GLB-treated group showed no histopathological changes, while the HLEHA (150 and 300 mg/kg) treated groups showed reduced fibroplasia of the Bowman's capsule and peritubular spaces and mild degeneration when compared with 5% tween-20–treated group (Fig. 2). The hearts of the 5% tween-20–treated group showed areas of muscle fibre necrosis and hypertrophy, while HLEHA 150 mg/kg treated rats showed mild hypertrophy and degeneration of muscle fibres when compared with the heart structure of the 5% tween-20–treated group. The GLB- and HLEHA 600 mg/kg treated rats showed normal cardiac muscle histological structure with prominent muscle striations (Fig. 3).

Table 2. Effect of HLEHA treatment on leukocytic profile (mean ±SEM)

Treatment	5% tween-20, 5 mL/kg	GLB 2 mg/kg	HLEHA 150 mg/kg	HLEHA 300 mg/kg	HLEHA 600 mg/kg
WBC (x10^3/mL)	11.68 ± 0.58	9.73 ± 0.43	10.89 ± 0.36	10.9 ± 0.68	9.85 ± 1.26
Re neutrophil (%)	37.0 ± 2.66	21.4 ± 1.57*	28.0 ± 0.53*	18.0 ± 2.28*	14.67 ± 1.65*
Re lymphocyte (%)	60.0 ± 3.12	75.8 ± 1.6*	68.0 ± 1.31*	77.67 ± 2.74*	78.0 ± 2.22*
Re eosinophil (%)	0.5 ± 0.19	2.4 ± 0.54*	1.5 ± 0.42	2.0 ± 0.37*	2.67 ± 0.56*
Re monocytes (%)	1.75 ±0.49	0.2 ± 0.13*	2.5 ± 0.78	1.67 ± 0.42	5.67 ± 1.05*
Re basophils (%)	0.75 ± 0.31	0.2 ± 0.13*	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
Ab neutrophil (×10^3/mL)	4.43 ± 0.73	2.1 ± 0.3*	3.04 ± 0.12*	1.87 ± 0.42*	1.55 ± 0.53*
Ab lymphocyte (×10^3/mL)	6.88 ± 0.13	7.37 ± 0.54*	7.41 ± 0.49*	7.97 ± 0.91*	7.55 ± 0.27*
Ab eosinophil (per mL)	53.5 ± 20.7	219.0 ± 41.92*	154.75 ± 42.70	220.33 ± 53.84*	244.0 ± 50.21*
Ab monocytes (per mL)	220.13 ± 61.98	20.2 ± 13.47*	278.00 ± 84.67	170.17 ± 42.23	573.17 ± 133.96*
Ab basophils (per mL)	92.88 ± 38.73	20.2 ± 13.47*	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
NLR	0.64 ± 0.06	0.29 ± 0.02*	0.41 ± 0.01*	0.24 ± 0.03*	0.19 ± 0.02*

*P < 0.05 when compared with 5% tween-20–treated group, SEM – standard error of mean, GLB – glibenclamide, HLEHA – hydromethanol leaf extract of *Helianthus annuus*. WBC – white blood cell count, Re – relative, Ab – absolute, NLR – neutrophil-lymphocyte ratio

Table 3. Effect of HLEHA treatment on serum markers of kidney function (mean ± SEM)

Treatment	Urea (mg/dL)	Creatinine (mg/dL)	Urea/creatinine ratio
5% tween-20, 5 mL/kg	85.43 ± 1.60	1.15 ± 0.03	75.26 ± 4.94
GLB, 2 mg/kg	45.19 ± 4.42*	1.05 ± 0.01	43.09 ± 6.15*
HLEHA, 150 mg/kg	48.94 ± 4.26*	1.22 ± 0.03	40.91 ± 7.10*
HLEHA, 300 mg/kg	53.84 ± 4.01*	1.08 ± 0.04	49.37 ± 3.16*
HLEHA, 600 mg/kg	49.69 ± 2.73*	1.10 ± 0.06	47.89 ± 8.42*

*P < 0.05 when compared with 5% tween-20–treated group, SEM – standard error of mean, GLB – glibenclamide, HLEHA – hydromethanol leaf extract of *Helianthus annuus*

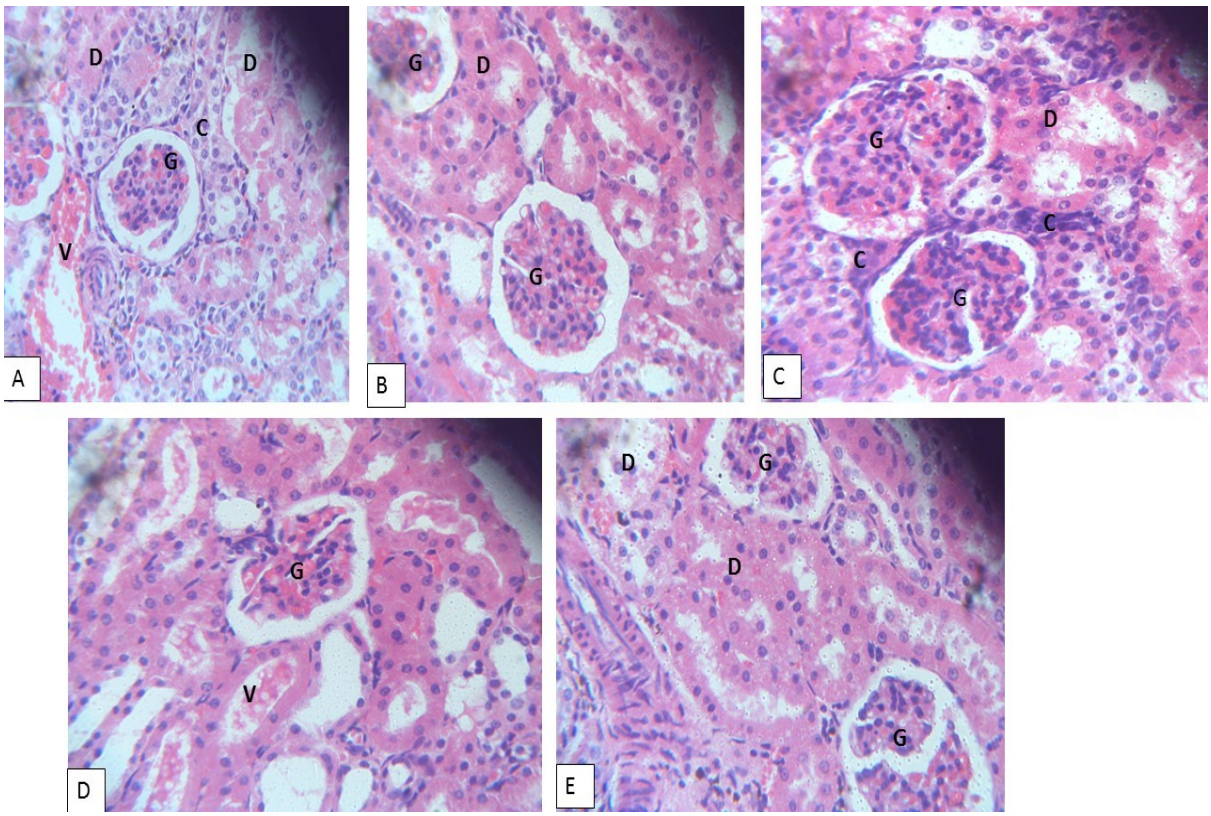

Fig. 2. Photomicrograph of the kidney, H & E, 400×
The V shows congested blood vessel, D shows renal tubular degeneration, G shows the glomerulus and line arrow is pointing at fibroblastic cells. A – 5% tween-20–treated group, B – glibenclamide treated group, C – HLEHA 150 mg/kg treated group, D – HLEHA 300 mg/kg treated group, and E – HLEHA 600 mg/kg treated group

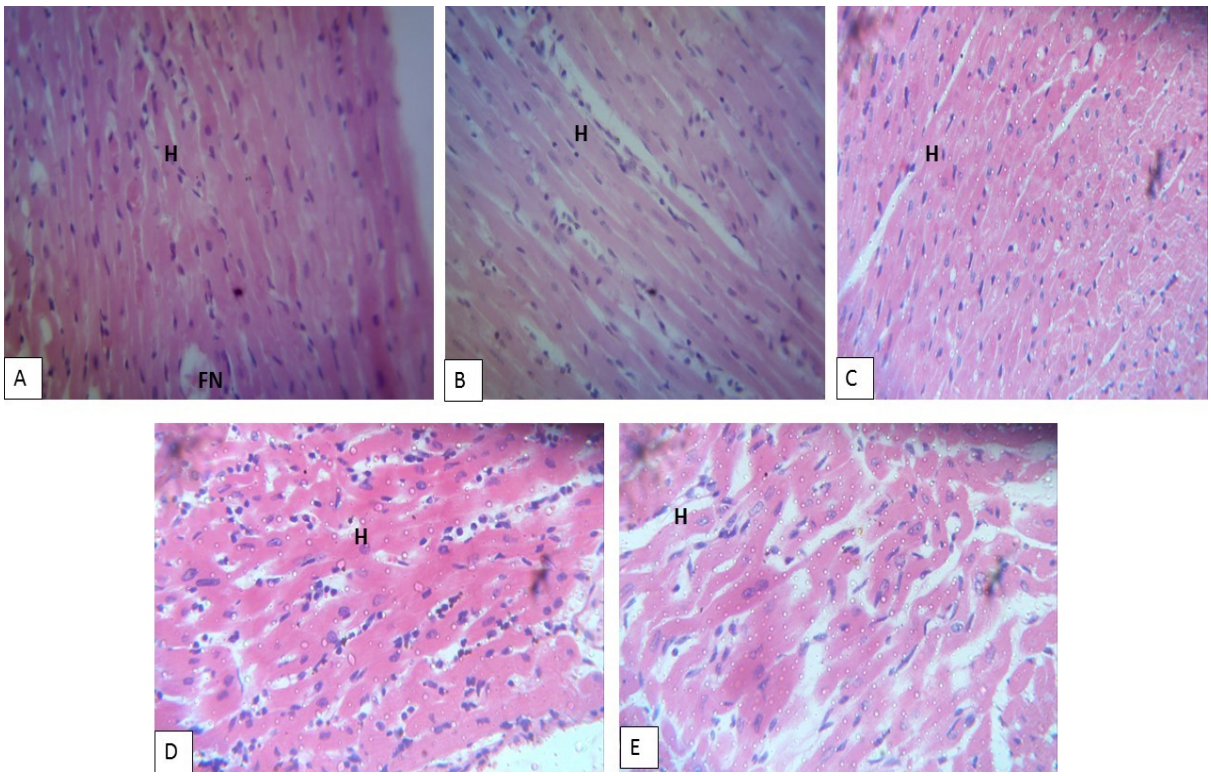

Fig. 3. Photomicrograph of the heart muscle, H & E, 400×
The FN shows focal necrosis and H shows hypertrophic muscle fibre in 5% tween-20–treated group. A – 5% tween-20–treated group, B – glibenclamide-treated group, C – HLEHA 150 mg/kg treated group, D – HLEHA 300 mg/kg treated group, and E – HLEHA 600 mg/kg treated group

Discussion

The ameliorative effects of hydromethanol leaf extract of *Helianthus annuus* on nephrotoxicity, cardiac and haematologic disorders in alloxan-induced hyperglycaemia were investigated in rats. Hydromethanol leaf extract of *H. annuus* produced nephroprotective, cardioprotective, and haematoprotective effects in alloxan-induced diabetic rats. The effects were caused by the phytoconstituents of *H. annuus* such as alkaloids, tannins, flavonoids, glycosides, and saponins, which have been documented (26). These effects have also been reported by other authors (27, 32).

The treatment of the rats with GLB and HLEHA reversed weight loss (Fig. 1) and dehydration (Table 3) induced by alloxan. Weight loss in alloxan induced DM is linked to polyuria and muscle wasting as a result of negative protein balance (11). Dehydration in alloxan induced DM is linked solely to polyuria and is associated with hypovolaemia, haemoconcentration (relative polycythaemia), and elevated serum urea and urea : creatinine ratio levels (30, 33). In this study, the serum urea and urea : creatinine ratio levels of GLB and HLEHA-treatment groups were lower ($P < 0.05$) than the 5% tween-20–treated group (Table 3).

Anaemia and diabetic nephropathy are common complications of both clinical and alloxan-induced DM. The anaemia is attributed to the non-enzymatic glycation of RBC and haemoglobin, and it shortens life-span of RBC as a result of an increased rate of apoptosis (7, 24, 25). Anaemia may also be due to impaired formation of RBC linked to protein glycation (34). Anaemia is also linked to reduced erythropoietin production due to diabetic nephropathy (21). The 5% tween-20–treated group had anaemia which was masked by dehydration-induced relative polycythaemia (2). Aside from anaemia, the haemogram of the 5% tween-20–treated group ought to have been elevated as a result of the dehydration. The absence of any significant ($P > 0.05$) difference in the erythrocyte profile (PCV, RBC, and HB) of the 5% tween-20–treated group was due to relative polycythaemia (18, 20). The antianaemic activity of HLEHA corroborates the reversal of kidney damage induced by alloxan. This implies that the antianaemic activity of the extract may be attributed to possibly improved erythropoietin production by the kidneys (3).

The treatment of the rats with GLB and HLEHA ameliorated inflammatory and stress responses in the alloxan-induced DM. GLB and HLEHA treatment reduced ($P < 0.05$) the neutrophil count when compared with 5% tween-20–treated group, while the lymphocyte counts of GLB and HLEHA treated groups were higher ($P < 0.05$) than the 5% tween-20–treated group (Table 2). The number of circulating neutrophils is elevated in inflammatory conditions, while a decrease in the number of circulating lymphocytes is associated with the stress response in rodents (19). Diabetes mellitus is described as a chronic inflammatory and stressful condition (12). The elevated WBC count in the 5% tween-20–treated group is in agreement with the report of Grossmann *et al*. (15). Stress response is a common leukocyte response (persistent lymphopenia and neutrophilia) mediated by released steroid hormones that accompanies renal failure, diabetic keto-acidosis, dehydration, traumatic pain, and inflammation (22). The suppressors of systemic inflammatory mediators has been shown to improve glycaemia in diabetic population (29).

The GLB and HLEHA reversed alloxan-induced DN in the treated rats. Alloxan administration produced elevated serum urea and urea : creatinine ratio (Table 3) as well as the degeneration and fibroplasia of Bowman's capsule and peritubular spaces (Fig. 2). Alloxan-induced nephrotoxicity is well documented; however, its mechanism is not well understood. The generation of free radicals from the redox cycling of dialuric acid has been proposed to explain the mechanism (28). These free radicals react with cellular substances which include nucleic acid, proteins, and lipids, causing cell damage (24). Although the mechanism of nephroprotective effects of *H. annuus* is not known, it could be attributed to the scavenging of free radicals and anti-inflammatory activities (17). The antioxidant and anti-inflammatory activities of *H. annuus* leaf have been reported (9, 26). Yankuzo *et al*. (35) associated the improvement of renal function of streptozotocin-induced diabetic rats treated with leaf extract of *Murraya Koenigii* to antioxidant activity. The findings of this study agree with the reports of Chen *et al*. (5) and Hua *et al*. (16) on the nephroprotective effects of *Smilax china* and a modified *Simiao* decoction, respectively.

The cardiac muscles of the 5% tween-20–treated group showed hypertrophy of the fibres and focal degeneration. Hypertrophy of muscle fibre is a characteristic feature of diabetes and chronic kidney disease, especially DN, and is linked to hypertension and anaemia (1). The treatment of the diabetic rats with GLB and HLEHA reversed the histopathologic changes in the heart. This indicates that GLB and HLEHA ameliorate cardiovascular complications associated with diabetes mellitus.

In conclusion, hydromethanol leaf extract of *H. annuus* produces nephroprotective, cardioprotective, and haematoprotective effects and can prevent the advancement of diabetic complications such as diabetic nephropathy and cardiovascular diseases. These findings support the ethnomedical use of *H. annuus* in the management of diabetes and its complications. Further studies should be undertaken to isolate the bioactive compound(s).

Conflict of Interests Statement: The authors declare no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: This study was not supported by any donor agency.

Animal Rights Statement: The experimental protocol was approved by the Animal Ethical Committee.

Acknowledgement: The authors are grateful to A.O. Ozioko and technical staff of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture Umudike.

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