

Original Research Article

Hypoglycemic, antidyslipidemic, hepatoprotective and anti-lipid peroxidation activities of hydromethanol leaf extract of *Helianthus annuus* in alloxan-induced diabetic rats

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Abstract

Purpose: To investigate the hypoglycemic, antidyslipidemic, hepatoprotective and anti-lipid peroxidation effects of hydromethanol leaf extract of *Helianthus annuus* (HLEHA) in alloxan-induced diabetic rats.

Methods: The extract was administered once daily at 150, 300 and 600 mg/kg for 21 consecutive days. Glibenclamide (GLB) 2 mg/kg was used as a reference drug (positive control) and 5 % Tween-20 solution in water was used as negative control. The fasting blood glucose (FBG) and body weights were measured on days 7, 14 and 21 while glycosylated haemoglobin concentration (HbA1c), serum markers of liver function, lipid profile, antioxidant status, histopathological changes in pancreas and liver were determined 24 h after the last dosing on day 21.

Results: The GLB and HLEHA caused significant ($p < 0.05$) time-dependent weight gains in the treated rats when compared with 5 % Tween-20 treated rats. The HLEHA and GLB caused significant ($p < 0.05$) decreases in FBG, HbA1c, malondialdehyde, and lipid profile levels in the treated rats when compared with rats in 5% tween-20 treated group. Alloxan-induced pancreatic and hepatic degeneration were reversed in GLB- and HLEHA-treated rats.

Conclusion: *Helianthus annuus* demonstrates potent antidiabetic, antioxidant and antidyslipidemic activities in rats.

Keywords: Antidiabetic, Antidyslipidemic, *Helianthus annuus*, Medicinal plant, Traditional medicine

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INTRODUCTION

Diabetes mellitus is a complex metabolic and endocrine disorder characterized by chronic hyperglycemia due to defective insulin secretion, insulin action or both [1]. Diabetes mellitus is usually associated with micro-and

macrovascular complications which have correlation to hyperglycemia [2]. Hyperglycemia induces vascular damage through enhanced polyol activity, resulting in sorbitol and fructose accumulation; elevated formation of advanced glycation end products; increased hexosaminase pathway, activation of protein

kinase C and nuclear factor-kappa beta [3].

The activation of the aforementioned processes by hyperglycemia leads to excessive release of superoxide and other reactive oxygen species from the mitochondria via nicotinamide adenine dinucleotide phosphate-oxidase; culminating in oxidative stress which play a critical role in the pathogenesis of diabetic complication [3-5]. Oxidative stress triggers the release of pro-inflammatory mediators and endothelial dysfunction which are linked in the progression of diabetes mellitus [6,7]. Oxidative stress also leads to lipid peroxidation and complications such as retinopathy, nephropathy, neuropathy and deoxyribonucleic acid (DNA) damage [8].

Helianthus annuus Linn. (Asteraceae) commonly called "sunflower" is an annual plant native to America and has a cosmopolitan distribution [9]. The leaves of *H. annuus* are used as major component of ethnomedical herbal mixture used in the traditional management of diabetes mellitus in South-Eastern Nigeria. The effect of a single dose of *H. annuus* on the fasting blood glucose level of alloxan-induced diabetic rats and *in vitro* antioxidant activities have been reported [9]. There is dearth of information on the sub-acute antidiabetic effects of *H. annuus* on the diabetic animal model and potential for amelioration of oxidative stress and dyslipidemia that accompany diabetes mellitus. In this study, the effects of sub-acute treatment of *H. annuus* extract on fasting blood glucose, serum lipid profile, *in vivo* antioxidant activity, and its safety on alloxan-induced diabetic rat model were investigated.

EXPERIMENTAL

Plant collection and extract preparation

The leaves of *Helianthus annuus* were collected from the wild in Nsukka, Enugu State, Nigeria and identified by Mr AO Ozioko. They were dried under shed at ambient temperature (25 – 27 °C). Hydromethanol extract of *H. annuus* was prepared using cold maceration method as described by Onoja and Anaga [9]. The extract was referred to as hydromethanol leaf extract of *H. annuus* (HLEHA) and was stored at 4 °C in a refrigerator while the experiment lasted.

Animals

Forty (40) male albino Wistar rats (100 - 120 g) used in the study were housed in aluminium cages in a well-ventilated room at ambient

temperature (25 – 27 °C) and natural light/darkness cycle. The rats were fed standard rat chow (Grower, Vital feed PLC Jos, Nigeria) *ad libitum* and had free access to clean drinking water except when fasting was required. They were acclimatized for 2 weeks and the experimental protocol was approved by the institution animal ethical committee.

Experimental design

Thirty (30) diabetic albino Wistar rats were randomly assigned to 5 groups (A – E, n = 6). Group A received vehicle (5 % Tween-20, 5 ml/kg); Group B received the reference drug, glibenclamide (GLB) (German Nigerian Chemist, Nigeria) (2 mg/kg) while Groups C-E received HLEHA at 150, 300 and 600 mg/kg, respectively. All treatments were administered orally once daily for 21 consecutive days. The FBG was determined on day 7, 14 and 21. After the last treatment, the rats were fasted for 16 h and blood samples collected via the retro-orbital venous plexus into plain and EDTA-coated sample bottles. Thereafter, all the animals were sacrificed by cervical dislocation; the liver was excised. Ten percent liver homogenate in phosphate buffer saline (pH 7.0) was used for estimation of lipid peroxidation, catalase and superoxide activity. The pancreas and liver portions were fixed in 10 % formal saline for histopathologic examination. The relative weights of kidney, liver and heart were also determined.

Induction of experimental diabetes mellitus

Diabetes was induced in each rat with a single intraperitoneal (i.p.) administration of 160 mg/kg of alloxan monohydrate (Sigma-Aldrich, Germany). Briefly, the rats were fasted for 16 h, after which the normal fasting blood glucose (FBG) levels were determined using Accu-Chek active (Roche Diagnostic, Germany) followed by alloxan monohydrate administration. Seven days post alloxan monohydrate administration the FBG was examined and rats with FBG greater than 126 mg/dl were considered diabetic and were used for the study [10].

Determination of glycosylated haemoglobin concentration (HbA1c)

Ion-exchange resin method was used in the determination of HbA1c [11,12]. A commercial reagent kit obtained from Spectrum diagnostic Laboratories (Egyptian Company of Biotechnology, Cairo Egypt) was used in this study.

Biochemical analysis

The blood collected in plain glass sample bottles were allowed to clot and the serum samples were harvested. The serum samples were used to determine the concentrations of total protein, albumin, total cholesterol (TC), triacylglycerol (TG) and high density lipoprotein cholesterol (HDL-C), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. These parameters were measured spectrophotometrically, using commercial assay kits (Randox Laboratory Diagnostics, United Kingdom) according to the manufacturer's procedures. Serum low density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's equation as in Eq 1 [13,14].

$$LDL-C = [TC - \{HDL-C + (TG/5)\}] \dots\dots (1)$$

while very low density lipoprotein-cholesterol (VLDL-C) was computed as in Eq 2.

$$VLDL-C = (TG/5) \dots\dots\dots (2)$$

Evaluation of *in vivo* antioxidant status

The liver homogenate was used to determine the level of the thiobarbituric acid reactive substances (TBARS) as described by Draper and Hadley [15] while superoxide dismutase (SOD) [16] and catalase activities were evaluated as described by Xin *et al* [16] and Alam and Bristi [17].

Histopathology

The histopathology sections of pancreas and liver of the rats were prepared as described by Ezeja *et al* [18]. Photomicrographs were captured at 400 x magnifications with an Olympus photomicroscope (Olympus Scientific Equipment, Ashburn, VA).

Statistical analysis

The obtained data were analysed using one-way ANOVA, followed by least significant difference test using SPSS software. The mean values were considered significant when $p < 0.05$.

RESULTS

Effect of HLEHA on body weight of alloxan-induced diabetic rats

The 5% tween-20 treated group showed significantly ($p < 0.05$) time-dependent loss of body weight while the GLB and HLEHA treated

groups had significant ($p < 0.05$) time-dependent weight gain as long as the experiment lasted. On day 21, the mean body weight of 5% tween-20 treated rats was significantly ($p < 0.05$) lower than the body weight of GLB- and HLEHA-treated groups (Figure 1).

Sub-acute effects of HLEHA on FBG of alloxan-induced diabetic rats

The FBG levels of GLB (2 mg/kg) and HLEHA (150 mg/kg) treated groups were significantly ($p < 0.05$) lower than the group treated with 5% tween-20 on days 7, 14 and 21 of treatment. On day 21, the FBG levels of GLB and HLEHA-treated groups (150, 300 and 600 mg/kg) were within the normal FBG range (< 100 mg/dl) while the FBG level of the 5% tween-20 treated group (untreated diabetic control) was still in the diabetic range (> 126 mg/dl) (Figure 2).

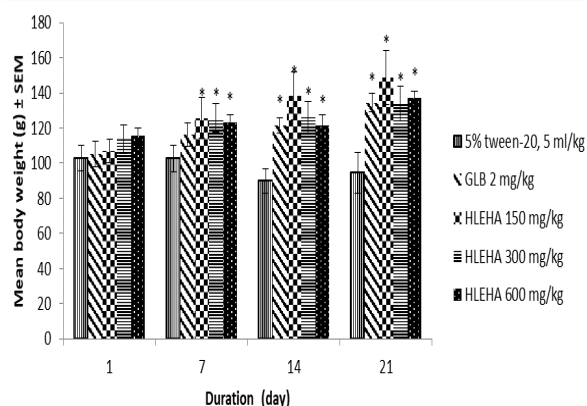


Figure 1: Effect of HLEHA on body weights of alloxan-induced diabetic rats. * $P < 0.05$ when compared with 5% tween-20 treated group, HLEHA = hydromethanol leaf extract of *Helianthus annuus*

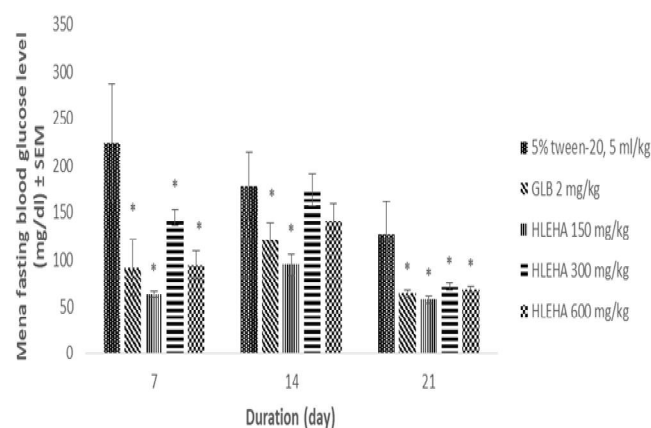


Figure 2: Sub-acute effects HLEHA on the FBG of alloxan-induced diabetic rats. * $P < 0.05$ when compared with 5% tween-20 treated group, HLEHA = hydromethanol leaf extract of *Helianthus annuus*

Effect of HLEHA on HbA1c in alloxan-induced diabetic rats

The HbA1c of GLB and HLEHA-treated groups (150, 300 and 600 mg/kg) were significantly ($p < 0.05$) lower when compared with 5 % tween-20 (5 ml/kg) treated group.

Table 1: Effects of HLEHA on Hba1c of alloxan-induced diabetic rat

Treatment	Hba1c (%)
5% tween-20, 5 ml/kg	7.37 ± 0.17
Glibenclamide, 2 mg/kg	5.67 ± 0.17*
HLEHA, 150 mg/kg	5.48 ± 0.25*
HLEHA, 300 mg/kg	6.57 ± 0.31*
HLEHA, 600 mg/kg	6.31 ± 0.37*

* $P < 0.05$ when compared with 5% tween-20 treated group; Hba1c = glycosylated haemoglobin concentration; HLEHA = hydromethanol leaf extract of *H. annuus*

Effects of HLEHA on liver function of alloxan-induced diabetic rats

The serum activities of AST, ALT and ALP of GLB and HLEHA-treated groups were significantly ($p < 0.05$) lower when compared

Table 2: Effect of HLEHA on liver function markers of alloxan-induced diabetic rats

Treatment	ALP (U/L)	AST (U/L)	ALT (U/L)
5% tween-20, 5 ml/kg	355.35 ± 9.59	173.36 ± 11.94	78.14 ± 5.11
Glibenclamide, 2 mg/kg	134.55 ± 6.44*	91.28 ± 2.88*	52.20 ± 1.56*
HLEHA, 150 mg/kg	132.48 ± 21.73*	100.28 ± 10.07*	51.90 ± 4.05*
HLEHA, 300 mg/kg	154.56 ± 8.88*	88.40 ± 5.02*	53.28 ± 1.96*
HLEHA, 600 mg/kg	138.00 ± 17.44*	95.70 ± 2.02*	50.38 ± 1.00*

* $P < 0.05$ when compared with 5% tween-20 treated group, HLEHA = hydromethanol leaf extract of *Helianthus annuus*, ALP = alkaline phosphatase, AST = aspartate aminotransferase, ALT = alanine aminotransferase

Table 3: Effect of HLEHA on serum lipid profile of alloxan-induced diabetic rats

Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)
5% tween-20, 5 ml/kg	98.80 ± 1.93	92.31 ± 5.70	18.52 ± 2.30	18.46 ± 1.14	73.91 ± 7.30
Glibenclamide 2 mg/kg	86.35 ± 2.63*	82.69 ± 3.33	26.52 ± 3.41	16.54 ± 0.67	49.65 ± 0.99*
HLEHA 150 mg/kg	84.94 ± 4.12*	66.67 ± 4.49*	20.71 ± 2.21	13.33 ± 0.90*	54.23 ± 4.94*
HLEHA 300 mg/kg	84.34 ± 2.53*	62.82 ± 2.20*	36.53 ± 3.47*	12.56 ± 0.44*	44.37 ± 4.12*
HLEHA 600 mg/kg	70.48 ± 4.67*	67.09 ± 5.31*	32.07 ± 3.43*	13.42 ± 1.06*	28.35 ± 3.07*

* $P < 0.05$ when compared to 5% tween-20 treated group, HLEHA = hydromethanol leaf extract of *Helianthus annuus*, HDL-C = high density lipoprotein cholesterol, VLDL-C = very low density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol.

Table 4: *In vivo* antioxidant activities of HLEHA in alloxan-induced diabetic rats

Group	TBARS (µmole/g protein)	SOD (U/g protein)	Catalase (millimole/ g protein)
5% tween-20, 5 ml/kg	0.95 ± 0.10	7.99 ± 0.50	1.73 ± 0.10
Glibenclamide, 2 mg/kg	0.65 ± 0.07*	8.37 ± 0.86	6.73 ± 1.02*
HLEHA, 150 mg/kg	0.71 ± 0.05	9.69 ± 1.28	4.77 ± 0.58*
HLEHA, 300 mg/kg	0.48 ± 0.03*	8.43 ± 0.65	3.11 ± 0.25
HLEHA, 600 mg/kg	0.46 ± 0.04*	5.47 ± 0.33	2.57 ± 0.58

* $P < 0.05$ when compared to 5% tween-20 treated group, HLEHA = hydromethanol leaf extract of *Helianthus annuus*, TBARS = Thiobarbituric acid reactive substance, SOD = superoxide dismutase

with 5% tween-20 treated group (Table 2).

Effects of HLEHA on lipid profile of alloxan-induced diabetic rats

The concentrations of the total serum cholesterol, triglyceride, VLDL-C and LDL-C of 5% tween-20 treated rats were higher ($p < 0.05$) when compared with GLB and HLEHA treated rats. The HDL-C of HLEHA 300 and 600 mg/kg - treated groups were higher ($p < 0.05$) when compared with 5% tween-20 treated group (Table 3).

In vivo antioxidant activities of HLEHA in alloxan-induced diabetic rats

The concentration of TBARS of 5% tween-20 treated group was significantly ($p < 0.05$) higher than GLB and HLEHA (300 and 600 mg/kg) treated groups. The SOD activities of all the groups did not vary significantly ($p > 0.05$) while the catalase activity of 5% tween-20 treated group was significantly ($p < 0.05$) lower when compared with GLB and HLEHA (150 and 300 mg/kg) treated groups (Table 4).

Histopathology

The photomicrograph section of the pancreas of alloxan-induced diabetic rats is presented in Figure 3. The pancreas of 5% tween-20 treated group (Figure 3A) showed shrunken and atrophied pancreatic islets with few degenerating cells. The GLB treated group (Figure 3B) showed larger pancreatic islets and regenerating cells when compared with 5% tween-20 treated group. The extract treated

groups (Figure 3C-E) showed dose-dependent increase in numbers and sizes of pancreatic islets with evidence of regenerating cells when compared with 5% tween-20 treated group.

In the liver, the 5% tween-20 treated group (Figure 4A) showed multifocal areas of mononuclear cells infiltration on the connective tissue of the portal triad, bile duct hyperplasia and fibroplasia. The GLB-treated group (Figure 4B) showed no histopathological lesion. The

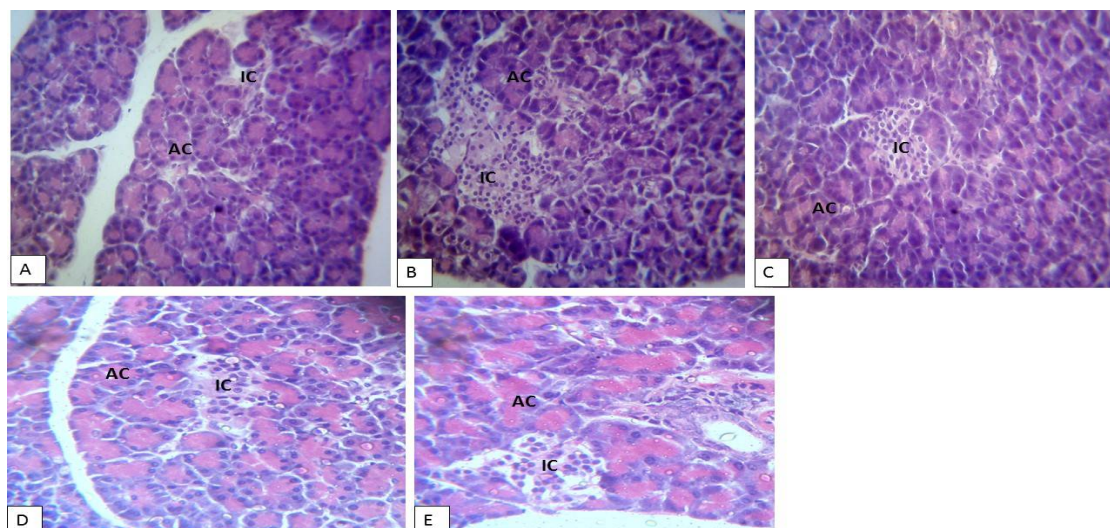


Figure 3: Photomicrograph section (H & E, x400) of pancreas of alloxan-induced diabetic rats. The 'IC' shows the pancreatic islet and the 'AC' shows the pancreatic acini. 3A = 5 % tween-20 treated group; 3B = Glibenclamide treated group; 3C = HLEHA 150 mg/kg treated group; 3D = HLEHA 300 mg/kg treated group and 3E = HLEHA 600 mg/kg treated group. The pancreas of 5 % tween-20 treated group (A) showed shrunken and atrophied pancreatic islet with few degenerating cells. The glibenclamide (B) and HLEHA (C-E) treated groups showed larger pancreatic islet and regenerating cells when compared with 5 % tween-20 treated group.

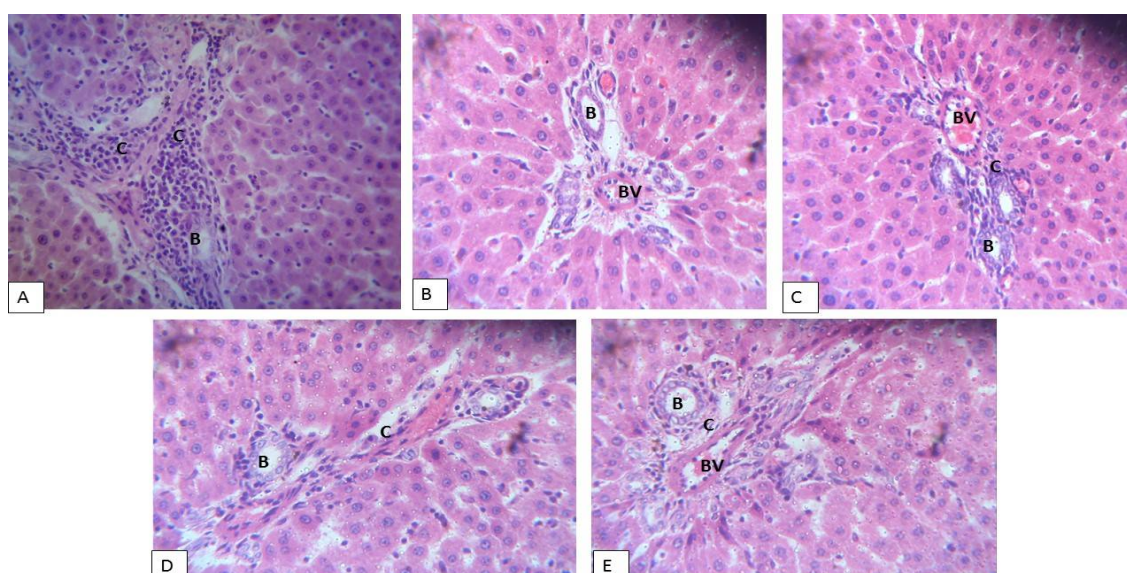


Figure 4: Photomicrograph section (H & E, x400) of liver of alloxan-induced diabetic rats. Legend: The 'B' shows the bile duct, 'BV' shows blood vessel while the 'C' shows cellular infiltrations. 4A = 5 % tween-20 treated group; 4B = Glibenclamide treated group; 4C = HLEHA 150 mg/kg treated group; 4D = HLEHA 300 mg/kg treated group and 4E = HLEHA 600 mg/kg treated group

extract-treated groups (Figure 4C-E) showed reduced proliferation of bile duct and mild mononuclear cells infiltration when compared with 5% tween-20 treated groups. There was no fibrosis in the extract-treated groups.

DISCUSSION

The extract treatment produced hypoglycemic, antidyslipidemic, hepatoprotective and anti-lipid peroxidation activities as well as reversed alloxan-induced histopathological changes in the pancreas and liver. HLEHA and GLB treatment caused significant decreases in the levels of FBG and HbA1c in the treated rats.

The antidiabetic activity of the extract could be linked to its ability to regenerate pancreatic beta cells (Figure 3) with possibly increased insulin secretion [19]. The result of HLEHA 150 mg/kg treated group compares favourably with that of the GLB (the reference drug) suggesting that the extract may have similar mechanism of action with glibenclamide (a sulfonylurea) which acts through the stimulation of insulin production from the β -cells [9]. Observed reduction in the concentration of HbA1c suggests that HLEHA and GLB have the potential to mitigate the development and progression of diabetic complication [20]. Elevated level of HbA1c predicts high risk of micro-vascular diabetic complications (retinopathy, neuropathy and nephropathy), development and progression [21].

The treatment of the animals with alloxan caused a persistent weight loss in 5% tween-20 treated group, which is attributed to dehydration, catabolism of protein and fat [22] (Figure. 1). The findings of this study indicate that HLEHA reversed the negative effects of alloxan on muscle metabolism by reducing the degeneration of adipose and muscle tissues through enhanced glucose metabolism [23].

The significantly reduced activities of ALT, AST and ALP in rats treated with HLEHA and GLB suggests that they alleviated hepatic and pancreatic toxicity induced by alloxan [24]. This was supported by the reversed histopathological lesions observed in the hepatic sections of the HLEHA and GLB-treated groups. Liver plays a critical role in carbohydrate, fat and protein metabolism [25]. A possible reason for this finding is that HLEHA elicited hypolipidemic effects which might be via the amelioration of the hyperlipidemia induced by alloxan administration and also exhibited the potential to prevent the development of cardiovascular complication in

diabetic patients. Possibly, HLEHA may have caused reduction in intestinal cholesterol absorption, bile acid sequestration and inhibition of 3-hydroxy-3-methylglutaryl coenzyme A to have produced the antidyslipidemic activities [26].

In general, alloxan and chronic hyperglycemia alter mitochondrial electron transfer chain, impair prooxidant/antioxidant balance and reduce antioxidant enzyme level via glycosylation, causing excessive generation of ROS which lead to oxidative stress [20,23]. Oxidative stress stimulates the release of pro-inflammatory mediators which lead to β -cell dysfunction and insulin deficiency [20]. Oxidative stress has also been linked to the development of diabetic complications and other chronic disease conditions [27]. Glibenclamide and HLEHA treatment elevated catalase and SOD activities but decreased the TBARS level when compared with 5% tween-20 treated group. This indicates that HLEHA can mitigate oxidative stress and its consequences in diabetes mellitus patients. The antioxidant activities of HLEHA may be responsible for the reversal of the alloxan-induced hepatic and pancreatic toxicities [28]. The regeneration of pancreatic islets and hepatocytes in the glibenclamide and HLEHA treated groups corroborated with reduced FBG, HbA1c level, liver enzymes and lipid profile in these groups. This finding is in agreement with reports of hypoglycemic and hypolipidemic activities of aqueous green tea extract in normal and alloxan-induced diabetic rats [23].

The antidiabetic, antioxidant and antidyslipidemic activities of *H. annuus* may have been mediated by the phytochemical constituents [29]. Previous studies have reported the presence of flavonoids, terpenes, saponins, alkaloids, tannins and glycoside in *H. annuus* leaf⁹. The antioxidant, antidiabetic and antidyslipidemic properties of these phytochemical constituents have been documented [30].

CONCLUSION

H. annuus has demonstrated potent antidiabetic, antioxidant and antidyslipidemic activities and has justified the pharmacological basis for the ethnomedical use in the treatment of diabetic patients. Possible mechanisms of the antidiabetic activity may have involved both pancreatic and extra-pancreatic processes. Further work should be aimed at the isolation, characterization and identification of the bioactive

compound(s) responsible for the reported pharmacological activities.

DECLARATIONS

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Conflict of interest

The authors declare that no conflict of interest is associated with this study.

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Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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