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ORIGINAL ARTICLE

Anti-hyperglycaemic effect of n-hexane fraction of *Lepidagathis alopecuroides* leaves in alloxan-induced diabetic rats

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Abstract

Diabetes remains a prominent cause of mortality in numerous developing nations. Hitherto, there is no cure for diabetes. Since time immemorial, medicinal plants have been explored for the treatment and management of diabetes. This study aimed to explore the anti-hyperglycaemic effects of the n-hexane fraction extracted from *L. alopecuroides* leaves in alloxan-induced diabetic rats. Twenty-four adult Wister rats were utilized, distributed across six groups, each consisting of four rats. Group 1 served as the normal control (i.e. were neither inducted nor treated), group 2 as the positive control (were inducted but were not treated) and group 3 as the standard control (were inducted and treated with the standard drug; glibenclamide). Groups 4, 5 and 6 received doses of 100, 200 and 400 mg/kg b.w of the *L. alopecuroides* leaf fraction. The treatment spanned 14 days, during which blood glucose levels, insulin levels, haematological parameters, histological features and other biochemical indicators were assessed. The findings indicated a significant reduction in blood glucose levels, significant increase in insulin levels, proper modulation of the haematological parameters and the restoration of normal pancreatic histoarchitecture in diabetic rats treated with *L. alopecuroides* leaf extract. This suggests that n-hexane fraction of *L. alopecuroides* possesses potential as an anti-diabetic agent.

Keywords Anti-hyperglycaemia · Diabetes mellitus · Histology · Insulin · *Lepidagathis alopecuroides*

Background

Diabetes mellitus (DM) is a prevalent condition characterized by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action or both (Arumugam et al. 2013). DM is a QA prevalent disease affecting the citizens of both developed and developing countries (Shiferaw et al. 2020). The prevalence of DM is on the high side. For instance, in 2021, there were 529 million people living with diabetes worldwide; this represents 10.5% of the world's population. The total number is predicted to rise to 643 million (11.3%) by 2030 and to 783 million (12.2%) by 2045 (Sun et al. 2022). The disease poses a significant health threat due to its association with severe complications such as blindness, kidney failure, cardiovascular diseases, amputations and increased mortality (Sun et al. 2022). Classic symptoms of untreated DM include weight loss, polyuria, blurry vision, headaches, fatigue, slow wound healing and itchy skin (WHO 2018). Despite the discovery of various medications to mitigate its effects, a definitive cure for DM remains elusive (WHO 2018). Thus, there is a growing need to explore alternative, cost-effective sources of treatment.

In many African countries, traditional medicinal plants are employed to manage DM. In Nigeria, herbal therapies hold a special place in healthcare, particularly among rural communities, owing to their easy accessibility and affordability (Iwueke et al. 2008). Recent studies indicate that over 80% of the African population relies on medicinal plants for primary healthcare (Ngbolua et al. 2016), with reported hypoglycaemic effects of some plants used in anti-diabetic remedies (Arumugam et al. 2013; Odoemena et al. 2010).

Lepidagathis alopecuroides (Vahl) is a tropical shrub belonging to the Acanthaceae family, commonly found in the coastal regions of West Africa. The plant is recognized for its antimicrobial properties and is traditionally used for treating abdominal pains and diarrhoea (Ekanem et al. 2003; Obomanu et al. 2010). Phytochemical analysis has identified alkaloids, tannins, saponins, glycosides and flavonoids in the plant

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(Obomanu et al. 2005, 2006). Although some herbalists in the Rivers State of Nigeria use the leaves of *L. alopecuroides* for DM treatment, there is a lack of scientific investigation to validate the efficacy of this leaf extract as an anti-diabetic agent (Gabriel and Okey 2009; Keremah et al. 2010; Edori et al. 2016). Therefore, this study aims to explore the anti-diabetic potential of *L. alopecuroides* in alloxan-induced diabetic rats.

Materials and methods

Materials

Chemicals and reagents

All chemicals and reagents utilized in this research were of analytical grade and procured from Sigma-Aldrich Co., St. Louis, Missouri, USA.

Animals

A total of twenty-four (24) adult male rats were sourced for, were housed under standard conditions (25 ± 2 °C and 12-h light/dark cycle) and provided with standard pellets (Grand Cereals Ltd., Enugu, Nigeria) twice a day.

Ethics for the use of laboratory animals

The study adhered to the guidelines for the care and use of laboratory animals as outlined by the Indian Council of Medical Research in 2001 and was approved by the Ethical Committee of Biological Sciences, University of Nigeria, Nsukka, with the approval number UNN/FBS/EC/3470.

Plant material

Lepidagathis alopecuroides leaves used in this study were collected from Abanwan community in Erei, Biasel local government area of Cross River State, Nigeria. The identification, confirmation and authentication of the plant material was done in the Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka. The plant specimen was then placed in the herbarium with the voucher number PCG/UNN/ 2758.

Methods

Experimental design

A total of twenty-four (24) Wistar albino male rats, 4 weeks old, weighing 150 g each, underwent a seven-day acclimatization period with free access to feed and water. The rats were fed with standard grower's mash rat pellets (Grand Cereals LTD, Enugu) and water. The basic constituent of the feed includes digestible protein, fibre, vitamins, minerals and essential amino acids. Following acclimatization, the rats were evenly divided into six (6) groups, each consisting of four rats. The oral route was employed for the administration of the extracts. The animals were fasted overnight (for about 12 h) on the last day of the experimental model before the induction of anaesthesia or the collection of blood samples.

Group 1: Normal control: animals were neither inducted nor treated
Group 2: Untreated group: animals were inducted but received no treatment
Group 3: Alloxan-induced diabetic rat + 5 mg/kg body weight of glibenclamide (standard drug)
Group 4: Alloxan-induced diabetic rat + 100 mg/kg body weight of n-hexane leaf fraction
Group 5: Alloxan-induced diabetic rat + 200 mg/kg body weight of n-hexane leaf fraction
Group 6: Alloxan-induced diabetic rat + 400 mg/kg body weight of n-hexane leaf fraction

The duration for the treatment was fourteen days and the doses used for the present study were based on the previous study reported by Obomanu et al. (2005).

Preparation of plant material

The fresh leaves of *L. alopecuroides* were gathered during summer, cleaned and air-dried until a constant weight was achieved at room temperature (29–35 °C) over a span of three weeks. Subsequently, the dried leaves were coarsely pulverized using a Crestor high-speed milling machine.

Extraction of plant materials

A total of 634.91 g of *L. alopecuroides* underwent soaking and maceration in 95% ethanol (Sigma-Aldrich Co., St. Louis, Missouri, USA) for 72 h, followed by filtration using muslin cloth and Whatman filter paper. The resulting clear filtrate was concentrated through a rotary evaporator. In a 500-mL separating funnel, 20 g of the crude extract was dissolved in 200 mL of 20% ethanol, followed by partitioning with 200 mL of n-hexane to obtain the n-hexane fraction. Further concentration of the n-hexane fraction took place using an oven at 45 °C. The sample was then stored in the refrigerator for 1 week before use.

Qualitative phytochemical analysis of the n-hexane fraction of *L. alopecuroides*

Phytochemical analysis of the n-hexane fraction of *L. alopecuroides* was conducted following the procedures outlined by Sofowora (1993) and Trease and Evans (2002) to identify its active constituents.

Acute toxicity studies

The acute toxicity of the n-hexane fraction of *L. alopecuroides* was assessed in adult male Wistar rats using the Lorke method (1983).

Determination of hematological and biochemical parameters

Determination of body weight The body weight of the animals was assessed using an electronic weighing balance.

Determination of blood glucose levels of experimental animals Blood glucose levels were determined according to the method of Frode and Medeiros (2008).

Determination of insulin level Insulin concentration was determined using the ELISA method as described by Sacks and Tietz (1994).

Haematological indices Haematological indices were measured using standard procedures outlined by Ochei and Kolhatkar (2008) and Dacie and Lewis (2000).

Determination of oxidative stress parameters Lipid peroxidation was estimated by spectrophotometrically measuring the level of the lipid peroxidation product, malondialdehyde (MDA), following the method of Wallin et al. (1993). Superoxide dismutase activity was assayed according to the method of Xin et al. (1991). Catalase activity was determined using Aebi's method (1983). Glutathione peroxidase activity was assessed following the procedure described by King and Wootton (1959).

Determination of lipid profile Serum total cholesterol concentration was determined according to the method described by Allian et al. (1974). Low-density lipoprotein (LDL) concentration was determined following the method of Albers et al. (1978) as per the Randox commercial kits. Serum high-density lipoprotein (HDL) concentration was determined according to the method of Albers et al. (1978) using the Randox commercial kit. Serum triacylglycerol (TAG) concentration was determined following the method of Albers et al. (1978) using the Randox commercial kit.

Determination of liver function parameters Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity were assayed using the Reitman and Frankel method (1975) as outlined in the Randox enzyme kit. Serum alkaline phosphatase (ALP) activity was assayed according to the method of Klein et al. (1960) as per the Randox enzyme kit.

Kidney function test The concentration of serum urea and creatinine was determined using the Tietz method (1994) as outlined in the Randox kit. Histological examination of the pancreas tissues of Wistar albino rats was conducted following the method described by Dury et al. (1991).

Table 1 Qualitative result of phytochemical composition of n-hexane fraction of *L. alopecuroides*

Phytochemical component	n-Hexane fraction of *L. alopecuroides*
Terpenoids	+++
Alkaloids	+++
Saponins	+++
Flavonoids	+++
Tannin	++
Phenol	++
Glycosides	+

+ = slightly present, ++ = moderately present, +++ = very much

Statistical analysis Results were analysed using the Statistical Package for the Social Sciences, SPSS version 22 (International Business Machine Corp., Armonk, New York, USA). Specific tests between means were carried out using the Duncan Multiple Range Test (DMRT). Generally, statistical significance was carried out at $p < 0.05$. Results were shown as mean ± standard deviation (SD).

Results

Qualitative phytochemical analysis of n-hexane fraction of *L. alopecuroides*

The screening of the n-hexane Fraction of *L. alopecuroides* revealed a substantial presence of bioactive compounds, including alkaloids, terpenoids, flavonoids and saponins, which were notably abundant. Phenol and tannin exhibited a moderate presence, while glycosides were observed to be slightly present in the fraction (see Table 1).

Weights of animals before and after administration of treatments

The outcomes demonstrated a noteworthy ($p < 0.05$) increase in the body weight of the treated groups compared to the untreated group (Fig. 1).

Effect of n-hexane leaf fraction of *L. alopecuroides* on blood glucose level of diabetic rats

The findings indicated a concentration and time-dependent substantial ($p < 0.05$) decrease in blood sugar levels in the treated groups compared to the untreated group. The most

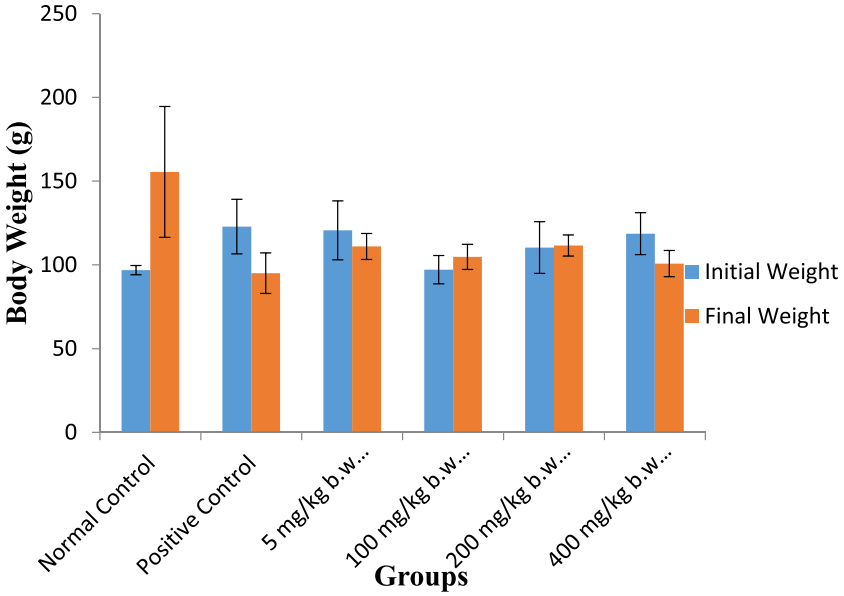

Fig. 1 Weight of animals before and after administration of treatments

significant reduction in blood sugar was observed at the 200 mg/kg treatment (Fig. 2).

Effect of n-hexane leaf fraction of *L. alopecuroides* on insulin levels of diabetic rats

The results demonstrated a notable ($p < 0.05$) increase in insulin concentration in the treated groups compared to the untreated group (Fig. 3).

Effect of n-hexane leaf fraction of *L. alopecuroides* on liver function enzymes of diabetic rats

The findings showed that various doses of the fraction resulted in a significant ($p < 0.05$) decrease in liver function enzymes (alkaline phosphatase, aspartate aminotransferase, alanine amino transferase) compared to the untreated group (see Table 2).

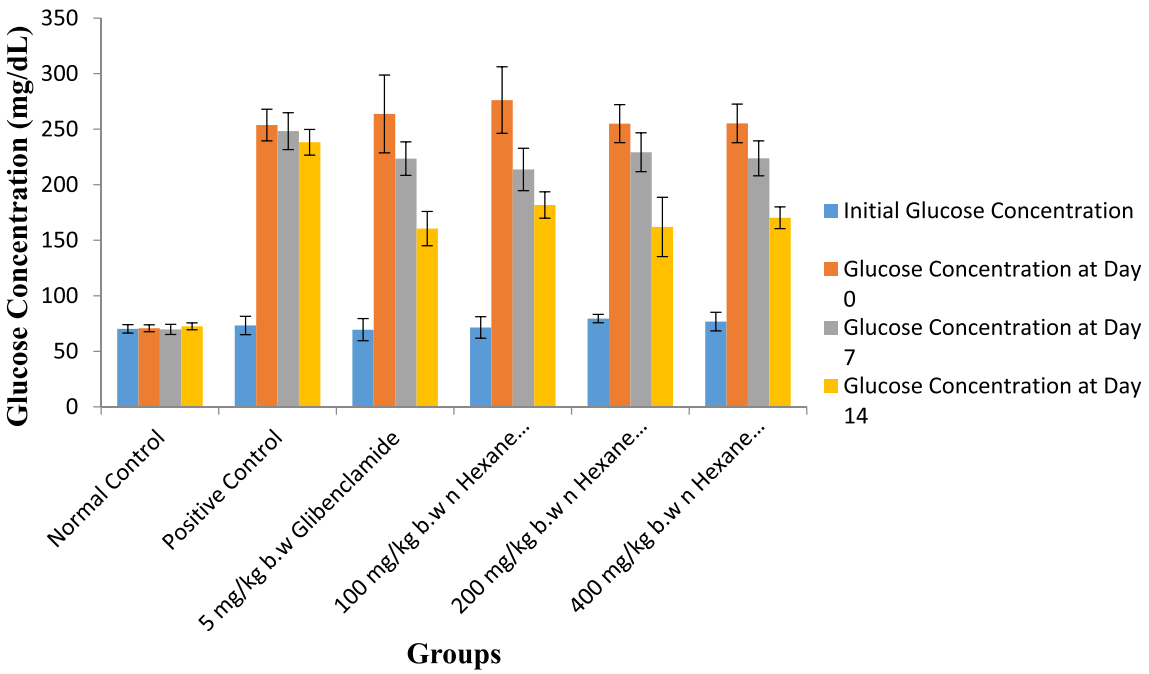

Fig. 2 Effect of n-hexane leaf fraction of *L. alopecuroides* on blood glucose levels of diabetic rats

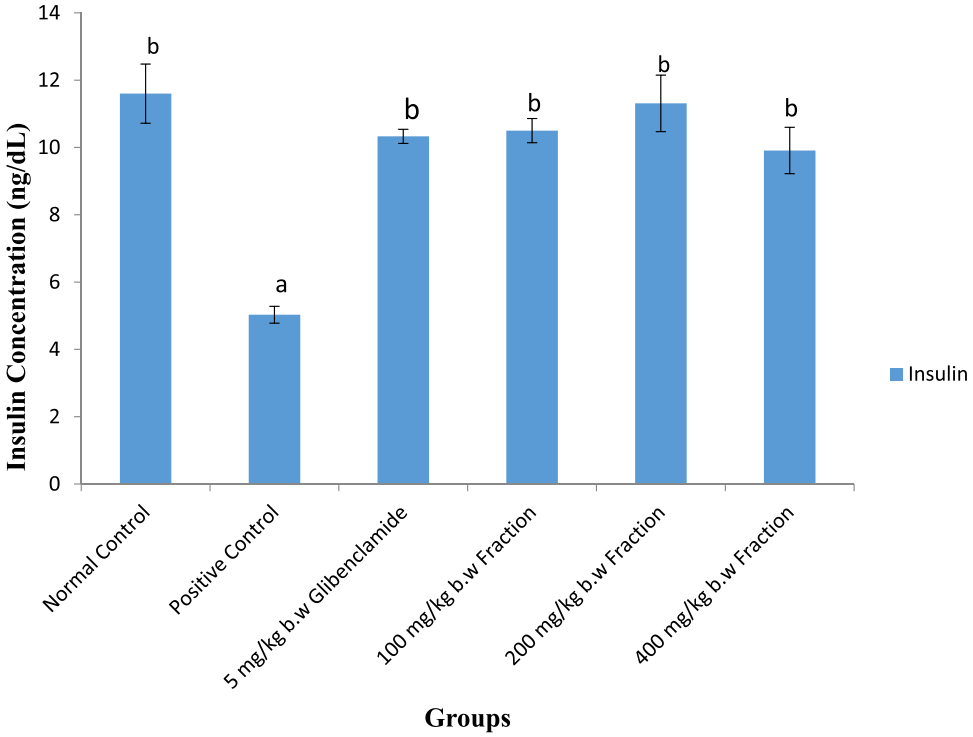

Fig. 3 Effect of n-hexane leaf fraction of *L. alopecuroides* on insulin levels of diabetic rats

Effect of n-hexane leaf fraction of *L. alopecuroides* on kidney function parameters

The result revealed that different doses of the fraction led to a concentration and time-dependent significant ($p < 0.05$) decrease in kidney function parameters (urea and creatinine) compared to the untreated group (Table 3).

Effect of n-hexane leaf fraction of *L. alopecuroides* on lipid peroxidation marker: malondialdehyde (MDA)

The results showed that the fraction caused a significant ($p < 0.05$) decrease in malondialdehyde levels in the treated group compared to the untreated group (Figs. 4 and 5).

Effect of n-hexane leaf fraction of *L. alopecuroides* on antioxidant enzyme activities in diabetic rats

The findings showed that the fraction led to a concentration and time-dependent significant ($p < 0.05$) increase in antioxidant enzymes (catalase, superoxide dismutase, glutathione) in the treated groups compared to the untreated group (Table 4).

Effects of n-hexane leaf fraction of *L. alopecuroides* on lipid profile

The results demonstrated a concentration and time-dependent significant ($p < 0.05$) decrease in LDL, TAG and total cholesterol in the treated groups compared to the untreated

Table 2 Reduction of liver function enzymes of diabetic rats by n-hexane leaf fraction of *L. alopecuroides*

Groups	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Normal control	17.50 ± 1.29^{b}	30.00 ± 3.27^{a}	19.00 ± 0.82^{a}
Positive control	26.25 ± 3.30^{c}	46.25 ± 1.71^{c}	26.00 ± 0.82^{d}
5 mg/kg b.w glibenclamide	11.25 ± 2.87^{a}	35.25 ± 3.77^{b}	20.25 ± 3.69^{ab}
100 mg/kg b.w n-hexane leaf fraction	22.25 ± 0.96^{bc}	42.75 ± 2.87^{c}	24.25 ± 0.96^{cd}
200 mg/kg b.w n-hexane leaf fraction	18.00 ± 7.26^{b}	37.25 ± 5.74^{b}	20.75 ± 1.89^{ab}
400 mg/kg b.w n-hexane leaf fraction	19.25 ± 0.96^{b}	37.50 ± 1.91^{b}	22.00 ± 0.82^{bc}

$n = 4$. Results are presented as mean ± STD. Mean values with different letters as superscripts across columns are considered significant at $p < 0.05$

ALP alkaline phosphatase, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

Table 3 Reduction of renal function parameters of diabetic rats by n-hexane leaf fraction of *L. alopecuroides*

Groups	UREA (mg/dL)	Creatinine (mg/dL)
Normal control	57.75 ± 4.03^{a}	2.04 ± 0.09^{ab}
Positive control	72.00 ± 4.97^{b}	2.16 ± 0.01^{c}
5 mg/kg b.w glibenclamide	60.00 ± 5.89^{a}	2.03 ± 0.10^{a}
100 mg/kg b.w n-hexane leaf fraction	69.50 ± 3.32^{b}	2.15 ± 0.03^{bc}
200 mg/kg b.w n-hexane leaf fraction	61.50 ± 2.08^{a}	2.08 ± 0.07^{abc}
400 mg/kg b.w n-hexane leaf fraction	69.25 ± 4.50^{b}	2.13 ± 0.04^{abc}

$n = 4$. Results are presented as mean ± STD. Mean values with different letters as superscripts across columns are considered significant at $p < 0.05$

group. Conversely, a significant ($p < 0.05$) increase in HDL was observed in the treated group compared to the untreated group (Table 5).

Effect of n-hexane leaf fraction of *L. alopecuroides* on haematological indices of diabetic rats

The outcomes related to haematological parameters in Table 6 revealed a significant ($p < 0.05$) decrease in the untreated groups across haematological parameters (WBC, RBC, Hb and PVC). Specifically, the WBC result showed a significant ($p < 0.05$) decrease in the positive control compared to the normal control. Standard control (5 mg/kg glibenclamide) and the treatment groups receiving 100 and 400 mg/kg displayed a significant ($p < 0.05$) increase in WBC concentration compared to the positive control (Table 5). The reference drug also demonstrated comparability with the normal control.

For RBC, a significant ($p < 0.05$) decrease was observed in the untreated diabetic group compared to the normal control. However, both the standard control (5 mg/kg glibenclamide) and the group receiving graded doses of leaf fraction significantly ($p > 0.05$) increased RBC count compared to the positive control. Moreover, the observed increase was comparable to the normal control group. Haemoglobin levels showed a significant ($p < 0.05$) decrease in the untreated diabetic group compared to the normal control, while a significant ($p < 0.05$) elevation was observed

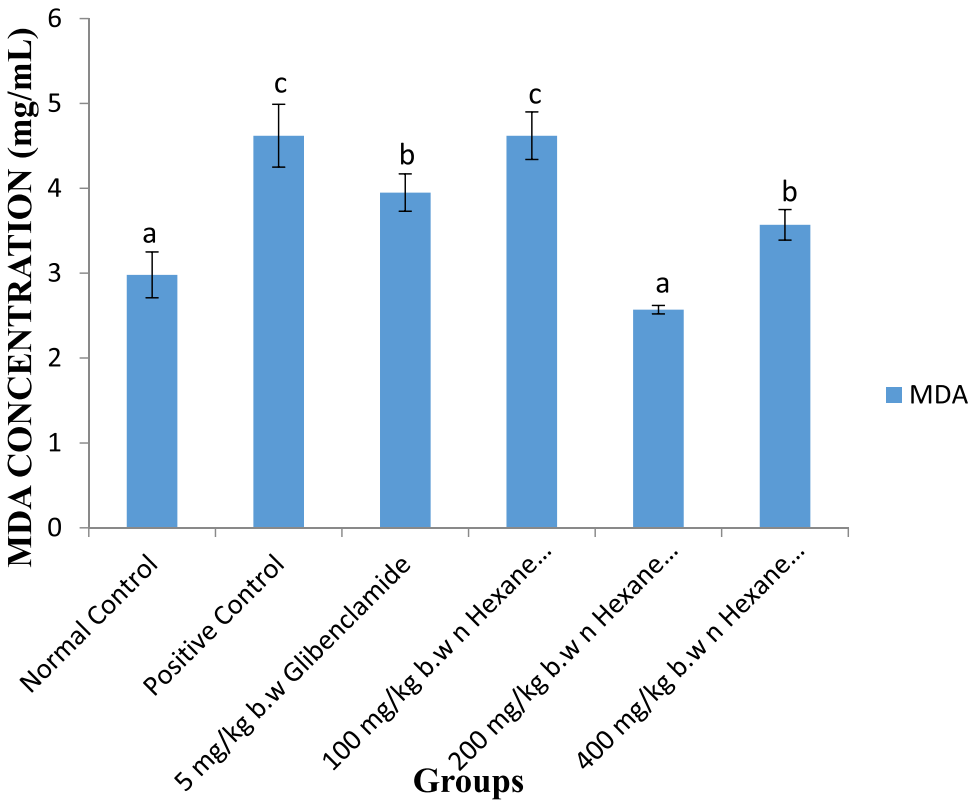

Fig. 4 Effect of n-hexane leaf fraction of *L. alopecuroides* on malondialdehyde (MDA)

in both the standard control (5 mg/kg glibenclamide) and the groups receiving graded doses of 100 and 400 mg/kg compared to the positive control. These increases were comparable to the normal control. PVC results indicated a significant ($p < 0.05$) decrease in the positive control compared to the normal control. No significant ($p > 0.05$) difference was observed between groups 2 and 4. Groups 3, 5 and 6 also showed no significant ($p > 0.05$) difference compared to the positive control. Similarly, comparing group 3 to the normal control showed no significant ($p > 0.05$) difference.

Histological examination of the pancreas of rats

Histological examination of the pancreas of rats in group 1 (normal control) not induced; not treated

Sections of the pancreas presented in plate 1 exhibited the normal histomorphology of the endocrine pancreas. Normal pancreatic islets (black arrow) were observed, randomly embedded between the acinar cells of the exocrine pancreas (Pas-Trichome × 400).

Histological examination of the pancreas of rats in group 2 (positive control) induced with 140 mg/kg b.w alloxan but not treated

Sections of the pancreas presented in plate 2 showed a marked decrease in the number and size of the pancreatic islets. The islet cells exhibited significant vacuolar degeneration and necrosis. The degenerate cells were markedly swollen with foamy cytoplasm (white arrow), admixed with eosinophilic necrotic debris (black arrow). (Pas-Trichome × 400).

Histological examination of the pancreas of rats in group 3 (standard control) induced and treated with (5 mg/kg b.w glibenclamide)

Sections of the pancreas presented in plate 3 revealed a reduction in the number and size of the pancreatic islets (black arrow). The islets that were present were mostly small and inconspicuous (Pas-Trichome × 400).

Histological examination of the pancreas of rats in group 4 induced and treated with (100 mg/kg b.w n-hexane fraction)

Sections of the pancreas presented in plate 4 displayed a reduction in the number and size of the pancreatic islets (black arrow). The islets that were present were mostly small and inconspicuous. Some of the islets also exhibited necrosis with fibrosis (white arrow) (Pas-Trichome × 400).

Histological examination of the pancreas of rats in group 5 induced and treated with (200 mg/kg b.w n-hexane fraction)

Sections of the pancreas presented in plate 5 showcased a marked proliferation of the α-cells (black arrow) in the pancreatic islets. The α-cells, characterized by small size, scant cytoplasm and heterochromatic nuclei, were predominantly located at the borders of the pancreatic islet. The slide displayed a less abundant β-cell located at the center of the pancreatic islets (white arrow) (Pas-Trichome × 400).

Histological examination of the pancreas of rats in group 6 induced and treated with (400 mg/kg b.w n-hexane fraction)

Sections of the pancreas presented in plate 6 demonstrated the normal histomorphology of the endocrine pancreas. Normal-sized pancreatic islet cells with apparent proliferation of the α-cells were observed (black arrow). However, multifocal areas of necrosis of the acinar cells of the pancreas were noted (white arrow) (Pas-Trichome × 400).

Discussion

The need for bioprospecting and the development of ethnomedicinal plants as hypoglycaemic agents is imperative, especially now that most diabetic patients find it increasingly difficult to manage hyperglycaemic conditions due to the high cost of synthetic anti-diabetic drugs with their consequent side effects. There are numerous traditional plants reported to have anti-hyperglycaemic properties.

The phytochemical screening of the n-hexane Fraction of *L. alopecuroides* indicated the presence of bioactive compounds such as phenol, alkaloids, terpenoids, flavonoids, saponins and tannins, while glycosides were slightly present in the leaf extract. The presence of these biologically active compounds suggests that these plants could exert some biological activities when taken by animals (Okafor 1983). Phytochemicals are secondary metabolites of plants known to exhibit diverse pharmacological and biochemical effects on living organisms (Trease and Evans 2002). Many studies have implicated that alkaloids and flavonoids such as (quercetin, kaempferol and caffeoyl glucoside) have all been demonstrated to inhibit hyperglycaemia in animal models (Shimizu et al. 2001; Ezekwe et al. 2014).

Flavonoids have aroused considerable interest recently because of their potential benefits on human health; they are potential anti-diabetic agents because they exert multiple actions that are both hypoglycaemic (insulinomimetic action) and anti-hyperglycaemic (insulin secretagogue)

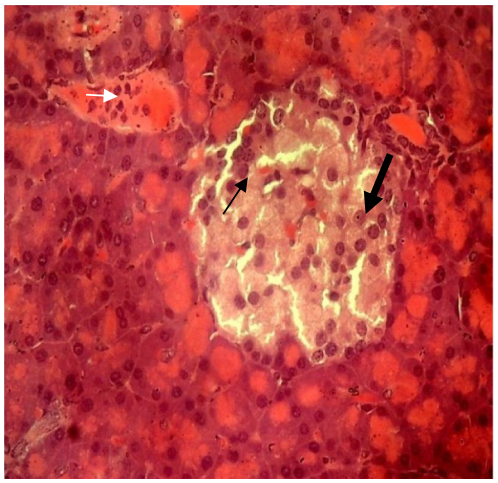

Plate 1: Photomicrograph of normalhistomorphology of the endocrine pancreas, of group 1 (normal control) showing normal pancreatic islets. (Pas-Trichome x 400).

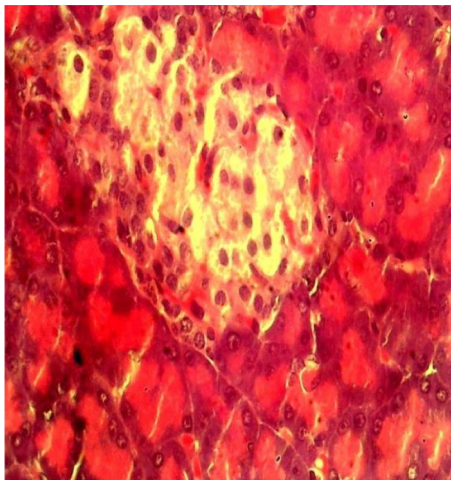

Plate 2: Photomicrograph of group 2 (positive control) showing a marked decrease in the number and sizes of the pancreatic islets, with marked vacuolar degeneration and necrosis of normal histomorphology of the endocrine pancreas, admixed with eosinophilc necrotic debris. (Pas-Trichome x 400).

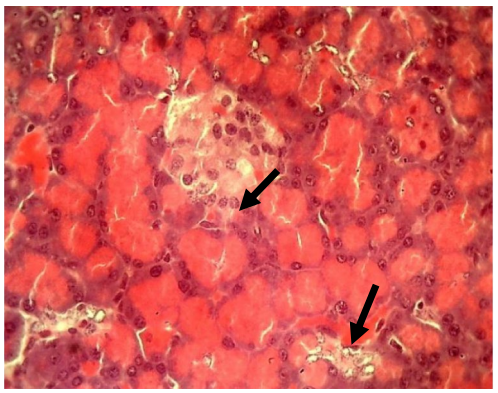

Plate 3: Photomicrograph of group 3 (standard control) showing a reduction in the number and sizes of the pancreatic islets. (Pas-Trichome x 400).

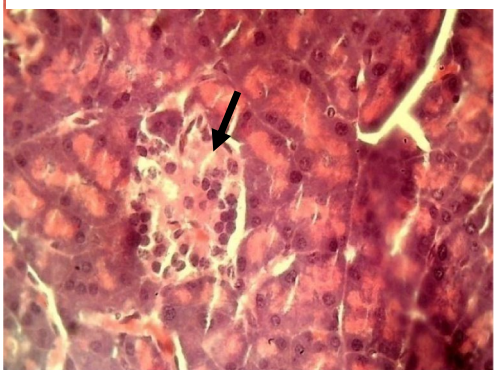

Plate 4: Photomicrograph of group 4 (100mg/kg fraction) showing a reduction in the number and sizes of the pancreatic islets. Some of the islets are also necrotic with fibrosis. (Pas-Trichome x 400).

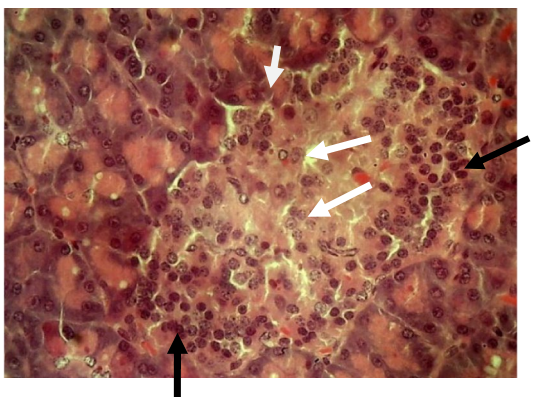

Plate 5: Photomicrograph of the pancreas of group 5 (200 mg/kg fraction) rat showing a marked proliferation of the α cells in the pancreatic islets with less abundant-βell located at the centre of the pancreatic islets. (Pas-Trichome x 400).

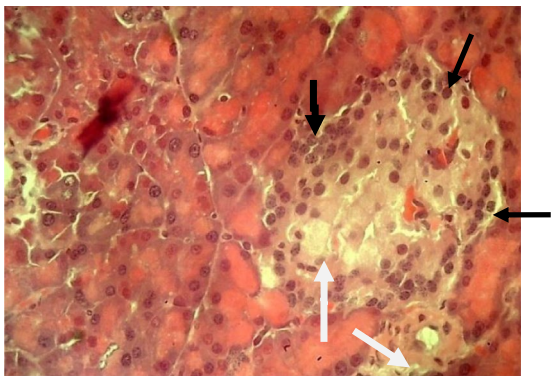

Plate 6: Photomicrograph of the pancreas of group 6 (400 mg/kg fraction) rats showing Normal sized pancreatic islet cells with seemingly proliferation of the αcells. However, multifocal areas of necrosis of the acinar cells of the pancreas were observed. (Pas-Trichome x 400).

(Cazarolli et al. 2008) and regenerate the damaged beta cells of pancreas (Tiwari and Rao 2002). In addition, flavonoids have been reported to possess antioxidant activity (Midddleton 2008) and thus, are capable of protecting cell membranes from peroxidative actions of free radicals. Flavonoids have also been reported to have anti-viral, anti-platelet, anti-tumour and anti-inflammatory effects (Middleton and Kardaswami 1992). Saponins are reported to

◂**Fig. 5** Histological examination of the animals' pancreas. **a** Photomicrograph of normal histomorphology of the endocrine pancreas, of group 1 (normal control) showing normal pancreatic islets. **b** Photomicrograph of group 2 (positive control) showing a marked decrease in the number and sizes of the pancreatic islets, with marked vacuolar degeneration and necrosis of normal histomorphology of the endocrine pancreas, admixed with eosinophilc necrotic debris. **c** Photomicrograph of group 3 (standard control) showing a reduction in the number and sizes of the pancreatic islets. **d** Photomicrograph of group 4 (100 mg/kg fraction) showing a reduction in the number and sizes of the pancreatic islets. Some of the islets are also necrotic with fibrosis. **e** Photomicrograph of the pancreas of group 5 (200 mg/kg fraction) rat showing a marked proliferation of the α-cells in the pancreatic islets with less abundant β-cell located at the centre of the pancreatic islets **f** Photomicrograph of the pancreas of group 6 (400 mg/kg fraction) rats showing Normal-sized pancreatic islet cells with seemingly proliferation of the α-cells. However, multifocal areas of necrosis of the acinar cells of the pancreas were observed

possess anti-diabetic and anti-carcinogenic, hypotensive and cardiac depressant properties and lower cholesterol levels (Trease and Evans 2002; Olaleye 2007).

There was a significant ($p < 0.05$) increase in body weight of both standard control and groups that received the graded doses of the fraction compared to the untreated group. This observation is true because, since the animals were fed properly and had no diabetes that could negatively impact their metabolism, there is tendency for them to add weight unlike the diabetic rats. Body weight loss represents one of the most common signs of DM. Despite the increased appetite, insulin deficiency reduces all anabolic processes and accelerates catabolic processes, contributing further to body weight loss (Association 2014).

Insulin is produced by the beta cells of the pancreas; it is the principal hormone regulating glucose metabolism. Its presence enhances glucose uptake and metabolism through various tissue cells including skeletal muscle, white adipose tissue and the liver (Barros et al. 2006). During non-pathological states, glucose is the stimulus for insulin secretion in pancreatic beta cells. Glucose entry into the pancreatic cells is through GLUT-2 transporters (Herman and Kahn 2005). Oxidation of glucose and the subsequent increase in ATP/ADP ratio in the cells triggers the closure of ATP-sensitive potassium channels (Doyle and Egan 2003). Inhibition of potassium efflux results in cell depolarization, leading to an influx of voltage-dependent calcium ions that stimulate the extrusion of insulin. A significant ($p < 0.05$) decrease in insulin concentration was observed in the untreated group when compared to the normal control. In contrast, there was no significant ($p > 0.05$) decrease in insulin concentrations of the groups that received the graded doses of the fraction when compared to normal control. The result further implies that the n-hexane fraction of *L. alopecuroides* has both protective and simulative effects on the pancreas. This could be as a result of the phytoconstituents such as flavonoids, alkaloids, tannin and phenol present in the plant as reported by Gupta et al. (2012); Tungmunnithum et al. 2018).

Hyperglycaemia is known to compromise the function of the liver. This is usually indicative by the alteration of the liver function parameters (Drotman and Lawhorn 1978). ALT, AST and ALP increased in activities in the alloxan intoxicated rats (positive control). The increased levels of serum marker enzymes are indicative of cellular leakage and loss of functional integrity of the cellular membrane in the liver (Drotman and Lawhorn 1978). In this study, the administration of graded doses of n-hexane leaf fraction of *L. alopecuroides* to respective groups of diabetic rats suppressed the elevated serum levels of ALT, AST and ALP. This clearly indicates that on continued treatment, the plant fraction may possibly stabilize the plasma membrane as well as help in the healing of the hepatic tissue damage (Effiong and Akpan 2015). Reduction in serum liver markers enzyme activities recorded in treatment groups is suggestive of cellular membrane/hepatocellular membrane protective effects of the fraction. ALP functions as a biochemical marker enzyme for maintaining membrane integrity. The increase in its plasma activity indicates lipid peroxidation of the cell membrane which occurs during DM mellitus (Akanji et al. 1993). The hepatocellular protection evidence in the present study might be due to the presence of flavonoids in the plant extract. According to Steffen and Menzel (2009), a significant increase in the serum activities of liver enzymes is an indication of hepatic injury, since there was a significant

Table 4 Increase of antioxidant enzyme activities of diabetic rats by n-hexane leaf fraction of *L. alopecuroides*

Groups	CAT (U/mg)	SOD (U/mg)	GSH (mg/dL)
Normal control	4.77 ± 0.34^{c}	11.40 ± 0.09^{ab}	5.73 ± 0.24^{c}
Positive control	2.44 ± 0.20^{a}	11.29 ± 0.09^{a}	3.57 ± 0.07^{a}
5 mg/kg b.w glibenclamide	3.58 ± 0.16^{b}	11.44 ± 0.04^{b}	5.72 ± 0.32^{c}
100 mg/kg b.w n-hexane leaf fraction	2.91 ± 0.08^{ab}	11.31 ± 0.14^{ab}	3.70 ± 0.28^{a}
200 mg/kg b.w n-hexane leaf fraction	3.18 ± 1.05^{ab}	11.36 ± 0.07^{ab}	4.73 ± 0.51^{b}
400 mg/kg b.w n-hexane leaf fraction	3.03 ± 0.38^{ab}	11.37 ± 0.07^{ab}	5.15 ± 0.34^{b}

$n = 4$. Results are presented as mean ± STD. Mean values with different letters as superscripts across columns are considered significant at $p < 0.05$

CAT catalase, *SOD* superoxide dismutase, *GSH* glutathione

Table 5 Improvement of the lipid profile of diabetic rats by n-hexane leaf fraction of *L. alopecuroides*

Groups	LDL (mmol/l)	TAG (mmol/l)	HDL (mmol/l)	T-CHOL (mmol/l)
Normal control	1.38 ± 0.11^{a}	1.08 ± 0.02^{a}	2.27 ± 0.13^{d}	4.40 ± 0.20^{a}
Positive control	2.27 ± 0.13^{d}	1.49 ± 0.36^{c}	1.38 ± 0.11^{a}	4.90 ± 0.24^{b}
5 mg/kg b.w glibenclamide	2.09 ± 0.08^{cd}	1.20 ± 0.00^{ab}	2.24 ± 0.19^{cd}	4.40 ± 0.16^{a}
100 mg/kg b.w n-hexane leaf fraction	2.24 ± 0.19^{cd}	1.39 ± 0.17^{bc}	2.05 ± 0.09^{c}	4.85 ± 0.25^{b}
200 mg/kg b.w n-hexane leaf fraction	2.05 ± 0.09^{c}	1.15 ± 0.07^{ab}	2.09 ± 0.08^{cd}	4.35 ± 0.39^{a}
400 mg/kg b.w n-hexane leaf fraction	1.60 ± 0.13^{b}	1.23 ± 0.14^{ab}	1.60 ± 0.13^{b}	4.85 ± 0.13^{b}

$n = 4$. Results are presented as mean ± STD. Mean values with different letters as superscripts in columns are considered significant at $p < 0.05$

LDL low-density lipoprotein, *TAG* triacylglyceride, *HDL* high-density lipoprotein, *T-CHOL* cholesterol

reduction in the serum concentration of these enzymes when compared with the untreated group, it suggests that *L. alopecuroides* are safe to be used as anti-diabetic drugs.

Kidneys are the major excretory organs and renal function tests are devised to detect possible renal damage. Increased serum levels of urea and creatinine are among the most sensitive indicators of kidney injury. Hyperglycaemia is known to induce the elevation of urea and creatinine (Alarcon et al. 2002). Alarcon et al. (2002) reported that alloxan-induced hyperglycaemia elevates the serum urea and creatinine. High serum urea in diabetic control rats can be attributed to the stimulation of gluconeogenesis as an alternative glucose source as a result of insulin deficiency (Abdulazeez et al. 2013). In this study, urea and creatinine levels were significantly ($p < 0.05$) higher in diabetic control rats indicating renal impairment. However, the administration of *L. alopecuroides* n-hexane leaf fraction for 14 days significantly lowered these indices. The stabilization of these parameters indicates improvement in renal function which could be attributed to the anti-hyperglycaemic effect of the fraction and thus increased insulin effect causing a decline in proteolysis. Similar observation has been reported (Shah and Khan 2014).

Various studies have shown that DM is associated with increased formation of free radicals and suppression of antioxidant enzyme systems. Significant ($p < 0.05$) decreases in serum SOD and CAT activities and a significant ($p < 0.05$) increase in MDA concentration were observed in the untreated rats when compared to the normal group. On the other hand, the administrations of *L. alopecuroides* leaf fraction improved the endogenous antioxidant system and suppressed the generation of free radicals evidenced by the decrease in MDA concentration in the treated groups. The antioxidant effect of *L. alopecuroides* fraction could be explained by two mechanisms. Firstly, the *L. alopecuroides* leaf fraction possibly prevented protein glycosylation and peroxidation by scavenging free radicals, thereby inhibiting their damaging effects. In addition, *L. alopecuroides* leaf fraction possibly induced protein synthesis of antioxidant enzymes. Based on previous studies, it has been reported that polyphenolic compounds increased enzyme expression of SOD and GPx at the transcriptional level (Vina et al. 2006; Ben et al. 2017). Furthermore, GSH is a major non-protein in living organisms which plays a central role in coordinating the body's antioxidant defence processes. Perturbation of GSH status in the biological system has been reported to lead to serious consequences (Uday et al. 1999). The decline in GSH content in the serum of diabetic-induced rats and their subsequent return towards near normal in leaf fraction treated groups reveals the antioxidant effect of *L. alopecuroides* leaves. The possible mechanisms underlying the antioxidant properties of most drugs include the prevention of GSH depletion and the destruction of free radicals (Valenzuela et al. 1985). These two factors may possibly be attributed to the antioxidant properties of *L. alopecuroides.*

Table 6 n-hexane leaf fraction of *L. alopecuroides* improves haematological indices of diabetic rats

Groups	WBC ($\times 10^9$/L)	RBC ($\times 10^{12}$/L)	Hb (g/dL)	PCV (%)
Normal control	76.00 ± 3.27^{c}	4.50 ± 0.08^{bc}	10.24 ± 0.7^{b}	40.25 ± 1.89^{c}
Positive control	62.75 ± 1.89^{a}	4.20 ± 0.22^{a}	8.15 ± 0.46^{a}	33.25 ± 2.75^{a}
5 mg/kg b.w glibenclamide	73.25 ± 0.96^{bc}	4.55 ± 0.10^{c}	9.81 ± 1.39^{b}	38.75 ± 2.87^{bc}
100 mg/kg b.w n-hexane leaf fraction	70.00 ± 4.32^{b}	4.50 ± 0.14^{bc}	9.75 ± 0.48^{b}	33.25 ± 2.75^{a}
200 mg/kg b.w n-hexane leaf fraction	64.00 ± 1.63^{a}	4.25 ± 0.25^{ab}	9.05 ± 0.40^{ab}	36.50 ± 3.42^{ab}
400 mg/kg b.w n-hexane leaf fraction	70.00 ± 3.27^{b}	4.40 ± 0.16^{ab}	10.05 ± 0.54^{b}	34.00 ± 4.24^{ab}

$n = 4$. Results are presented as mean ± STD. Mean values with different letters as superscripts across columns are considered significant at $p < 0.05$

WBC white blood cell, *RBC* red blood cell, *Hb* haemoglobin, *PCV* packed cell volume

The total serum lipids (cholesterol, LDL-chol and TAG) levels in treated animals were significantly ($p < 0.05$) lower compared to the untreated group. The increase in HDL-cholesterol concentrations of the treated rats could account for the use of the plant in traditional medicine for the treatment of DM and hypertension. The results of this study clearly indicate that the administration of n-hexane leaf fraction of *L. alopecuroides* produces hypolipidaemic effects and may prevent cardiovascular diseases, which have a correlation with DM A similar decrease in serum lipids was also reported by Matter and Halal (2001) and Longe et al. (2015). The serum levels of triacylglycerol in the standard control group and groups that received 200 and 400 mg/kg leaf fraction respectively were not significantly different from normal control. The decrease in total lipids, therefore, may be due to a comparable decrease in free fatty acids (Harper et al. 1993). Such a decrease is expected in the case of accumulation of fat in adipocytes, both by synthesis and decreased liberation of fatty acids. It may also be due to a number of other mechanisms including the inhibition of rate limiting enzymes of cholesterol biosynthesis, HMG, COA reductase into bile acids and inhibition of cholesterol absorption from the intestine due to the formation of complexes with compounds such as glycosides and saponins (Gamousi et al. 2010).

Alteration in the various haematological parameters and the immune system during the course of DM has been reported (Mansi and Lehham 2008). Also, toxicological studies revealed that ingestion of medicinal plants and drugs can alter normal haematological values (Ajagbonna et al. 1999). Therefore, haematological parameters could be an important tool in the assessment of deleterious effects of drugs as well as medicinal plants and their extracts (Yakubu et al. 2007). There was a non-significant ($p > 0.05$) decrease of RBC count of the groups that received the graded doses of the fraction compared to the normal control. The white blood cell (WBC), packed cell volume (PCV) and haemoglobin (Hb) concentration decreased in diabetic rats compared to the group that received the graded doses of the fraction. This is in line with previous work where the occurrence of anaemia in DM has been documented (Thomas et al. 2005).

The histological analysis of the experimental rats showed that the untreated group had a marked decrease in the number and sizes of the pancreatic islets. The islet cells showed marked vacuolar degeneration and necrosis, swollen with foamy cytoplasm and eosinophilic necrotic debris when compared to the normal control, which showed the normal histomorphology of the endocrine pancreas, and normal pancreatic islets that are randomly embedded in-between the acinar cells of the pancreatic. There was also a marked decrease in the number and size of the pancreatic islets in groups that received 5 mg/kg b.w glibenclamide (standard control) and 100 mg/kg b.w fraction. However, the groups that received 200 and 400 mg/kg b.w fraction showed a marked proliferation of the α-cells in the pancreatic islets, with less abundant β-cells located at the centre of the pancreatic islets. Recent studies have associated the proliferation of the α-cells of the islet with β-cell regeneration via the process of trans-differentiation (Sangan and Tosh 2010). Several lines of evidence have also indicated an important role of α-cells as direct progenitors of β-cells both in the embryonic development of the islets and in the regeneration of islets in the adult pancreas (Sangan and Tosh 2010; Courtney et al. 2011). The ability of *L. alopecuroides* fractions to induce the proliferation of β-cells supports other results in this work and also serves as an indication that *L. alopecuroides* could serve as a potent anti-diabetic drug.

Conclusions

The results of this study showed that the n-hexane fraction of *L. alopecuroides* significantly reduced the hyperglycaemia, increased the insulin level, improved the lipid profile, the antioxidant status, the liver function parameters, the kidney function parameters and the haematological parameters in the alloxan-induced diabetic rats. This signifies that while n-hexane fraction of *L. alopecuroides* has the ability as an anti-diabetic agent, it can as well properly modulate the liver and kidney functions without any side effects on these key organs of the body. However, further studies need to be done to isolate the active principle in n-hexane fraction of *L. alopecuroides* responsible for its ability as an anti-diabetic agent.

Abbreviation DM: Diabetes

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Informed consent For this type of study, informed consent is not required.

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