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Anti-diabetic Properties of Crude Methanolic Leaf Extract of *Launaea taraxacifolia* (Wild Lettuce)

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Abstract: *Launaea taraxacifolia*, commonly called wild lettuce has been claimed to have anti-hyperglycaemic property but there is no sufficient scientific data to justify it. Thirty male Wistar rats used were divided into five groups of six animals each. Group 1 received food and water only serving as control. Diabetes was induced in Groups 2, 3 and 4 with 150 mg/kg alloxan; thereafter, distilled water, 100 mg/kg body weight extract, 200 mg/kg body weight of extract and 100 mg/kg body weight glibenclamide were administered once daily respectively. At the end of the third week, the rats were fasted overnight and sacrificed, their blood was collected and organs excised. Cholesterol, triglycerides, LDL-cholesterol, HDL-Cholesterol, urea and creatinine were analysed spectrophotometrically. Gas Chromatography- Mass Spectrometry (GC-MS) analysis of the extract was done. The GC-MS analysis revealed the presence of 8 compounds. The most and least abundant were cis-10-pentadecen-01-ol (15.6%) and stearic acid (4.4%) respectively. The extract of *L. taraxacifolia* significantly ($p<0.05$) reduced the blood glucose levels from day 14 onwards and also significantly ($p<0.05$) decreased total cholesterol, triglyceride and LDL- cholesterol levels in plasma, liver and kidney while significant increase ($p<0.05$) in HDL cholesterol levels in plasma and kidney was obtained when compared with the diabetic control. The extract also significantly decreased ($p<0.05$) creatinine and urea levels compared to the untreated control animals. The photomicrographs of the liver and kidney revealed that the histoarchitecture of the animals treated with the extract were not significantly different from the distilled water treated control animals. This study has shown that *L.taraxacifolia* leaves are potent and effective as anti-hyperglycaemic, hypocholesterolemic, hepatoprotective and renoprotective agent due to the action of inherent bioactive compounds.

KEYWORDS: *Launaea taraxacifolia*, Diabetes, Cholesterol, Blood Glucose

1.0 Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by elevated blood glucose levels (hyperglycaemia) resulting from defects in insulin secretion, insulin action or both. It may present with clinical symptoms which include thirst, polyuria, blurring of vision and weight loss, and may develop into coma and ultimately death in its severe form (WHO, 1999).

In search for the treatment of hyperglycaemia, several plants have been screened and reported to have antidiabetic effects. Concentrated extract of onions bulb exerted a week hypoglycemic action in healthy and alloxan diabetic animals. Garlic has also be found to possess antidiabetic and hypocholesterolaemic actions. Administrations of *Alstonia boonei* stem bark to diabetic mice caused a decrease in blood glucose level close to that of control and also reduced the specific activity of hepatic glucose-6-phosphatase and fructose-1, 6-diphosphatase with concomitant increased activity of glucose-6-phospahate dehydrogenase.

Launaea taraxacifolia, formerly known as *Lactuca taraxacifolia*, is a medicinal plant found in Europe, Asia, Central and South America, North and West Africa. It is common in West Africa particularly Ghana, Benin, Senegal and Nigeria. It is known as wild lettuce and locally called *efo yanrin* in the South Western region of Nigeria (Adebisi and Ladipo, 2000).

L. taraxacifolia is one of the undervalued

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reservoirs of diversity of African leafy vegetables which are extremely important for food security, nutrition and poverty alleviation throughout the continent (Sakpere *et al.*, 2011). The plant serves for food and medicinal purposes. The leaves are eaten fresh as salad or used in soups. The leaves of the plant have fertility potentials in that it is used to induce multiple births in rabbits, sheep and goats. The plant is rich in vitamins, minerals, proteins, essential fatty acids, fibre contents and flavonoids (Adinortey *et al.*, 2012; Adejuwon *et al.*, 2014).

L. taraxacifolia leaves have protective roles against cisplastin-induced micronuclei in bone marrow erythrocytes of Wistar rats (Adejuwon *et al.*, 2014). Previous finding has revealed the antioxidant and hypolipidemic effects of *Launaea taraxacifolia* leaves on cell lines (Koukoui *et al.*, 2015). There is increasing demand for plants with potential activities in the therapeutic field of medicine. Because plants have phytochemicals which on their own or by interaction with other chemicals may cause toxicity, therefore, there is need to investigate the safety use of plants for medicinal purposes.

However, there are no data on the ameliorative potentials of *Launaea taraxacifolia* leaves against alloxan-induced diabetic animals. In addition, there are no available findings on the in-depth analysis of the chemical constituents of *L. taraxacifolia* leaves. This study seek to address these lacuna.

2.0 Materials and Methods

2.1 Materials

2.1.1 Plant

The fresh leaves of *Launaea taraxacifolia* were bought from a market at Ota, Ogun State, Nigeria and authenticated by Dr. P. I. Oni of the Department of Biological Sciences, Bells University of Technology, Ota.

2.1.2 Experimental Animals

Thirty male albino rats weighing from 100-170 g obtained from a recognised breeder were used for the experiments. They were housed 6 rats per cage, maintained under proper temperature and ventilation at the Experimental Animal House, Bells University of Technology, Ota, Nigeria. The rats were housed in polypropylene cages, maintained under standard conditions; 12 hour light and 12 hour dark cycle, 25±5 °C and 40-60% humidity. They were fasted for 12 hours before the commencement of the experiment but were allowed free access to water. The rat were fed standard pelleted diet, given water freely and allowed to acclimatize for 10 days before commencement of the experiment.

2.2 Methods

2.2.1 Preparation of Extract

The leaves were rinsed in distilled water to free them from dust and other unwanted particles. Leaves of *L. taraxacifolia* were then dried for 4 days at room temperature, pulversied, extracted in methanol and kept at 60°C for 24 hours. The resulting mixture was filtered and concentrated using a rotary evaporator. The extracts were then kept in a glass container.

2.2.2 Induction of Diabetes

Diabetes was induced by a single intraperitonial injection of 150 mg/kg body weight of alloxan monohydrate in normal saline solution. After 3 days, the surviving rats with fasting blood glucose level of more than 200 mg/dL were considered as alloxan-induced diabetic rats (Gidado *et al.*, 2005).

2.2.3 Experimental Design

The rats were divided into five groups. Each group consisted of 6 rats. Table 1 shows the summary of the experimental treatment of the individual groups. Blood glucose levels were determined using Accu-chek™ glucose strips and Accu-chek™ test metre before treatment and after treatment at the interval of 7 days.

2.2.4 Collection of Samples

After 21 days, the rats were subjected to an overnight fast and blood glucose levels were

determined. Blood samples were then collected into heparinized tubes by retroorbital plexus puncture method. Liver and kidney were removed for biochemical analyses. The samples were centrifuged immediately at 4000 rpm for 10 minutes to separate plasma from erythrocytes. All samples were stored at -20°C until required.

Table 1: Animal grouping and extract administration

Groups	Treatment
Group I (Control)	Normal, given feed and water only
Group II (Diabetic Control)	Diabetic, given feed and water only
Group III (*L. taraxacifolia* Dose I)	Diabetic, given feed, water and treated orally with 100 mg/kg body weight/day of methanolic extract of *Launaea taraxacifolia* leaves
Group IV (*L. taraxacifolia* Dose II)	Diabetic, given feed, water and treated orally with 200 mg/kg body weight/day of methanolic extract of *Launaea taraxacifolia* leaves
Group V	Diabetic, given feed, water and treated with 100 mg/kg body weight/day of the reference drug, Glibenclamide.

2.2.5 Biochemical Analyses

2.2.5.1 Preparation of HDL Extract

Aliquots of plasma samples were taken for HDL- extraction. The procedure involved the precipitation of Very Low Density Lipoprotein (VLDL) and Low Density Lipoprotein (LDL) with precipitant, phosphotungstic acid in the presence of magnesium ions using commercial kit (Randox Laboratories Limited, United Kingdom). The resultant mixture was vortexed and left at room temperature for 10 minutes. The mixture was then centrifuged at 4000 rpm for 10 minutes; the supernatant which contains the HDL extract was carefully decanted into Eppendorf tubes and stored until analyzed.

2.2.5.2 Determination of Lipid Profile

Plasma concentrations of total cholesterol and triglycerides were determined using commercial (Randox Laboratories, United Kingdom) kits. Total Cholesterol was determined spectrophotometrically, according to the methods of Allain *et al* (1974). Triglyceride was determined spectrophotomerically using the appropriate kits, according to the method of Buccolo and David (1973). HDL-Cholesterol was determined in plasma with same commercial kits for total cholesterol after very low density lipoproteins (VLDL) and LDL-C were precipitated with heparin-$MnCl_2$ solution as previously described. LDL-Cholesterol values were calculated using the values of total cholesterol, HDL-Cholesterol and triglyceride according to the method of Friedewald *et al* (1972).

2.2.5.3 Determination of Urea and Creatinine

Urea concentration was determined according to the procedure described by Weatherburn (1967). Creatinine concentration was determined as described by Bartels *et al* (1972).

2.2.6 Histopathological Examination

Histological studies were carried out on the liver and kidney as described by Krause (2001). After sacrifice, the tissues were immediately removed and portions of the tissues were fixed in 10% buffered formalin. Fixed tissue samples were dehydrated, cleared and embedded in paraffin wax (Sigma Chemical, St. Louis, MO, USA). The formed paraffin block together with the tissues was then sectioned into very thin sliced sections. Sections were stained with haematoxylin and eosin (Sigma Chemical, St. Louis, MO, USA) and then examined under a microscope using a magnification of x40.

2.2.7 Gas Chromatography Mass Spectrometric Analysis

The crude extract was analyzed using a Shimadzu gas chromatograph (model QP2010 plus), equipped with Flame Thermionic Detector (FTD) and capillary column (30 m x 0.25 mm, film thickness, 0.25 µm). Injector and detector temperatures were set at 220 and 290, respectively. Column oven temperature was programmed from 80-220 at the rate of 4 min^{-1}; initial and final temperatures were held for 3 and 10 minutes, respectively. Helium was used as a carrier gas with a flow of 1.5 mL min^{-1}. A sample of 1.0 µL was injected, using slit mode (split ratio, 1:100). Quantification was completed by built-in data-handling software supplied by the manufacturer of the gas chromatograph. The results (composition) were reported as a relative percentage of the total peak area.

GC-MS analysis of the essential oils was performed using a gas chromatograph (model QP 2010 plus, Shidmazu Corporation, Kyoto, Japan), gas chromatographic (GC) system, equipped with a Mass selective detector and auto injector. Compounds were separated on capillary column (30 m x 0.25 mm, film thickness 0.25 µm). A sample of 1.0 µL was injected using the split mode (split ratio 1:100). For GC/MS detection, an electron ionization system, with ionization energy of 70 eV, was used. Column oven temperature programme was the same as previously used in GC analysis. Helium was used as a carrier gas at a flow rate of 1.5 mL min^{-1}. Mass scanning range was 40-700 *m/z* while injector and MS transfer line temperatures were set at 220 and 290 °C, respectively.

The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library, as well as on comparison of their retention indices either with those of authentic compounds or with literature values.

2.2.9 Statistical Analysis

Data were expressed as mean ± S.D. One way analysis of variance (ANOVA) followed by Duncan Multiple Comparisons was used to analyze the results with $p<0.05$ considered significant.

3.0 Results

Figure 1 presents the glucose levels of the rats before and after treatment. There was a general decrease in the glucose levels in all the treated groups. The cholesterol levels of the rats are depicted in Figure 2. Generally, there was a significant decrease ($p<0.05$) in the plasma, liver and kidney total cholesterol levels in the treated diabetic animals compared with the untreated diabetic rats.

Figure 3 summarizes triglyceride levels in all the animals. A general trend of significant decrease in plasma, liver and kidney triglyceride levels was observed in the treated diabetic animals compared with the untreated diabetic rats.

Figure 4 shows the HDL-Cholesterol levels in all the animals. There was a significant increase ($p<0.05$) in the plasma and kidney HDL-Cholesterol levels in the treated diabetic rats compared with the untreated diabetic animals.

LDL-Cholesterol levels of the animals are summarized in Figure 5. A significant decrease ($p<0.05$) in plasma and liver LDL cholesterol levels was observed in the treated diabetic animals compared to the untreated diabetic rats.

Urea levels in the rats are depicted in Figure 6. A significant ($p<0.05$) decrease in the plasma and liver urea levels was observed in the treated diabetic rats compared with the untreated diabetic animals. There was a decrease in the kidney urea levels in the diabetic animals only compared with the untreated diabetic rats.

Figure 7 shows the levels of creatinine in the animals. There was a significant ($p<0.05$) decrease in plasma and liver levels of creatinine in the treated rats compared with the untreated diabetic animals.

The GC-MS analysis of the leaves of *L. taraxacifolia* revealed the presence of 8 compounds (Table 2). The most abundant component is *cis*-10-pentadecen-1-ol (15.6%). The other major compound present in the extract were *trans*-9-tetradecenoic acid (15.0%), *cis*-11-hexadecenoic acid (15.0%), *trans*-2-octadecadecen-1-ol (15.0%), oleic acid (15.0%),

palmitic acid (13.3%), margaric acid (4.4%) and stearic acid (4.4%).

The control liver shows moderate vacuolar change of centrilobular hepatocytes. Induction of diabetes caused multiple foci of coagulative hepatocellular necrosis with influx of macrophages and moderate Kupffer cell hyperplasia. The liver sections of the animals treated with 100 mg/kg body weight of *L. teraxacifolia* generally have a finely reticulated appearance with no visible lesion. Multifocal thinning of hepatic cords; random foci of single-cell hepatocellular necrosis with moderate Kupffer cell hyperplasia were evident when the animals were treated with 200 mg/kg body weight of *L. teraxacifolia*. Treatment with glibenclamide showed no visible lesion except for mild congestion of hepatic sinusoids (Plates A1-E1).

The kidney sections of control animals showed moderate congestion of glomerular capillaries with marked sloughing off of epithelium of tubules, acute tubular nephropathy. Extensive severe sloughing-off of tubular epithelium at the cortico-medullary junction and cortex with mild multifocal interstitial nephritis were observed in the sections of diabetic animals. Treatment with 100 mg/kg body weight of *L. teraxacifolia* show no visible lesion. Marked sloughing-off of tubular epithelium gave the tubules distended cyst-like appearance in the kidney sections of animals treated with 200 mg/kg body weight of *L. teraxacifolia*. There was evidence of mild swelling of tubular epithelial cells in section of the kidney of rats treated with glibenclamide.

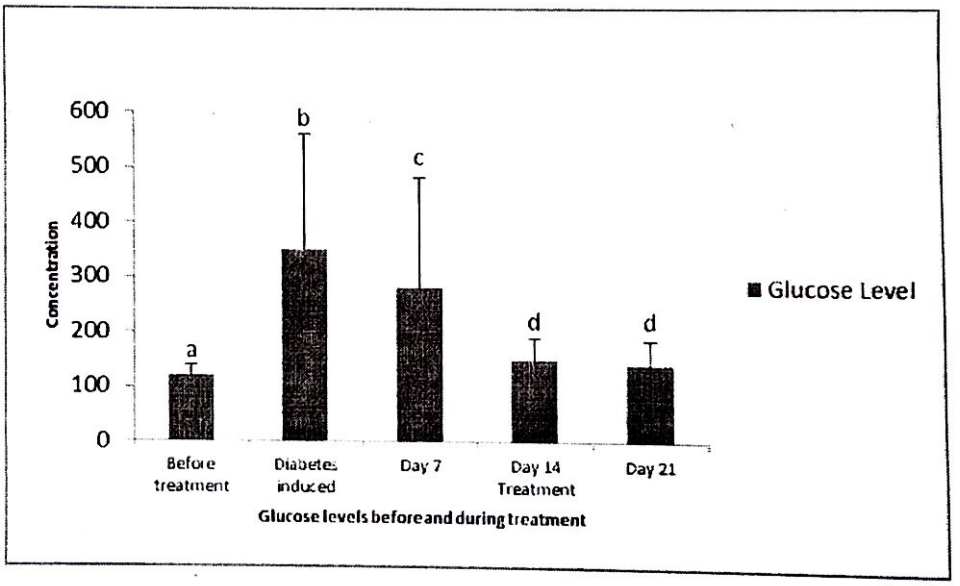


Figure 1: Glucose levels of the rats before and during treatment. Each bar represents the mean ± SD of 6 rats. Bar with different alphabets are significantly different at $p<0.05$

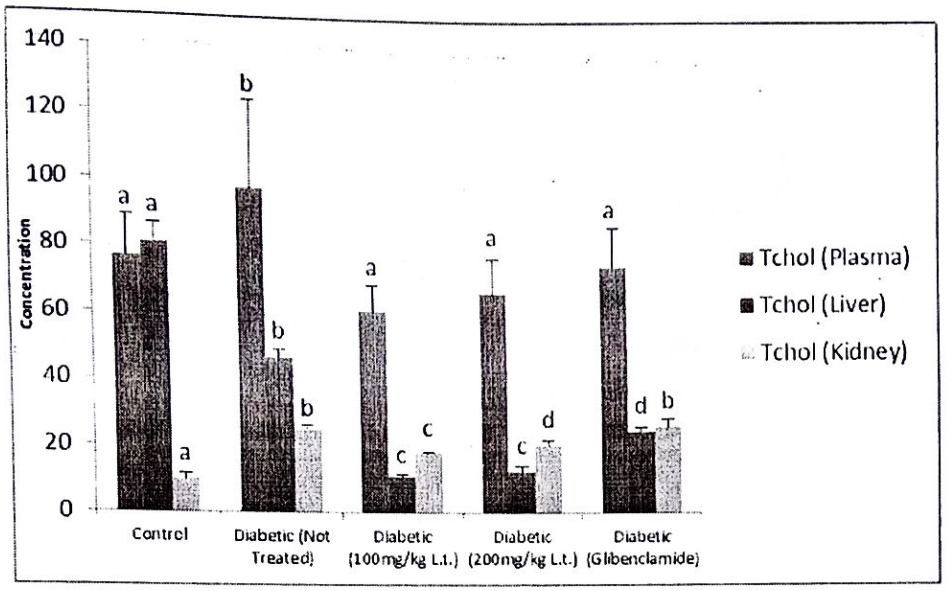


Figure 2: Total Cholesterol levels of diabetic rats administered methanolic extract of *L. taraxacifolia* leaves. Each bar represents the mean ± SD of 6 rats. Bar with different alphabets are significantly different at $p<0.05$

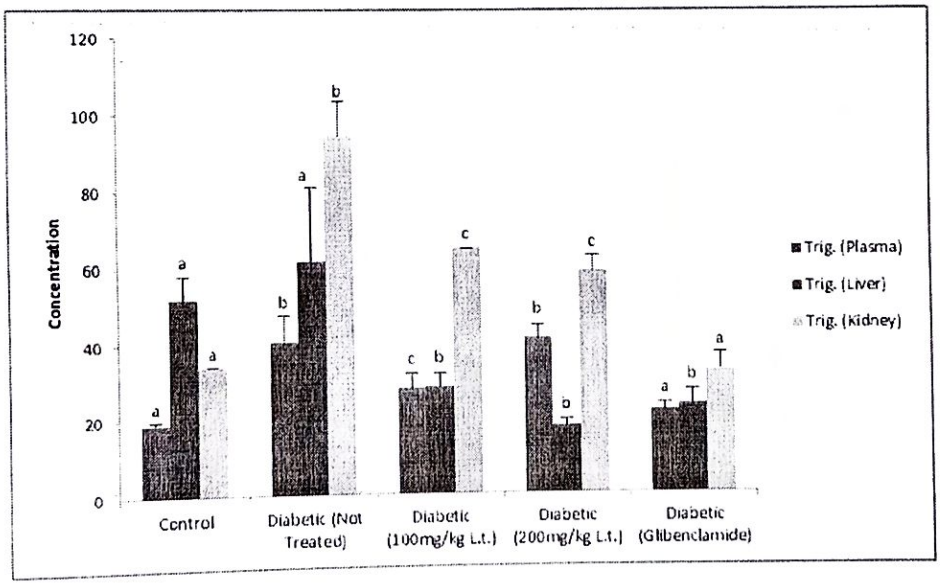


Figure 3: Triglyceride levels of diabetic rats administered methanolic extract of *L. taraxacifolia* leaves. Each bar represents the mean ± SD of 6 rats. Bars with different alphabets are significantly different at $p<0.05$

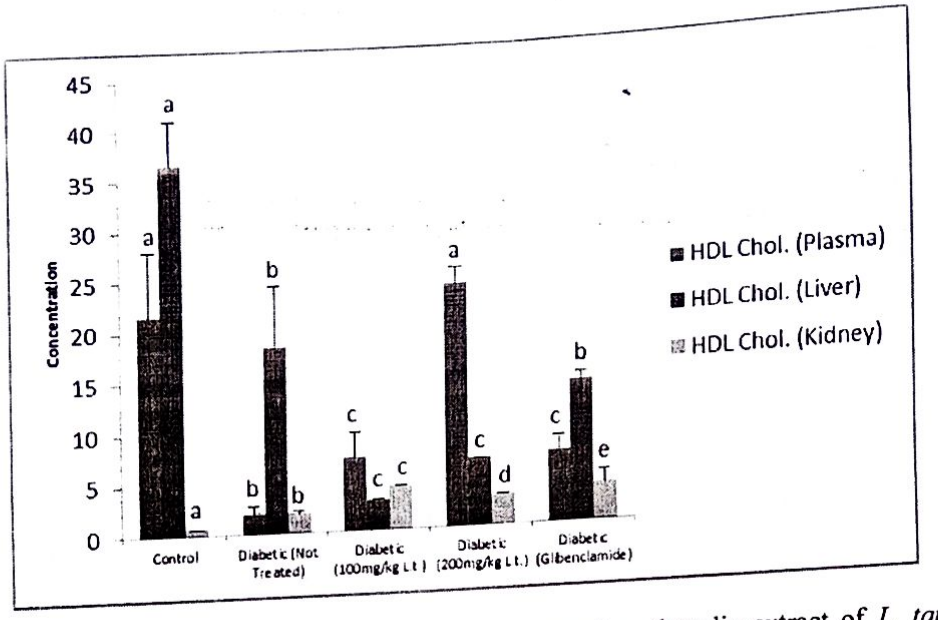


Figure 4: HDL Cholesterol levels of diabetic rats administered methanolic extract of *L. taraxacifolia* leaves. Each bar represents the mean ± SD of 6 rats. Bars with different alphabets are significantly different at $p<0.05$

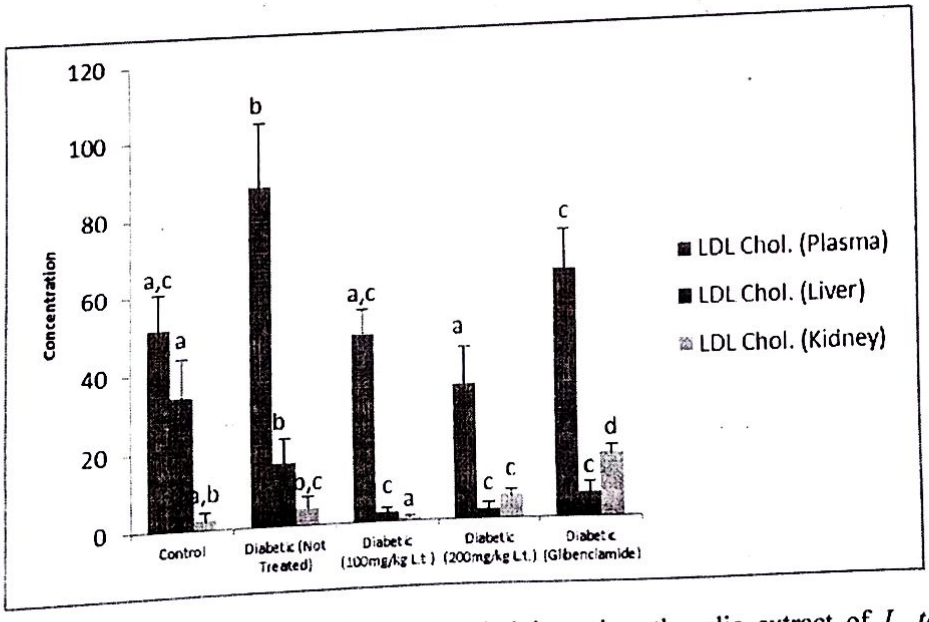


Figure 5: LDL-Cholesterol levels of diabetic rats administered methanolic extract of *L. taraxacifolia* leaves. Each bar represents the mean ± SD of 6 rats. Bars with different alphabets are significantly different at $p<0.05$

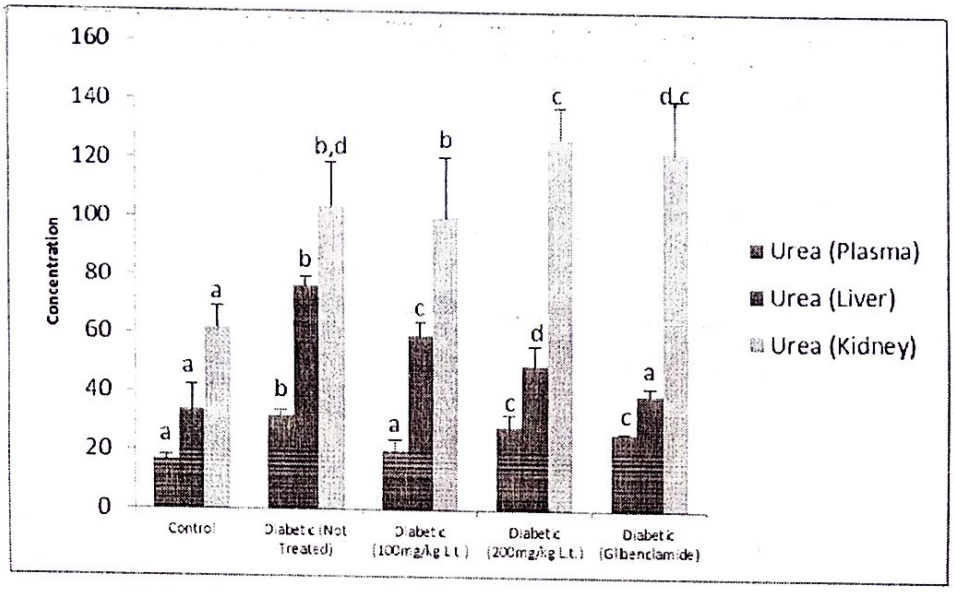


Figure 6: Urea levels of diabetic rats administered methanolic extract of *L. taraxacifolia* leaves. Each bar represents the mean ± SD of 6 rats. Bars with different alphabets are significantly different at $p<0.05$

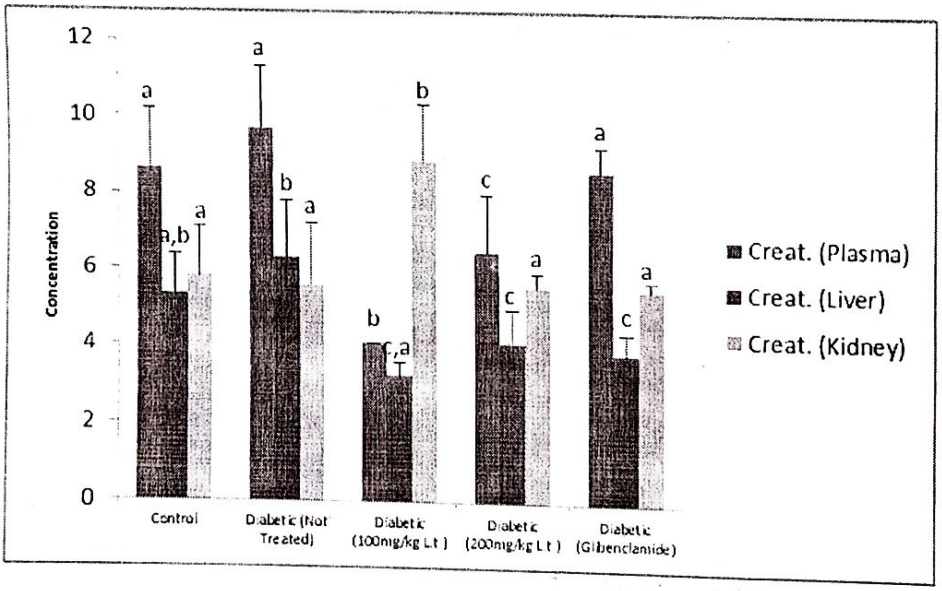
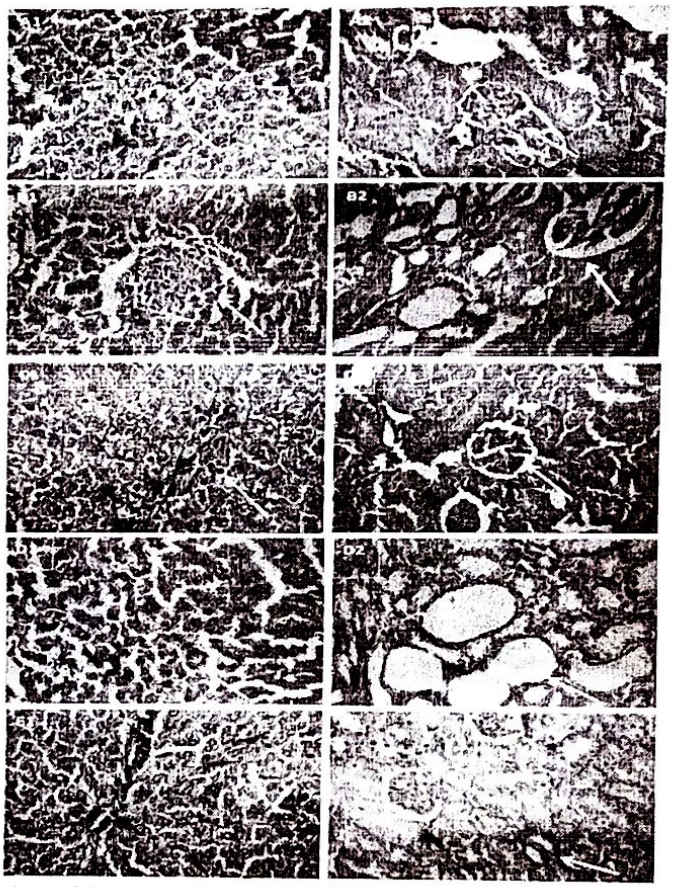


Figure 7: Creatinine levels of diabetic rats administered methanolic extract of *L. taraxacifolia* leaves. Each bar represents the mean ± SD of 6 rats. Bars with different alphabets are significantly different at $p<0.05$

Table 2: Chemical constituents of *Launaea taraxacifolia* leaves

Compounds	Retention Index	Percentage Composition
cis-10-pentadecen-1-ol	1763	15.6
trans-9-tetradecenoic acid	1777	15.0
Palmitic acid	1968	13.3
cis-11-hexadecenoic acid	1976	15.0
trans-2-octadecadecen-1-ol	2061	15.0
Margaric acid	2067	4.4
Stearic acid	2167	4.4
Oleic acid	2175	15.0
Percentage Total		**97.7**



Plates A1-E2: Cross section of liver and kidney of diabetic rats administered methanolic extract of *L. taraxacifolia* leaves. (H & E; x40)

A1 Liver of normal rats
B1 Liver of diabetic rats
C1 Liver of diabetic, L.t., 100mg/kg b.w.
D1 Liver of diabetic, L.t., 200mg/kg b.w.
E1 Liver of diabetic, GBC, 100mg/kg b.w.
L.t.- *Launaea taraxacifolia*, GBC- Glibenclamide

A2 Kidney of normal rats
B2 Kidney of diabetic rats
C2 Kidney of diabetic, L.t., 100mg/kg b.w.
D2 Kidney of diabetic, L.t., 200mg/kg b.w.
E2 Kidney of diabetic, GBC, 100mg/kg b.w.

Discussion

The results of this study indicate that extracts of *L. taraxacifolia* significantly reduced glucose levels in alloxan-induced diabetic animals. The observed changes in concentrations of the major lipids and kidney function parameters are characteristic alterations which occur in diabetic conditions. Elevated serum cholesterol and triglyceride levels have long been associated with the diabetic state (Bradley *et al.*, 1971).

In addition, the findings of this study have also revealed that the extracts have remarkable effects on the metabolism of lipids in the different compartments as shown by the increased and or/ decreased concentrations of cholesterol and triacylglyceride. Significant decreased concentration of plasma, liver and kidney total cholesterol and triacylglyceride, significant decreased levels of plasma and liver LDL-cholesterol and significant increase in HDL-cholesterol concentrations were observed in all groups of animals. This indicates potential ameliorative effects of *L. taraxacifolia* in diabetic -induced hypercholesterolemia and hypertriglyceridaemia in the experimental animals. This finding is consistent with previous work which used ethanol-aqueous extracts of *L. taraxacifolia* on HepG2 cells (Koukoui *et al.*, 2015).

In the circulation, triglyceride is gradually removed from lipoproteins by the action of lipoprotein lipase which is present in the capillaries of a number of tissues, predominantly adipose tissue and skeletal muscle. As it loses triglyceride, the chylomicron becomes smaller and deflated with folds of redundant surface material. These remnants are removed by the liver. The cholesterol may be utilized by the liver to form cell membrane components or bile acids, or may be excreted by which cholesterol leaves the body. The liver synthesizes VLDL particles which undergo delipidation as chylomicrons by the action of lipoprotein lipase. This results in the formation of an intermediate density lipoprotein (IDL) which becomes low density lipoprotein (LDL) when further dilapidated. LDL may be removed from the circulation by the high affinity LDL receptor or by other scavenger routes. HDL particles are derived from both liver and gut. They act as cholesteryl ester shuttles, removing the sterol from the peripheral tissues and returning it to the liver. The HDL is taken up either directly by the liver, or indirectly by being transferred to other circulating lipoproteins, which then return it to the liver. This process is thought to be anti-atherogenic and an elevated HDL-cholesterol level has been shown to confer a decreased risk of coronary heart disease (Gaw *et al.*, 1999).

The results of this study also showed that plasma and liver urea and creatinine levels decreased significantly as a result of treatment with the extract, indicating possible reno-protective potentials of *L. taraxacifolia*. Creatinine is a nitrogenous waste product derived from creatine and creatine phosphate in muscle tissue. Creatinine is not re-utilized but excreted from the body in the urine through the kidney. The plasma level of creatinine is independent of protein ingestion, water intake, rate of urine production and exercise. Therefore, creatinine measurement is used primarily in the assessment of kidney function.

Previous studies have shown that the leaves of *L. taraxacifolia* are rich in tannins and flavonoids (Arawande *et al.*, 2013; Koukoui *et al.*, 2015). The results on gas chromatography mass spectrometry analysis showed that leaves of *L. taraxacifolia* grown in Nigeria have various medicinally active compounds to treat a great variety of human diseases such as diabetes, coronary heart disease and cancer.

Conclusion

This study has shown that *Launaea taraxacifolia* extract has possible cholesterol lowering activities and anti-hyperglycemic activities in alloxan-induced diabetes. However, it is recommended that further work should be done to know the actual component(s) of the extract responsible for the observed activities.

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