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Launaea taraxacifolia leaf partitions ameliorate alloxan-induced pathophysiological complications via antioxidant mechanisms in diabetic rats

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ABSTRACT

Launaea taraxacifolia (Wild.) Amin ex. Jeffery belongs to family Asteracaea. The plant is used for treatment of diseases and eaten as vegetable in Nigeria. This study investigated the ameliorative potentials of *L. taraxacifolia* leaf partitions in alloxan induced diabetic complications. Male Albino rats were divided into eleven groups of five rats each. Diabetes was induced following intraperitoneal administration of 150 mg/kg alloxan monohydrate and was treated with 200 and 300 mg/kg of each partitioned fractions. Hyperglycemia was reversed in all treated rats within seven days of treatments. Rats treated with the partitions showed significant increase in hematological parameters compared with diabetic control. N-hexane fraction had the best overall effect against oxidative stress particularly on heart and pancreas reduced glutathione (GSH), superoxide dismutase (SOD) and kidney glutathione S-transferase (GST) activities. The various degrees of degeneration observed in the kidney, liver, pancreas and heart of the untreated diabetic rats were milder in rats treated with partitions. The results therefore revealed the ameliorative potentials of the partitioned fractions of *L. taraxacifolia* leaf extract against diabetes mellitus complications via activation of the antioxidant enzymes.

1. Introduction

Mitochondrial production of free radicals induced by hyperglycemia has been suggested as a key episode in the progress of diabetic complications [1]. Studies in diabetic patients have shown that elevated generation of free radicals with concomitant decline in antioxidant responses could be responsible for the onset, and promote the development of various pathophysiological complications associated with the disease [2]. These complications are wide ranging and are due at least in part to chronic elevation of blood glucose levels, which results in complications grouped under microvascular disease (a disease due to damage to small blood vessels) and macrovascular disease (a disease due to damage to the arteries) [3]. Microvascular complications include retinopathy, nephropathy, and neuropathy, whereas macrovascular complications include accelerated cardiovascular disease resulting in myocardial infarction and cerebrovascular disease manifesting as strokes [4–6].

Previous researches have been conducted to investigate the efficacy of varieties of drugs as potent therapies against the various complications of diabetes mellitus. However, very few of them are clinically available because of undesirable side effects and poor pharmacokinetics [7]. This has led to an increased search for newer therapies with mild or no side effects. Herbal medicines are popularly used remedies for many diseases by a vast majority of the world's population and herbal formulations have attained widespread acceptability as anti-diabetics [8]. Available literatures show that there are more than 400 plant species showing anti-diabetic activity with the possible use in the treatment of diabetes complications [9,10].

Launaea taraxacifolia is an annual West Africa tropical herb commonly known as wild lettuce. It is an economically valued plant which is utilized as food, fodders for animal where it serves to increase milk yield in cattle and induce multiple births in livestock, and it is burnt into ash as vegetable salt. In Nigeria, the plant has been used for centuries as cure for several complaints such as eye

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diseases (conjunctivitis), yaws and measles. An extract from the leaf mixed with breast milk of a nursing mother is employed in the treatment of partial blindness resulting from snake bite [11,12]. Studies have also attributed the anti-atherogenic effects of ethanol leaf extract of *L. taraxacifolia* to its chemical compositions which confer antioxidant, hypolipidemic, hypotensive, hypoglycemic and free radical scavenging properties [13–15].

Traditional claims and scientists suggested that *L. taraxacifolia* can be useful in the management of diabetes and other health problems, but studies on the anti-diabetic potentials of the plant and its ameliorative efficacies on various pathophysiological complications are yet to be elucidated. This study therefore investigated the anti-diabetic activity of various fractions of ethanol leaf extract of *L. taraxacifolia* on alloxan-induced diabetic rats.

2. Materials and methods

2.1. Collection of plant material

Fresh leaves of *L. taraxacifolia* were collected from local herbal market (Oje market) in Ibadan, Nigeria. The identity of the plant was confirmed at the University of Ibadan Herbarium (UIH). The plant sample was air-dried, powdered and stored in a clean air-tight glass container for further use.

2.2. Preparation of ethanol extract

The powdered sample was extracted in absolute ethanol. The plant sample (1500 g) was extracted in 10 L of ethanol, decanted and re-soaked. The extract was concentrated to dryness in a rotary vacuum evaporator at 50 °C, air-dried to constant weight and was refrigerated prior to use.

2.3. Partitioning of ethanol extract

A portion of the ethanolic extract was soaked in n-hexane, and the solution was continually stirred and decanted until clear supernatant was obtained. This procedure was repeated for chloroform and ethyl acetate as solvents. All extracts obtained were concentrated using vacuum rotary evaporator at 50 °C, air-dried to constant weight and was refrigerated prior to use.

2.4. Phytochemical screening

Powdered sample and extracts were screened for the presence of alkaloids, flavonoids, anthraquinones, saponins, tannins and cardiac glycosides [16].

2.5. Experimental animals

Adult albino male rats weighing 120–150 g were purchased from the animal house of the Department of Physiology, University of Ibadan, Nigeria. They were transported to the animal house of the Department of Zoology, University of Ibadan. The animals were kept in well ventilated, pathogen free cages at room temperature (25–27 °C) and were feed with food (commercial pellet) and water *ad libitum*. The animals were acclimatized for two weeks before the commencement of the experiment.

2.6. Induction of diabetes mellitus

The animals were fasted overnight after which the basal blood glucose level of all animals were checked using Accu-check glucometer, prior to induction of diabetes with alloxan. Each experimental rat except the normal control group was administered a single intraperitoneal dose of 150 mg/kg of alloxan monohydrate dissolved in 0.9% normal saline, to induce diabetes [17]. After 48 h, the blood glucose level was checked and animals with glucose levels greater than 100 mg/dL were termed diabetic and were used for the experiment.

2.7. Experimental design

Experimental rats were divided into eleven (11) groups of five (5) each. All groups except group 11 which served as normal control group were diabetic. Following induction of diabetes, groups 1 and 2 were treated with 200 and 300 mg/kg body weight of n-hexane extract, groups 3 and 4 were treated with 200 and 300 mg/kg body weight of ethyl acetate extract, groups 5 and 6 were treated with 200 and 300 mg/kg body weight of ethanol extract, groups 7 and 8 were treated with 200 and 300 mg/kg body weight of chloroform extract, 9 and 10 were diabetic untreated and glibenclamide (10 mg/kg) treated groups respectively. Treatment was administered daily for seven consecutive days.

2.8. Blood collection and dissection

Twenty 4 h after the final administration (7 days), rats were sacrificed and blood samples were collected from each rat by cardiac puncture into clean heparinized tubes and processed for hematological studies. Blood samples were also collected in plain tubes, centrifuged at 3000 rpm for 10 min to obtain the serum used for the estimation of some biochemical parameters. Pancreas, liver, heart and kidney were also harvested, rinsed in normal saline and blotted dry. Part of them were excised, weighed and homogenized in 0.1 M ice-cold phosphate buffer (pH 7.4). Homogenization was followed by centrifugation at 5000 rpm for 10 min and the resulting supernatants were aliquoted in Eppendorf tubes and used for the estimations of oxidative stress parameters. The remaining organs were fixed in 10% formalin and processed for histopathological studies.

2.9. Hematological studies

Packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC), red blood cell (RBC), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were determined according to Dacie and Lewis [18].

2.10. Biochemical analysis

The activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT) and concentration total bilirubin were estimated to assess liver function, while concentrations of albumin and creatinine were also determined to assess kidney function using commercial diagnostic kits.

2.11. Estimation of liver, kidney, heart and pancreas GSH, SOD and GST activities

GSH level was determined according to the method of Moron et al. [19], where the colour developed was read at 412 nm. SOD activity was determined by the method of Misra and Fridovich [20]. The method is based on the ability of superoxide dismutase to inhibit auto-oxidation of adrenaline to adrenochrome at alkaline pH. The unit of enzyme activity is defined as the enzyme required for 50% inhibition of adrenaline auto-oxidation. GST activity was determined by the method of Habig et al. [21] based on enzyme-

catalyzed condensation of glutathione with the model substrate, 1-chloro-2,4-dinitrobenzene. The product formed (2,4-dinitrophenylglutathione) absorbs light at 340 nm.

2.12. Histopathological studies

The liver, kidney, heart and pancreas sections fixed in 10% formalin solution were washed in 10 mmol/L phosphate buffer pH 7.4 at 4 °C for 12 h. After dehydration, the tissue was embedded in paraffin, cut into sections, stained with haematoxylin–eosin dye, and finally observed at x 100 magnification under a microscope.

2.13. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by least significant difference (LSD) to test for significant differences among the groups of rats using Statistical Package for Social Sciences program version 17.0. Data were expressed as mean ± standard error of mean. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Qualitative and quantitative phytochemical contents of L. taraxacifolia leaf extracts

The n-hexane fraction contained flavonoids only; chloroform fraction had alkaloids, cardiac glycosides, and anthraquinones contents; ethyl acetate fraction contained flavonoids and tannins; whereas ethanol extract was rich in flavonoids, alkaloids, cardiac glycosides, and anthraquinones. In all the fractions, saponin was not detected (Table 1). Overall, the powdered sample contained alkaloids, flavonoids, and tannins in appreciable quantities (Table 2).

3.2. Blood glucose level

The blood glucose concentrations of rats prior to induction ranged from 60 to 78 mg/dL. The level rose to the range of 109–289 mg/dL after induction of diabetes. Following treatments, a significant ($p < 0.05$) decrease in fasting blood glucose level was noted in diabetic rats treated with extracts of *L. taraxacifolia* after each day of treatment. The mean blood glucose reduction of the different extracts at the concentration of 200 mg/kg and 300 mg/kg were 57.7, 50.6, 49.6 and 26.3 mg/dl for n-hexane, ethyl acetate, ethanol and chloroform respectively while glibenclamide reduced the blood glucose level by 36.6 mg/dl. Generally, chloroform extract at 300 mg/kg had greater blood glucose lowering effect than the standard drug (Fig. 1).

3.3. Hematological studies

There was a non-significant ($p > 0.05$) increase in the counts of all the hematological parameters following treatments with the various fractions of the extract compared with the diabetic rats and the standard drug treated rats (Table 3).

Table 1
Qualitative phytochemical screening of various fractions of *L. taraxacifolia*.

Phytochemicals	N-hexane	Chloroform	Ethyl acetate	Ethanol
Flavonoids	+	–	+	+
Aklanoids	–	+	–	+
Tannins	–	–	+	–
Cardaic glycosides	–	+	–	+
Saponins	–	–	–	–
Anthraquinones	–	+	–	+

Key: + Presence; - Absence.

Table 2
Quantitative phytochemical screening of *L. taraxacifolia* extract.

Phytochemical	Component (mg/100g)
Alkaloids	631.7 ± 17.56
Flavonoids	648.3 ± 12.58
Tannins	631.7 ± 16.07
Anthraquinones	20.0 ± 5.00
Cardiac glycosides	23.3 ± 2.89

Values are mean of 3 replicates.

3.4. Biochemical studies

There was a significant ($p < 0.05$) increase in the activities of AST, ALP, GGT as well as level of total bilirubin of rats treated with extracts compared with glibenclamide treated and normal control rats (Table 4). Rats treated with the extracts showed no significant difference ($p > 0.05$) in albumin and creatinine concentrations compared with the control groups (Table 5).

3.5. Effect of treatments on oxidative stress parameters

The results of concentrations of liver and kidney GSH showed that there was no significant difference ($p > 0.05$) in the groups (Table 6). Only treatments with 300 mg/kg n-hexane, ethyl acetate and 200 mg/kg chloroform extracts significantly ($p < 0.05$) decreased heart GSH concentration while in the pancreas, GSH level was also significantly ($p < 0.05$) increased by 300 mg/kg n-hexane, ethyl acetate and 200 mg/kg ethanol extract compared with diabetic untreated rats (Table 6). Liver GST activity was significantly ($p < 0.05$) increased by the two tested doses of ethyl acetate and chloroform extracts compared with diabetic untreated rats (Table 7). For the kidney, 200 mg/kg n-hexane and chloroform extracts as well as 300 mg/kg n-hexane, ethyl acetate, ethanol and standard drug significantly ($p < 0.05$) increased the GST activity compared with diabetic untreated (Table 7). Heart GST activity was increased significantly ($p < 0.05$) by 200 mg/kg and 300 mg/kg ethyl acetate and ethanol extracts respectively, while only 300 mg/kg ethanol extract significantly increased ($p < 0.05$) the enzyme compared with diabetic untreated rats (Table 7). Liver SOD activity was significantly ($p < 0.05$) reduced by both doses of n-hexane extract and significantly ($p < 0.05$) increased by 300 mg/kg ethanol extract and both doses chloroform extract compared with diabetic untreated rats (Table 8). In kidney, all treatments significantly ($p < 0.05$) increased the SOD activity except 200 mg/kg ethanol and 300 mg/kg chloroform extracts compared with diabetic untreated (Table 8). Standard drug, both doses of n-hexane extracts and 300 mg/kg ethyl acetate extract significantly ($p < 0.05$) reduced heart SOD while also in pancreas, both doses of n-hexane extract and 200 mg/kg ethyl acetate extract significantly ($p < 0.05$) increased SOD activity compared with diabetic untreated rats (Table 8).

3.6. Tissues histopathology of normal, treated and untreated diabetic rats

Kidney of the normal and all extract treated rats showed a normal appearance with the glomerular, renal institum and tubules having no visible lesions (Fig. 2). There are multiple dense aggregates of mononuclear inflammatory cells in the renal interstitium of the untreated diabetic rats when compared with the treated

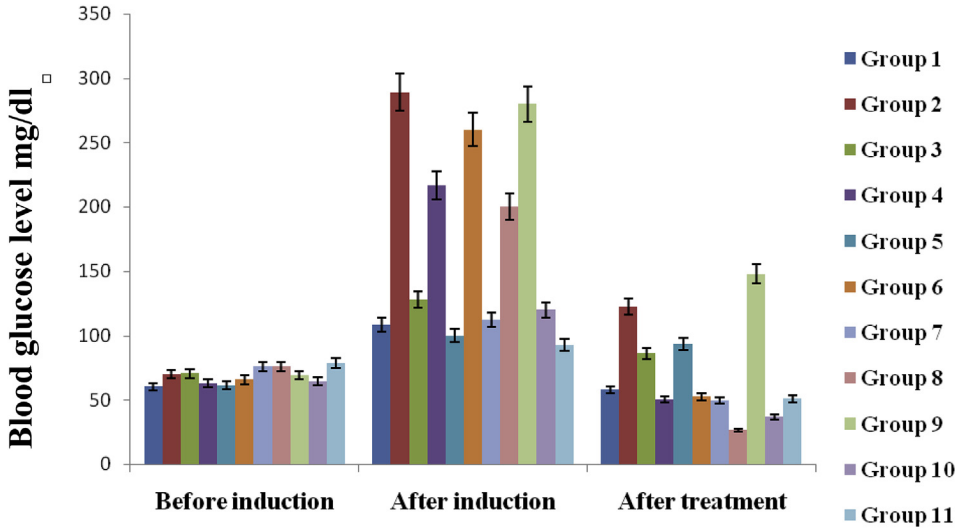

Fig. 1. Blood glucose level before induction, after induction and after treatment of treated and untreated alloxan-induced diabetic rats.

Table 3
Hematology of normal, treated and untreated diabetic rats.

Parameters	200 nHE	300 nHE	200 EAE	300 EAE	200 EE	300 EE	200 CE	300 CE	DC	SD	NC
PCV (%)	34.6 ± 19.3^a	42.2 ± 2.6^a	44.6 ± 2.1^a	42.8 ± 3.3^a	40.2 ± 3.7^a	41.8 ± 2.7^a	42.2 ± 1.6^a	45.2 ± 4.0^a	33.4 ± 19.0^a	44.0 ± 3.6^a	42.2 ± 1.8^a
HB(g/cell)	11.5 ± 6.4^a	14.0 ± 1.0^a	14.9 ± 0.6^a	14.6 ± 1.2^a	13.7 ± 1.3^a	14.3 ± 1.3^a	14.1 ± 0.4^a	14.6 ± 1.5^a	10.9 ± 6.2^a	14.8 ± 1.5^a	13.7 ± 1.5^a
RBC(cell/L)	6.0 ± 3.3^a	7.0 ± 0.5^b	7.6 ± 0.3^b	7.2 ± 0.5^b	6.5 ± 0.6^b	7.0 ± 0.6^b	6.9 ± 0.3^b	8.6 ± 3.8^b	5.6 ± 3.1^b	7.1 ± 0.7^b	7.2 ± 0.3^b
WBC($x10^3$ cell/L)	4.1 ± 3.1^b	9.3 ± 1.4^b	8.0 ± 2.8^b	6.3 ± 1.1^c	6.1 ± 2.1^c	7.5 ± 8.9^c	6.3 ± 2.8^c	6.4 ± 32.0^c	4.5 ± 32.0^c	6.5 ± 32.0^c	4.7 ± 32.0^c
MCH (pg/cell)	15.5 ± 8.7^a	19.5 ± 1.1^a	19.7 ± 0.8^a	19.7 ± 0.8^a	20.2 ± 0.4^a	21.0 ± 0.8^a	20.4 ± 0.7^a	20.1 ± 1.2^a	15.7 ± 1.2^a	20.8 ± 1.6^a	19.3 ± 1.2^a
MCHC (g/dl)	26.8 ± 15.0^a	33.2 ± 0.8^a	33.4 ± 0.9^a	34.1 ± 0.3^a	34.0 ± 1.1^a	34.1 ± 0.7^a	33.4 ± 1.2^a	32.3 ± 1.8^a	26.3 ± 1.8^a	34.1 ± 1.8^a	32.6 ± 1.8^a

Values are expressed as mean ± S.E.M. Values along the same column having different superscripts are significantly different ($p < 0.05$). 200 nHE = 200 mg/kg n-hexane extract, 300 nHE = 300 mg/kg n-hexane extract, 200 EAE = 200 mg/kg ethyl acetate extract, 300 EAE = 300 mg/kg ethyl acetate extract, 200 EE = 200 mg/kg ethanol extract, 300 EE = 300 mg/kg ethanol extract, 200 CE = 200 mg/kg chloroform extract, 300 CE = 300 mg/kg chloroform extract, DC = diabetic control, SD = standard drug, NC = normal control.

Table 4
Activities of AST, ALT and ALP in normal, treated and untreated diabetic rats.

Parameters	200 nHE	300 nHE	200 EAE	300 EAE	200 EE	300 EE	200 CE	300 CE	DC	SD	NC
AST (IU/L)	31.2 ± 174^a	40.6 ± 2.9^a	36.8 ± 1.1^a	40.8 ± 4.3^a	38.8 ± 3.6^a	36.8 ± 1.7^a	37.8 ± 1.3^a	41.0 ± 1.4^a	34.8 ± 4.0^a	38.2 ± 4.0^a	38.8 ± 4.0^a
ALT (IU/L)	22.6 ± 12.7^a	29.6 ± 2.7^b	27.2 ± 1.9^b	30.0 ± 3.9^b	27.4 ± 2.8^b	26.2 ± 1.6^b	25.8 ± 1.3^a	29.4 ± 1.5^b	24.2 ± 1.5^a	27.2 ± 1.5^a	40.6 ± 1.5^a
ALP (IU/L)	93.2 ± 52.7^a	114.8 ± 83^b	89.2 ± 1.1^a	103.8 ± 1.5^a	96.4 ± 2.1^a	101.2 ± 2.1^a	103.6 ± 12.8^a	107.4 ± 1.9^a	74.0 ± 3.8^b	99.6 ± 2.3^b	92.0 ± 1.8^b
Total bulburin (mg/dl)	12.3 ± 6.9^a	15.8 ± 0.9^a	15.5 ± 0.5^a	15.6 ± 0.8^a	16.5 ± 0.6^a	15.9 ± 0.5^a	16.1 ± 0.3^a	15.3 ± 1.5^a	12.4 ± 32.0^c	15.4 ± 32.0^c	15.0 ± 32^c
GGT (IU/L)	1.2 ± 0.9^a	1.7 ± 0.9^b	3.0 ± 0.6^b	1.4 ± 0.5^a	2.4 ± 0.6^b	2.1 ± 0.5^b	1.3 ± 0.7^a	2.0 ± 1.0^b	2.0 ± 2.6^b	1.3 ± 1.9^b	2.0 ± 2.6^b

Values are expressed as mean ± S.E.M. Values along the same column having different superscripts are significantly different ($p < 0.05$). 200 nHE = 200 mg/kg n-hexane extract, 300 nHE = 300 mg/kg n-hexane extract, 200 EAE = 200 mg/kg ethyl acetate extract, 300 EAE = 300 mg/kg ethyl acetate extract, 200 EE = 200 mg/kg ethanol extract, 300 EE = 300 mg/kg ethanol extract, 200 CE = 200 mg/kg chloroform extract, 300 CE = 300 mg/kg chloroform extract, DC = diabetic control, SD = standard drug, NC = normal control.

Table 5
Renal parameters of normal, treated and untreated diabetic rats.

Parameters	200 nHE	300 nHE	200 EAE	300 EAE	200 EE	300 EE	200 CE	300 CE	DC	SD	NC
Creatinine (mg/dl)	0.6 ± 0.4^a	0.7 ± 0.2^a	0.6 ± 0.1^a	0.7 ± 0.3^a	0.8 ± 0.2^a	0.8 ± 0.3^a	0.8 ± 0.2^a	0.7 ± 0.7^a	0.6 ± 0.7^a	0.7 ± 0.1^a	0.8 ± 1.0^a
Albumin (mg/dl)	2.5 ± 1.5^a	2.9 ± 0.5^a	2.8 ± 0.5^a	3.1 ± 0.8^a	3.2 ± 0.5^a	3.3 ± 0.5^a	3.0 ± 0.4^a	2.8 ± 0.4^a	2.2 ± 0.2^a	3.2 ± 0.3^a	3.2 ± 1.5^a

Values are expressed as mean ± S.E.M. Values along the same column having different superscripts are significantly different ($p < 0.05$). 200 nHE = 200 mg/kg n-hexane extract, 300 nHE = 300 mg/kg n-hexane extract, 200 EAE = 200 mg/kg ethyl acetate extract, 300 EAE = 300 mg/kg ethyl acetate extract, 200 EE = 200 mg/kg ethanol extract, 300 EE = 300 mg/kg ethanol extract, 200 CE = 200 mg/kg chloroform extract, 300 CE = 300 mg/kg chloroform extract, DC = diabetic control, SD = standard drug, NC = normal control.

diabetic rats. Also, there is a sign of regeneration in the glomerular spaces of the treated diabetic groups when compared with the untreated diabetic rats (Fig. 2). Liver histopathology of normal control rats appears normal, with hepatocytes closely packed together (Fig. 3) while the liver of rats treated with 200 mg/kg of n-hexane and standard drug appears normal when compared with untreated diabetic rats that reveal a few foci of moderate thinning of hepatic plates and random foci of single-cell hepatocellular

Table 6
Liver, kidney, heart, and pancreas GSH concentrations of normal, treated and untreated rats.

>GROUPS	GSH (μg/g)			
	Liver	Kidney	Heart	Pancreas
200 nHE	4.27 ± 0.32^{acde}	13.93 ± 0.70^{abc}	15.60 ± 0.96^{a}	13.43 ± 1.23^{a}
300 nHE	4.68 ± 0.88^{abcde}	14.00 ± 0.33^{ab}	6.66 ± 1.60^{c}	25.60 ± 0.75^{b}
200 EAE	6.39 ± 0.83^{bc}	10.70 ± 1.17^{a}	13.29 ± 1.32^{a}	11.37 ± 1.06^{a}
300 EAE	4.30 ± 0.87^{de}	16.06 ± 2.28^{abc}	7.83 ± 1.06^{c}	22.19 ± 4.36^{b}
200 EE	3.53 ± 0.60^{d}	14.35 ± 2.07^{abc}	12.65 ± 1.69^{a}	21.88 ± 8.94^{b}
300 EE	4.34 ± 1.07^{de}	11.77 ± 0.11^{abc}	13.26 ± 0.11^{a}	14.21 ± 1.82^{a}
200 CE	4.90 ± 0.48^{bcde}	18.79 ± 2.24^{b}	10.31 ± 1.18^{cb}	12.73 ± 0.43^{a}
300 CE	5.83 ± 0.27^{be}	18.26 ± 2.45^{bc}	13.86 ± 1.47^{a}	12.51 ± 1.06^{a}
DC	5.70 ± 0.32^{bcde}	15.35 ± 3.70^{abc}	14.92 ± 2.11^{a}	14.54 ± 2.45^{a}
SD	6.68 ± 0.74^{b}	18.68 ± 2.79^{bc}	14.71 ± 1.63^{a}	14.78 ± 1.80^{a}
NC	4.98 ± 0.68^{bcde}	11.80 ± 1.60^{a}	11.80 ± 1.96^{ab}	14.29 ± 1.68^{a}

Values are expressed as mean ± S.E.M. Values along the same column having different superscripts are significantly different ($p < 0.05$). 200 nHE = 200 mg/kg n-hexane extract, 300 nHE = 300 mg/kg n-hexane extract, 200 EAE = 200 mg/kg ethyl acetate extract, 300 EAE = 300 mg/kg ethyl acetate extract, 200 EE = 200 mg/kg ethanol extract, 300 EE = 300 mg/kg ethanol extract, 200 CE = 200 mg/kg chloroform extract, 300 CE = 300 mg/kg chloroform extract, DC = diabetic control, SD = standard drug, NC = normal control.

Table 7
Liver, kidney, heart, and pancreas GST activities of normal, treated and untreated rats.

GROUPS	GST (nmol/min/mgprotein)			
	Liver	Kidney	Heart	Pancreas
200 nHE	45.75 ± 3.65^{a}	26.30 ± 2.27^{bc}	4.48 ± 1.09^{a}	1.87 ± 0.12^{abc}
300 nHE	41.09 ± 7.51^{a}	35.27 ± 15.00^{c}	3.80 ± 1.02^{a}	1.84 ± 0.31^{abc}
200 EAE	172.34 ± 32.50^{bc}	7.22 ± 1.47^{ad}	19.99 ± 6.00^{c}	1.42 ± 0.09^{a}
300 EAE	207.38 ± 45.35^{bc}	18.53 ± 3.00^{ab}	5.63 ± 2.68^{a}	2.48 ± 0.12^{bc}
200 EE	90.19 ± 8.27^{a}	8.00 ± 1.29^{ad}	3.53 ± 0.55^{a}	1.99 ± 0.48^{abc}
300 EE	77.99 ± 11.80^{a}	13.03 ± 3.73^{ab}	11.04 ± 0.27^{b}	3.79 ± 0.13^{d}
200 CE	160.07 ± 0.10^{b}	12.08 ± 2.61^{a}	5.14 ± 1.68^{a}	2.48 ± 0.06^{bc}
300 CE	231.50 ± 41.55^{c}	3.02 ± 0.30^{d}	7.38 ± 1.42^{ab}	2.78 ± 0.47^{c}
DC	47.85 ± 13.28^{a}	3.97 ± 0.82^{d}	5.38 ± 0.61^{a}	2.10 ± 0.37^{abc}
SD	45.17 ± 7.08^{a}	11.44 ± 4.31^{a}	2.93 ± 0.47^{a}	1.63 ± 0.10^{ab}
NC	77.76 ± 17.91^{a}	18.46 ± 4.17^{ab}	2.94 ± 0.62^{a}	2.60 ± 0.37^{c}

Values are expressed as mean ± S.E.M. Values along the same column having different superscripts are significantly different ($p < 0.05$). 200 nHE = 200 mg/kg n-hexane extract, 300 nHE = 300 mg/kg n-hexane extract, 200 EAE = 200 mg/kg ethyl acetate extract, 300 EAE = 300 mg/kg ethyl acetate extract, 200 EE = 200 mg/kg ethanol extract, 300 EE = 300 mg/kg ethanol extract, 200 CE = 200 mg/kg chloroform extract, 300 CE = 300 mg/kg chloroform extract, DC = diabetic control, SD = standard drug, NC = normal control.

necrosis (Fig. 3). The pancreas of the untreated diabetic rats shows a less number of islets (Fig. 4). Pancreas of rats treated with standard drug, 200 mg/kg ethanol extract and 300 mg/kg n-hexane extract shows moderate expansion of cellular population and size of islet cells when compared with the untreated diabetic rats (Fig. 4). Multiple foci of cardiomyocytes are observed in heart histopathological examination of untreated diabetic rats compared with normal control and treated diabetic rats (Fig. 5). The untreated diabetic rats showed marked areas of degeneration of the myofibers with diffuse vacuolar while mycocytes of treated diabetic rats appears normal (Fig. 5).

4. Discussion

In this study, the hypoglycemic effects of n-hexane, ethyl acetate, chloroform fractions from ethanolic extract of *Launaea taraxacifolia* leaf were evaluated on both the normal and alloxan-induced diabetic rats. All partitions lowered the mean blood glucose levels of all treated diabetic rats significantly ($p < 0.05$)

Table 8
Liver, kidney, heart, and pancreas SOD activities of normal, treated and untreated rats.

GROUP	SOD (Unit/mg protein)			
	Liver	Kidney	Heart	Pancreas
200 nHE	0.55 ± 0.14^{a}	1.02 ± 0.07^{bc}	0.22 ± 0.07^{a}	0.77 ± 0.21^{e}
300 nHE	0.60 ± 0.08^{a}	1.59 ± 0.05^{cde}	0.92 ± 0.10^{ab}	0.90 ± 0.09^{d}
200 EAE	1.02 ± 0.03^{b}	1.61 ± 0.12^{cde}	2.68 ± 0.79^{cd}	0.66 ± 0.08^{c}
300 EAE	0.96 ± 0.38^{ab}	1.75 ± 0.09^{de}	0.69 ± 0.25^{a}	0.58 ± 0.03^{bc}
200 EE	0.86 ± 0.03^{ab}	0.90 ± 0.24^{ab}	2.40 ± 0.06^{cd}	0.51 ± 0.01^{abc}
300 EE	1.96 ± 0.48^{d}	1.88 ± 0.14^{e}	2.85 ± 0.26^{d}	0.57 ± 0.05^{bc}
200 CE	2.05 ± 0.18^{de}	1.86 ± 0.10^{e}	2.49 ± 0.25^{cd}	0.40 ± 0.02^{a}
300 CE	3.39 ± 0.74^{c}	0.68 ± 0.54^{ab}	2.42 ± 0.24^{cd}	0.38 ± 0.03^{a}
DC	1.37 ± 0.22^{b}	0.28 ± 0.14^{a}	2.66 ± 0.26^{cd}	0.46 ± 0.05^{ab}
SD	1.49 ± 0.49^{b}	1.14 ± 0.13^{bcd}	1.09 ± 0.01^{ab}	0.38 ± 0.02^{a}
NC	2.00 ± 0.97^{e}	0.73 ± 0.28^{ab}	1.80 ± 0.22^{bc}	0.43 ± 0.07^{ab}

Values are expressed as mean ± S.E.M. Values along the same column having different superscripts are significantly different ($p < 0.05$). 200 nHE = 200 mg/kg n-hexane extract, 300 nHE = 300 mg/kg n-hexane extract, 200 EAE = 200 mg/kg ethyl acetate extract, 300 EAE = 300 mg/kg ethyl acetate extract, 200 EE = 200 mg/kg ethanol extract, 300 EE = 300 mg/kg ethanol extract, 200 CE = 200 mg/kg chloroform extract, 300 CE = 300 mg/kg chloroform extract, DC = diabetic control, SD = standard drug, NC = normal control.

when compared with the standard drug (glibenclamide), thus confirming the hypoglycemic potential of *L. taraxacifolia* extract for the treatment of diabetes. The blood glucose lowering effect of the partitions on alloxan-induced diabetes may be attributed to the ability of the extract to produce glucose as source of energy to the tissue, increasing glucose removal from the blood, decrease in the release of glucagon by stimulating direct glycolysis in peripheral tissues or reduce glucose absorption from the gastro-intetestinal tract as reported in some anti-hypoglycemic plants in existing literatures [22–24]. Also, it may be due to inhibitory action of the extract on α-glucosidase which is an enzyme found in the brush border of the intestine and is responsible for the conversion of polysaccharide into simple sugars [25]. This study is in line with the study by Ghosh et al. [26], who reported that ethanolic extract of the bark of *Ficus hispida* caused a better hypoglycemic effect than glibenclamide 2 h after administration. Adeyi et al. [27] also reported on the aqueous leaf extract of *Ficus exasperata* which has hypoglycemic effect when compared with glibenclamide after administration.

The evaluation on the phytochemical constituents revealed a higher concentration of flavonoids followed by alkaloids and tannins while cardiac glycosides and anthraquinones were very low. Phenolic compounds such as flavonoids, alkaloids, tannins etc., have been isolated from anti-diabetic medicinal plants which have been found to stimulate secretion of or possess an insulin like-effect [28].

Also in this study, treatment with the extract increased the values of packed cell volume (PCV), hemoglobin (HB) and red blood cell count (RBC) when compared to the untreated alloxan-induced diabetic rats and rats treated with glibenclamide. The decrease in red blood cell count of the untreated alloxan-induced diabetic rats might be due to the destruction of matured red blood cells leading to low hemoglobin count which is an indication of in-balance between its synthesis and destruction [29,30]. Excellent performance of the all fractions (especially 300 mg/kg ethyl acetate, 200 mg/kg ethanol, 200 mg/kg and 300 mg/kg chloroform) in reversing all this irregularities in the hematological parameters may be ascribes to the presence of iron and calcium in the plant extract, as vitamins and minerals are essential requirements for many enzymes function in cells and also form part of heme structure in hemoglobin (Fe) as reported by Ajiboye*et al.* [24] which might be essential in improving the weak immune system due to the generation of

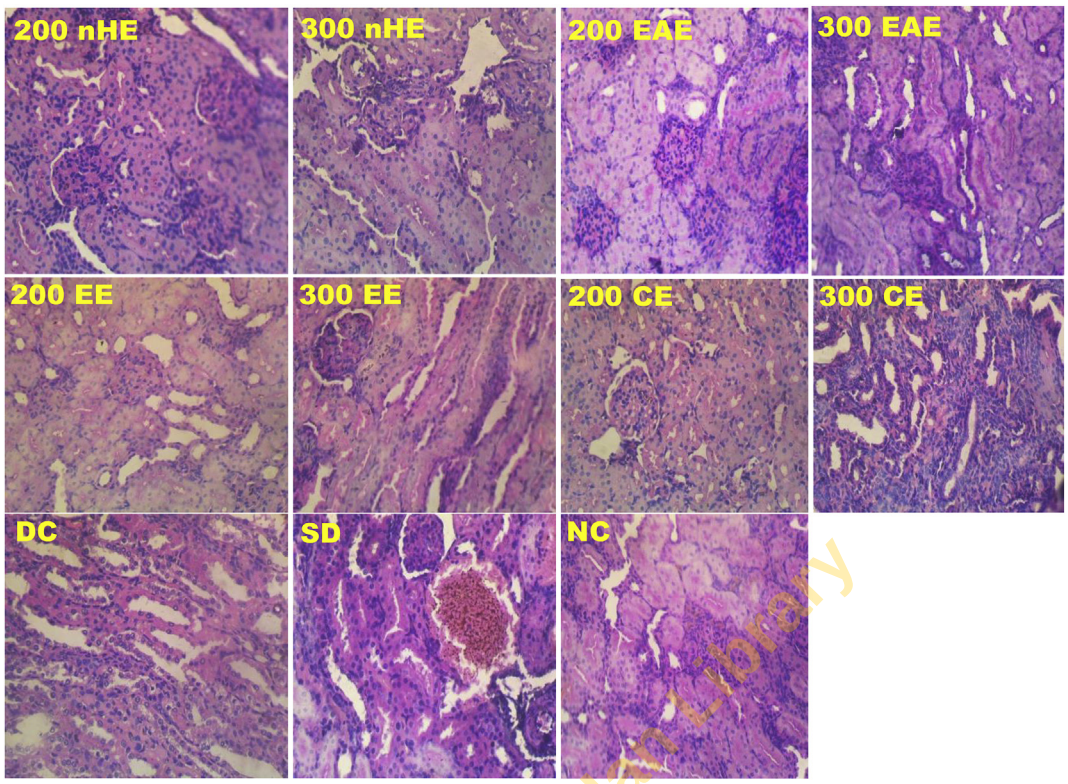

Fig. 2. Kidney histopathology of normal, treated and untreated rats (100 X magnification). 200 nHE = 200 mg/kg n-hexane extract showing sign of regeneration in the glomerular spaces; 300 nHE = 300 mg/kg n-hexane extract showing sign of regeneration in the glomerular spaces; 200 EAE = 200 mg/kg ethyl acetate extract showing sign of regeneration in the glomerular spaces; 300 EAE = 300 mg/kg ethyl acetate extract showing sign of regeneration in the glomerular spaces; 200 EE = 200 mg/kg ethanol extract showing sign of regeneration in the glomerular spaces; 300 EE = 300 mg/kg ethanol extract showing sign of regeneration in the glomerular spaces. 200 CE = 200 mg/kg chloroform extract showing sign of regeneration in the glomerular spaces; 300 CE = 300 mg/kg chloroform extract showing sign of regeneration in the glomerular spaces; DC = diabetic control showing multiple dense aggregates of mononuclear inflammatory cells in the renal interstitium; SD = standard drug showing sign of regeneration in the glomerular spaces; NC = normal control showing normal appearance.

reactive oxygen species following alloxan-induced diabetes.

The liver is known to play an important role in the metabolism of carbohydrate and so its cellular integrity can be compromised in diabetes [31]. There was significant increase in the activities of AST, ALP, total bilirubin and GGT of rats treated with extracts compared with rats treated with glibenclamide and normal control rats. The value for ALT was higher in the normal control than all other groups. An increase in creatinine and albumin levels of normal control and treated diabetic rats against the untreated diabetic rats is an indication of the positive impact of treatments on the glomerular filtration rate in which an increase in creatinine level is an indication of well functioned kidney and vice-versa [24]. The ability of the fractions of *L. taraxacifolia* leaf extract to increase the level of creatinine and albumin suggests its ability to ameliorate diabetic nephropathy.

The key intracellular antioxidant defense enzymes are SOD, catalase (CAT), glutathione peroxidase (GPx) [32], and non-enzymatic antioxidants like GSH [33]. These antioxidant enzymes guard against oxidative tissue damage by free radicals [34]. Both GPx and CAT catalyze the decomposition of H_2O_2 to oxygen and water. While CAT does not require oxidation of GSH to GSSG in the detoxification process, this is however a requirement for GPx [35]. In this study, n-hexane fraction had the greatest effect against tissue oxidative stress and out of the two tested doses; 300 mg/kg n-hexane extract was more effective. Overall, 300 mg/kg of the extract of *L taraxacifolia* had better effects on tissues oxidative stress than 200 mg/kg of the extract. Quantitative phytochemical screening conducted in this study revealed the presence of high amounts of flavonoids. Flavonoids have antioxidant properties and these may explain the recorded effect of the leaf extract against tissue oxidative stress.

Marked vacuolations of the Islets of Langerhans observed in the pancreas of untreated diabetic rats is due to toxic action of alloxan. Mild vacuolations of the islets of langerhans was however observed in pancreas of rats treated with the plant extract which is probably an indication that the extract was able to restore the degenerations caused by alloxan-induced diabetes mellitus [36,37]. Severe degeneration of the hepatocytes and glomeruli of the liver and kidney respectively was observed in the untreated diabetic rats. Report by Malmberg [38] stated that diabetic nephropathy is an important cause of mortality and morbidity and among the most common causes of end-stage renal failure. The potentials of the extract to ameliorate the pathological effects of diabetes mellitus was also demonstrated as rats treated with the extract showed mild degeneration compared to rats treated with glibenclamide. Also the histopathology of heart sections of treated diabetic rats shows no visible lesion in the cardiomyocytes which was similar with that of the normal control. This shows the ameliorative properties of the partitions which may probably be due to the presence of several bioactive compounds like flavonoids, saponins, alkaloids and tannins [37].

It can be noted that the partitions of ethanolic leaf extract of *L. taraxacifolia* has proved to be effective in reducing blood glucose levels, protecting hepatocytes against injury caused by oxidative stress and stimulating blood cell formation. It has also showed ameliorative and restorative effects on organs during diabetic

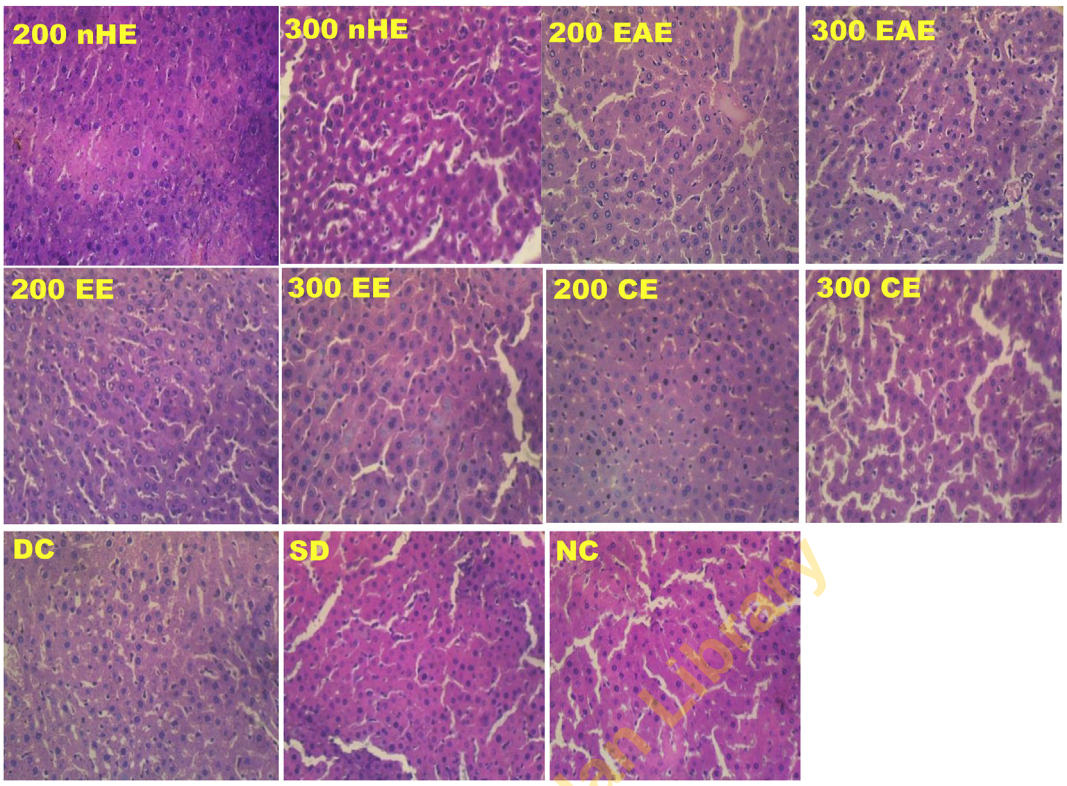

Fig. 3. Liver histopathology of normal, treated and untreated rats (100 X magnification). 200 nHE = 200 mg/kg n-hexane extract appearing normal; 300 nHE = 300 mg/kg n-hexane extract; 200 EAE = 200 mg/kg ethyl acetate extract; 300 EAE = 300 mg/kg ethyl acetate extract; 200 EE = 200 mg/kg ethanol extract; 300 EE = 300 mg/kg ethanol extract; 200 CE = 200 mg/kg chloroform extract; 300 CE = 300 mg/kg chloroform extract; DC = diabetic controlrevealing a few foci of moderate thinning of hepatic plates and random foci of single-cell hepatocellular necrosis; SD = standard drug showing normal hepatocytes; NC = normal control showing normal appearance with hepatocytes closely packed together.

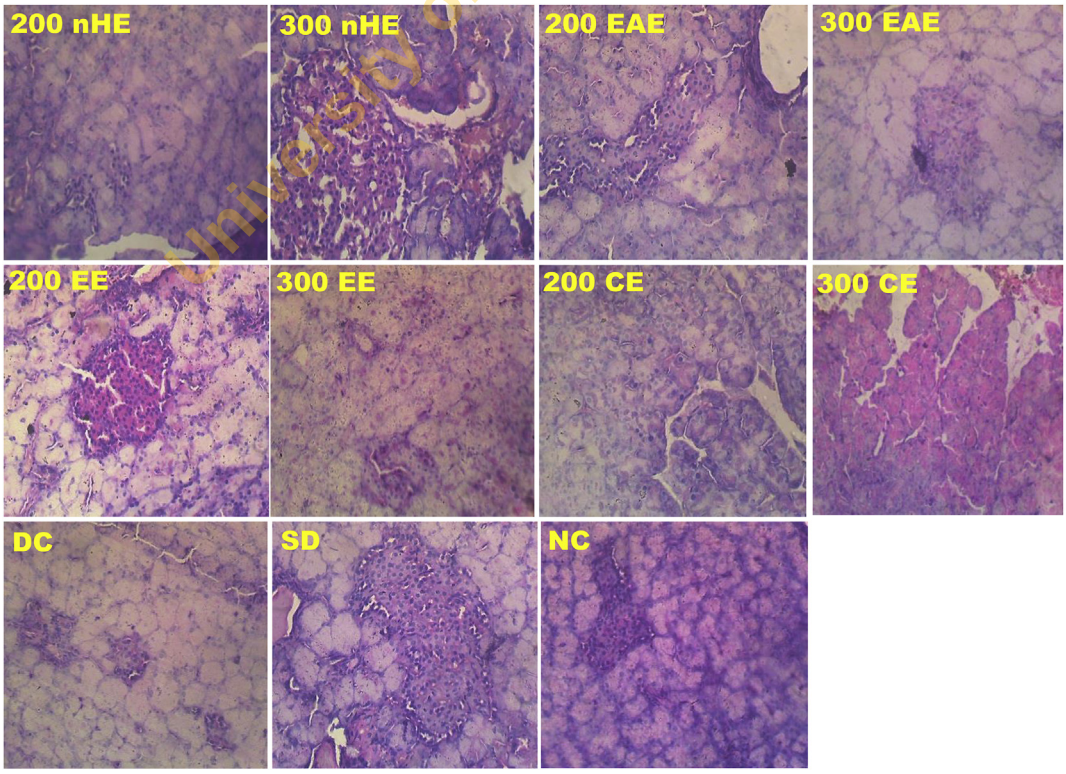

Fig. 4. Pancreas histopathology of normal, treated and untreated rats (100 X magnification). 200 nHE = 200 mg/kg n-hexane extract showing moderate expansion of cellular population and size of islet cells; 300 nHE = 300 mg/kg n-hexane extract showing moderate expansion of cellular population and size of islet cells; 200 EAE = 200 mg/kg ethyl acetate extract; 300 EAE = 300 mg/kg ethyl acetate extract; 200 EE = 200 mg/kg ethanol extract; 300 EE = 300 mg/kg ethanol extract; 200 CE = 200 mg/kg chloroform extract; 300 CE = 300 mg/kg chloroform extract; DC = diabetic control showing a less number of islets; SD = standard drug showing moderate expansion of cellular population and size of islet cells; NC = normal control showing normal appearance.

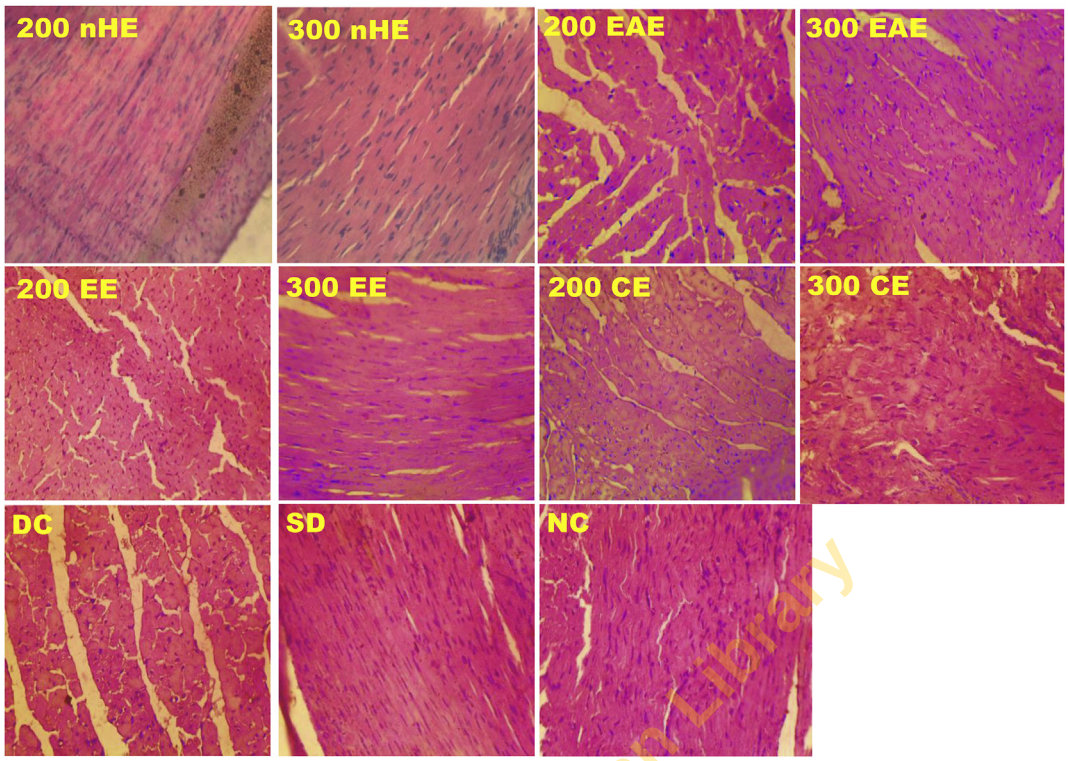

Fig. 5. Heart histopathology of normal, treated and untreated rats (100 X magnification). 200 nHE = 200 mg/kg n-hexane extract showing normal mycocytes; 300 nHE = 300 mg/kg n-hexane extract showing normal mycocytes; 200 EAE = 200 mg/kg ethyl acetate extract showing normal mycocytes; 300 EAE = 300 mg/kg ethyl acetate extract showing normal mycocytes; 200 EE = 200 mg/kg ethanol extract showing normal mycocytes; 300 EE = 300 mg/kg ethanol extract showing normal mycocytes; 200 CE = 200 mg/kg chloroform extract showing normal mycocytes; 300 CE = 300 mg/kg chloroform extract showing normal mycocytes; DC = diabetic control showing multiple foci of cardiomyocytes and marked areas of degeneration of the myofibers with diffuse vacuolar; SD = standard drug showing normal mycocytes; NC = normal control.

complications.

In conclusion, *L. taraxacifolia*to ameliorated the pathophysiological and histopathological complications caused by diabetes mellitus. A further study is therefore necessary to isolate the active compounds in the extract and examine its effects in the treatment and management of diabetes mellitus.

Declaration of competing interest

None to declare.

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