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Gamma-sitosterol–rich fraction from the methanolic extract of *Ficus exasperata* restores diabetes associated pathophysiological alterations in an alloxan-induced diabetic rats

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ABSTRACT: *Ficus exasperata* has been reported to have hypoglycemic and antidiabetic effects which are even better than some standard antidiabetic drugs. However, key compounds behind these effects are still unknown. This study was therefore designed to isolate and conduct preliminary characterization of the components of *F. exasperata* that are responsible for its antidiabetic effects. Methanol extract of *F. exasperata* was partitioned using ethyl acetate and n-hexane when ethyl acetate extract was found to have more hypoglycemic potential in animal model. Hence, the ethyl acetate fraction was used for the *in vivo* study. Adult male rats were divided into 4 treatment and one control groups (n=5). Diabetes was induced by a single intraperitoneal injection of alloxan (150mg/kg body weight). The effects of the fractions and a standard antidiabetic drug (glibenclamide) on blood glucose, haematological parameters, liver enzymes, lipid profile and histopathology of some organs were studied thereafter. All treated rats responded positively to treatment with the fraction and hyperglycemia was reversed within 7 days of treatment. Treatment with the fraction induced significantly better ($p<0.05$) haematopoetic values and lower hyperlipidemia than the standard antidiabetic drug. The degrees of diabetes related degeneration in the pancreas, kidney, liver and heart of the treated groups were significantly lower compared to the rats treated with glibenclamide. The fraction contained 7 compounds and the most prominent compound was gamma-Sitosterol with a percentage of 25.49. The results of this study suggest that *Ficus exasperata* (Ethyl Acetate fraction) is a good candidate for the treatment of diabetes mellitus.

Keywords: Diabetes mellitus, *Ficus exasperata*, hypoglycemic, histopathology, rats

Abbreviations
DM: Diabetes mellitus; T1D: Type 1 diabetes; T2D: Type 2 diabetes; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; TLC: Thin layer chromatography ;GC-MS :Gas Chromatography–Mass Spectrometry; PCV: Packed Cell volume; Hb: Hemoglobin; WBC: White blood cell; RBC: Red blood cell; MCH: Mean corpuscular Hemoglobin; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin concentration; AST:

Aspartate Transaminase; ALT: Alanine Transaminase; ALP: Alkaline Phosphatase; GGT: Gamma Glutamyl Transferase; BILI: Bilirubin; HP:Hepatocytes; CCR: Creatinine Clearance Rate; GFR: Glomerular Filtration Rate.

Introduction

Diabetes mellitus (DM) is a metabolic disorder of multiple aetiologies characterized by chronic hyperglycaemia, glycosuria and negative nitrogen balance. Pathophysiology of diabetes involves a complex cascade of several interrelated mechanisms resulting from lack of insulin secretion from the beta cells of pancreas and desensitization of insulin receptors at cell surface with disturbances of carbohydrate, fat and protein metabolism [1]. The global prevalence of diabetes in adults has been increasing over the recent decades. The International Diabetes Federation (IDF) estimated the global prevalence was 151 million in 2000 [2] which has been increased to 425 million by 2017 [3]. This is expected to increase by 48% in 2048 with a total of 629 million diabetics [3].

The etiology of DM has been associated with the inability of the pancreatic cells to secret insulin, regarded as type 1 diabetes (T1D) or inability of the body to utilize the insulin synthesized by the pancreatic β-cells, a condition termed type 2 diabetes (T2D) [4]. Occurrence of the former has been linked to genetic, environmental and immunological factors (T1D), with daily injection of insulin as the major treatment [5].

In recent years, there has been a growing interest in the plant-based natural anti-diabetic medicines not only due to their lower cost but also for their less or no side effects. This became more apparent following the recommendation of World Health Organization [6] regarding the development and evaluation of better pharmacological agents for improving insulin secretion, enhancing insulin sensitivity, preventing pancreatic beta cells destruction, promoting beta cells regeneration and ameliorating pathways that lead to the various complications of diabetes [6]. In many Nigerian communities, various herbs are being used in the treatment and management of DM [7]. Amongst such herbs is *Ficus exasperata.*

Ficus exasperata is a commonly used medicinal plant for various ailments. Different parts of the plant are used for the folkloric treatment of ulcers, anaemia, piles, jaundice, haemorrhage of the nose and mouth, DM and various diseases of the blood [8]. Studies by [9] reported that the aqueous extract of *F. exasperata* exhibited better hypoglycemic effects than glibenclamide in experimentally induced diabetic rats. Additionally, treatment with the extract increased the values of PCV, Hb and RBC compared to the untreated rats and rats treated with glibenclamide. Concentrations of total cholesterol, triglyceride, HDL-/LDL-cholesterol ratio and CRI were also markedly reduced when diabetic groups were treated with the extract compared to those treated with glibenclamide. From the results of this study, [9] suggested the isolation and characterization of the active components of the plant that may be responsible for these effects.

Hence, the current study was designed to examine the effects on restoring diabetes related alterations of *F. exasperata* ethyl acetate fraction in an alloxan-induced diabetes model of rats and characterization of the bioactive compounds that maybe responsible for its hypoglycemic as well as antidiabetic activities.

Materials and Methods

Collection of plants materials and extract preparation

Fresh leaves of *F. exasperata* were collected from the University of Ibadan, Ibadan, Nigeria during the month of December 2014. The plant materials were identified and authenticated by Mr. Donatus, Department of Botany, University of Ibadan, Nigeria. A sample specimen was deposited at the University herbarium, after assigning a voucher number UIH - 22438.

The leaves were shade-dried and grounded to coarse powder using an industrial mill. The blended sample (2849.45g) was extracted with methanol by soaking in 10L of the extracting solvent for 5 days.

The obtained solution was decanted, filtered with Whatmann filter paper (no. 1) and concentrated *in vacou* with a rotary evaporator (Heidolph, Germany).

The methanolic extract was further fractionated with n-hexane and ethyl acetate. Both fractions obtained were concentrated using above-mentioned vaccum rotary evaporator.

Qualitative phytochemical screening of the extract

The procedure described by [10] was adopted for the determination of tannins, cardiac glycosides, anthraquinones, terpenoids, alkaloids, saponins, flavonoids in the methanolic extract of the leaf of *F. exasperata.*

***In vivo* hypoglycemic studies**

A preliminary *in vivo* study was conducted with the methanolic extract and ethyl acetate and n-hexane fractions to identify the extract or fraction with best hypoglycemic effect.

Characterization of compounds from *F. exasperata fraction*

The ethyl acetate fraction having the best hypoglycemic effect in diabetic rats was further separated by column chromatography. The fraction was successfully eluted with stepwise gradient of *n*-hexane and ethyl acetate solvent system (100: 0; 97: 3; 95:5; 90: 10; 85: 15; 80: 20; 70: 30; 50: 50) on a silica gel column. Fractions obtained were checked on thin layer chromatography (TLC) plates. Dark green sticky eluents were obtained in Hexane, Ethyl Acetate solvents in the ratio 90: 10. This fraction was used for further studies. It was checked on thin layer chromatography (TLC) and showed a single spot and used for an *in vivo* study. It was also subjected to gas chromatography–mass spectrometry (GC–MS) analysis. The analysis was performed on a JEOL GCMATE II GC–MS system in EI/CI mode equipped with a split/split less injector (220°C), at a split ratio of 1/10, using a VF-1MS fused-silica capillary column (30 m × 0.25 mm i.d.; film thickness: 0.25 mm). The oven temperature was programmed from 60°C to 280°C in 5 min at an increment rate of 4°C/min and held at the temperature for 10 min. Helium was used as a carrier gas at a flow rate of 0.8 ml/min.

Animals

Adult male Wistar rats weighing between 120 to 140g were obtained from the Central Animal House of the Department of Anatomy, University of Ibadan, Nigeria. They were transported to the animal house of the department of Zoology, University of Ibadan, Nigeria where the animal study was conducted. The animals were kept in rat cages at room temperature (25-27^{o}C) where food (Ladokun commercial pellet) and water was given to them *ad libitum.* They were allowed to adapt for a week prior to the induction of diabetes. The protocol for this study was approved by the Animal Care and Use Committee of the University of Ibadan.

Animal experimental design

The animals were randomly divided into four groups of 5 animals as follows:

Group A: Non-diabetic animals treated with distilled water
Group B: Diabetic animals treated with glibenclamide 10 mg/kg
Group C: Diabetic animals treated with 200mg/kg of the 90:10, hexane:ethyl acetate fraction
Group D: Diabetic rats treated with distilled water only.

Induction of diabetes mellitus

After an overnight fast, diabetes mellitus was induced in the rats by a single intraperitoneal injection of 0.2 ml ofalloxan in normal saline at a dose of 150mg /kg body weight. After a period of 48 hours, the blood glucose level of the animals was checked using a portable glucometer (Accu-chek, Roche Diagnostics, Mannheim, Germany).Blood glucose levels above 200mg/dl were considered as diabetic and used for the further experiment.The intervention trial was lasted for 7 days when the blood glucose level of rats were measured daily during the entire intervention period.

All experiment was conducted according to the National Institute of Health guidelines of care and use of laboratory animals (NIH 1985).

Sacrifice and organ collection

At the end of the experiment, rats were fasted overnight. Blood samples were collected from the retro orbital venous plexus of conscious animals by using heparinized capillary tubes. A portion of whole blood was used for the haematological analysis. The remainingblood samples were allowed to clot and then centrifuged at 2300 rpm for 10 min to obtain serum. The serum samples were preserved at -30^{o}C until further analysis of biochemical parameters.The animals were then sacrificed by cervical dislocation and dissected as described by [11]. Urine was collected from their bladder after dissection into sample bottles for biochemical analysis. The liver, heart, kidneys, and pancreas of each animal were collected and a small piece of each was stored in 10% formalin solution for histopathological studies.

Haematological studies

The haematological parameters such as Packed Cell volume (PCV), Hemoglobin (Hb), White blood cell (WBC), Red blood cell (RBC), Mean corpuscular Hemoglobin (MCH), Mean corpuscular volume (MCV) and Mean corpuscular hemoglobin concentration (MCHC) were measured in the whole blood according to the methods described by [12].

Determination of serum biochemical parameters

The levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and total bilirubin in the serum was determined using Randox test kits (Randox Laboratories Ltd., Crumlin, England) according to manufacturer's protocols.

Serum lipid profile

Total cholesterol, HDL-cholesterol and triglycerides in the serum were determined using commercially available kits (Randox Laboratories Ltd., Crumlin, England). Low density lipoprotein (LDL) cholesterol was calculated according to the following formula:

LDL-Cholesterol = [Total cholesterol – (HDL-cholesterol + TG/5)]

Where TG/5 is equivalent to the concentration of VLDL-cholesterol.

Urine analysis

The albumin concentration in the urine sample was determined by using a commercially available reagent (Bromocresol green solution), the urea concentration of the urine samples was determined using the urease – Berthelot (enzymatic) colorimetric method, while the creatinine content was determined using commercially available kits (Randox Laboratories Ltd., Crumlin, England).

Statistical analysis

Data obtained were expressed as mean ± SEM. Significant difference between test and control groups was determined by using one way analysis of variance(ANOVA)and $p < 0.05$ were considered as significantly different(SPSS for Windows, version 16.0, USA)

Results

Phytochemicals in methanolic extract

Phytochemical screening of the methanolic extract of *F. exasperata* leaf revealed the presence of alkaloids, saponins, flavonoids and phenolics while tannins, anthraquinones, cardiac glycosides and steroids were not detected (Table 1).

Table 1: Qualitative phytochemical constituents of methanolic extract of *F. exasperata* leaves

Phytochemicals	**Inference**
Alkaloids	+
Saponins	+
Tannins	+
Anthraquinones	+
Cardiac glycosides	–
Flavonoids	+
Terpenoids	+

Key: + = Detected; - = Not detected

GC-MS analysis

GC-CM analysis of the 90:10, hexane:ethyl acetate fraction revealed the presence of 6 compounds, with gamma Sitosterol being the most abundant (35.87%) as shown in Figure 1 and Table 2.

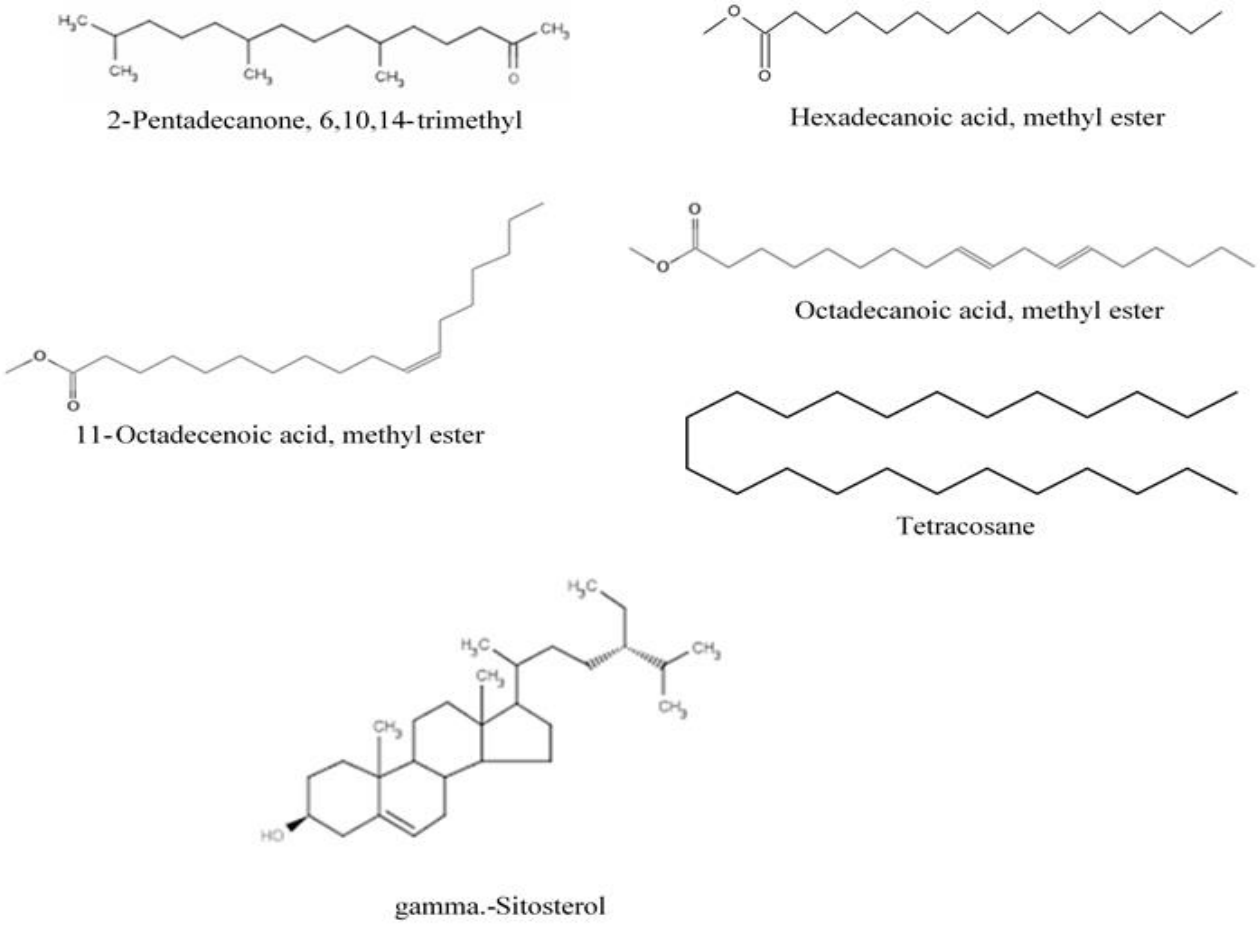


Figure 1 GC-MS identified compounds in 90:10 hexane : ethyl acetate fraction

Table 2: GC-MS identified compounds in 90:10 hexane:ethyl acetate fraction

Pk#	RT	Area%	Library/ID	CAS#	Quality
1	15.953	2.15	2-Pentadecanone, 6,10,14-trimethyl	112036	000502-69-2 96
2	16.805	11.52	Hexadecanoic acid, methyl ester	113690	000112-39-0 98
3	18.574	3.45	11-Octadecenoic acid, methyl ester	133708	052380-33-3 99
4	18.814	1.59	Octadecanoic acid, methyl ester	135381	000112-61-8 98
5	21.452	15.21	Tetracosane	164289	000646-31-1 96
6	35.877	25.49	gamma.-Sitosterol	199879	000083-47-6 99

Blood glucose concentration

The blood glucose concentrations prior to the induction of diabetes were between 61 and 78.50 mg/dl (Figure 2). Marked increase in blood glucose concentrations (ranging from 267 to 400mg/dl) were recorded after alloxan injection, indicating an induction of DM. Treatment of diabetic rats with the fraction and glibenclamide caused marked reduction in glucose concentration after each day of treatment. Rats treated with the fraction had significantly ($p < 0.05$) lower blood glucose levels compared to rats treated with glibenclamide. Hyperglycemia was reversed in all treated rats by the 6th day of treatment.

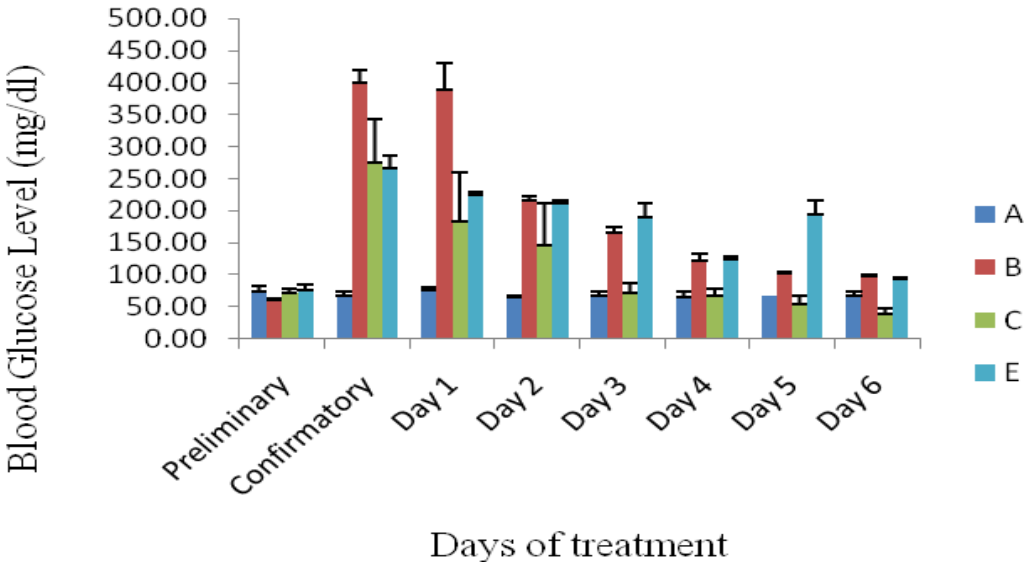


Figure 2: Blood glucose concentration in different animal groups during the intervention period

Haematological parameters

There was no significant difference ($p < 0.05$) in the values of the PCV, Hb and platelet count of the untreated group when compared with the treated groups (Table 3). The MCV and MCH values of the untreated group were significantly lower ($p < 0.05$) than the treated group. There was however no significant difference in MCHC levels in all the groups.

Table 3: The levels of various haematological parameters indifferent animal groups at the end of the study

Treatment Groups	PCV (%)	Hb (g/dL)	RBC (cell/L)	Platelet (10^5µL)	MCV (femtoliter)	MCH (pg)	MCHC (g/dL)
A	44.50±0.5^a	14.90±0.3^a	7.31±0.05^a	9.9±6.5^a	60.92±0.3^a	20.39±0.51^c	33.48±0.3^a
B	42.00±4.0^a	14.05±1.5^a	7.16±0.6^a	8.9±22.5^b	60.69±2.5^a	19.59±1.25^b	33.40±0.5^a
C	44.50±1.5^a	14.90±0.6^a	6.65±0.6^b	9.3±7.0^a	67.31±3.8^a	22.52±1.2^c	33.48±0.2^a
D	42.50.±1.5^a	14.00±0.5^a	7.74±0.4^a	8.6±5.5^a	55.15±4.7^b	18.17±1.6^a	32.95±0.0^a

Data are presented as mean ± SEM ($n \leq 5$); Values in the same column with different superscript letters are significantly different from each other group of animals ($p<0.05$).
Group A, Non-diabetic animals treated with distilled water only; **Group B**,Diabetic animals treated with glibenclamide 10 mg/kg bw;**Group C**, Diabetic animals treated with 200mg/kg of 90:10 hexane:ethyl acetate fraction;**Group D**, Diabetic rats treated with distilled

Levels of various blood cells

The values of the WBC, lymphocytes and neutrophils were significantly higher ($p<0.05$) in the untreated group than in the treated and control groups (Table 4). However, there was no significant difference ($p<0.05$) in the counts of monocytes. The eosinophils count in the untreated rats was significantly lower ($p<0.05$) than the treatment groups.

Table 4: Levels of various blood cellsin different animal groups at the end of the study

Treatment groups	White Blood Cell (10^3µL)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophils (%)
A	3.6±1.5^a	62.00±2.0^d	34.00±3.0^a	3.0±0.0^a	2.0±0.0^a
B	5.5±1.3^b	62.00±6.0^d	33.00±5.0^a	2.0±1.0^b	3.0±0.0^b
C	5.7±1.0^b	61.00±2.0^d	36.00±4.0^a	2.0±1.0^b	3.0±1.0^b
D	6.9±2.2^c	65.50±3.5^d	29.50±4.5^b	2.0±1.0^b	2.0±1.0^a

Values are shown as mean±SEM ($n \leq 5$); Values in the same column with different superscript letters are significantly different from each other group of animals ($p<0.05$).

Liver function tests

The results of the liver function tests are presented in Table 5.The result shows no significant difference ($p<0.05$) in AST level of the untreated, glibenclamide and fraction treated diabetic rats (group B-D). There was also no significant difference ($p<0.05$) in the activities of ALT in the plasma of the untreated and fraction treated group but was significantly reduced ($p<0.05$) in the glibenclamide treated group.

There was a significant reduction ($p<0.05$) in the concentration of alkaline phosphatase (ALP) in the treated group (C) compared to the diabetic control group (B). The total bilirubin concentration of the rats treated with the fraction was reduced significantly ($p<0.05$) compared to all other groups (Table 5).

Table 5: Levels of liver function related parameters in the serum of different rat groups at the end of the study

Treatment groups	AST	ALT	ALP	GGT	Total Bilirubin (mg/dL)
	(IU/L)				
A	38.00 ± 2.0^{c}	30.00 ± 1.0^{a}	109.00 ± 1.0^{b}	0.15 ± 0.05^{a}	0.40 ± 0.1^{c}
B	40.50 ± 0.5^{a}	29.50 ± 0.5^{b}	111.50 ± 11.5^{c}	0.20 ± 0.00^{b}	0.40 ± 0.1^{c}
C	41.00 ± 5.0^{a}	30.50 ± 2.5^{a}	109.00 ± 4.0^{b}	0.25 ± 0.05^{b}	0.35 ± 0.05^{b}
D	44.50 ± 1.5^{a}	33.00 ± 1.0^{a}	122.50 ± 1.5^{a}	0.30 ± 0.06^{c}	0.45 ± 0.05^{c}

Values are shown as mean±SEM ($n \leq 5$); Values in the same column with different superscript letters are significantly different from each other ($p<0.05$).
Group A,Non-diabetic animals treated with distilled water only; **Group B**,Diabetic animals treated with glibenclamide 10 mg/kg bw;**Group C**, Diabetic animals treated with 200mg/kg of 90:10 hexane:ethyl acetate fraction; **Group D**, Diabetic rats treated with distilled water only; **AST**, aspartate transaminase; **ALT**, alanine transaminase; **ALP**, alkaline phosphatase; **GGT**, gamma glutamyl transferase.

Serum lipid profile

Table 6 shows the results of serum total cholesterol, triglyceride, LDL-cholesterol and HDL-cholesterol levels in the serum of different experimental groups. It was observed that the levels of total cholesterol, triglyceride and LDL-cholesterol were reduced significantly ($p<0.05$) in the treated group (C) compared to the untreated group (D). However, the HDL cholesterol level was significantly increased ($p<0.05$) in treated group compared to the untreatment group (Table 6).

Table 6: Serum lipid profile in different animal groups at the end of the study

Treatment groups	Total cholesterol	Triglycerides	HDL-cholesterol	LDL-cholesterol
	(mg/dL)			
A	58.50 ± 7.5^{b}	41.00 ± 1.0^{c}	38.50 ± 0.5^{a}	11.80 ± 0.4^{c}
B	63.00 ± 0.0^{a}	43.00 ± 2.0^{c}	33.00 ± 6.0^{a}	21.40 ± 0.6^{b}
C	60.50 ± 7.5^{a}	40.50 ± 2.5^{c}	30.00 ± 6.0^{a}	22.40 ± 1.0^{b}
D	72.00 ± 1.0^{c}	52.50 ± 3.5^{b}	27.00 ± 7.0^{b}	34.50 ± 0.7^{a}

Values are shown as mean ± SEM, $n \leq 5$. Values within a column having different superscript letters are significantly different from each other group of animals, $p<0.05$.
Group A,Non-diabetic animals treated with distilled water only;**Group B**,Diabetic animals treated with glibenclamide 10 mg/kg bw;**Group C**, Diabetic animals treated with 200mg/kg of 90:10 hexane:ethyl acetate fraction;**Group D**, Diabetic rats treated with distilled water only; **HDL**, high density lipoprotein; **LDL**, low density cholesterol.

Urine Analysis

The urea, creatinine and albumin levels in the urine of all animal groups are shown in Table 7. In this study, the level of urea was significantly decreased in the urine of fraction treated group compared to all other groups. There was no significant difference ($p<0.05$) in the levels of creatinine and albumin among the diabetic groups.

Table 7: The levels of renal function related parameters in the urine of different animal groups at the end of the study

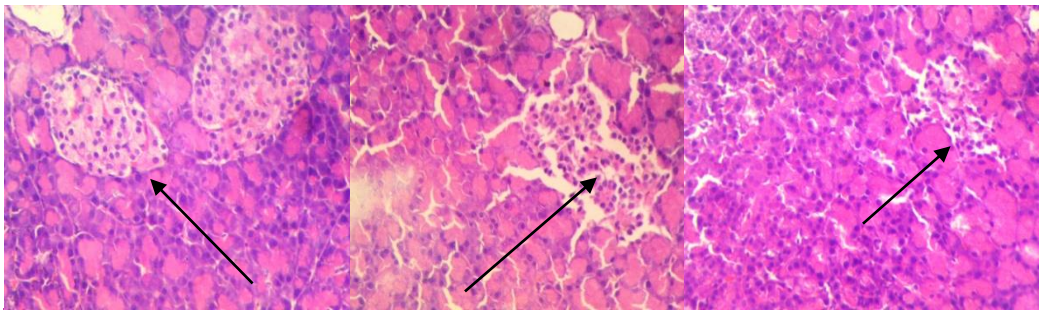
Treatment groups	Urea (mg/dL)	Creatinine	Albumin
A	9.25 ± 0.45^{a}	38.50 ± 1.50^{c}	2.15 ± 0.05^{a}
B	11.20 ± 1.00^{b}	26.50 ± 1.50^{b}	2.15 ± 0.05^{a}
C	8.85 ± 0.75^{c}	29.00 ± 1.00^{b}	2.55 ± 0.05^{a}
D	12.15 ± 0.65^{d}	25.50 ± 1.50^{b}	2.40 ± 0.10^{a}

Values are mean ± SEM, $n \leq 5$. Values within a column having different superscript letters are significantly different from each other group of animals, $p<0.05$.
Group A, Non-diabetic animals treated with distilled water only;**Group B**,Diabetic animals treated with glibenclamide 10 mg/kg bw;**Group C**, Diabetic animals treated with 200mg/kg of 90:10 hexane:ethyl acetate fraction;**Group D**, Diabetic rats treated with distilled water only.

Histopathological Studies

Pancreas

The pancreatic tissue of the diabetic control group showed normal appearance of the islets of Langerhans scattered throughout the tissue (Fig. 3A). That of the diabetic group treated with plant fraction showed mild vacoulation of the islets of Langerhans (Fig. 3B). Untreated diabetic rat shows abnormal and fewer islets of Langerhans that are barely seen (Fig. 3C)



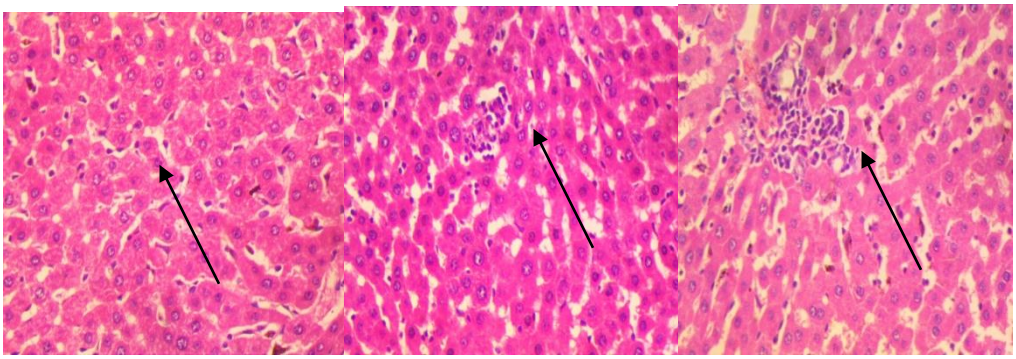
A **B** **C**

Figure 3. (A) Pancreas of control rat showing normal appearance of the islet of langerhans ×400 H and E; ×400 H and E; (B) pancreas of alloxan-induced diabetic rat treated with plant fractionshowing mild vacoulation of the islets of Langerhans ×400 H and E. (C) pancreas of untreated alloxan-induced diabetic rat, showing marked degeneration of the Islets of Langerhans.

The Liver

Histopathological examination of the liver of the control group appeared normal with a proper arrangement of the hepatocytes (Fig. 4A). That of the diabetic group alloxan-treated with the plant

fraction revealed a mild degeneration of the hepatocytes (Fig. 4B), the untreated diabetic group showed severe degeneration of the hepatocytes (Fig.4C).

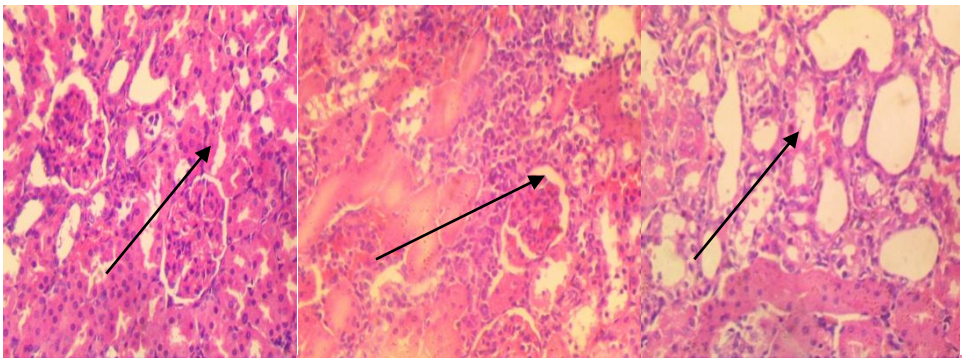


A **B** **C**

Figure 4. (A) Liver of control rat, showing normal arrangement of the hepatocytes with no visible lesion. ×400 H and E; (B) liver of alloxan-induced diabetic rat treated with the plant fraction revealed a mild degeneration of the hepatocytes ×400 H and E. (C) Liver of untreated alloxan-induced rat showing severe degeneration of the hepatocytes (HP) with numerous vacuolations. ×400 H and E

The kidney

Histopathology of the kidney of the control groups showed normal appearance of the organ with no visible lesions (Fig. 5A). The glomeruli are also surrounded by narrow and normal bowman's spaces. The kidney diabetic rat treated with the plant fraction showed normal glomerulus with mild vacuolations of the tissue (Fig. 5B). The glomerular spaces in the treated group were noticed to show some signs of regenerations in them, with their glomerular spaces appearing narrower than that of the diabetic untreated rat (Fig. 5C).

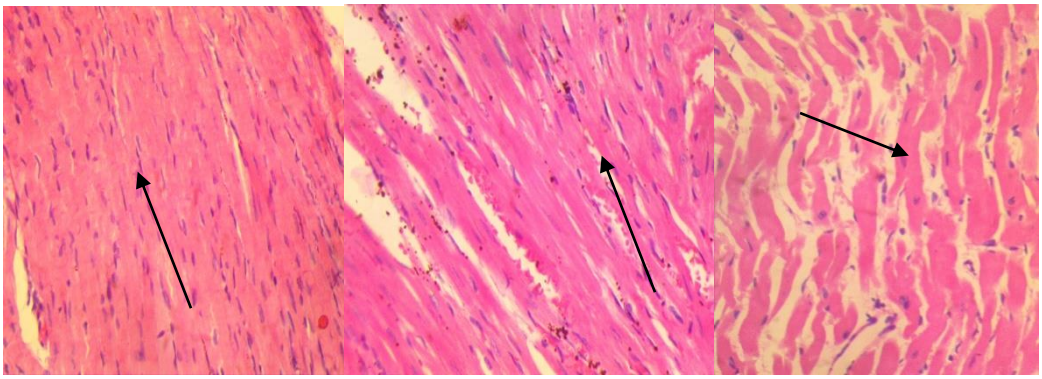


A **B** **C**

Figure 5. (A) Kidney of normal rat showing normal appearance of the glomeruli. ×400 H and E; (B) kidney of alloxan-induced rats treated with plant fraction showing normal glomerulus with mild vacoulations of the tissue. ×400 H and E (C) kidney of untreated alloxan-induced diabetic rat showing vacoulation of the kidney ×400 H and E

The heart

A normal appearance of the endothelium which is supported by a layer of collagenous tissue was observed in the histological examination of the heart of the control group (Fig. 6A). In the diabetic untreated group (E), marked areas of degeneration of the myofibres with diffuse vacoular degenerations of the myocytes were revealed (Fig. 6C). Also, a depletion of the cardiac blood tissues was visibly observed. Whereas, that of the diabetic treated groups revealed a normal appearance of the myocytes and likewise, a form of regeneration was identified in the cardiac muscle / tissues (Fig. 6B).



A **B** **C**

Figure 6. (A) Heart of normal rat showing normal appearance of the endothelium. ×400 H and E; (B) Heart of alloxan-induced rats treated with plant fraction showing regeneration of the cardiac tissue. ×400 H and E (C) Heart of untreated alloxan-induced diabetic rat showing severe degeneration of the myocytes ×400 H and E

Discussion

Diabetes is referred to as a chronic disease marked by high levels of sugar in the blood [13]. The geometric increase in glucose concentration observed in all rats after a single intraperitoneal injection of alloxan monohydrate confirmed the induction of diabetes. Alloxan induces "chemical diabetes" in a wide variety of animal species by damaging the insulin secreting pancreatic β-cell of the islets of Langherhans, resulting in reduced synthesis and release of endogenous insulin characteristically similar to type 1 diabetes in humans [14].

The phytochemicals present in the methanol extract of *F. exasperata* showed flavonoids, tannins, saponin, alkaloids, terepenoids and anthraquinone. Flavonoids is one of the most diverse and widespread group of natural compounds, the presence of hydroxyl groups confers scavenging ability and also plays an important role in preventing lipid peroxidation [15]. Saponins are glycosides of triterpenes, steroids or alkaloids. Saponins may have a glucagon decreasing effect and may enhance glucose utilization lowering blood glucose as well as stimulate insulin release from the pancreas [16]. Tannin is composed of a central glucose molecule derivatized at its hydroxyl groups with one or more galloyl residue and in the presence of copper ions; act as an antioxidant suppressing hydroxyl radical formation [17]. Anthraquinones derivatives have also been found to play an important role in the treatment of tumors, diabetes, ulcer and cancer [18]. Thus, the phytochemical constituents indicate that the methanol extract of *F. exasperata* could have potentials to be an antidiabetic agent which is in agreement with a previous study [9].

The preliminary study showed that the ethyl acetate fraction of the methanolic crude extract has the best hypoglycemic effect when compared to the methanolic crude extract and the n-hexane fraction in alloxan-induced diabetic rats. The components were therefore separated using column chromatography. Fractions received from the column chromatography at ratio 90:10 ethyl acetate to n-hexane was used to treat alloxan-induced diabetic rats in the main study. Results obtained from this study show that the fraction significantly ($P<0.05$) lowered blood glucose levels of diabetic rats (Figure 2). This action of the fraction on blood glucose in diabetic rats is similar to that of Gilbenclamide (10mg/kg bw), a potent hypoglycemic agent, and suggested that the fraction contain active principles with potent hypoglycemic property. The fractions may have achieved this hypoglycemic property via increased insulin secretion, increased peripheral utilization of glucose, inhibition of endogenous glucose production or by inhibition of intestinal glucose absorption as reported in some previous studies [19,20]. This result supports the hypoglycemic and antidiabetic potential of the *F. exasperata* for the treatment of diabetes mellitus.

In diabetes, the value of PCV, Hb, RBC, MCV, MCH and MCHC are reduced due to lyses of blood cells caused by reactive oxygen species (ROS) and the resulting oxidative stress [21,22,23] leading to anaemia [24]. This was the trend observed in this study (Table 3). However, fraction treatment caused significant ($p<0.05$) amelioration in the value of these parameters such that it brought about a significant increase in the MCH value, a factor that measures the rate of erythrocyte synthesis. It therefore can be deduced that extract was able to reverse the lytic effect of ROS and so reduced or rather completely prevented oxidative stress thereby giving room for the regeneration of erythropoietic cells, a process mediated by erythropoietin secretion from the bone marrow [21,25]. The overall effect is the restoration of the oxygen carrying capacity of the RBC as a result of the inhibition of the process of lipid peroxidation in the membrane of RBC [26].

Changes in TWBC have been associated with insulin resistance and complications of CVD [22,23]. The result of this study showed a significant ($p<0.05$) increase in the value of TWBC in the diabetic control group which reduced significantly on fraction treatment. This may be interpreted to mean the fraction's ability to restore insulin sensitivity to the cells [23].

The liver is known to play an important role in the metabolism of carbohydrate and so its cellular integrity can be compromised in diabetes and related disease [27]. Therefore, some liver function tests were performed to assess its pathological condition. Prominent among these tests are the analysis of AST, ALT, ALP activities and Bilirubin levels. Results show that the plant fraction reduced ALP activities and the level of total Bilirubin. Reduction of ALP activity as shown by fractions treatment is suggestive of the fractions' ability to protect the cell from cytotoxic injury. Bilirubin is a breakdown product of blood with biological and diagnostic values [28]. Mild decrease in bilirubin levels in the treated group compared to untreated group has been proposed to have a null protective effect on cells [29].

The diabetic control groups had elevated total cholesterol, triglycerides, decreased high density lipoprotein cholesterol (HDL-cholesterol). The results agreed with [30], who reported that high levels of triglycerides, LDL-cholesterol and low levels of HDL-cholesterol have been associated with heart disease, insulin resistance and diabetes mellitus. On the other hand, HDL is often referred to as 'Good Cholesterol' with high levels associated with a decreased risk of myocardial infarction. HDL removes cholesterol from non-hepatic tissues to liver through the process known as reverse cholesterol transport [31]. The plant fraction therefore has shown hypolipidemic effect in diabetic rats. This is in agreement with the results of [9].

The significant increase in urine creatinine for both normal and treated diabetic rats is a strong indication of the positive impact treatment with of the fraction may have on the glomerular filtration rate. Creatinine levels in blood and urine are usually used to calculate the creatinine clearance rate (CCR) which reflects the glomerular filtration rate (GFR). The GFR is important clinically because it is a measure of the renal function. High creatinine level in the urine indicates the ideal, while a low urine creatinine level may indicate malfunctioning of the kidneys. GFR is so important in assessing the excretory function of the kidneys [32]. The ability of the fraction of the methanolic leaf extract of *F. exasperata* to increase the levels of creatinine, reduce the level of urea and albumin in urine suggest that the fraction may ameliorate diabetic nephropathy.

Alloxan monohydrate has been described as a toxic glucose analogue which selectively destroys the insulin-producing beta cells of the pancreas [33]. Mild vacuolations of the islets was however observed in pancreas of rats treated with the plant extract fractions which is probably indicative of the ability of the extract to restore the degenerations cause by alloxan-induced diabetes mellitus this is in agreement with [34,35]

The potentials of the extract to ameliorate the pathological effects of diabetes mellitus was demonstrated as rats treated with the plant fraction showed mild degeneration of liver compared to rats treated with glibenclamide.

An ameliorative property of the fraction is seen as the kidney of rats treated with the fraction show normal glomeruli with mild vacuolation while the histology of diabetic heart section of the treatment groups (B &C) show no alteration with the cytoarchitecture similar to that of normal control. The fraction

has ameliorative effect on the heart of the diabetic rat. The section of the heart of diabetic control showing myocytes with their intercalated discs and interdigitations.

It is worthy to note that treatment with the plant extract fraction has proved to be effective in the reduction of blood glucose levels, is capable of stimulating blood cell formation (erythropoiesis) and confer protection to hepatocyte against cell injury due the effect of oxidative stress it has hypolipidemic potentials and has also showed ameliorative and restorative effects on structures during diabetic complications. The GC-MS result shows that the most prominent compound in this plant fraction is β sitosterol which has been reported to exhibit anti-inflammatory activity in carragenaan paw oedema model [36] and in mice induced by phorbol derivative [37].

In conclusion, this study demonstrates the hypoglycaemic and hypolipidemic potentials of *F. exasperate* in T1D model of rats, which can be attributed to the synergistic effect of the identified compounds particularly gamma-sitosterol in the fraction. This further gives credence to the use of the plant in the management of diabetes and its complications.

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References

1. Bennett PH, Knowler WC: Definition, Diagnosis and Classification of Diabetes Mellitus and Glucose Homeostasis, in Joslin's Diabetes Mellitus, 14th ed., 2004.
2. International Diabetes Federation. IDF Diabetes Atlas. 1st ed. Brussels, Belgium: International Diabetes Federation; 2000.
3. International Federation of Diabetes (IDF). 2018. IDF Diabetes Atlas, 8th ed. International Diabetes Federation.
4. Erukainure OL, Hafizur R, Kabir N, Choudhary I, Atolani O, Banerjee P, Preissner R, Chukwuma CI, Muhammad A, Amonsou E. Suppressive Effects of Clerodendrum volubile P Beauv.[Labiatae] Methanolic Extract and Its Fractions on Type 2 Diabetes and Its Complications. Frontiers in Pharmacology. 2018; 9, 8.
5. Dyck P. Severity and staging of diabetic polyneuropathy. Textbook of Diabetic Neuropathy.2003;170-175.
6. World Health Organisation. WHO Study Group Report on Prevention of Diabetes Mellitus. WHO, Geneva.1994; pp: 1- 92.
7. Gidado A, Ameh DA, Atawodi SE. Effects of Nuclea ratifolia leaves aqueous extracts on blood glucose levels of normal and alloxan-induced diabetic rats. Afr. J. Biotechnol.2000; 4(1):91-93.
8. Joseph B, Raj SJ. Phytopharmacological and phytochemical properties of three *Ficus* species - An overview. Int J Pharma Bio Sci. 2010; 1:246-53.
9. Adeyi A.O, Idowu, AB, Mafiana CF, Oluwalana SA. Ajayi OL. Effects of aqueous leaf extract of *Ficus exasperata* on pathophysiology and histopathology of alloxan-induced diabetic albino rats. J Med Plants Res.2012; 6(46): 5730-5736.
10. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO. Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. Trop J Pharmaceutical Res.2008; 7 (3): 1019-1024
11. Rowett HGO. Dissecting guides of rats with notes on mouse. Bulter and tanner LTD. London.1977; 111:5-23.
12. Cheesbrough, M. District Laboratory Practice in Tropical Countries, part 2. Cambridge University Press. 2004; pp 249-258.
13. El-Wakf A, Tarek MA, Rizk AE, Wafaa A. Role of Hypertension and Metabolic Abnormalities in the Development of Diabetic Nephropathy among Egyptian Patients with Type 2 Diabetes. Nature and Science. 2011;7(9): 220-228.
14. Jorns A, Munday R, Tiedge M, Lenzen S. Comparative toxicity of alloxan, Nalkylalloxans and ninhydrin to isolated pancreatic islets in vitro. J. Endocrinol. 1997;155: 283-293.

15. Mayur B, Sandesh S, Shruti S, Sung-Yum S. Antioxidant and α-glucosidase inhibitory properties of Carpesium abrotanoides .L J. Medicine in Plant Research.2010; 4: 1547-1553.
16. Norberg A, Hao NK, Liepinsh E, Phan DV, Thuan ND, Jornvall H. A novel insulin releasing substance, phanoside, from the plant *Gynostemma pentaphyllum*. J. Biol. Chemistry. 2004*;* **279**(40): 41361-41367.
17. Andrade A. Becerra-jimenez J, Cardenas-Vazquez R. The antioxidant property of tannin. J Ethanopharmacology.2005; 116: 27-32.
18. Rajendran AV, Gnanarel I. Evaluation of *Aloe vera* sap in diabetes and treating wounds and inflammation in animals. J. Appl. Scs. Resea.2011; 3(11): 1434-1436.
19. Adeneye AA, Olagunju JA, Elias SO, Olatunbosun DO, Mustafa AO, Adeshile OI, Ashaolu AO, Laoye TA, Bamigboye AO, Adeoye AO. Protective activities of the aqueous root extract of *Harungana madagascariensis* in acute and repeated acetaminophen hepatotoxic rats. Intl. J. App R in Natl. Products.2008; Vol. 1(3), pp. 29-42.
20. Eddouks M, Jouad H, Maghrani M, Lemhadri A, Burcelin R. Inhibition of endogenous glucose production accounts for hypoglycemic effect of Spergularia purpurea in streptozotocin mice. Phytomedicine, 2003;10: 594-599.
21. Oyedemi SO, Adewusi EA, Aiyegoro OA Akinpelu DA. Antidiabetic and Haematological Effect of Aqueous Extract of Stem Bark of *Afzelia africana* (Smith) on Streptozotocin-Induced Diabetic Wistar Rats. Asian Pacific Journal of Tropical Biomedicine 2011;1(5):353-58.
22. Mohammed RK, Ibrahim S, Atawodi SE, Eze ED, Suleiman JB, Malgwi IS. Anti-diabetic and Haematological Effects of n-Butanol Fraction of *alchornea cordifolia* Leaf Extract in Streptozotocin-Induced Diabetic Wistar Rats. Scientific J Bio Sci.2013; 2; 3.
23. Uko EK, Erhabor O, Isaac IZ, Abdulrahaman Y, Adias TC, Sani Y, Shehu RS, Liman HM, Dalhtu, MK Mainasara AS. Some Haematological Parameters in Patients with Type – I Diabetes in Sokoto, North Western Nigeria. J Blood Lymph,2013*;* 3(110):2165-7831.
24. Kothari, R and Bokariya, P. A Comparative Study of Haematological Parameters in Type 1 Diabetes Mellitus Patients & Healthy Young Adolescents. 2012; Int. J.Biol Med Res. 3(4): 2429-32.
25. Ohisson A, Aher SM. Early Erythropoietin for Preventing Red Blood Cell Transfusion in Preterm and/or Low Birth Weight Infants. Cochrane Database System Rev.*2006; 3. CDoo4863.*
26. Ashafa AO, Yakubu MT, Grierson DS, Afolayan AJ. Toxicological Evaluation of the Aqueous Extract of *Felicia muricata* Thiemb Leaves in Wistar rats. African J Biotech. 2009; *6 (4): 949 – 954.*
27. Harris M. National Diabetes: Data Group National institutes of health. Diabetes and Digestive and Kidney Diseases. "Diabetes in America" 2nd edition NIH1995*;* pp.1395-1468.
28. Chowdhury JR, Wolkoff AW, Arias IM. In: ScriverCR, Beaudet AL, Sly WS, Valle D (eds.) The metabolicbasis of inherited diseases. Vol. 1, Part 8, NewYork: McGraw Hill. 1982; p.1367-1408.
29. Vitek L.The Role of Bilirubin in Diabetes, Metabolic Syndrome, and Cardiovascular Diseases. Frontier in Pharmacol.2012*;3:55.*
30. Akah JA, Lemji JA, Salawa OA, Okoye TC, Offiah NV. Effects of *Vernonia amygdalina* on Biochemical and Haematological Parameters in Diabetic Rats. Asian J Med Sc. 2009;1(3): 108-113.
31. Khan, A, Safdar, M, and Ali khan MM. Effect of various doses of cinnamon on lipid profile in diabetic individuals. Pakistan J nutrition. 2003; 2: 313-319.
32. Gross JL, De Azevedo MJ, Silveiro SP, Canani LH, Caramori ML. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care 2005;28: 164–176.
33. Bansal R, Ahmandu R, Kiduai SR. Alloxan – glucose interaction. Effect of Incorporation of 14 C - leucine into pancreatic islets of rats. Acta. Diabetologica Latina. 1980; 17:135 -143.
34. Ghosh S, Surawanshi SA. Effect of Vinca rosea extracts in treatment of alloxan diabetes in male albina rats. Indian J Pharmacology, 2001;8, 748 – 759.
35. Thakran S, Siddiqui MR., Baquer NZ. *Trigonella foenum graecum* seed powder protects against histopathological abnormalities in tissues of diabetic rats. Mol Cell Biochem ,2004; 266 (1-2), 151- 159.
36. Safayli H, Sailer ER. Anti-inflammatory actions of pentacyclic triterpenes Planta Medica, 1997;63: 487-493.
37. Yasukawa K, Akihisa T, Yoshida Z Takido M. Inhibitory effect of euphol, a triterpene alcohol from the roots of *Euphorbia kansui*, on tumour promotion by 12-o-tetradecanoyl phorbol 13 acetate in two stages carcinogensis in mouse skin. J Pharmacy Pharmacology 2000*;* 52: 119-124.