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Evaluation of the anti-diabetic and toxicological profile of the leaves of *Stachytarpheta jamaicensis* (L.) Vahl (Verbenaceae) on alloxan-induced diabetic rats

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Abstract
This study is aimed at ascertaining the scientific basis for the ethnomedicinal uses of *Stachytarpheta jamaicensis* (SJ) leaf extracts in diabetes mellitus, determining the phytoconstituents present in the plant and the safety (beneficial/toxicity) of the plant. The methanol extract of *S. jamaicensis* (MESJ) was fractionated into n-hexane (HFSJ), ethyl acetate (EFSJ), n-butanol (BFSJ) and water fractions (WFSJ). The MESJ and fractions were screened for anti-diabetic activities using oral glucose tolerance test, normoglycemic and hyperglycemic models. Other parameters e.g. blood, liver, renal and lipid profiles were determined in normal and alloxan induced diabetic rats after oral administration of MESJ for 28 days. Histopathological changes in normal and diabetic rat organs (pancreas, liver and kidney) were also observed after MESJ treatment. The LD_{50} of MESJ was >5000 mg/kg. The phytochemical analysis showed the presence of flavonoids, resins, alkaloids, glycosides, steroids, terpenoids, reducing sugars and saponins. Preliminary anti-diabetic study revealed a non-dose dependent reduction in the blood glucose level of alloxan-induced diabetic rats. MESJ showed significant ($P< 0.01$) hypoglycemic/anti-hyperglycemic effects (25.40 and 55.80%) against glibenclamide (12.60 and 51.40%) in normal rats for acute and chronic studies respectively and (22.00 and 86.90%) against glibenclamide (19.37 and 85.50%) in diabetic rats for acute and chronic studies respectively. It also improved body weight, blood parameters, kidney, liver functions and hyperlipidaemia in alloxan-induced diabetic animals. MESJ also showed improvement in blood parameters and non significant increases in liver, kidney and lipid profile of normoglycemic rats. Furthermore, MESJ also had a favorable effect on the histopathological changes of the pancreas, liver and kidney in alloxan-induced diabetes. Histopathological pictures of the pancreas, liver and kidney of normoglycemic rats were normal. *Stachytarpheta jamaicensis* thus posses anti-diabetic property and is also not toxic to body organs on long term administration.

Keywords: *Stachytarpheta jamaicensis*, Hyperglycemia, Diabetes mellitus, histopathology, phytochemical analysis

Introduction
Diabetes mellitus is a disorder of glucose metabolism that results from an absolute or relative lack of insulin in the body. It describes a group of disorders of varying etiology and pathogenesis usually characterized by elevated blood glucose concentration, reduced insulin action or absolute insulin deficiency (Edward and Raffaela, 1996) [6].

WHO reports diabetes mellitus as one of the most common public health problems which will affect a total population of 220 million people worldwide in the year 2020 (Aurbert *et al.*,1998; Amos *et al.*, 1997) [3, 1]. The increasing prevalence of diabetes mellitus worldwide is a major societal issue because diabetes is a complex and multifactorial origin disease. Management of diabetes without any side effects is still a challenge for the medical system. This leads to an increasing search for improved antidiabetic drugs. Few of plant treatments used in traditional medicine for diabetes have received scientific scrutiny, and the World Health Organisation has recommended that this area warrants attention (WHO, 1980) [27]. Some of these plants include: *Ceiba pentandra* (Odoh *et al*, 2016) [15], *Combretum dolichopetalum* (Uzor *et al*., 2015) [25], *Acalypha wilkesiana* (Odoh *et al*., 2014) [16], and *Loranthus micranthus* (Osadebe *et al*., 2010) [17].

S. jamaicensis (L.) Vahl (Verbenaceae) is a potent medicinal plant commonly used in treatment of infections in West African (Iwu, 1982) [12]. Native throughout the Caribbean, the species is commonly known as blue porter weed or Jamaica vervain, a medicinal plant commonly used in the Phillipines as a vermifuge, for cough, fever, as a diuretic, laxative, for diabetes and for maternal cases.

Hypoglycemic effect of ethanol leaf extract of *S. jamaicensis* on blood glucose level of streptozotocin-induced diabetic rats has been reported (Silambujanaki *et al*., 2009) [23]. The present work was therefore aimed at ascertaining the scientific basis for the ethnomedicinal uses of its leaf extracts in diabetes mellitus in Nigeria and determining the safety (beneficial/toxicity) of the leaves.

Materials and Methods

Collection and Preparation of Plant Material

Fresh leaves of *Stachytarpheta jamaicensis* (L.) Vahl were collected in June, 2014 form Orba, Udenu LGA, Enugu State, Nigeria and identified by Mr. A.O Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State, Nigeria. The voucher specimen (UNN/PCG/14/022) was deposited in the Herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. The leaves were air dried under shade for three weeks. The dried leaves were then ground into powder using a grinding machine.

Extraction and fractionation

The grinded leaf power was exhaustively macerated with absolute methanol for 72 hours with intermittent shaking. It was filtered and the filtrate concentrated *in vacuo* using a rotary evaporator. A 200 g of the crude extract was dissolved in water and poured into a separating funnel. It was then partitioned successively using n-hexane, ethyl-acetate and n-butanol to get the different fractions which were also dried *in vacuo* to remove the excess solvents. The percentage yield of the extract and fractions were calculated.

Preliminary phytochemical analysis

The test carried out was based on procedures outlined by Harborne (1973) [10], Trease and Evans (1996) and Iwu (1978) [12]. The tests were carried out on the leaf powder, methanol extract and fractions of *Stachytarpheta jamaicensis*.

Animals

Adult female Swiss albino rats (180-200 g) and mice (24-30 g) used in this experiment was sourced from the Department of Zoology, University of Nigeria, Nsukka. Animals were fed with standard feed and water *ad libitum.* They were kept in cages until commencement of the experiment.

Pharmacological evaluation

Acute toxicity studies

The LD_{50} of the extract in albino mice was determined using Lorke's method (Lorke, 1983) [14]. White albino mice (20-30 kg) were fasted overnight for 12 h and doses of the extracts (10, 100 and 1000 mg/kg) were administered orally to the groups of mice (n=3), and observed for 24-48 hr where no death was observed, subsequent doses (1600, 2900 and 5000 mg/kg body weight) of the extract was administered to fresh groups of mice (n=3) and observed for another 24-48 hr. The mice that served as control received normal saline only. The LD_{50} for the extract was calculated as the geometric mean of the dose killing none of the three animals in the group and the dose killing all the animals in the group.

√ (Dose killing all the animals in the group) x (dose killing none of the animals in the group)

Oral Glucose Tolerance Test (OGTT)

Animals used were fasted for 16 hr before the OGTT. Glucose (1 g/kg body weight) was administered orally to the animals 30 min after administration of 200 mg/kg of *S. jamaicensis* methanol extract. Glibenclamide at dose of 5 mg/kg was used as a reference drug. Blood glucose level was measured at 0, 30, 60, 120 and 180 min after administration of glucose (Vessal *et al*., 2003) [26].

Hypoglycemic effect of extract and fractions of *S. jamaicensis* on blood glucose of Normal rats

Swiss albino rats (180-200 g) were randomly divided into eight groups (n=5) and fasted for 12 hr before the administration of the extracts. The experimental design is as shown below:

Group 1: Normal rats given the extract
Group II: Normal rats given n-hexane fraction
Group III: Normal rats given ethylacetate fraction
Group IV: Normal rats given n-butanol fraction
Group V: Normal rats given water fraction
Group VI: Normal rats given glibenclamide (positive control)
Group VII: Normal rats given only vehicle (distilled water) (negative control)
Group VIII: Normal healthy control.

A 200 mg/kg each of the extract and fractions were administered orally for 28 days and the fasting blood glucose level was monitored periodically during the treatment with the tail prick method using Accucheck Active Glucometer and strips (Roche Diagnostics GmbH, Germany.). The blood glucose level was checked after 1, 3, 6 and 24 hours of treatment and on the 7^{th}, 14^{th}, 21^{st} and 28^{th} day of treatment and was measured in milligrams per deciliter

Induction of Diabetes Mellitus

The *in-vivo* study was conducted on albino rats (180-200 g) at an ambient temperature of 25 ± 2 ºC and standard food and water *ad libitum*. The rats were made diabetic with a single intravenous injection of Alloxan monohydrate (150 mg/body weight) dissolved in distilled water. This dose produced diabetes within 48 hr with blood glucose level of approximately (200 - 600 mg/dL). The entire procedure was carried out as per stated guidelines of Institutional Animal Ethical Committee.

Anti-hyperglycemic Effects of extract and fractions of *S. jamaicensis* on the blood glucose of Alloxan-induced diabetic Rats: The experimental design and procedure was the same as that of the normoglycemic model.

Body weight: The body weights of the animals were measured before and after the experiment.

Biochemical Parameters

Blood samples were collected on the 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of treatment for analysis of the following paraments; serum alanine transaminase (ALT), serum Aspartate transaminase (AST), serum alkaline phosphatase (AST), serum Urea, serum creatinine, serum cholesterol, low density lipoproteins (LDL), High density lipo-proteins (HDL), packed cell volume (PCV) Hb, blood differential, total WBC and RBC.

Histochemical Analysis

On the 28th day of treatment, pancreas, liver and kidney were collected from sacrificed animals of the extract treatment group and control of the normoglycemic and hyperglycemic groups and were fixed in 4% paraformaldehyde. Micrometer thick sections were taken on a rotary microtome and used for immunohistochemical analysis.

Statistical analysis

All values of results are presented as mean ± standard error of mean (S.E.M). One way analysis of variance (ANOVA) followed by Duncan's multiple range test was used for statistical comparison between control and various treatment groups. Statistical significance was accepted at the $P< 0.01$ values.

Results and Discussion

Phyto-chemical analysis of the methanol extract and fractions of the leaves of *S. jamaicensis* showed the presence of flavonoids, resins, alkaloids, glycosides, steroids, terpenoids, carbohydrates, reducing sugars, tannins, proteins and saponins. Acidic compounds were absent in the methanol extract and fractions (Table 1). The pharmacological activities of most plant extracts can be traced to these bioactive constituents. For instance, various compounds belonging to the terpenoid and flavonoid groups are known to be biologically active (Puntero *et al*., 1997). Alkaloids, glycosides and flavonoids have been implicated in anti-diabetic activities of plant extracts (Leven *et al*., 1979) and their presence in the extract and fractions only supported their observed pharmacological activities. The presence of these phyto-chemical constituents has been reported in *S. jamaicensis* (Idu *et al*., 2007) [11].

In the acute toxicity and lethality tests, results indicated no death in the two phases of the tests. The LD_{50} was thus established to be >5000 mg/kg. This implies that *S. jamaicensis* leaves are non toxic.

The results of the normoglycemic study demonstrated that MESJ had a significant hypoglycemic effect in normoglycemic rats. MESJ also showed significant increases in the haematological parameters of the normoglycemic rats. Significant decreases were observed in the liver and kidney enzymes and also in the cholesterol levels of the normoglycemic MESJ treated group.

MESJ showed a non dose-dependent reduction in blood glucose concentration of alloxan-induced diabetic rats. Alloxan acts as a diabetogenic by the destruction of β-cells of the islets of langerhans and causes massive reduction in insulin release, thereby inducing hyperglycemia (Grover *et al*., 2000) [9]. Insulin deficiency leads to various metabolic alterations in the animals *viz* increased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases and decreased levels of PCV, Hb and other blood parameters etc. (Shanmugasundaram *et al*., 1983; Begum and Shanmugasundaram, 1978) [22, 4]. MESJ, BFSJ, WFSJ and glibenclamide were found to reduce the elevated glucose level significantly in alloxan induced diabetic animals during 28 days treatment.

Diabetes mellitus induced by alloxan is associated with anaemia. This might not be unconnected with both the effects of alloxan on rapidly dividing haemopoietic cells and suppression of haemopoiesis as a result of insulin deficiency occasioned by the selective destruction of the β-cells in the islets of langerhans of the pancreas by alloxan (Phillips *et al*., 2004) [20]. Significant increase in differential lymphocytes and neutrophils count in the diabetic rats must have also resulted from the stress induced by diabetes in accordance with stress induced lymphocytosis and neutrophilia in avian species (Forbes *et al*., 2002) [7]. Following treatment of alloxan – induced diabetic rats with MESJ, there was significant increases in the PCV, RBC, WBC and Hb and decrease in lymphocytes and neutrophils values.

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Shanmugasundaram *et al*., 1990) [21]. Repeated administration of MESJ for 28 days significantly ($P< 0.01$) decreased hypercholesterolemia, the observed hypolipidemic effect may be due to decreased cholesterologenesis and fatty acid synthesis (Chi and Koh, 1982; Patel *et al*., 2011; Thirumalai *et al*., 2011; Oyedemi *et al*., 2011; Arokiyaraj *et al*., 2011; Kumar *et al*., 2011) [5, 19, 24, 18, 2, 13, 19]. HDL cholesterol level was significantly improved by MESJ.

Liver enzymes e.g. AST, ALT and ALP levels were increased in diabetic rats which is responsible for the liver damage. The elevated serum level of these enzymes was significantly reduced by MESJ treatment. The diabetic complications such as increase gluconeogenesis and ketogenesis may be due to elevated enzymes (Ghosh and Suryawansi, 2001) [8].

MESJ also improved renal functions in diabetic rats by reducing serum urea and creatinine.

The histopathological examination revealed normal tissues of the pancreas, kidney and liver in the normoglycemic and hyperglycemic treatment groups. Generally, these studies have shown that MESJ produced marked hypoglycemic and anti-hyperglycemic activity. Its anti-diabetic activity is comparable to Glibenclamide. The histopathological examination also laid credence to its long term usage with no damage to body tissues. This suggests that extract of *S. jamaicensis* could be recommended for use in the treatment of diabetes. Further studies will focus on discovering and isolation of the active principle(s) responsible for the anti-diabetic activity of MESJ.

Table 1: Results of phyto-chemical analysis of the crude powder, methanol extract and fractions of *S. jamaicensis* leaves.

S/N	Test	SJ mPowder	MESJ	HFSJ	EFSJ	BFSJ	WFSJ
1	Carbohydrates	+	+	–	–	+	+
2	Reducing sugars	+	+	–	–	+	+
3	Alkaloids	+	+	-	+	+	+
4	Glycosides	+	+	–	+	+	+
5	Saponins	+	+	–	+	+	+
6	Tannins	+	+	–	+	+	+
7	Flavonoids	+	+	–	+	+	–
8	Resins	+	+	+	+	–	–
9	Proteins	+	+	–	+	+	+
10	Oils	+	+	+	–	–	–
11	Steroids	+	+	+	+	+	+
12	Terpenoids	+	+	+	–	–	–
13	Acidic compounds	–	–	–	–	–	–

Key: –: absent, +: present

Competing Interest

The authors declare that they have no competing interest.

Table 2: Effect of *S.jamaicensis* leaves extract and fractions on oral glucose tolerance of normal rats

Treatment group	Mean fasting blood sugar (mg/dl)					Percentage reduction
	0mins	30mins	60mins	120mins	180mins	
MESJ	118.6± 0.78	116.3± 0.71^{a}	93.4± 0.76^{b}	86.5± 0.76^{a}	77.3± 0.84^{a}	34.82
HFSJ	136.8± 0.63	143.6± 0.71	138.9± 0.56	129.7± 0.84^{a}	121.4± 0.78^{b}	11.26
EFSJ	121.3± 0.71	133.5± 0.56	129.3± 0.71	119.6± 0.46^{b}	106.4± 0.75^{b}	12.28
BFSJ	115.6± 0.75	116.2± 0.84^{b}	102.3± 0.89^{a}	100.6± 0.71^{b}	92.3± 0.76^{b}	20.16
WFSJ	120.3± 0.84	118.6± 0.56^{b}	110.3± 0.71^{b}	100.5± 0.54^{b}	100.7± 0.64^{b}	16.29
Glibenclamide (5 mg/kg)	110.5± 0.71	115.6± 0.56^{a}	80.6± 0.89^{a}	80.1± 0.56^{b}	79.3± 0.78^{b}	28.24
Control (distilled water)	128.6± 0.56	130.6± 0.78^{c}	129.2± 0.56^{c}	136.5± 0.58	141.8± 0.78^{c}	-10.26

Values are mean ± SEM, n= 5, $^{c}P< 0.001$, $^{b}P< 0.01$, $^{a}P< 0.05$ significantly different when compared with control

Table 3: Effect of *S. jamaicensis* extract and fractions on mean fasting blood sugar of normorglycemic rats (acute test)

Treatment group	Mean fasting blood sugar (mg/dl)					Percentage reduction (%)
	0 h	1 h	3 h	6 h	24 h	
MESJ	114.5 ± 1.45	103.6 ± 0.71^{**a}	97.6 ± 0.78^{**a}	92.8± 0.84^{**a}	85.4± 0.71^{**b}	25.4
HFSJ	121.7 ± 1.75	118.1 ± 1.73^{**b}	115.5 ± 1.70	113.9 ± 1.75*	109.6 ± 1.76*	9.9
EFSJ	114.8 ± 1.74	111.6 ± 1.69^{*b}	106.9 ± 1.76*	102.3 ± 1.73**	100.2 ±1.75^{**b}	12.7
BFSJ	118.7 ± 1.73	114.3 ± 1.69*	108.9 ±1.76^{**a}	102.7 ± 1.73**	100.2 ±1.75^{**a}	15.5
WFSJ	122.9 ± 1.75	119.7 ± 1.52*	116.7 ± 1.65^{*a}	111.3 ±1.75^{**a}	107.6 ± 1.63**	12.4
Gliben clamide (5 mg/kg)	120.6 ± 1.66	118.2 ±1.71*	115.6 ± 1.76*	110.7 ± 1.76	105.3 ± 1.69**	12.6
Control (distilled water)	120.6 ± 1.78	122.1 ±1.65^{d}	126.8 ± 1.75	123.5 ± 1.68	116.5 ± 1.69^{d}	3.3

Values are mean ± SEM, n= 5, $^{***}P< 0.001$, $^{**}P< 0.01$, $^{*}P< O.05$ significantly different compared with control; $^{c}P< 0.001$, $^{b}P< 0.01$, $^{a}P< 0.05$ significantly different when compared with Glibenclamide

Table 4: Effect of *S. jamaicensis* fractions on blood glucose concentration of normoglycemic rats (chronic test)

Treatment group	Mean fasting blood sugar (mg/dl)					Percentage reduction
	0 day	7th day	14thday	21st day	28th day	
MESJ	114.5 ± 1.45	81.0 ± 0.00	74.0 ±1.10^{**a}	70.6± 0.27^{***a}	50.6 ±1.90***	55.8
HFSJ	121.7 ± 1.75	103.4 ± 1.73	100.6 ± 1.76*	93.6 ± 1.75**	90.3 ± 1.70	25.5
EFSJ	121.9 ± 1.74	97.6 ± 1.73	92.6 ± 1.75**	89.2 ± 1.70**	86.6 ±1.68***	28.9
BFSJ	118.7 ± 1.73	92.6 ± 1.62	86.5 ± 1.42**	79.5 ± 1.63**	60.9 ±1.75***	48.7
WFSJ	122.9 ± 1.75	100.7 ± 1.49^{c}	83.2 ± 1.66	83.2 ± 1.66**	70.8 ±1.70**	42.4
Glibenclamide (5 mg/kg)	120.6 ± 1.66	98.7 ±1.58^{bc}	81.6 ± 1.60	76.2 ± 1.63**	58.6 ±1.68***	51.4
Control ((distilled water)	120.6 ± 1.78	117.8 ± 1.66	116.2 ± 1.75	117.7 ± 1.73	115.7 ±1.68^{a}	4.06

Values are mean ± SEM, n= 5, $^{***}P< 0.001$, $^{**}P< 0.01$, $^{*}P< 0.05$ significantly different compared with control; $^{c}P< 0.001$, $^{b}P< 0.01$, $^{a}P< 0.05$ significantly different when compared with Glibenclamide

Table 5: Effect of *S. jamaicensis* extract and fractions on blood glucose concentration of hyperglycemic rats (acute test)

Treatment	Mean fasting blood sugar (mg/dl)					Percentage reduction (%)
	0 h	1 h	3 h	6 h	24 h	
MESJ	569.8 ± 005	537.4 ± 0.71*	524.4 ± 0.71*	495.2 ± 0.56*a	444.4 ± 0.71*a	22.00
HFSJ	487.6 ± 0.78	478.0 ±0.00**b	485.2 ±0.78**b	500.4 ± 0.71*	504.8 ± 1.46*	3.52
EFSJ	519.4 ± 0.71	367.0 ± 0.00*	335.6 ± 0.78*a	331.8 ± 0.84*a	323.8 ± 0.84*a	23.61
BFSJ	462.8 ± 0.71	444.6 ± 0.78*a	416.6 ± 0.78*a	394.8 ± 0.84*b	357.2 ±0.56**b	22.81
WFSJ	529.4 ± 0.71	494.4 ± 0.71*a	485.4 ± 0.71*a	445.8 ±0.84**a	405.4 ±0.71**a	23.42
Glibenclamide (5 mg/kg)	565.6 ± 0.78	547.8 ± 0.84*	532.4 ± 0.71*	514.6 ± 0.78*	456.0 ± 0.00*	19.37
Control (distilled water)	600.0 ± 0.71	600.0 ± 0.71	600.2 ± 0.78	596.8 ± 0.56c	583.6 ± 0.84	2.73

Values are mean ± SEM, n= 5, ***P< 0.001, **P< 0.01, *P< O.05 significantly different compared with control; cP< 0.001, bP< 0.01, aP< 0.05 significantly different when compared with Glibenclamide.

Table 6: Effect of *S. jamaicensis* leaves extract and fractions on blood glucose concentration of hyperglycemic rats (chronic test)

Treatment	Mean fasting blood sugar (mg/dl)					Percentage Reduction (%)
	0 day	7thday	14th day	21st day	28th day	
MESJ	569.8 ± 005	350.6 ± 0.78	278.6 ±0.78***a	170.8 ± 0.84***	74.4 ± 0.56a	86.9
HFSJ	487.6 ± 0.78	500.2± 1.67c	456.4 ± 0.71*	352.4 ± 1.59**	258.8 ± 1.89	46.9
EFSJ	519.4 ± 0.71	286.4 ± 0.75	220.2 ± 0.56**b	157.4 ± 0.71**	109.3 ±0.00***a	78.8
BFSJ	462.8 ± 0.71	259.6 ± 0.78	219.4 ± 1.72**a	131.2 ±0.56***a	87.2 ± 0.56	81.9
WFSJ	529.4 ± 0.71	304.8 ± 0.84	251.6 ± 0.78**a	150.6 ± 0.89**	95.4 ±0.71***a	81.1
Gliben Clamide (5 mg/kg)	565.6 ± 0.78	337.2 ± 0.56	233.0 ± 0.30***	161.0 ± 0.32***	81.8 ± 0.84***	85.5
Control (distilled water)	600.0 ± 0.71	594.8 ± 0.78	595.8 ± 071	585.8 ± 0.00	-	2.4

Values are mean ± SEM, n= 5, ***P< 0.001, **P< 0.01, *P< O.05 significantly different compared with control; cP< 0.001, bP< 0.01, aP< 0.05 significantly different when compared with Glibenclamide

Table 7: Effect of *S. jamaicensis* leaves extract on hematological parameters of normoglycemic rats

Parameters	Treatment group	0 day	7th day	14th day	21st day	28th day
PCV	MESJ	51.63 ± 0.48	52.3 ± 0.51	53.63 ± 0.48	55.23 ± 0.61*	56.34 ± 0.78*
	Control	53.18 ± 0.36	53.62 ± 0.78	54.65 ± 0.18	52.73 ± 0.35	53.61 ± 0.62
Hb	MESJ	10.68 ± 0.86	12.62 ± 0.86	12.68 ± 0.81	13.51 ± 0.51*	14.86 ± 0.36*
	Control	12.41 ± 0.65	12.13 ± 0.16	12.11 ± 0.18	12.18 ± 0.15	12.36 ± 0.23
WBC	MESJ	4896 ± 0.41	4965 ± 0.81	5163 ± 0.78	5348 ± 0.65*	5678 ± 0.78*
	Control	4973 ± 0.83	4971 ± 0.75	4998 ± 0.81	4892 ± 0.63	4918 ± 0.61
RBC	MESJ	4.83 ± 0.15	4.98 ± 0.71	5.15 ± 0.68	5.23 ± 0.28	5.83 ± 0.38*
	Control	5.62 ± 0.71	5.61 ± 0.63	5.32 ± 0.31	5.48 ± 0.45	5.57 ± 0.23
Neutrophils (%)	MESJ	48.14 ± 0.65	49.63 ± 0.86	51.58 ± 0.71	56.23 ± 0.38	58.61 ± 0.15*
	Control	48.56 ± 0.81	47.15 ± 0.65	48.15 ± 0.23	48.23 ± 0.71	47.63 ± 0.68
Lymphocytes (%)	MESJ	38.63 ± 0.73	40.65 ± 0.13	41.31 ± 0.18*	41.65 ± 0.71*	43.25 ± 0.18*
	Control	35.23 ± 0.52	35.18 ± 0.68	36.00 ± 0.27	34.23 ± 0.86	33.18 ± 0.56

Values are mean ± SEM, n=5, *P<0.01 significantly different from control

Table 8: Effect of *S. jamaicensis* leaves extract on serum liver profile of normoglycemic rats

Parameters	Treatment group	0 day	7th day	14th day	21st day	28th day
AST (IU/L)	MESJ	18.51 ± 0.83	18.58 ± 0.65	19.23 ± 0.68	19.68 ± 0.53	20.15 ± 0.68*
	Control	19.63 ± 1.68	19.65 ± 0.98	19.63 ± 0.71	19.81 ± 0.48	19.83 ± 0.65
ALT (IU/L)	MESJ	16.68 ± 0.63	16.75 ± 1.48	17.34 ± 1.52	17.98 ± 1.78*	18.38 ± 0.68*
	Control	18.23 ± 1.75	18.24 ± 0.14	18.36 ± 0.18	18.45 ± 1.63	18.49 ± 1.63
ALP (IU/L)	MESJ	48.63 ± 0.48	48.52 ± 0.63	48.94 ± 0.51	49.48 ± 0.71	49.82 ± 1.83*
	Control	51.68 ± 0.71	52.65 ± 0.23	49.78 ± 0.68	49.95 ± 0.63	50.05 ± 1.67

Values are mean ± SEM, n=5, *P< 0.01 significantly different from control

Table 9: Effect of *S. jamaicensis* leaves extract on serum kidney profile of normoglycemic rats

Parameters	Treatment group	0 day	7th day	14th day	21st day	28th day
Urea (mg/dL)	MESJ	7.81 ± 0.84	7.83 ± 0.56	7.80 ± 0.18	7.63 ± 0.73	7.14 ± 0.68*
	Control	6.61 ± 0.36	6.73 ± 0.56	6.84 ± 0.13	6.86 ± 0.56	6.92 ± 0.61
Creatinine (mg/dL)	MESJ	0.65 ± 0.81	0.61 ± 0.56	0.58 ± 0.63	0.56 ± 0.71	0.51 ± 0.83*
	Control	0.63 ± 1.63	0.68 ± 0.51	0.70 ± 0.86	0.70 ± 0.33	0.71 ± 0.65

Values are mean ± SEM, n=5, *P< 0.01 significantly different from control

Table 10: Effect of *S. jamaicensis* leaves extract on serum lipid profile of normoglycemic rats

Parameters	Treatment	0 day	7th day	14th day	21st day	28th day
Total Cholesterol (mmol/L)	MESJ	9.98 ± 0.65	9.83 ± 0.71	9.68 ± 0.65	9.15 ± 0.68	8.86 ± 0.73*
	Control	10.63 ± 0.78	10.65 ± 0.68	10.71 ± 0.62	10.84 ± 0.62	10.96 ± 1.32
LDL (mmol/L)	MESJ	2.53 ± 0.63	2.50 ± 1.12	2.43 ± 0.56	2.18 ± 0.18	2.13 ± 0.68*
	Control	2.93 ± 0.86	2.96 ± 0.15	2.95 ± 0.95	2.96 ± 0.71	2.94 ± 0.67
HDL (mmol/L)	MESJ	1.12 ± 0.76	1.41 ± 0.53*	1.21 ± 1.62	1.18 ± 0.18	1.08 ± 0.15*
	Control	1.86 ± 0.15	1.81 ± 0.63	1.83 ± 0.18	1.85 ± 0.71	1.96 ± 0.63

Values are mean ± SEM, n=5, *P< 0.01 significantly different from control

Table 11: Effect of *S. jamaicensis* leaves extract on body weight of normoglycemic rats

Treatment group	Body weight (g)	
	0 day	28th day
MESJ	175.54 ± 2.70	188.36 ± 1.35*
Control	178.65 ± 0.65	183.23 ± 0.48

Values are mean ± SEM, n=5, *P< 0.01 significantly different from control

Table 12: Effect of *S. jamaicensis* leaves extract on hematological parameters of hyperglycemic rats

Parameters	Treatment	0 day	7th day	14th day	21st day	28th day
PCV	MESJ	28.31 ± 0.23	33.52 ± 0.68*	35.76 ± 0.75*	36.85 ± 0.35*	39.43 ± 0.81
	Control	27.36 ± 0.68	27.22 ± 0.71	27.15 ± 0.18	-	-
Hb	MESJ	9.68 ± 0.78	11.35 ± 0.75*	12.65 ± 0.34*	13.78 ± 0.54*	14.65 ± 0.56
	Control	10.23 ± 0.15	9.23 ± 0.78	9.18 ± 0.23	-	-
WBC	MESJ	3682 ± 0.54	3994 ± 0.98*	4003 ± 0.25*	4567 ± 0.67*	4990 ± 0.89
	Control	3778 ± 0.65	3557 ± 0.98	3309 ± 0.15	-	-
RBC	MESJ	2.86 ± 0.71	3.67 ± 0.78*	4.01 ± 0.56*	5.25 ± 0.65*	6.18 ± 0.33
	Control	2.36 ± 0.34	2.18 ± 0.54	2.10 ± 0.32	-	-
Neutrophils (%)	MESJ	54.6 ± 0.71	50.6 ± 0.19	48.2 ± 0.76*	42.5 ± 0.36*	38.3 ± 0.23
	Control	32.6 ± 0.65	36.8 ± 0.23	41.80 ± 0.45*	-	-
Lymphocytes (%)	MESJ	39.60 ± 0.56	36.56 ± 0.45	34.43 ± 0.98*	31.98 ± 0.78*	29.60 ± 0.71
	Control	31.78 ± 0.71	33.65 ± 0.65	38.23 ± 0.68*	-	-

Values are mean ± SEM, n=5, *P< 0.01 significantly different from control

Table 13: Effect of *S. jamaicensis* leaves extract on serum liver profile of hyperglycemic rats

Parameter	Treatment	0 day	7th day	14th day	21st day	28th day
AST (IU/L)	MESJ	116.7 ± 0.68	98.6 ± 0.56*	87.9 ± 0.71*	73.9 ± 0.84*	67.4 ± 0.75
	Control	108.9 ± 0.89	126.4 ± 0.78*	128.6 ± 0.86*	-	-
ALT (IU/L)	MESJ	119.3 ± 0.78	102.4 ± 0.71*	98.6 ± 0.56*	73.6 ± 0.34*	55.6 ± 0.78
	Control	121.4 ± 0.67	128.6 ± 0.56 *	129.9 ± 0.45*	-	-
ALP (IU/L)	MESJ	239.6 ± 0.78	218.9 ± 0.84	198.6 ± 0.68*	176.3 ± 0.65*	156.8 ± 0.34
	Control	213.9 ± 0.63	218.8 ± 0.76	220.7 ± 0.71*	-	-

Values are mean ± SEM, n=5, *P< 0.01 significantly different from control

Table 14: Effect of *S. jamaicensis* leaves extract on serum kidney profile of hyperglycemic rats

Parameters	Treatment	0 day	7th day	14th day	21st day	28th day
Urea (mg/dL)	MESJ	60.6 ± 0.56	51.3 ± 0.78*	43.9 ± 0.56*	34.8 ± 0.71*	22.8 ± 0.54
	Control	63.8 ± 0.71	65.7 ± 0.65	68.5 ± 0.47	-	-
Creatinine (mg/dL)	MESJ	4.10 ± 0.65	3.48 ± 0.78*	2.65 ± 0.86*	2.17 ± 0.52*	1.63 ± 0.78
	Control	4.23 ± 0.66	4.25 ± 0.71	4.31 ± 0.83	-	-

Values are mean ± SEM, n=5, *P<0.01 significantly different from control

Table 15: Effect of *S. jamaicensis* leaves extract on serum lipid profile of hyperglycemic rats

Parameters	Treatment	0 day	7th day	14th day	21st day	28th day
Total Cholesterol (mmol/L)	MESJ	25.10 ± 0.65	21.52 ± 0.58	18.41 ± 0.89*	14.18 ± 0.17*	11.63 ± 0.84*
	Control	23.36 ± 0.58	23.12 ± 0.65	24.36 ± 0.56	25.09 ± 0.72	-
LDL (mmol/L)	MESJ	18.61 ± 0.71	15.23 ± 0.78*	13.93 ± 0.43*	10.25 ± 0.27*	8.63 ± 0.65*
	Control	17.65 ± 0.78	17.53 ± 0.45	18.18 ± 0.87	-	-

HDL (mmol/L)	MESJ	0.56 ± 0.65	0.83 ± 0.68	1.97 ± 0.25*	2.11 ± 0.76*	2.68 ± 0.53*
	Control	0.65 ± 0.76	0.59 ± 0.26	0.56 ± 0.76	-	-

Values are mean ± SEM, n=5, *P< 0.01 significantly different from control

Table 16: Effect of *S. jamaicensis* leaves extract on body weight of hyperglycemic rats

Treatment group	Body weight (g)	
	0 day	28th day
MESJ	187.54 ± 2.70	193.36 ± 1.35*
Control	178.65 ± 0.65	–

Values are mean ± SEM, n=5, *P< 0.01 significantly different from control

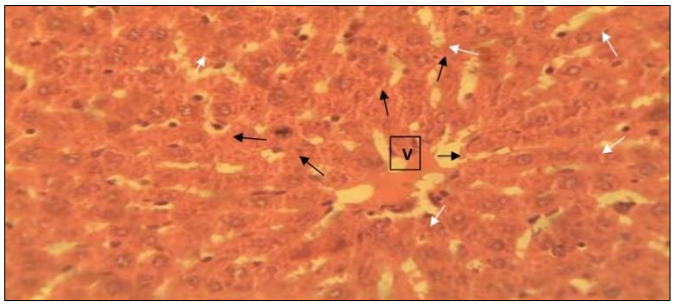

Plate 1: A photomicrograph of a section of the liver of normoglycemic rat showing the central vein (V), surrounded by normal hepatocytes arranged in chords (black arrow). The spaces in between the hepatic chords are the sinusoids (white arrow). H&Ex400.

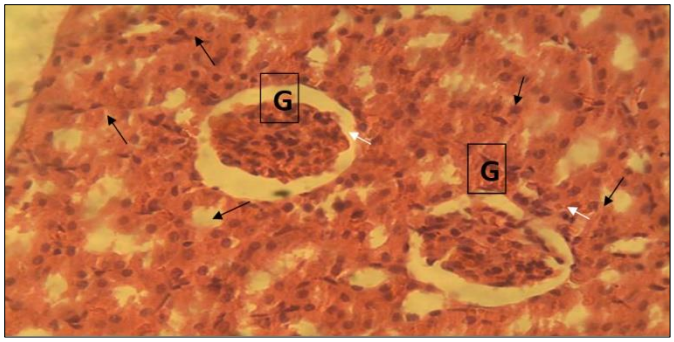

Plate 2: A photomicrograph of a section of the kidney of normoglycemic rat showing normal glomeruli (G) in Bowman's capsule (white arrow) and normal renal tubules (black arrow). H&Ex400.

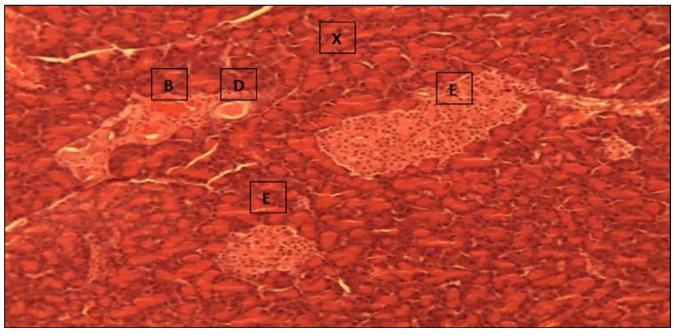

Plate 3: A photomicrograph of a section of the pancreas of normoglycemic rat showing normal exocrine pancreas (X) and endocrine pancreas (E). Intralobular duct (D), Blood vessel (B). H&Ex 100].

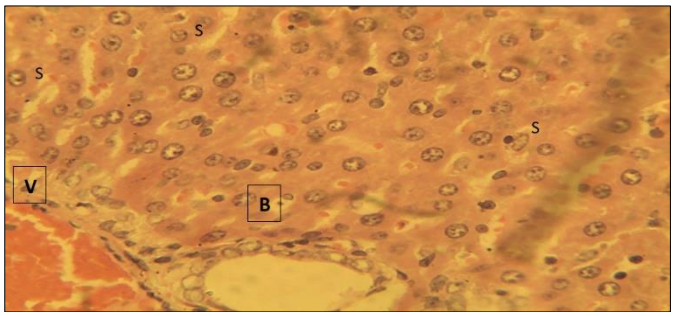

Plate 4: A photomicrograph of a section of the liver from hyperglycemic rat showing normal hepatocytes arranged in chords around the portal area. Hepatic sinusoids (s), Bile duct (B), Hepatic vein (V), H&EX400.

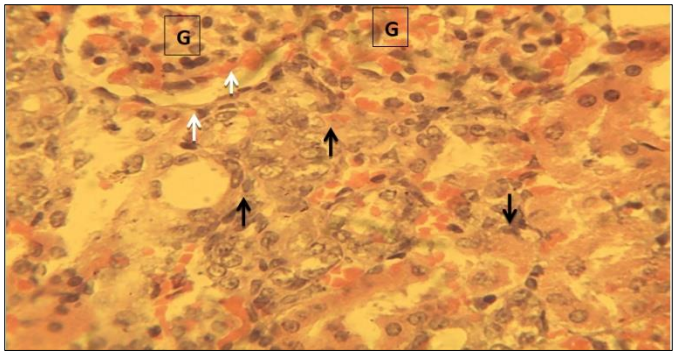

Plate 5: A photomicrograph of the kidney from hyperglycemic rat showing vacuolar degeneration of tubular epithelial cells of proximal convoluted tubule (P) and pars recta (R). H&EX400.

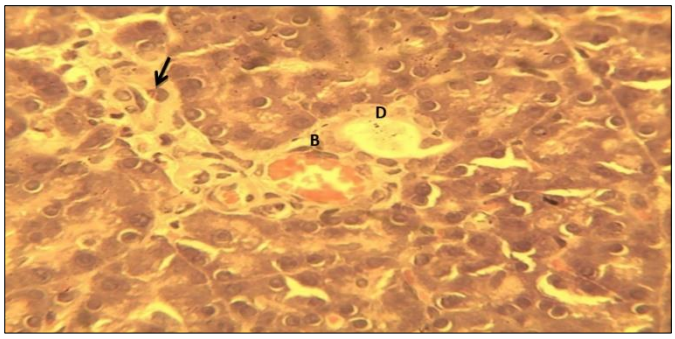

Plate 6: A photomicrograph of a section of the pancreas from hyperglycemic rat showing normal exocrine pancreas and inconspicuous endocrine pancreas. The available pancreatic islets appeared atrophied (arrow). Pancreatic duct (D), Blood vessel (B). H&E X100, X400.

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