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Original Research Article

Hydro-Ethanol Extract of the Aerial Parts of *Secamone afzelii* Ameliorates Diabetic Status of Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus as the most prevalent metabolic disease has become a global health challenge. This study evaluated the hypoglycemic potential of *Secamone afzelii (Apocynaceae)* aerial part extract in streptozotocin-induced diabetic rats. Four groups of five diabetic rats each were treated orally once daily for 21 days with *S. afzelii* extract (100 and 200 mg/kg), metformin (150 mg/kg), or normal saline (10 mL/kg), while a fifth group as non-diabetic group received distilled water. Fasting blood glucose (FBG) levels were monitored weekly, and blood samples were analyzed for serum enzymes, lipids (total cholesterol, triglycerides, HDL, LDL), hemoglobin, and blood parameters on day 21. The histopathology of the cardiac, hepatic and renal tissues was also examined. The *S. afzelii* extract at both doses significantly reduced glucose levels ($p<0.05$) of diabetic rats compared to the standard metformin after 21 days of treatment. HDL levels slightly increased ($p>0.05$) without altering LDL, while Triglyceride (TG) and Total Cholesterol (TC) levels were lower in extract treated groups than in metformin-treated group. Serum biomarkers of liver (ALT and AST) and kidney (creatinine and urea) were reduced in extract-treated groups when compared to untreated diabetic group. Hematological parameters, such as red and white blood cell counts, improved. Histopathology revealed that the extract restored damage to heart, liver, and kidney tissues caused by hyperglycemia. The findings demonstrate that the *S. afzelii* hydro-ethanolic extract exhibits notable hypoglycemic activity, improves lipid profile, and mitigates organ damage in diabetic rats. Further studies are needed to confirm its potential for antidiabetic therapy development.

Keywords: *Secamone afzelii*, Hydro-ethanol extract, Diabetes, Hyperglycemia, Streptozotocin, Hematology, Hepatic, Renal

Introduction

Diabetes mellitus (DM) is a metabolic disorder with characteristic chronic hyperglycemia, abnormalities in the metabolism of lipids, carbohydrate, and protein due to defects in insulin secretion, action, or both.[1] From 108 million cases in 1980 to 589 million cases and 3.5 million fatalities in 2024, the number of adult cases of diabetes worldwide has increased significantly. The majority of the world's diabetes cases are found in low- and middle-income nations, and by 2050, there will likely be 853 million diabetics globally.[2] Diabetes prevalence is significantly rising in Sub-Saharan Africa. As per the 2024 data of the International Diabetic Federation, the number of adults with diabetes in the Africa Region was 24.6 million (4.17%).[3] In 2024, an estimated 2.9 million people in Nigeria alone are thought to have diabetes, and the prevalence of the disease is significantly higher throughout the African continent.[3]

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Type 1 diabetes is known by an absolute lack of insulin due to auto-destruction of pancreatic beta cells, while type 2 diabetes is due to defects in the ability of pancreas to secrete insulin or by increased insulin resistance in peripheral tissue.[4] Due to inadequate insulin secretion or activity, patients with diabetes have a decreased ability to metabolize glucose and are unable to generate triglycerides and free fatty acids (FFA) from amino acids or carbohydrates. Enzymes in glycolysis, the pentose phosphate, and lipid pathways are suppressed when gluconeogenesis, glycogenolysis, and lipolysis are increased, reversing the metabolic pathway in non-diabetic individuals since the cells lack the ability to be able to detect and use plasma glucose.[5] Insulin resistance eventually leads to elevated insulin levels and thus causes dyslipidemia, hypertension, and alteration in the metabolism of glucose.[6] Insulin resistance can further strain and breakdown the signals that regulate hunger, fullness and appetite. However, the upregulation of inflammation and the emergence of insulin resistance, can elevate FFA in circulation and in fatty tissues; all of which alter the storage capacity of adipose tissue for fat calories.[7,8] There is strong evidence from research that diabetes can cause structural, neurochemical, and neurophysiological abnormalities in the central nervous system (CNS), which is also known as diabetic encephalopathy. The two major pronged approach in treating Type 2 DM targets increased insulin secretion by agents like metformin, thiazolidinediones and body cell increased sensitization by sulfonylureas and sitagliptins. Others such as dapagliflozins block the reabsorption of glucose from the kidney while incretins mimetics such as exenatide target Glucagon-Like Peptide 1 receptor (GLP1R).[9] While most of these drugs have yielded very good results, there is still need to develop more potent antidiabetics with

lesser side effects. *Secamone afzelii* is a small herb with adequate documentation of its medicinal properties and use in traditional medicine for different ailments.[10] It is a climbing woody vine with smooth foliage that produces a milky latex upon damage. *Secamone afzelii* (Roem. &Schult.) K. Schum, a creeping wood and plant climber of *Asclepiadaceae* family, is used in traditional Ivorian medicine to treat painful manifestations of gastric origin, colic, dysentery and kidney problems.[11] It is also reported as a remedy for diabetes in different parts of Nigeria.[12] Before now, many phytochemical studies have shown that *Secamone afzelii* is rich in secondary metabolites like coumarins, flavonoids and tannins.[11] This study is therefore set to evaluate the effect of hydro-ethanolic extract of *Secamone afzelii* on diabetic and lipid profile in streptozotocin induced diabetic rats, thereby validating the folkloric use of this plant.

Materials and Methods

Collection and Preparation of Plant Sample

The aerial parts of *Secamone afzelii* was collected in June 2023, at the Botanical Garden, University of Ibadan and was submitted for authentication at the Botany department of University of Ibadan, with voucher number *(UIH-23246)*, given. The plant was dusted and air-dried for 4 weeks at room temperature and then pulverized using milling machine.

Plant Extraction

Maceration method of extraction was employed for the extraction of the pulverized plant part. Eight hundred and fourteen grams (0.814kg) of the pulverized plant was extracted with 80% ethanol of total volume of 5 litres. Extract obtained was concentrated using a Buchi Rotary evaporator ((Heidolph HB digital, Germany) at 40ºC. Further drying of the concentrated extract was carried out on water bath to obtain solid crude extract yielding 84.5 g weight of extract. The weight and percentage yield of the crude extract was determined and extract was kept for further analysis.

Experimental Animals

Twenty-five (25) Munich-Wistar rats of 150–220g weight were acquired from Suramos farms, Ikere-Ekiti, Ekiti State, Nigeria and housed at the Animal Research Center of Afe Babalola University, Ado-Ekiti, Ekiti State. The rats were acclimated for two weeks before the experiment, during which they had free access to a standard rat diet and water and were kept at room temperature. Each rat was housed individually in polypropylene cages containing paddy husk bedding. The maintenance of animals was carried out under standard laboratory conditions, including a consistent 12-hour light/dark cycle and a controlled temperature of 25 ± 2°C.[13]

Induction of Diabetes

The animals were fasted overnight to ensure consistent basal glucose levels. Then, the animals were given an intraperitoneal administration (i.p) with 40 mg/kg body weight of streptozotocin, dissolved in 0.1 M Citrate buffer (pH=4.5), followed by 5% glucose for 24 h. The fasting plasma glucose level of the animals were checked after two days post induction using *Accuchek*® test glucometer by dripping blood from the tail vein. With their fasting blood glucose level ≥ 200 mg/dL, the animals were proven diabetic.[14]

Grouping of the animals

The animals were distributed into five groups of 5 animals having the rats not induced with diabetes (normal control) in group 1, the rats induced with diabetes and not treated (diabetic control) in group 2, rats induced with diabetes but treated with 100 mg/kg of *S. afzelii* extract in group 3, rats induced with diabetes but treated with 200 mg/kg of *S. afzelii* extract in group 4, and the last group 5 are the rats with diabetes which received 150 mg/kg of metformin.

Estimation of effective dose of *extract of Secamone afzelii (ESA)* in STZ-induced DM in male adult Wistar albino rats was done. After the diabetes mellitus phenotype manifested, the rats already received the *single dose of extract/metformin by oral route on day 3*, and fasting blood glucose (FBG) monitored on days *7, 14 and 21*.[15]

Weight, polyphagia and polydipsia of the animals

The weights of all animals in each group were taken and recorded before the induction by STZ, and then on days 7, day 14 and day 21. The water and food consumption of the animals were evaluated daily for the period of the experiment.[16]

Blood collection and measurement of biochemical parameters

After the experiment which lasted for 3 weeks, the rats were euthanized via cervical dislocation. Whole blood (2 mL) was collected by cardiac puncture into EDTA and lithium heparin sample bottles (BD Diagnostics, pre-analytical systems, Midland, USA) for the lipid profile analyses.

Serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured to evaluate liver function. The activities of AST and ALT were determined following the standard method described by Reitman and Frankel[15,16,17], while the method described by Roy was used to evaluate the ALP activity.[16,18]

Total Cholesterol (TC) and Triglyceride (TG) concentrations were evaluated according to methods in Trinder[19] with little modification by Oyedemi et al.[16] using commercial kits purchased from Randox Laboratories Ltd. (Crumlin, UK). Uric acid was evaluated using Randox kit by the methods reported in Fossati *et al*.[20] High Density Lipoprotein (HDL) was evaluated using the techniques by Warnick and Albers while Freidewald formula was employed to estimate serum low-density lipoprotein (LDL).[15]

Hematological parameters of red blood cells (RBC) and its related indices were determined. These included hemoglobin (Hb), packed cell volume (PCV)/ hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RCDW). White blood cell (WBC) and platelet were also analyzed.[16]

Histopathological Analysis

The histopathology analysis outlined involves a series of steps to prepare, analyze, and visualize tissue (cardiac, hepatic and renal) samples under a microscope. Initially, the tissues were carefully processed, including fixation, dehydration, clearing and infiltrating with paraffin wax. The processed tissues were then embedded, sectioned, and mounted on slides. Staining with hematoxylin and eosin was performed to enhance cellular visualization. Finally, the slides were thoroughly dried, mounted with coverslips, and viewed under a microscope for detailed examination.[21,22]

Statistical analyses

We expressed our results and data as mean ± S.E.M. t-test and one-way analysis of variance (ANOVA) were used in the inferential analysis to spot significant differences among the group. We employed the statistical package, Graph Pad Prism (7.0) for all analysis. Data were converted to figures using Graph Pad Prism (7.0). Results were considered to be significant at $p < 0.05$.

Ethical consideration

Ethics approval was sought and obtained from the Ethics Committee on Animals Use of Afe Babalola University (ECAU- ABUAD) with number ABUADHREC/I6/04/2024/341.

Results and Discussion

Hypoglycemic effects of hydroethanolic extract of Secamone afzelii

Table 1 reveals the effect of the hydroethanolic extract of *S. afzelii* on FBG level of STZ-induced diabetic rats. Compared to the normal control, streptozotocin effectively modelled diabetes in all other groups as evidenced by the elevated glucose levels ($p<0.05$) 48hrs post induction. The glucose levels of animals in diabetic control continued to rise post 48hrs mainly due to lack of intervention but fell back after

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14days and even much closer to the basal level after 21days. The hydroethanolic extract of *S. afzelii* showed a dose dependent decrease ($p>0.05$) in the glucose levels of the diabetic animals after 7 days of treatment. However, only the 200mg/kg dose showed comparable results to the group treated with metformin. A significant observation showed a further rise in the glucose level of animals treated with 200mg/kg dose of extract from day 7 to 14 and finally fell to normal pre-induction states. The effect of treatment on the weight of the rats were examined as seen in Table 2. Treatment with the extracts did not significantly impact ($p>0.05$) the weight of the diabetic animals. Even for the standard drug (metformin), treatment did not markedly affect the weight of the animals. In the pattern of water and feed intake of STZ-induced diabetic rats, the treated group animals feed intake was reduced ($p>0.05$) as compared to the untreated diabetic rats. The feed intakes of the diabetic rats were significantly decreased ($p<0.05$) as compared with the normal rats as show in the Figure 1, while the water intakes of the animals with hyperglycemia have significant increase comparing with the normal rats as show in the Figure 2.

Table 1: Effect of hydro-ethanolic extract of *S. afzelii*on glucose level of STZ-induced diabetes

Treatment	Glucose level (mg/dL)				
	Basal	**48 hrs. of induction**	**7 days**	**14 days**	**21 days**
Normal control	75.25 ± 12.47	79.25 ± 15.63	82.50 ± 8.35	95.00 ± 3.16	97.32 ± 2.45
Diabetic control	69.5 ± 2.38	312.75 ± 8.02#	591.25 ± 4.11	313.50 ± 15.26	80.25 ± 8.62
Diabetic + *S. afzelii* (100 mg/kg)	77 ± 4.97	457.25 ± 94.97#	246.75 ± 29.78**	128.75 ± 5.85**	83.75 ± 15.54
Diabetic + *S. afzelii* (200 mg/kg)	74.5 ± 5.97	435.5 ± 11.44#	118.75 ± 5.56*	204.5 ± 5.45**	99.5 ± 13.70
Diabetic + metformin (150 mg/kg)	69 ± 5.71	332.75 ± 18.14#	99 ± 9.13*	95.75 ± 12.99*	77.25 ± 7.93

*P<0.05 versus diabetic control, **P>0.05 versus diabetic control. #P<0.05 versus basal control. Data are shown as mean ± SEM, n=5

Table 2: Body weight of the STZ-induced diabetic rats during the study

Treatment	Body weight (g)			
	Basal	**7 days**	**14 days**	**21 days**
Normal control	258.80 ± 6.92	258.45 ± 9.18	265.6 ± 9.09	242.14 ± 15.7
Diabetic control	208.12 ± 1.29	195.01 ± 14.01	201.67 ± 24.02	194.23 ± 29.31
Diabetic + *S. afzelii* (100 mg/kg)	204.68 ± 4.18	183.80 ± 11.71	189 ± 23.99	205.45 ± 43.46
Diabetic + *S. afzelii* (200 mg/kg)	179.31 ± 15.65	164.85 ± 7.05	173.7 ± 12.79	178.28 ± 20.43
Diabetic + metformin (150 mg/kg)	149.99 ± 9.51	147.47 ± 11.62	149.5 ± 17.11	145.87 ± 15.38

Data are shown as mean ± SEM, n=5

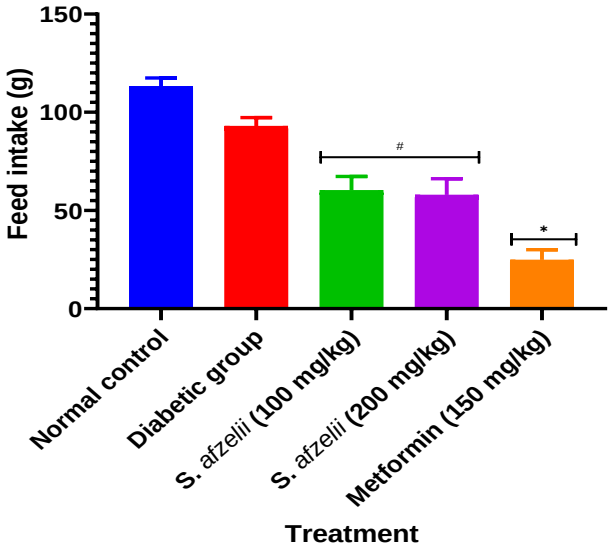

Figure 1: Pattern of feed intake of STZ-induced diabetic rats. *P < 0.05 compared to the diabetic group; #P > 0.05 compared to the diabetic group

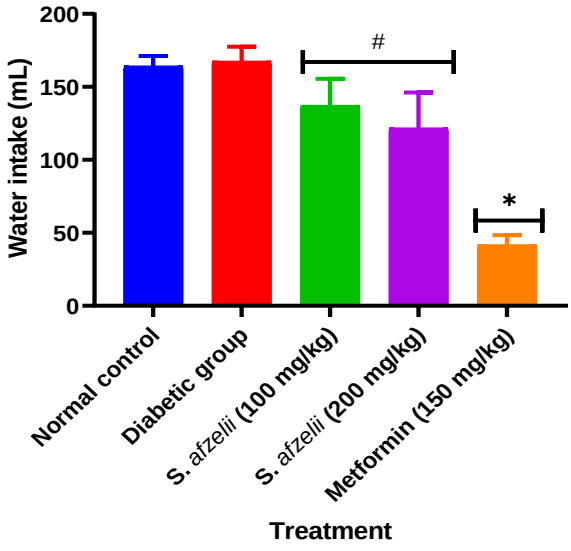

Figure 2: Pattern of water intake of STZ-induced diabetic rats. *P < 0.05 compared to the diabetic group; #P > 0.05 compared to the diabetic group

Effect of S. afzelii hydroethanolic extract on some biochemical indices

The performance of lipid parameters in STZ-induced diabetic rats after treatment was revealed in Table 3. HDL level was slightly increased ($p>0.05$) without an increase or decrease in LDL, while levels of TC show a decrease ($p>0.05$) relative to those in the group without diabetes, though none of these were significant. The metformin treated group displayed the highest level of Total Glyceride (TG) while they also possessed a Total Cholesterol (TC) level comparable to that of the normal control. The kidney parameters after treatment remained relatively same across the groups as shown in Table 4.

In Table 5, the treatment effect on the hematological parameters of STZ-induced diabetic rats were shown. The parameters remained essentially the same across all the groups that received treatment relative to the normal control. Though the WBC levels of animals treated with 200mg/kg of SA was lower compared to others. Also, among all the important red cell parameters examined, diabetic rats treated with 200mg/kg of SA showed lower values compared to others though the difference was not significant. The key liver function parameters including ALT, AST and ALP all rose significantly immediately after induction of diabetes as shown in Table 6. Treatment that followed reversed the levels though at different rates. The 200mg/kg SA treated group reduced the AST and ALT more than the group treated with Metformin (150 mg/kg). The reverse was however the case for the levels of ALP.

Table 3: Performance of lipid parameters in STZ-induced diabetic rats after treatment

Parameters	Normal	Diabetic	SA (100 mg/kg)	SA (200 mg/kg)	Metformin (150 mg/kg)
TC (mmol/L)	2.37 ± 0.78	2.03 ± 0.08	1.69 ± 0.08*	1.79 ± 0.80*	2.25 ± 0.49*
HDL (mmol/L)	0.43 ± 0.12	0.54 ± 0.15	0.48 ± 0.01*	0.61 ± 0.32*	0.48 ± 0.04*
LDL (mmol/L)	0.35 ± 0.11	0.39 ± 0.08	0.35 ± 0.13*	0.34 ± 0.11*	0.44 ± 0.04*
TG (mmol/L)	0.68 ± 0.13	0.86 ± 0.18	0.79 ± 0.30*	0.75 ± 0.25*	1.20 ± 0.42*

Note: SA- *Secamone afzelii*, TC- total cholesterol, HDL- high density lipoprotein, LDL- low density lipoprotein, TG- triglyceride. Data are shown as mean ± SEM. *P > 0.05 compared to the diabetic group

Table 4: Performance of kidney parameters in STZ-induced diabetic rats after treatment

Parameters	Normal	Diabetic	SA (100 mg/kg)	SA (200 mg/kg)	Metformin (150 mg/kg)
Creatinine (mg/dL)	37.65 ± 8.56	27.95 ± 0.35	38.50 ± 4.52*	39.15 ± 7.99*	36.30 ± 2.12*
Urea (mg/dL)	8.15 ± 0.12	10.05 ± 3.75	7.550 ± 0.49*	10.55 ± 2.47*	9.700 ± 0.56*
Na (mg/dL)	103.0 ± 1.41	99.00 ± 7.07	98.00 ± 2.83*	97.00 ± 1.41*	100.0 ± 4.24*
Cl (mg/dL)	138.5 ± 0.71	136.0 ± 2.83	139.0 ± 5.66*	135.0 ± 0.00*	142.5 ± 0.71*

Note: SA- *Secamone afzelii*, Na- sodium, Cl- chloride. Data are shown as mean ± SEM. *P > 0.05 compared to the diabetic group

Table 5: Performance of blood parameters in STZ-induced diabetic rats after treatment

Parameters	Normal	Diabetic	SA (100 mg/kg)	SA (200 mg/kg)	Metformin (150 mg/kg)
WBC (10^9/L)	3.80 ± 1.27	8.15 ± 0.35	6.00 ± 0.56	3.85 ± 0.49	8.650 ± 0.21
RBC (10^{12}/L)	7.28 ± 1.35	8.10 ± 0.27	7.32 ± 0.95	6.53 ± 0.58	7.220 ± 0.14
HGB (g/dL)	13.80 ± 1.56	14.15 ± 0.92	13.70 ± 2.83	8.35 ± 5.59	13.50 ± 0.14
HCT (%)	41.30 ± 6.50	44.85 ± 0.92	38.60 ± 4.81	35.60 ± 4.10	40.55 ± 0.35
PLT (10^9/L)	974.0 ± 4.24	612.5 ± 6.36	731.0 ± 5.66	737.0 ± 31.11	760.0 ± 1.41
MCH (pg)	19.00 ± 1.41	18.45 ± 0.35	18.55 ± 1.48	12.40 ± 7.49	18.65 ± 0.21
MCHC (g/dL)	33.50 ± 1.56	33.90 ± 0.57	32.30 ± 1.27	22.65 ± 13.08	33.70 ± 0.57
MCV (fL)	56.95 ± 1.63	54.50 ± 1.84	57.80 ± 6.79	54.55 ± 1.48	55.75 ± 0.07

Note: SA- *Secamone afzelii*, WBC- white blood cells, RBC- red blood cells, HGB- hemoglobin, HCT- hematocrit, PLT- platelet, MCH- mean corpuscular hemoglobin, MCHC- mean corpuscular hemoglobin concentration, MCV- mean corpuscular volume. Data are shown as mean ± SEM

Table 6: Performance of liver function parameters in STZ-induced diabetic rats after treatment

Parameters	Normal	Diabetic	SA (100 mg/kg)	SA (200 mg/kg)	Metformin (150 mg/kg)
AST (IU/L)	406.0 ± 7.07	652.5 ± 6.36	914.0 ± 1.41	344.0 ± 9.9	915.0 ± 2.83
ALT (IU/L)	87.0 ± 2.83	142.0 ± 4.24	468.5 ± 13.44	48.50 ± 6.36	135.5 ± 3.54
ALP (IU/L)	85.0 ± 2.83	115.0 ± 2.83	149.0 ± 4.24	241.0 ± 5.66	141.0 ± 5.66
TBIL (µmol/L)	0.45 ± 0.21	0.30± 0.14	0.1 ± 0.00	0.65 ± 0.78	0.2 ± 0.14
TP (g/L)	100.0 ± 4.24	81.00 ± 5.66	68.50 ± 3.54	83.50 ± 19.09	86.50 ± 14.85
GGT (UI)	7.5 ± 2.12	11.50 ± 2.12	7.0 ± 2.83	11.00 ± 1.41	5.50 ± 2.12

Note: SA- *Secamone afzelii*, AST- aspartate amiotrasferase, ALT- alanine aminotransferase, ALP- alkaline phosphatase, TBIL- total bilirubin, TP- total protein, GGT- gamma glutamyl transferase. Data are shown as mean ± SEM

Effect of S. afzelii hydroethanolic extract on the histoarchitecture of critical organs

In Figure 3, the histoarchitecture of the heart was revealed, the normal control (G1) revealed normal slender muscle fibers that were densely packed and stained pink with centrally placed nuclei. The diabetic control (G2) and 100mg/kg dose of *S. afzelii* (G3) revealed few extra-cellular spaces. The 200mg/kg dose of *S. afzelii* (G4) revealed distinct nuclei with evidence of myocardium infraction. 150mg/kg dose of Metformin (G5) revealed normal muscular fibers and little extra-cellular space. The photomicrograph in figure 4 shows the histoarchitecture of the liver, revealing the hepatocytes and the portal triad. The normal control (G1) revealed shrunken portal vein and moderate vacuolar degeneration. The diabetic control (G2) shows normal portal area and moderate vacuolar degeneration. Diabetic + ESA 100 mg/kg (G3) shows normal architecture of the liver. Diabetic + 200 mg/kg (G4) shows slightly congested portal vein. Diabetic + metformin 150 mg/kg (G5) shows normal arrangement of the hepatocyte, but congested portal vein. Figure 5 shows the histoarchitecture of the kidney for all groups, revealing the glomerulus, proximal and distal convoluted tubules. The normal control (G1) revealed normal arrangement of the nephron with loss of urinary space. The diabetic control (G2) shows a moderate alteration of the renal tubules and appearance of the urinary tubules. Diabetic + ESA 100 mg/kg (G3) shows loss of glomerulus, mild congestion, and moderate alteration of the renal tubules. Diabetic + 200 mg/kg (G4) shows mild congestion. Diabetic + metformin 150 mg/kg (G5) shows normal renal tubules, but a shrunken glomerulus.

In this work, the hypoglycemic potential of *Secamone afzelii* in STZ-induced diabetic rat was investigated. Furthermore, the impact of various treatment groups on the vital organs such as liver, heart and kidney were also examined. We injected the experimental animals with 40mg/kg of STZ to induce hyperglycemia and waited for 48hrs for the features of hyperglycemia to develop. Streptozotocin (STZ) is a chemical generally employed in the induction of insulin-dependent diabetes mellitus during experimental hypoglycemic studies due to its toxic effects on islet beta cells.[23] The direct effect of irreparable injury to the pancreatic beta cells, which causes degranulation and loss of insulin secretory capacity, is the ability to cause hyperglycemia. When laboratory rats receive several sub-diabetogenic doses of STZ intravenously or intraperitoneally, the result is severe pancreatic insulitis that eventually destroys insulin-secreting beta cells and results in diabetes mellitus.[24] All the animals that received STZ in the different groups developed hyperglycemia after 48hrs as shown in Table 1, and thus had established diabetes mellitus consistent with the use of STZ.

As shown in the results in table 1, the glucose levels of animals in diabetic control continued to rise post 48hrs, mainly due to lack of intervention but fell back after 14 days and even much closer to the basal level after 21 days. This is easily explained by the self-recovery ability of the pancreas after STZ shock. Previous reports have shown that self-recovery occurs in diabetic rats after induction with intravenous dose of low dose streptozotocin at 40mg/kg.[25] Above this dose, the ability of the pancreas to recover becomes a question of debate and one influenced by so many variables. Lower doses of STZ still have ability to induce and maintain diabetes mellitus in rats if injected at multiple doses over an interval.[26] The species and strain of the animals and possibly the health status has important effects on self-recovery.[27] A combination of natural endogenous antioxidants and reactive oxygen species produced by the diabetogenic agents could be responsible for the self-recovery.[28] Self-recovery has clinical importance in the experimental diabetic studies as it can also blunt the effect of our treatment or become a confounding variable making it difficult to establish significant and reliable results.[26] Normo-glycemia, which is a fall in the blood glucose levels towards the normal range is always the target of treatment in diabetes mellitus.[5] Continuous administration of the hydroethanolic extract to the diabetic rats led to the reduction of the glucose levels of these animals. The 200mg/kg dose had a higher effect than the 100mg/kg dose up to the 7 days post-induction (435.5 to 118.75 md/dL and 457.25 to 246.75 mg/dL respectively), showing a dose dependent effect, though not significant. The group treated with metformin had a reduced glucose levels than those treated with extract but the differences were not significant. This can be partly explained by the mechanism of action of metformin which does not stimulate release of insulin from pancreatic cells.[29] Other antidiabetic drugs like glibenclamide and glimepiride would have given a better hypoglycemic model since they act on the beta cells.

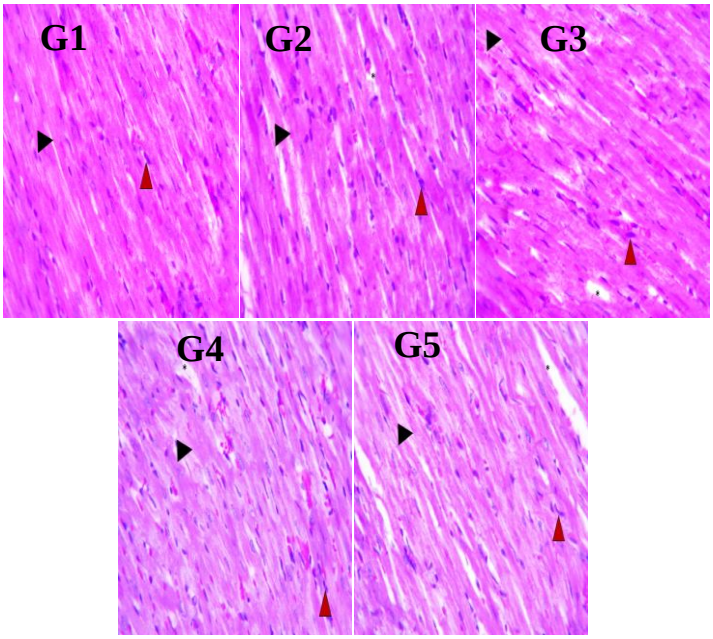

Figure 3: Histoarchitecture of the Heart showing the cardiac muscle. Stained with H&E (Mg. x800). (Red arrowhead: Nuclei, Black arrowhead: muscle fibers). G1- normal control; G2- diabetic control; G3- diabetic + ESA 100 mg/kg; G4- diabetic + 200 mg/kg; G5- diabetic + metformin 150 mg/kg.

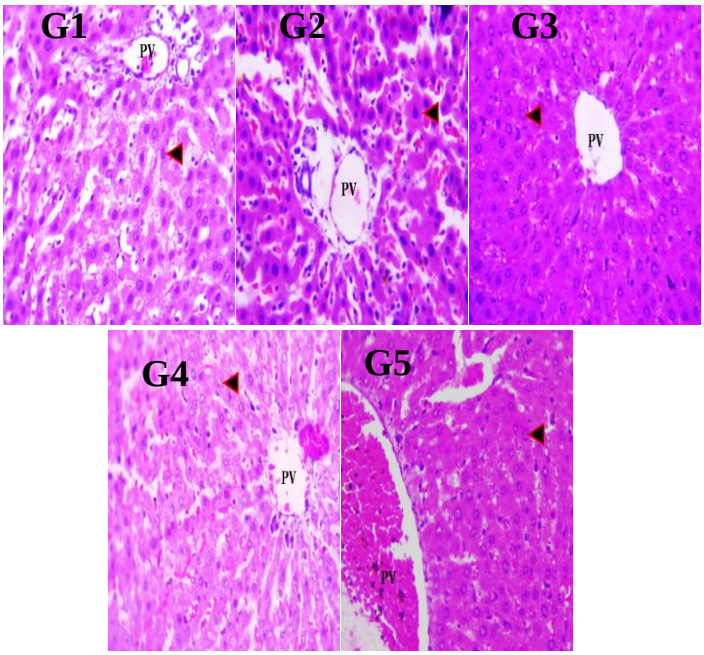

Figure 4: Photomicrograph showing the histoarchitecture of the Liver: hepatocytes and the portal triad. Stained with H&E (Mg x800). (Black arrowhead: Hepatocyte, PV: Portal vein). G1- normal control; G2- diabetic control; G3- diabetic + ESA 100 mg/kg; G4- diabetic + 200 mg/kg; G5- diabetic + metformin 150 mg/kg.

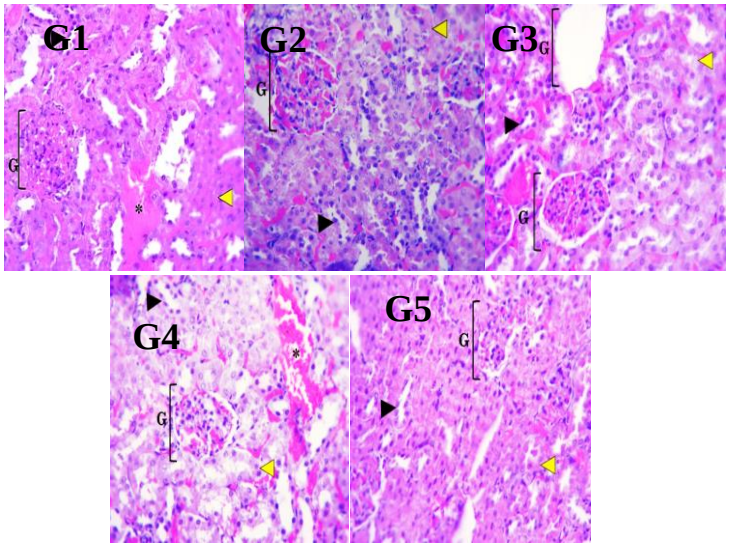

Figure 5: Histoarchitecture of the kidney showing the glomerulus, proximal and distal convoluted tubules. Stained with H&E (Mg. x800). (Yellow arrowhead: Proximal convoluted tubules, Black arrowhead: Distal convoluted tubules, G: Glomerulus).
G1- normal control; G2- diabetic control; G3- diabetic + ESA 100 mg/kg; G4- diabetic + 200 mg/kg; G5- diabetic + metformin 150 mg/kg.

Further away from the pancreas, STZ exert severe toxic effect on various organs and tissues of the body.[30] STZ causes alkylation of DNA producing hyperglycemia and necrotic lesions.[31] This effect usually has a significant impact on the weights of the animals. The body weight of an animal is vital as it relates to fat levels and insulin resistance in the species. Induction of hyperglycemia with STZ, as shown in table 2, resulted in severe loss in weights of the animals in the 7 days post-induction similar to the findings of previous studies.[24,32] This may be due to the initial damaging effect of the diabetogenic agent on the organs of the animals. All the diabetic groups that received treatment (extract) had their weights restored to the pre-induction level. Only the group treated with metformin got the weights to levels slightly below the basal line, though not significant. This provides insights to the ability of our plant extract to rapidly initiate healing from the cytotoxic effect of STZ. Our plant has been reported to be rich in tannins, coumarins and flavonoids especially the kaempferol glycosides which could be responsible for this effect observed.[33] Flavonoids and tannins are potent antioxidants which possess immense capabilities to neutralize the reactive oxygen species generated by STZ. The further reduction in weight beyond base-line for group treated with metformin is most likely due to the weight reduction action of metformin. Metformin most likely lowers hepatic glucose production and modulate mitochondrial energetics and redox potential leading to weight loss.[34] Restoration of weight to basal levels while significant is not productive at the long run. Increase in weight for diabetics is strongly correlated to complications. This also leads to abnormalities in the lipid profile and insulin resistance.[32]
As already established in the previous paragraph, STZ exerts its toxic effect on very many tissues including the kidney and liver mainly through the alkylation of DNA.[30,31] In diabetics, abnormalities in glucose metabolism and consequent impairment in lipid and protein metabolism puts so much pressure on the liver leading to hepatic damage. Leakage of important biomarker enzymes from the hepatic and renal tissues are always the sign of damage to these tissues.[35] Creatinine and Urea are key markers for kidney damage while AST, ALP and ALT do same for the liver. In this work, we report a significant increase in the levels of ALT, AST and ALP in the untreated (diabetic control) rats in comparison with the normal control providing evidence to damaging effects of hyperglycemia and STZ on the hepatic tissues. Treatment was quick to reverse the high concentration of AST and ALT found in the plasma post induction. This reversal was dose dependent for the hydroethanolic extract where the higher dose (200mg/kg) provided a quicker fall in these liver enzymes than the 100mg/kg dose. The extract even outperformed the group treated with metformin possibly due to difference in the mechanism of action. Rodents with diabetes induced by streptozotocin (STZ) often develop kidney changes that closely resemble the initial stages of diabetic kidney disease observed in humans.[36] Diabetic nephropathy is an important microvascular complication in diabetics. Our results showed that diabetic rats treated with STZ had significantly higher serum creatinine and blood urea, evidence of renal damage and functional decline. These increases reflect glomerular damage, which leads to decreased excretion of these substances by the kidneys.[37] Diabetes mellitus appears to elevate the generation of reactive oxygen species (ROS), leading to injury of oxygen-dependent mediators, disruption of glomerular filtration, and enhanced membrane permeability.[21] These serum parameters, however did not significantly change in the presence of treatment across the groups.
Dyslipidemia and lipid disorders are components of the triad in metabolic syndrome of which DM is a major contributor.[38] Insulin is a key hormone that regulates not only glucose but the metabolism of lipids and protein.[39] While the reduction in glucose levels is a key goal in DM, control of lipid metabolism is as well very vital to reduce events of complications.[40] Studies and practice have shown that disorders in lipid metabolism in DM Increases chances for vascular complications such as cardiovascular diseases, cardiovascular heart diseases and cerebrovascular accidents.[41] Apart from the fallout of reduced insulin levels, STZ used for the induction of DM in this study causes a significant increase in dyslipidemia parameters including LPO, FFA and sd-LDL. An important characteristic of dyslipidemia in diabetics is the triad of low HDL-cholesterol accompanied with hyper-triglycerides concentrated in VLHDL and atherogenic sd-LDL particles.[42] The groups treated with the hydroethanolic extract in this study showed a progressive rise in HDL values and decline in LDL and TG levels. Total Cholesterol and Total triglycerides were highest in groups treated with metformin. This could be a pointer to the differences in the mechanism of action of our extract from metformin.
The effect of treatments on the hematological parameters of rodents is vital in experimental antidiabetic studies. The presence of anemia in diabetes have been widely reported to be due to a rise in non-enzymatic glycosylation of red blood cells (RBC) membrane proteins [16]. This sets up a chain of reaction coupled with hyperglycemia which causes lipid peroxidation and hemolysis of RBC.[43] In this study, we investigated many red cell parameters such as RBC, HGB, HCT, MCH, MCHC, MCV to understand the effect of the extract on the anemic status of diabetic rats. The hydroethanolic extract of SA improved the MCH, MCHC and MCV parameters but did not significantly impact the RBC, HGB and HCT values (Ref- we need reference to literature for this). Now, at increasing dose, the effect of the extract on these parameters fell drastically. The 100mg/kg of the extract outperformed the 200mg/k dose in improving these red cell parameters. These findings lend support to the report by Atoe and Idu[44] in which the methanolic extract of *Secamone afzelii* improved red blood cell parameters in albino Wister rat with pre-eclampsia._Similar findings were also reported by Mbaka and Akala.[45] The group treated with metformin did not significantly show improved red cell parameters over the normal control. Studies have shown that metformin do not have much effect on hematological parameters but might even show increased risks of anemia in diabetic patients.[46]
Beyond vital organs, STZ continues its damaging effect on the body by attacking the white blood cells and thus halting the immune system.[47] The intraperitoneal injection of STZ leads to the reduction of white blood cells and its differential. This is primarily due to its action on suppression of bone marrow and thus poor defensive mechanisms against diseases. Here, we measured the overall white blood cell population instead of the differentials. The WBC levels rose drastically post induction of diabetes contrary to popular reports. In the work by Oyedemi et al [16], the WBC and its differentials were reduced drastically post induction and only returned to normal levels after treatment. Why our findings took the opposite direction is poorly understood. However, possible reasons might be due to the initial outburst in WBC activities

during an infection as a defensive mechanism. This is also supported by the return to normal levels for all the treatment groups as the hyperglycemia normalized. Diminished platelet aggregation ability has been reported in diabetics with poor control of glycemia owing to insufficient or lack of insulin.[48] Platelets are known to play critical role in blood clotting and repair of damaged tissues. The platelet level is linked to blood clothing, tissue repair and healing of wounds in diabetics. End stage diabetes always manifests with poor wound healing and sores especially at the extremities.[49] Plant extracts and natural products with properties capable of boosting immunity through enhanced platelet aggregation would be golden in the search of new hypoglycemic agents. In this study, the platelet level reduced significantly post induction in the diabetic group thus, confirming our theory. However, treatment with extract and metformin restored the levels closer to the normal groups though not to exact same values. There was no significant dose dependent effect in the groups treated with the hydroethanolic extract. Metformin however was superior in improving the platelets count compared with the extract groups. These findings show the capacity of the plant extract to stimulate synthesis of clotting factors.[50]

In the histopathology examination, treatment with increasing dose of hydroethanolic extract of SA (200mg/kg) shows signs of myocardial infarction. This could reveal potential cardiotoxicity of the plant extract. This is opposed to other studies which report no toxicity on the heart.[51] Groups treated with metformin displayed no significant variation from the normal control save for few extracellular spaces (import of EC spaces in cardiac muscles). Any alterations in the structure of the hepatocytes would have a profound effect on the function of the liver as a metabolic machinery.[21] The hepatocytes of untreated (diabetic) group showed normal portal area and moderate macular degeneration. This is consistent with liver damage marked by elevated levels of AST/ALT discussed above. Groups treated with metformin showed congested portal vein which might indicate blood cell infiltration. This however oppose studies in which metformin has a positive impact on the hepatocytes, repairing damaged tissues and suppressing carcinoma.[52] The kidney is at the important juncture that regulates excretion of metabolic wastes. Important morphological changes were observed in the kidney tissue of groups treated with extracts and metformin. This corroborates the rise in serum creatinine and urea- evidence of kidney damage.

Conclusion

The goal of our work was to evaluate the hypoglycemic effect of *Secamone afzelii* in order to validate the folkloric use of this plant in the remedy of diabetes. The hydroethanolic extract of *S. afzelii* has demonstrated sufficient hypoglycemic effect due to its ability to reduce the fasting blood glucose level in STZ-induced diabetic rats compared to metformin. It also had marked effect on the lipid and hematological profiles of the diabetic animals while retaining the ability to restore STZ and hyperglycemia-induced toxicity on cardiac, hepatic and renal tissues. In essence, the extract could prevent various complications due to diabetes as well as improve some hematological parameters. This study lends pharmacological support to the traditional use of the plant in treating diabetes. Further studies are necessary to understand the mechanism of action and the exact phytochemicals responsible for the hypoglycemic effect reported.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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