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Aqueous extract of *Persea americana* leaves ameliorates alloxan-induced hyperglycaemia and hyperlipidaemia in rats

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Decoctions of *Persea americana* Mill (Lauraceae) leaves are employed to manage diabetes mellitus among the Ibo communities of Eastern Nigeria. In this study, we studied the ameliorative effects of the aqueous extract of *P. americana* leaves (AEPAL) on alloxan-induced hyperglycaemia and hyperlipidaemia in Wistar rats. The oral median lethal dose (LD_{50}) of AEPAL was determined in rats. The effects of extract on blood glucose and lipids levels; and biochemical parameters were evaluated. The effects of AEPAL on relative organs weights, body weight changes as well as food and water consumption were monitored for 28 days in alloxan-induced hyperglycaemic rats and histopathological changes of the pancreas examined. The estimated oral LD_{50} of AEPAL was greater than 5000 mg kg^{-1}. AEPAL (125–500 mg kg^{-1}) significantly reduced the fasting blood glucose levels and hyperlipidaemia in the alloxan-induced hyperglycaemic rats. The extract significantly reversed the decreased body weight, increased food and water intake; and attenuated elevated levels of aspartate transaminase (AST), urea, total protein (TP), albumin (ALB), alanine transaminase (ALT) and alkaline phosphatase (ALP) in alloxan-induced hyperglycaemic rat. Histological examination of the pancreas showed regeneration of the β–cells of the Islet of Langerhans in the extract treated alloxan-induced hyperglycaemic rat. Our findings revealed that AEPAL contains biologically active components with potential hypoglycaemic activity, thus supporting its further development for the management of diabetic mellitus.

Key words: *Persea americana,* acute toxicity, antidiabetic, β–cells, metformin.

INTRODUCTION

Diabetic mellitus (DM), a group of metabolic disorders of carbohydrate, fat and protein resulting from defects in insulin's secretion, action or both (Triplitt et al*.,* 2008), is characterized by persistent elevated levels of blood glucose (hyperglycaemia), cholesterol, triglycerides and phospholipids as well as changes in lipoprotein composition (Tripathy et al., 2000). The development of DM involves several pathological processes including the

ones that destroy the β–cells of the pancreas resulting to insulin deficiency (Triplitt et al., 2008).

DM is classified into type 1 and type 2, with the type 1 accounting for 5-10% of the diabetic population (Whiting et al., 2011). The type 1 DM, which is diagnosed mainly in children (juvenile onset) and adolescents, is an autoimmune disorder where the body's own immune system attacks the β–cells of the Islets of Langerhans in the pancreas, destroying or damaging them sufficiently to reduce insulin production. Such diabetics are prone to ketoacidosis and often manifest the polytriad of diabetic symptoms including polyphagia, polyuria and polydipsia (Ukwe, 2006). The type 2 diabetes is characterized by relative insulin deficiency or insulin resistance (Triplitt et al., 2008).

The antidiabetic drugs currently in use do not provide cure nor prevent relapse and are often accompanied by serious adverse effects (Triplitt et al., 2008). The development of new pharmacological agents that can overcome these challenges is currently a major goal in diabetic research. The plant kingdom is the major area of interest in the search for safer and more efficacious drugs and lead compounds to treat this serious endocrine disorder.

For many years, preparations of *Persea americana* Mill (Lauraceae) have been used among the Ibo communities of Eastern Nigeria for the management of DM. In previous studies, the leaf extract of this plant demonstrated anti-ulcer (Oluwole et al., 2011), hypotensive (Adeboye et al., 1999) and hepatoprotective (Ekor et al., 2006) effects. The aim of this study was to evaluate the effects of the aqueous extract of *P. americana* leaves (AEPAL) in alloxan-induced hyperglycaemic Wistar rats to lend scientific support for the use of this plant in the Nigerian traditional medicine for the management of DM.

MATERIALS AND METHODS

Drugs and equipment

Alloxan monohydrate (Sigma-Aldrich Co. St. Louis,MO, USA), metformin (Merke Sante, France), chloroform, formalin (Sigma-Aldrich Co. St. Louis,MO, USA), ELITech reagent kits, (Vital Scientific, Netherlands), Glucometer and strips (Accu-check® Advantage, Roche Germany), hematocrit centrifuge (Denley BS400 centrifuge, England) and automated biochemical analyzer (Selectra XL, Vital Scientific, Netherlands) were used for the study.

Animals

Adult Wistar rats (150 to 200 g) of either sex obtained from Animal Facility Centre (AFC) of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used for this study. The animals were properly housed in transparent plastic cages padded with wood shavings, under standard conditions of temperature, relative humidity and light/dark cycles (12/12 h). They were fed with standard feeds and water *ad libitum* and were approved for use by the AFC ethical committee after reviewing the protocol (Ethical approval number: DAC/IW–OT/665/14). All the experiments were carried out in accordance with the National Health's Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23) revised 1996. Efforts were made to minimize the number of rats used and their suffering.

Plant material

Fresh leaves of *P. americana* were collected from Zaria in Kaduna State, Nigeria in July, 2015. The leaves were identified and authenticated by Mallam U.S Gallah, a taxonomist from the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen (No. 992) was deposited at the Departmental Herbarium for future reference.

Preparation of the extract

The leaves were washed, shade-dried, pulverized into a coarse powder and stored in an air tight container until needed. To 500 g of powdered leaf material, 2 L of cold distilled water was added and stirred in a conical flask until evenly mixed. The mixture was left to stand for 24 h with occasional shaking and stirring. The mixture was filtered using muslin cloth followed by Whatman filter paper No 1. The solvent was removed under reduced pressure using a rotary evaporator and yielded 9.93% (w/w) of extract that was used for the study.

Acute toxicity study

The median lethal dose (LD_{50}) of the extract was determined orally in rats using methods described in detail by Lorke (1983) and modified by Amos et al. (2002).

Induction of hyperglycaemia

Hyperglycaemia was induced by a single intraperitoneal injection of freshly prepared alloxan monohydrate (150 mg kg^{-1}) in 16 h fasted rats with free access to water. Six hours after that, the rats were treated with 20% glucose solution orally to prevent fatal hypoglycaemia and the rats were fed with 5% glucose to prevent hypoglycaemia (Dhandapani et al., 2002). Seventy-two hours after alloxan administration, blood glucose concentrations were measured with the aid of digital glucometer and strips (Accu-Chek® Advantage, Roche Diagnostic, Germany). Rats with fasting blood glucose level ≥ 200 mg dL^{-1} were selected for the study and allowed to stabilize for another 3 days before commencement of the experiment.

Treatment

Rats were randomly selected and divided into six groups of six

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(n=6) of rats per group as follows: Group I received normal saline to serve as normal control, while group II received only alloxan monohydrate (150 mg kg^{-1}) to serve as hyperglycaemic controls. The alloxan-induced hyperglycaemic rats in groups III, IV and V received graded doses (125, 250 and 500 mg kg^{-1}) of the extract, while Group VI (alloxan-induced hyperglycaemic rats) received metformin 250 mg kg^{-1} to serve as positive control (Maithili et al., 2011). Freshly prepared normal saline, graded doses of the extract and metformin were orally administered daily using an oral cannula for a period of 28 days. The blood glucose levels were analyzed at regular intervals of 0, 7, 14, 21 and 28 days of the experimental period. The body weight of the rats were taken individually before the commencement of treatment and thereafter taken once at day 7, 14, 21 and 28 throughout the treatment period. The food and water consumptionin each group were measured daily as the difference between the quantity of feed and water supplied and the amount remaining after 24 h.

Biochemical analysis

The animals were sacrificed on the 29th day under anesthesia by cervical dislocation. Blood samples were collected by cardiac puncture into plain bottles, allowed to clot and centrifuged. The supernatant was collected for evaluation of biochemical parameters. Alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) activities, total protein (TP), albumin (Alb), serum urea and creatinine, total cholesterol(TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were analyzed with an automated chemistry analyzer (Cobas Mira, Roche), using commercial kits obtained from Randox Laboratories, UK.

Histology of the pancreas

The pancreas was carefully dissected out, washed in ice cold saline immediately and fixed in 10% formalin fixative solution for 48 h, processed routinely and embedded in paraffin wax. Histological sections were cut at 5–6 µm; and stained with haematoxylin and eosin as described by Strate et al. (2005). The slides were viewed at magnification of ×250 and photomicrographs were taken.

Statistical analysis

All data were presented as mean ± SEM and analyzed by one-way ANOVA followed by Dunnett's *post hoc* test using Graph Prism version 4.00 (GraphPad Software, Inc., La Jolla, CA, U.S.A.). Results were considered significant at $p < 0.05$.

RESULTS

The estimated oral LD_{50} of AEPAL was greater than 5000 mg kg^{-1} with no recorded signs of toxicity or mortality during the 24 h observation period.

Effect of the extract on alloxan-induced hyperlipidaemia and hyperglycaemia

The hyperglycaemic control showed a rise in blood glucose levels, which was significantly ($p < 0.05$) reduced in AEPAL and metformin treated groups from the 7th to 28th day of the treatment period (Figure 1).

In Figure 2, hyperglycemic control showed significant hyperlipidaemia compared to normal control. Treatment with AEPAL significantly reversed the hyperglycaemic-induced hyperlipidaemia in a dose related fashion. AEPAL (125 mg kg^{-1}) significantly ($p < 0.05$) increased HDL and decreased TG and LDL. Rats treated with 250 and 500 mg kg^{-1} AEPAL and 250 mg kg^{-1} metformin significantly ($p < 0.001$) reversed all the lipid profile of the alloxan-induced hyperglycaemic rats.

Effects of the extract on body weights variations

AEPAL (250 mg kg^{-1}) significantly ($p < 0.05$) reversed the body weight reduction caused by alloxan-induced hyperglycaemia on day 28 and standard drug (metformin 250 mg kg^{1}) on day 21. Rats treated with 500 mg kg^{-1} of the extract and metformin 250 mg kg^{-1} showed a significant ($p < 0.01$) weight gain on the last day of treatment (Figure 3).

Food and water intake in alloxan hyperglycaemia

The hyperglycaemic control rats showed an increase in food and water intake, which are characteristics of DM. The extract significantly ($p < 0.05$) reduced the feed consumption after week 1 in metformin (250 mg kg^{-1}) treated group and week 2 in 250 and 500 mg kg^{-1} treated groups (Figure 4). A significant ($p < 0.001$) reduction in water intake was observed in standard drug (metformin 250 mg kg^{-1}) group and in all AEPAL treated groups (Figure 4).

Biochemical parameters in alloxan hyperglycaemia

A significant reduction in AST, creatinine, urea, TP, ALB, ALT and ALP were observed in hypoglycemic rats treated with both metformin and graded doses of the extract (Table 1).

Histology

The pancreas was intact in the normal control group (Plate 1A). Pancreatic sections of hyperglycemic control rats showed severe necrotic changes of pancreatic islets, especially in the center of islets (Plate 1B). There were increased sizes of islets and hyperchromic nucleus from lowest to the highest dose of extract compared to hyperglycaemic control (Plate 1C to E). The metformin treated group had a lesser effect than the extract (Plate 1F).

DISCUSSION

From the data presented in this study, there are

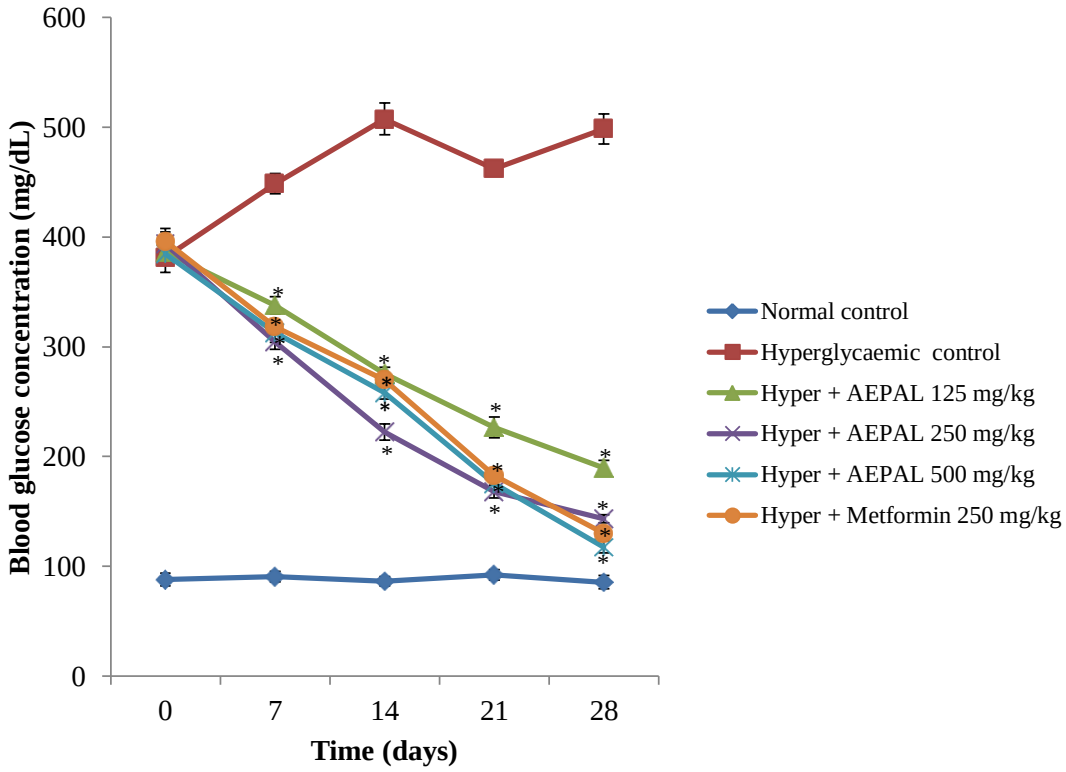


Figure 1. Effect of AEPAL on fasting blood glucose concentrations in alloxan-induced hyperglycemic Wistar rats. AEPAL, aqueous extract of *P. americana* leaf, n=6; values expressed as mean ± SEM.; data analyzed using one-way ANOVA followed by Turkey's post hoc test; *p < 0.05, statistically significant compared to hyperglycemic control.

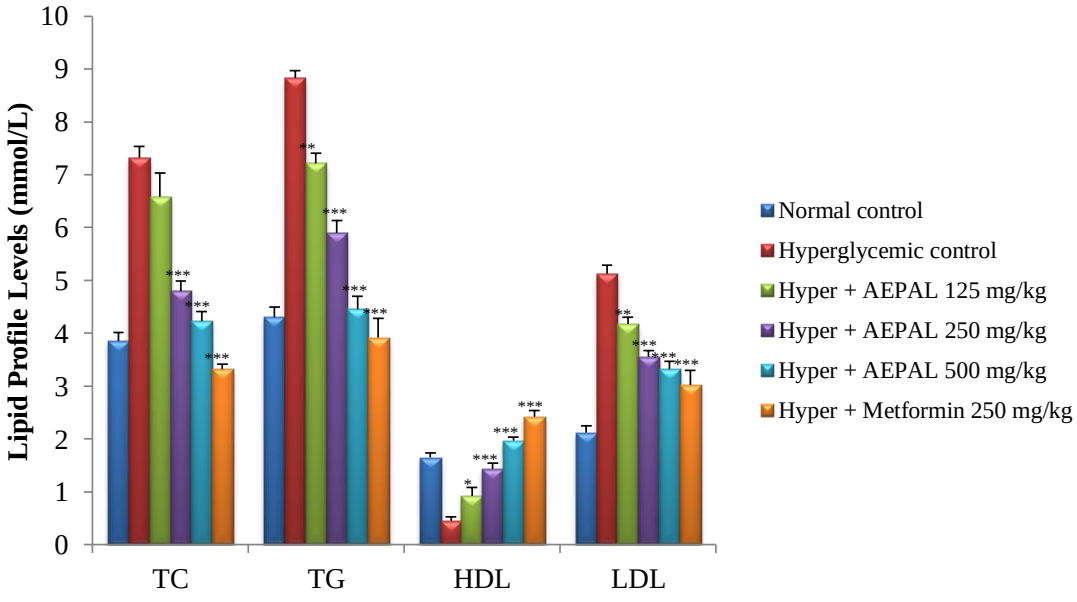


Figure 2. Effect of AEPAL on lipid profile levels in alloxan-induced hyperglycemic Wistar rats. AEPAL, aqueous extract of *P. americana* leaf; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein, n = 6; values expressed as mean ± SE. Data analyzed using one-way ANOVA followed by Turkey's post hoc test; *p < 0.05, **p < 0.01, ***p <0.001 statistically significant compared to hyperglycemic control.

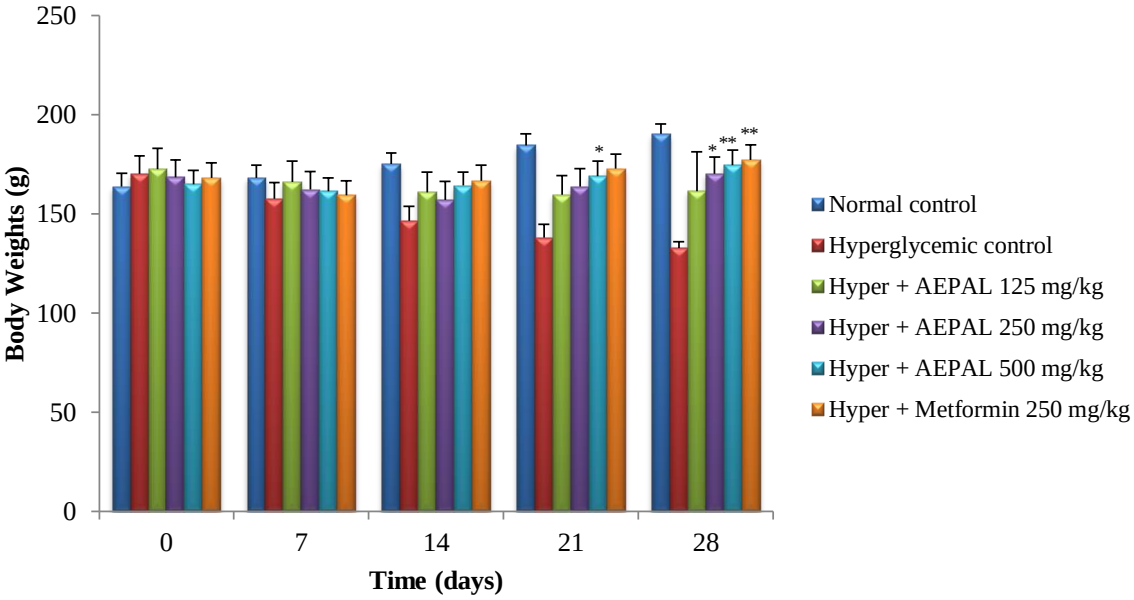


Figure 3. Effect of AEPAL on body weights of alloxan induced hyperglycemic rats. AEPAL – aqueous extract of *P. americana* leaf, n = 6; values expressed as mean ± SEM.; data analyzed using one-way ANOVA followed by Turkey's post hoc test; *p <0.05, **p <0.01, statistically significant compared to hyperglycemic control.

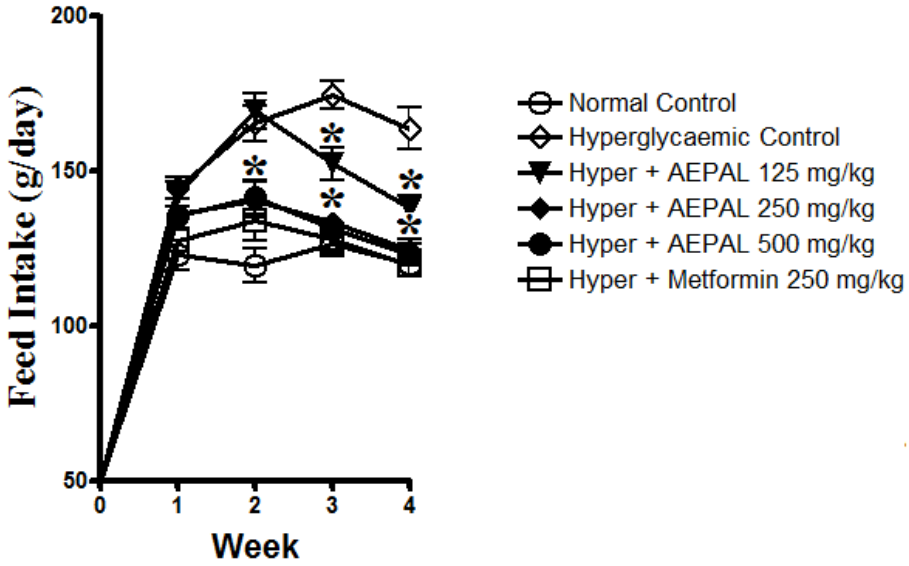
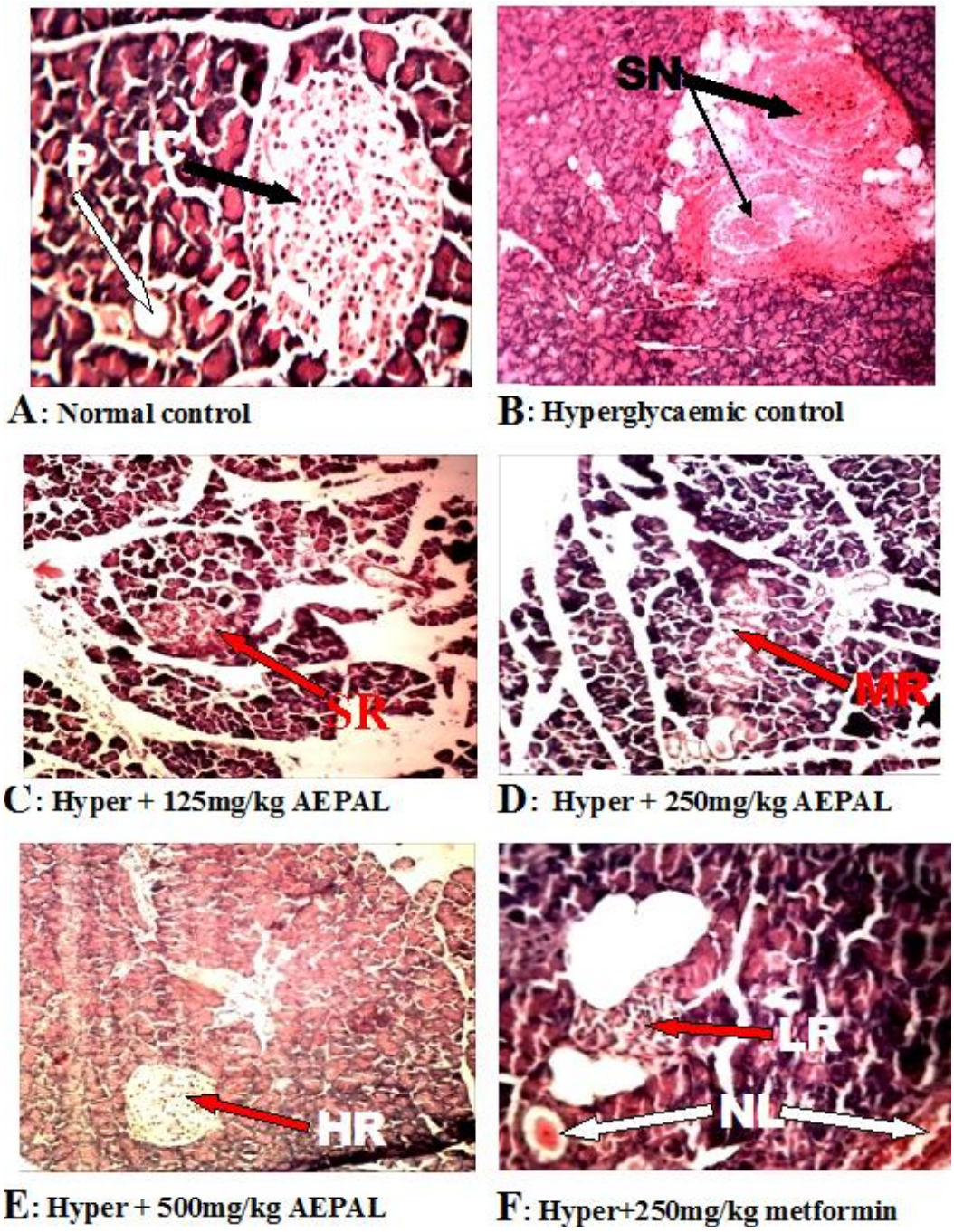


Figure 4. Effect of AEPAL on feed intake in alloxan-induced hyperglycemic Wistar rats. AEPAL, aqueous extract of *P. americana* leaf, n = 6; values expressed as mean ± SEM. Data were analyzed using one-way ANOVA followed by Turkey's post hoc test;*p<0.05, statistically significant compared to hyperglycemic control.

evidences that AEPAL contains biologically active constituents that are relevant in the management of diabetes mellitus. AEPAL significantly lowered blood glucose levels in alloxan-induced hyperglycaemic rats. Alloxan-induced hyperglycaemia is an acceptable model for Type I diabetes mellitus (Goldner and Gomori, 1944; Rohilla and Ali, 2012). Alloxan selectively destroys the insulin producing pancreatic beta cells (Szkudelski et al., 1998; Fasanmade and Alabi, 2008), resulting to various metabolic alterations including increased levels of blood



Plates 1. A, IC, Intact Islet cells; PL, normal pancreatic lobule. **B,** SN, Severe necrosis and atrophy of Islet cells; **C,** SR, slight regeneration of Islet cells; **D,** MR, moderate regeneration of Islet cells; **E,** HR, high regeneration and almost intact islet cells; **F,** LR, low regeneration of islet cells; NL, necrosis, lesion, distortion and atrophy still present.

glucose, cholesterol, alkaline phosphate and transaminases (Murugan et al., 2009). In this study, AEPAL (125 to 500 mg kg^{-1}) significantly decreased the blood glucose concentration, thus supporting the use of *P. americana* leaf preparations in folk medicine for the management of diabetes mellitus.

Hyperglycaemia, hyperlipidaemia and oxidative stress generally coexist in diabetic subjects (Mironova et al., 2000; Beckman et al., 2002). This study shows an increase in the concentration of total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C) and a decrease in HDL-C in hyperglycaemic control.

Table 1. Effect of AEPAL on biochemical parameters in alloxan-induced hyperglycaemic rats.

Treatment/dose (mg/kg)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TP (g/L)	ALB (g/L)	Urea (mmol/L)	Creatinine (µmol/L)
Normal control	40.83±4.67	47.67± 5.11	62.83 ±7.94	65.00 ±6.55	37.17 ± 6.68	2.18 ±0.17	49.50 ±1.48
Hyperglycaemic control	78.67±5.46	90.50 ±11.42	102.17 ±8.28	28.00 ±3.90	19.17 ±4.31	5.05±0.20	68.50±2.57
Hyperglycaemic + AEPAL125	54.33±3.9**	71.67±5.65	93.50 ±5.97	32.33±3.46	23.83 ±2.97	4.62 ±0.28	60.50 ±1.34*
Hyperglycaemic + AEPAL250	57.50±3.30*	64.67±7.62	77.33 ±7.50	42.00 ±4.76	30.50±2.57	3.72±0.26**	45.67±1.20***
Hyperglycaemic + AEPAL500	43.67±6.49***	46.50±4.69**	61.50 ± 6.20**	57.00 ± 7.40**	46.50 ± 5.06**	2.95 ±0.17***	43.67±2.03***
Hyperglycaemic + Metformin 250	39.83 ±1.35***	52.83 ± 5.53**	58.68 ± 8.02**	65.33 ± 1.63***	38.67± 4.76	2.63 ± 0.17***	47.67±1.84***

AEPAL, aqueous extract of *P. americana* leaf, n = 6, values expressed as mean ± SEM., *p < 0.05, **p < 0.01, ***p < 0.001, statistically significant compared to hyperglycaemic control.

Diabetic-induced hyperlipidaemia is attributable to excess mobilization of fat from the adipose tissue due to underutilization of glucose (Krishnakumar et al., 2000). The lack of insulin stimulates lipolysis and enhanced release of free fatty acids from adipose tissue (Subbiah et al., 2006), which are converted to triglyceride (Suryawanshi et al., 2006). Lowering of serum lipid levels through dietary or drugs therapy may be associated with a decrease in the risk of vascular disease in diabetes (Claudia et al., 2006). In this study, graded doses of AEPAL significantly reduced serum levels of total cholesterol, triglyceride, low-density lipoprotein and increased serum levels of high-density lipoproteininalloxan-induced hyperglycaemic treated rats, suggesting that the plant extract may be useful in reducing the complications of hyperlipidaemia and hypercholesterolemia thatare often comorbidities in diabetics (Sharma, 2003).

The current study showed a progressive decrease in body weights of alloxan treated hyperglycaemic animals consistent with the reports of Andrade and Wiedenfeld (2001) and Eze et al. (2015). The reduction in body weights may be associated with degradation of structural proteins and increased muscle wasting (Cheng et al., 2013; Eze et al., 2015). AEPAL significantly reversed the body weight reduction, decreased food and water intake in alloxan-induced hyperglycaemic rats, suggesting that the AEPAL countered the basic polytriad symptoms of diabetes mellitus, polyphagia, and polydipsia and weight loss.

Furthermore, the study showed elevated AST, ALT and ALP in hyperglycemic control and treatment of hyperglycaemic animals with AEPAL and metformin produced a significant reduction in the levels of AST, ALT and ALP. The level of serum protein and albumin were reduced in hyperglycaemic control. An improvement in the total protein and albumin content was observed in AEPAL and metformin treated groups, which may be attributable to an increased in protein synthesis.

The blood urea and creatinine levels increased significantly in hyperglycaemic control group and reversed in AEPAL and metformin treated group. Increase in serum urea and creatinine concentrations, which are considered as markers of kidney dysfunction (Gross et al., 2005) may indicate diminished ability of the kidneys to filter these waste products from the blood and excrete them in the urine (Gross et al., 2005).

Alloxan caused degeneration of pancreatic β-cells in hyperglycaemic animals. AEPAL at tested doses increased sizes of islets and hyperchromic nucleus, relative granulated and normal beta cells compared to hyperglycaemic control.

Conclusion

The AEPAL may contain bioactive substances with potential hypoglycaemic and hyperlipidaemic properties, thus supporting further the development of these substances as antidiabetic agents. Studies are in progress in our laboratories to isolate and mechanistically characterize the biologically active components from this important medicinal plant that is already in common use.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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