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Hydroethanol Extract of Amaranthus hybridus (Amaranthaceae) Leaves Ameliorates Type 2 Diabetes in an Animal Model

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ABSTRACT

Background: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia. Conventional antidiabetic medicines are associated with worrisome side-effects, hence the search for relatively more efficacious and better tolerated remedies from medicinal plants.
Objective(s): To evaluate the effects of *Amaranthus hybridus* (Amaranthaceae) hydroethanol leaf extract in alloxan-nicotinamide induced Type 2 diabetes.
Methods: Two groups of mice (n=5) received *A. hybridus* extract (2000 mg/kg) and distilled water (10 mL/kg) per oral (p.o.) respectively for the acute toxicity test. Several groups of rats were used in the determination of effects of the extract on normal and glucose-loaded rats (NG-OGTT), acute and subchronic antidiabetic study. Diabetes was induced in rats by intraperitoneal (i.p.) administration of Alloxan monohydrate (150 mg/kg) and Nicotinamide (90 mg/kg) 5 min later. In the subchronic antidiabetic study, rats in different groups were administered the extract (125, 250 and 500 mg/kg), and Metformin (100 mg/kg) p.o. for 35 days. Daily food and water intakes were determined, while body weight and blood glucose level were measured at 7-day interval. The rats were sacrificed on day 36 and blood samples were collected for determination of biochemical parameters. Vital organs (liver, kidneys, heart and pancreas) were harvested for assay of antioxidant indices and histologic assessment.
Results: The extract at the dose of 2000 mg/kg did not cause mortality and visible signs of delayed toxicity. In respect of NG-OGTT, the extract caused significant ($p<0.05$) reductions in blood glucose level comparable to Metformin. Acute treatment of diabetic rats with the extract and Metformin did not elicit significant reduction in glucose level. However, subchronic treatment of diabetic rats with the extract (250 mg/kg) resulted in significant reduction in glucose level on days 28 and 35, similar to the effect of Metformin. The extract did not elicit any significant effect on biochemical parameters. However, the extract enhanced *in-vivo* antioxidants while reducing MDA level in selected vital organs of diabetic rats. The extract preserved body weight gain in diabetic rats with no significant deleterious changes in respect of histology of selected vital organs.
Conclusion: The results obtained in this study suggest that the hydroethanol extract of *Amaranthus hybridus* possess beneficial effects in an animal model of Type 2 diabetes and may therefore be useful as nutraceutical agent in the management of diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia as a result of impaired insulin secretion, resistance to peripheral actions of insulin, or combination of these root courses.[1] It accounts for significant morbidity and mortality, hence high burden of disease globally. Based on International Diabetes Federation (IDF) data, 589 million adults (20-79 years) are living with diabetes worldwide with a projection that this number will rise to 853 million by 2050.[2] Within this context, diabetes was responsible for 3.4 million deaths in 2024 with at least USD 1 trillion dollars in health expenditure. Diabetic patients experience symptoms such as increased appetite, polydipsia, dysuria, weight loss, increased appetite, and vision problems etc.; if not properly managed these individuals may experience complications such as coma, confusion, and possibly death from ketoacidosis or nonketotic hyperosmolar syndrome.[3-5] In diabetic patients, chronic hyperglycemia with other metabolic aberrations may result in damage to organs and microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular complications with increased risk of cardiovascular diseases.[1]

In terms of classification, diabetes mellitus encompasses Type 1 diabetes, Type 2, gestational, monogenic and secondary diabetes. According to Goyal *et al.*,[1] Type 2 diabetes mellitus (T2DM) is associated with diminished response to insulin (insulin resistance) which accounts for approximately 90% of all diabetes cases. Beyond persons older than 45 years, T2DM is now increasingly diagnosed in children, adolescents, and younger adults. This worrisome trend has been linked to rising levels of obesity, physical inactivity, and energy-dense diets.[1] Current pharmacotherapeutic agents for T2DM are associated with significant side-effect. For example, Rosuvastatin - muscle pain, liver enzyme abnormalities, and gastrointestinal symptoms; Thiazolidinediones - weight gain, oedema, and risk of fractures; Metformin - gastrointestinal symptoms (diarrhea, nausea), vitamin B12 deficiency, and rarely lactic acidosis; Sodium-glucose cotransporter-2 inhibitors (SGLT2 inhibitors) - urinary tract infections, genital yeast infections, and increased urination; Insulin - hypoglycemia, injection site reactions, and weight gain etc.[5-10] These side effect profiles have warranted the search for newer antidiabetic agents with better efficacy and safety profile relative to conventional antidiabetic drugs.

The use of medicinal plants for the treatment of various diseases is an age-long practice. Herbal medicines form a significant proportion of traditional medicine which has over the years served the healthcare needs of populations of the world in rural and semi-urban areas owing to the absence or inadequacy of orthodox medicine facility. Plants have over the years proven to be very reliable sources of orthodox medicines with a long list of derived drugs, either as purely derived from plants or being modifications of plant phytoconstituents. According to Wachtel-Galor and Benzie,[11] plants and natural sources form the basis of today's modern medicine which contribute largely to the commercial drug preparations manufactured today; approximately 25% of drugs prescribed worldwide are derived from plants. There is a surge in the patronage of herbal medicines in developing and developed countries owing to the perceived acknowledgement of efficacy, safety, accessibility and affordability. In view of these facts, the scientific evaluations of medicinal plants, particularly edible plants such as *Amaranthus hybridus* Linn. (Amaranthaceae) used traditionally in combating diseases may be a worthwhile endeavour. *Amaranthus hybridus*, commonly known as Green Amaranth, is an erect annual plant with a stem that can be much-branched to nearly free of branches with an history of cultivation as a food crop for its edible leaves and seeds.[12] The plant, originally from the Americas and naturalized in Europe, is widespread in the tropics where it grows wild in cultivated fields and waste places. *Amaranthus hybridus* plant is widely consumed in Nigeria by diverse ethnic groups as a vegetable soup owing to its richness in nutrients and mineral elements.[13,14] The plant and its different parts have been reported to have various ethnomedicinal uses, including treatment of dysentery, diarrhoea, ulcers, bowel hemorrhage, diabetes, infection, inflammation, snake and scorpion bite.[15,16]

This study was conducted to evaluate the effects of *Amaranthus hybridus* (Amaranthaceae) hydroethanol leaf extract in alloxan-nicotinamide induced Type 2 diabetes.

MATERIALS AND METHODS

Drugs and chemicals

Alloxan monohydrate, ethanol (Sigma-Aldrich Corporation, St. Louis, USA), Metformin (Glucophage®, Merck Pharmaceuticals, Spain), and Nicotinamide (Puritan Pride, New York, USA).

Plant material and extraction

Amaranthus hybridus plant was purchased from Mushin market, Lagos State, Nigeria. The identification and authentication of the plant material was done by Mr. O.O. Oyebanji of the Department of Botany, University of Lagos, Nigeria. A voucher specimen with number LUH 7577 was deposited in the institutional herbarium. The fresh leaves of *A. hybridus* were separated from their stalks and were shade dried in the Pharmacology Laboratory of the

College of Medicine, University of Lagos, Lagos, Nigeria, until constant weight was obtained. The dried leaves were grounded using an electric grinder. The powdered *A. hybridus* material (1 kg) was then macerated with hydroethanol (ethanol: distilled water at a ratio of 1:1) with intermittent stirring. This was filtered after 72 h using muslin cloth and re-filtered with Whatman filter paper no. 4. The marc was re-macerated (× 2) in hydroethanol for exhaustive extraction and the combined filtrates was evaporated to dryness at 40°C. The yield (%) was calculated based on the derived weight of the dried extract and weight of the starting powdered plant material.

Yield (%) = (Weight of dried extract ÷ Weight of powdered plant material) × 100

= (76 g ÷ 1000 g) × 100 = 7.6%

Experiment animals

Healthy Wistar rats of either sex (140-200 g) and male Swiss mice (15-25 g) were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria. The animals were housed in plastic cages (males and females separately) at appropriate humidity, temperature and 12 h light/dark cycle. The animals were allowed to acclimatize for 2 weeks before the commencement of the experiment. The rodents were fed with standard diet (Livestock Feeds PLC, Lagos, Nigeria) and water *ad libitum*. Ethical approval was sought and obtained from the Animal Care and Use Research Ethics Committee (ACUREC) of the College of Medicine, University of Lagos with approval reference number CMUL/ACUREC/08/22/1092.

GC-MS analysis of *A. hybridus*

Gas Chromatography - Mass Spectrometry (GC-MS) analysis of the hydroethanolic leaf extract of *A. hybridus* was carried out using an Agilent HP-7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with HP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm, 0.25 µm film thickness; Restek, Bellefonte, PA, USA) equipped with an MSD detector and characterized as previously described by Adams[17] with some modifications by Okolie *et al*.[18] Oven temperature was maintained at 40°C for 3 min initially, and then raised at the rate of 3°C/min to 250°C. Injector and detector temperatures were set at 220°C and 290°C, respectively. Helium was used as carrier gas at a flow rate of 1 mL/min, and diluted samples (1/1000 in n-pentane, v/v) of 1.0 µl were injected manually in the splitless mode. Peak area percents were used for obtaining quantitative data. The constituent compounds were determined by comparing their retention times and mass weights with those of authentic samples obtained by GC as well as the mass spectra. The spectrum of the unknown components were compared with the spectrum of the known components stored in the database of National Institute of Standards and Technology (NIST) version 2.0 MS.

Acute toxicity test

The extract was administered orally (*p.o.*) to a group of 5 mice at the dose of 2000 mg/kg. Another group of 5 mice served as control and received distilled water (10 mL/kg, *p.o.*). The mice were observed for behavioural changes and signs of toxicity for 2 h post-treatment. Mortality in each group was checked 24 h after. Surviving mice were observed for further 14 days for signs of delayed toxicity.[19]

Determination of blood glucose level

The experimental rats were fasted overnight for a period of 16 h but had free access to clean and drinkable water. The fasting blood glucose level was determined by placing 3-4 drops of venous blood on glucose strip appropriately inserted into a glucose monitoring meter (Glucometer, Accu-chek®). The value observed on the digital display was recorded.

Effects of *A. hybridus* on normal and glucose-loaded rats (NG-OGTT)

The rats were randomly divided into 5 groups of 6 rats each and the fasting blood glucose level of each rat was determined. Thereafter, distilled water (10 mL/kg, *p.o.*) was given to one group which served as the control group. Metformin (standard drug; 100 mg/kg, *p.o.*) and *A. hybridus* extract (125, 250 and 500 mg/kg, *p.o.*) were administered *p.o.* to the remaining groups, respectively. The blood glucose levels were determined at 30, 60, 90, and 120 min post-treatment. Thereafter, the rats were orally loaded with 2 g/kg glucose and the determination of blood glucose level then continued at 150, 180, 240, 300, 360 and 480 min.[20,21]

Induction of diabetes

Baseline fasting blood glucose levels of 16 h fasted rats were determined. Diabetes was induced in the experimental rats by *i.p.* administration of Alloxan monohydrate (150 mg/kg, *i.p.*) and Nicotinamide (90 mg/kg, *i.p.*) 5 min later. The fasting blood glucose levels were determined 72 h thereafter. Rats with fasting blood glucose level >150 mg/dL were considered diabetic.

Acute antidiabetic test

After 72 h post-induction, diabetic rats were randomly divided into 5 groups (n=6). The different groups were treated orally with distilled water (10 mL/kg), Metformin (100 mg/kg), and *A. hybridus* extract at doses of 125, 250 and 500 mg/kg. A group

of rats served as non-diabetic control. Blood glucose level of each rat was determined at 30, 60, 120, 240 and 360 min post-treatment.[20,21]

Subchronic antidiabetic test

Diabetic rats were randomly allotted into 6 groups (n=6). The animals were fasted overnight for 16 h and fasting blood glucose level for each rat was determined. One of the groups received distilled water (10 mL/kg, *p.o.*), while the other groups received oral doses of *A. hybridus* extract at 125, 250, and 500 mg/kg, and Metformin (100 mg/kg). A group of rats served as non-diabetic control. Treatment was done for 35 days and the fasting blood glucose level and body weight of each rat were determined on days 7, 14, 21, 28 and 35. Daily food and water intakes of the experimental animals were measured and recorded. On the evening of the 34th day, all the experimental rats were fasted for 16 h and on the morning of day 35, blood samples were collected from the retro-orbital sinus under anaesthesia (through *i.p.* injection of 1% chloralose in 25% urethane (w/v), 5 mL/kg) into plain sample tubes. The sera obtained from the blood samples were used for biochemical and electrolytes analyses. Vital organs (liver, kidneys, heart and pancreas) were harvested from the sacrificed rats and weighed. Some vital organ samples across each of the groups were used for antioxidant indices analysis, while others were preserved in 10% buffered formalin for histopathology assessment.[21,22]

Biochemical analysis

The sera obtained from blood samples of the experimental rats were used for the determination of biochemical and lipid parameters, including aspartate transaminase, alanine transaminase, alkaline phosphatase, creatinine, total protein, albumin, urea, glucose, cholesterol, triglycerides, high density lipoprotein, and low density lipoprotein with the use of Roche/Hitachi 904 automated analyzer (Roche Diagnostics, Basel, Switzerland) and Roche and Cobas commercial kits (Roche Diagnostics, Basel, Switzerland). Serum electrolytes, including potassium, chloride and calcium were determined using a flame photometer (Sherwood, Model 410).[23]

Antioxidant indices

Antioxidant indices, including Catalase (CAT), Reduced glutathione (GSH), Superoxide dismutase (SOD), and Malondialdehyde (MDA) were determined using the supernatants derived from the homogenates of the liver, kidneys, heart and pancreas according to the methods earlier described by Akindele *et al.*[24] and Olatoye *et al.*[25]

Histopathology assessment

The vital organs (liver, kidneys, heart and pancreas) harvested from the sacrificed rats were fixed in 10% buffered formalin and duly processed using a tissue processor (Micron STP 125, Thermo-Fisher, USA). The processing time was 17 h during which the specimen was passed through a series of steps involving dehydration in graded alcohols, clearing in two changes of xylene, infiltration in molten paraffin wax, and then embedded. Cutting was then performed using a rotary microtome (Microm HM 325, Thermo-Fisher, USA), followed by staining and viewing under the microscope, as previously reported.[25,26]

Statistical analysis

All data are expressed as mean ± standard error of mean (S.E.M). Statistical analysis was done using one-way analysis of variance (ANOVA) with Tukey's multiple comparison test. $p<0.05$ was considered to be significant for the results. The software used was GraphPad Prism 6.0 version (GraphPad Software Inc., CA, USA).

RESULTS

GC-MS analysis of hydroethanolic leaf extract of *A. hybridus*

The GC-MS analysis of the hydroethanolic leaf extract of *A. hybridus* revealed the most abundant constituents to be Phytol; Octadecadienoic acid, ethyl ester; Ethyl oleate; 9-Octadecenoic acid (Z)-,2,3-dihydroxypropyl ester; 9,12-Octadecadienoic acid; Octadecadienoic acid, 2-hydroxy-1,3-propane; 12-Methyl-E,E-2,13-octadecadien-1-ol; 2-Methyl-Z,Z-3,13-octadecadienol; and Beta carotene (Figure 1; Table 1).

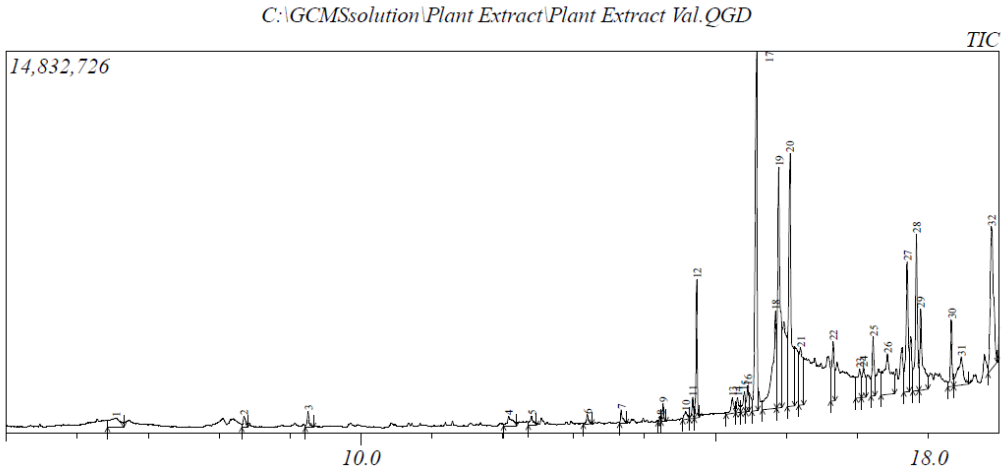

Figure 1: GC-MS profile of the hydroethanolic leaf extract of *A. hybridus*.

Table 1: GC-MS analysis of the hydroethanolic leaf extract of *A. hybridus* indicating the most abundant constituents.

Retention time (min.)	Chemical name	Peak area (%)	Nature
15.58	Phytol	11.91	Acyclic diterpene
16.05	Octadecadienoic acid, ethyl ester	11.78	Fatty acid
15.90	Ethyl oleate	11.04	Fatty acid
18.90	9-Octadecenoic acid (Z)-,2,3-dihydroxypropyl ester	7.95	Fatty acid
15.45	9,12-Octadecadienoic acid	6.88	Fatty acid
17.83	Octadecadienoic acid, 2-hydroxy-1,3-propane	5.88	Fatty acid
17.43	12-Methyl-E,E-2,13-octadecadien-1-ol	5.09	Fatty acid
17.70	2-Methyl-Z,Z-3,13-octadecadienol	4.88	Fatty acid
18.47	Beta carotene	2.72	Vitamin

Acute toxicity

The hydroethanolic leaf extract of *A. hybridus* did not produce any mortality when administered orally at the dose of 2000 mg/kg and no visible delayed toxicity or mortality was observed when animals were monitored for further 14 days. Behavioural manifestations observed with the extract within 2 h of administration include calmness, sensitivity to touch, rearing and assisted rearing, grooming, pupillary reaction, defecation, social interaction, and increased righting reflex compared to the control group.

Effect of hydroethanolic leaf extract of *A. hybridus* on normal and glucose-loaded rats

The extract at the dose of 125 mg/kg caused significant reductions ($p<0.05$) in glucose level pre- and post-glucose loading (at the various time intervals) relative to the baseline (0 min) value with the highest reduction (47.61%) produced at the 480 min interval. This trend of result was generally the same with the 250 mg/kg and 500 mg/kg doses of the extract, and Metformin, with peak glucose level reductions at the 480 min interval and values of 42.11%, 30.00%, and 44.02%, respectively. The peak reduction in glucose level value for the extract at 125 mg/kg (47.61%) was slightly higher than that of Metformin (44.02%) (Table 2).

Effect of hydroethanolic leaf extract of *A. hybridus* on diabetic rats in acute glucose treatment study

Comparing glucose level values at the different time intervals with baseline (0 min) values, there were no significant changes ($p>0.05$) in all the treatment groups (Table 3).

Effect of hydroethanolic leaf extract of *A. hybridus* on diabetic rats in subchronic treatment study

The extract at the dose of 250 mg/kg elicited significant reductions in glucose level on day 28 ($p<0.05$; 47.74%) and day 35 ($p<0.05$; 54.11%). Metformin caused significant reductions in glucose level on day 21 ($p<0.01$; 53.86%), day 28 ($p<0.001$; 62.72%) and day 35 ($p<0.0001$; 68.15%). Percentage glucose level reductions elicited by the extract at the dose of 250 mg/kg on days 28 and 35 were lower than those of Metformin at the same day intervals (Table 4).

Table 2: Effect of hydroethanolic leaf extract of *A. hybridus* on normal and glucose-loaded rats.

Treatment	Dose (mg/kg)	0 min	30 min	60 min	90 min	120 min	150 min	180 min	240 min	360 min	480 min
Control	-	74.17 ± 3.17	78.19 ± 3.91 (5.40%↑)	75.17 ± 3.78 (13.50% ↑)	70.53 ± 3.66 (1.58%)	70.33 ± 3.66 (5.18%)	97.50 ± 5.97 (31.45%↑)	84.83 ± 3.63 (14.37%)	74.67 ± 3.38 (5.16%)	78.00 ± 3.95 (5.26)	86.83 ± 3.63 (14.75%)
AH	125	80.50 ± 3.78	64.17 ± 2.30[a] (20.29%)	62.17 ± 2.23[b] (22.70%)	59.67 ± 2.35[c] (20.20%)	57.50 ± 2.54 (28.57%)	95.67 ± 2.81 (18.84%↑)	79.83 ± 5.32 (0.83%)	59.50 ± 2.95[c] (20.09%)	52.00 ± 3.49[d] (34.40%)	42.17 ± 2.93[d] (47.61%)
AH	250	85.50 ± 3.37	68.50 ± 5.43 (19.88%)	65.83 ± 5.20 (23.63%)	64.17 ± 5.42 (25.04%)	61.17 ± 5.20[a] (28.46%)	97.00 ± 5.63 (13.50%↑)	84.50 ± 5.90 (1.17%)	62.33 ± 2.53[a] (26.09%)	56.50 ± 3.00[b] (33.92%)	49.50 ± 2.95[d] (42.11%)
AH	500	76.67 ± 2.50	70.83 ± 4.43 (7.53%)	69.00 ± 4.34 (9.92%)	67.00 ± 4.51 (12.53%)	64.30 ± 4.54 (16.06%)	97.83 ± 2.27 (27.72%↑)	85.67 ± 3.90 (11.74%)	74.67 ± 3.41 (2.52%)	61.17 ± 3.91 (20.22%)	53.67 ± 4.75[b] (30.00%)
Metformin	100	86.33 ± 3.48	66.00 ± 3.94[a] (23.53%)	63.83 ± 3.74[c] (26.06%) i	61.00 ± 3.53[d] (0.34%)	61.00 ± 3.53[d] (29.34%)	104.80 ± 3.10 (21.39%↑)	93.50 ± 3.85 (8.31%)	90.33 ± 3.60 (4.63%)	61.67 ± 2.17[a] (25.09%)	48.33 ± 0.99[d] (44.02%)

Values are mean ± S.E.M. (n = 5). [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$, [d]$p<0.0001$ vs. 0 min (horizontal comparison) (One-way ANOVA followed by Tukey's multiple comparison test). AH - *A. hybridus*

Table 3: Effect of hydroethanolic leaf extract of *A. hybridus* on diabetic rats in acute treatment study.

Treatment	Dose (mg/kg)	0 min	30 min	60 min	120 min	240 min	360 min
Normal control	-	97.73±1.86	91.17±2.14 (6.71%)	81.83±2.44 (15.90%)	76.83±3.22 (21.39%)	74.17±2.76 (24.16%)	64.33±1.98 (34.18%)
Diabetic control	-	379.00±59.16	382.70±59.04 (0.98%↑)	387.70±58.97[d] (2.3%↑)	389.80±59.73 (2.85%↑)	390.70±59.70 (3.10%↑)	392.70±89.84 (3.61%↑)
AH	125	283.20±48.61	274.20±48.10 (3.18%)	258.70±47.74 (8.65%)	249.30±46.38 (11.97%)	236.20±46.89 (16.59%)	229.30±44.59 (19.03%)
AH	250	192.30±12.10	186.30±11.48 (3.12%)	249.20±19.20 (22.83%↑)	233.70±19.18 (17.72%↑)	213.20±17.99 (10.87%↑)	179.70±17.99 (6.55%)
AH	500	263.80±46.89	252.30±44.48 (4.36%)	240.50±44.40 (8.65%)	238.80±41.46 (0.47%)	220.80±43.03 (16.26%)	208.80±45.21 (0.48%)
Metformin	100	234.70±37.33	225.50±36.59 (3.92%)	220.70±34.22 (5.97%)	190.70±19.81 (18.75%)	177.30±16.24 (24.46%)	162.50±14.42 (30.70%)

Values are mean ± S.E.M. (n = 5). $p>0.05$ (One-way ANOVA). Values in parenthesis represent % reduction in glucose levels with reference to 0 min, except otherwise stated. AH - *A. hybridus*

Table 4: Effect of hydroethanolic leaf extract of *A. hybridus* on diabetic rats in subchronic treatment study.

Treatment	Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Normal control	-	97.73±1.81	82.71±3.31 (15.37%)	88.14±5.49 (9.81%)	87.86±5.93 (10.10%)	74.43± 3.20 (23.84%)	72.29± 3.48 (26.03%)
Diabetic control	-	379.00±59.15	409.50±62.49 (8.05%↑)	392.20±67.48 (0.35%↑)	354.00±68.60 (6.60%)	298.70±44.67 (21.19%)	356.00±35.00 (6.07%)
AH	125	283.20±48.69	190.30±27.14 (32.80%)	151.40±24.27 (46.54%)	136.50±22.03 (51.80%)	106.30±24.57 (62.46%)	94.25±24.85 (66.72%)
AH	250	192.30±12.10	157.70±15.00 (17.99%)	127.80±16.34 (33.54%)	98.17±19.00 (48.95%)	100.5±6.51[a] (47.74%)	88.25±6.97[a] (54.11%)
AH	500	263.80±46.09	182.50±45.50 (30.82%)	150.20±41.69 (43.06%)	167.35±51.25 (36.56%)	158.70±63.73 (39.84%)	144.00±61.61 (45.41%)
Metformin	100	234.70±37.30	148.50±10.32 (36.73%)	123.20±5.31 (47.51%)	108.30±4.03[b] (53.86%)	87.50±3.52[c] (62.72%)	74.75±5.76[d] (68.15%)

Values are mean ± S.E.M. (n = 5). [a]p<0.05, [b]p<0.01, [c]p<0.001, [d]p<0.0001 vs. 0 min (horizontal comparison) (One-way ANOVA followed by Tukey's multiple comparison test). Values in parenthesis represent % reduction in glucose levels with reference to 0 min, except otherwise stated. AH - *A. hybridus*

Effect of hydroethanolic leaf extract of *A. hybridus* on food intake of diabetic rats

The diabetic control group had significantly higher food intake on day 14 (p<0.0001) compared to normal control group. However, there were significantly lower food intakes on days 21, 28 and 35 (p<0.0001) relative to the normal control group. The extract at doses of 125, 250 and 500 mg/kg, and Metformin significantly increased the food intake relative to the diabetic control group (p<0.001, 0.0001). Values for the extract at the various doses are generally comparable to those of Metformin at the various day intervals (Table 5).

Effect of hydroethanolic leaf extract of *A. hybridus* on water intake of diabetic rats

The diabetic control group had significantly (p<0.0001) higher water intake on days 14 and 21, but significantly (p<0.0001) lower water intake on days 28 and 35 relative to the normal control group. The extract at the various doses (125, 250 and 500 mg/kg) and Metformin significantly (p<0.01) reduced the water intake relative to the diabetic control group on days 14 and 21, while significantly increasing the water intake relative to the diabetic control group on days 28 and 35 (p<0.01-0.0001). Values for the extract at the various doses are generally comparable to those of Metformin at the various day intervals (Table 6).

Table 5: Effect of hydroethanolic leaf extract of *A. hybridus* on food intake in alloxan-nicotinamide induced diabetic rats.

Treatment	Dose (mg/kg))	Day 7	Day 14	Day 21	Day 28	Day 35
Normal control	-	118.60±3.40	117.90±1.49	125.70±1.70↑	125.70±2.29	129.60±0.43
Diabetic control	-	117.10±2.86	124.11±2.00^{d}	97.14±5.55^{d}	80.00±3.7^{d}	70.01±4.36^{d}
AH	125	120.00±0.00	117.90±1.01$^{\beta}$	112.10±3.25$^{b,\beta}$	110.00±1.89$^{c,\beta}$	115.00±0.00$^{d,\beta}$
AH	250	122.90±1.80	122.90±1.80$^{\alpha}$	112.10±3.25$^{b,\beta}$	122.10±1.10$^{\beta}$	120.70±0.71$^{d,\beta}$
AH	500	120.00+0.00	115.70+1.30$^{\alpha}$	112.10+1.10$^{b,\beta}$	112.90+1.01$^{c,\beta}$	117.90±1.10$^{d,\beta}$
Metformin	100	119.30±0.70	115.00±0.00$^{\alpha}$	112.90±1.01$^{a,\beta}$	110.00±0.00$^{c,\beta}$	112.10±1.01$^{d,\beta}$

Values are mean ± S.E.M. (n = 5). $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$,$^{d}p<0.0001$ vs. normal control; $^{\alpha}p<0.001$, $^{\beta}p<0.0001$ vs. diabetic control (One-way ANOVA followed by Tukey's multiple comparison). AH - *A. hybridus*

Table 6: Effect of hydroethanolic leaf extract of *A. hybridus* on water intake in alloxan-nicotinamide induced diabetic rats.

Treatment	Dose (mg/kg)	Day 7	Day 14	Day 21	Day 28	Day 35
Normal control	-	141.40±3.22	138.60±1.43	142.90±2.86	139.30±1.70	145.70±2.02
Diabetic control	-	147.10±2.56	150.00±3.00^{d}	150.00±3.00^{d}	110.00±6.53^{d}	95.00±1.89 ^{d,a}
AH	125	142.9±+2.64	137.90±1.01$^{\Upsilon}$	128.60±2.37$^{b,\Upsilon}$	128.60±0.96$^{c,\Upsilon}$	115.00±0.00^{a}
AH	250	148.60±1.43	133.60±4.85$^{\beta}$	127.10±1.80$^{b,\Upsilon}$	114.30±1.70$^{\Upsilon}$	130.00±0.00^{a}
AH	500	144.30±2.02	128.60±3.03^{a}	120.70±3.69$^{d,\Upsilon}$	130.00±0.00$^{\Upsilon}$	130.00±0.00^{a}
Metformin	100	139.30±0.71	132.10±1.84$^{\alpha}$	127.90±2.64$^{b,\Upsilon}$	121.40±1.43$^{a,\Upsilon}$	129.30±2.02$^{d,\Upsilon}$

Values are mean ± S.E.M. (n = 5). $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$, $^{d}p<0.0001$ vs. normal control; $^{\alpha}p<0.01$, $^{\beta}p<0.001$, $^{\Upsilon}P<0.0001$ vs. diabetic control (One-way ANOVA followed by Tukey's multiple comparison). AH - *A. hybridus*

Effect of hydroethanolic leaf extract of *A. hybridus* on biochemical parameters in alloxan-nicotinamide induced diabetic rats

Compared to the normal control, there was no significant ($p>0.05$) change in the biochemical parameters and electrolytes in the diabetic control, extract and Metformin treatment groups. Also in comparison to the diabetic control, there was no significant ($p>0.05$) change in the biochemical parameters and electrolytes in the extract and Metformin treatment groups (Table 7).

Effect of the hydroethanolic leaf extract of *A. hybridus* on lipid profile in the subchronic study

Compared to the normal control, there was no significant ($p>0.05$) change in the lipid profile and glucose level in the diabetic control, extract and Metformin treatment groups. Also in comparison to the diabetic control, there was no significant ($p>0.05$) change in the lipid profile and glucose level in the extract and Metformin treatment groups (Table 8).

Effect of hydroethanolic leaf extract of *A. hybridus* on antioxidant indices and MDA level in the liver of alloxan-nicotinamide induced diabetic rats

The diabetic control had significantly lower GSH value ($p<0.0001$) compared with the normal control; values were not significantly different in respect of SOD, CAT and MDA relative to the normal control ($p>0.05$). The extract at the various doses significantly increased the values of GSH and SOD ($p<0.01$, 0.0001) with peak effects at the dose of 500 mg/kg and 250 mg/kg, respectively relative to the diabetic control. Conversely, the extract at the various doses caused significant reductions in the level of MDA ($p<0.0001$) with peak effect at the dose of 500 mg/kg compared to the diabetic control. Metformin significantly increased the value of GSH relative to the diabetic control ($p<0.0001$) but significantly reduced the levels of SOD and CAT compared with the diabetic control ($p<0.0001$) with no significant effect on the level of MDA. The values elicited by Metformin were comparable to those of the extract at 500 mg/kg and 250 mg/kg respectively for GSH and SOD. Values for MDA were also comparable (Table 9).

Effect of hydroethanolic leaf extract of *A. hybridus* on antioxidant indices and MDA level in the kidney of alloxan-nicotinamide induced diabetic rats

The diabetic control group had significantly lower GSH and CAT values ($p<0.05$, 0.01) with no significant change in SOD and MDA values ($p>0.05$) compared with the normal control group. Relative to the diabetic control group, the extract at various doses caused significant increase in the value of GSH ($p<0.0001$) with peak effect at the dose of 500 mg/kg. However, the extract elicited significant reductions in the level of SOD at 250 and 500 mg/kg ($p<0.001$, 0.0001) compared with the diabetic control. The extract at 125 mg/kg significantly increased the value of CAT ($p<0.0001$) relative to the diabetic control. The extract at the various doses elicited significant reduction in the level of MDA compared with the diabetic control with peak effect at the dose of 500 mg/kg ($p<0.05$, 0.01). Metformin caused significant increase in the value of GSH ($p<0.0001$) and reduction in the value of MDA ($p<0.0001$) relative to the diabetic control. However, it caused significant reduction in the level of SOD and CAT ($p<0.0001$) compared with the diabetic control. Values for the extract at the dose of 500 mg/kg were generally comparable to those of Metformin (Table 10).

Effect of the hydroethanolic leaf extract of *A. hybridus* on antioxidant indices and MDA level in the heart of alloxan-nicotinamide induced diabetic rats

The diabetic control group had significantly lower value of GSH ($p<0.01$) and higher value of MDA ($p<0.05$) relative to the normal control group. The extract at various doses caused significant increase in GSH value ($p<0.0001$) with peak effect at the dose of 500 mg/kg, and significantly lower MDA value ($p<0.0001$) with peak effect also at the dose of 500 mg/kg compared with the diabetic control group. The extract at 250 mg/kg elicited significant increase in SOD and CAT values ($p<0.0001$) compared with the diabetic control group. Metformin induced significant increase in GSH value ($p<0.0001$), but significant reduction in SOD and CAT values ($p<0.0001$) compared with the diabetic control group. Metformin also caused significant reduction in the value of MDA ($p<0.0001$) relative to the diabetic control group. The value for Metformin in respect of GSH was higher relative to the extract at the dose of 500 mg/kg, but lower in respect of MDA (Table 11).

Effect of the hydroethanolic leaf extract of *A. hybridus* on antioxidant indices and MDA level in the pancreas of alloxan-nicotinamide induced diabetic rats

The diabetic control group had significantly lower value of GSH and CAT, and higher value of MDA ($p<0.05$-0.0001) relative to the normal control group. The extract at the various doses caused significant increase in the value of GSH and CAT ($p<0.0001$) with peak effect at the dose of 500 mg/kg compared with the diabetic control. The extract at the dose of 125 mg/kg elicited significant increase in the value of SOD ($p<0.05$) relative to the diabetic control group. Although values were lower in respect of MDA at the various doses of the extract and Metformin compared

with the diabetic control value, these changes were not significant ($p>0.05$). Metformin elicited significant increase in the value of GSH ($p<0.0001$) relative to the diabetic control. However, it caused significant reduction in the value of CAT ($p<0.0001$). The GSH value for Metformin was slightly higher than that of the extract at the dose of 500 mg/kg (Table 12).

Effect of hydroethanolic leaf extract of *A. hybridus* on vital organs weight in alloxan-nicotinamide induced diabetic rats

A significant reduction ($p<0.05$) in liver weight was observed in the diabetic control compared with the normal control. The extract at the dose of 125 mg/kg produced significant increase in liver weight relative to the normal and diabetic control groups ($p<0.05$, 0.01). Values for the extract at 250 and 500 mg/kg, and Metformin were comparable to the normal control. For the kidney, there was generally no significant change in weight relative to the normal and diabetic controls. The same observation was the case for the heart except that the extract at 125 mg/kg elicited significant increase ($p<0.05$) in weight relative to the normal control. The extract at 250 mg/kg caused significant increase in the weight of the pancreas ($p<0.05$, 0.01) compared to the normal and diabetic controls (Table 13).

Effect of hydroethanolic leaf extract of *A. hybridus* on body weight in alloxan-nicotinamide induced diabetic rats

The normal control and extract treated group at 500 mg/kg groups had significantly higher ($p<0.05$) body weights on day 28 relative to day 7 values. All the treatment groups had significantly higher body weight values at day 35 ($p<0.05$-0.0001) compared with days 7, 14 and 21 values with the exception of the diabetic control which showed significantly reduced body weight value ($p<0.05$) relative to days 7, 14 and 21 values (Table 14).

Table 7: Effect of the hydroethanolic leaf extract of *A. hybridus on* biochemical parameters and electrolytes in alloxan-nicotinamide induced diabetic rats in the subchronic treatment study.

Treatment	Dose (mg/kg)	K^+ (mEq/L)	Cl^- (mEq/L))	Ca^{2+} (mg/dL)	Urea (mg/dL)	Cr (mg/dL)	Pro (mg/dL)	Albumin (mg/dL)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
Normal control	-	3.78±0.40	95.60±1.31	9.73±0.31	41.22±2.20	0.90±0.11	60.08±1.01	42.57±0.61	124.30±10.31	43.00±1.00	19.50±1.18
Diabetic control	-	3.78±0.40	95.60±1.31	9.730±0.31	43.40±5.00	0.90±0.11	59.80±1.00	44.00±0.80	107.30±15.01	44.00±2.00	21.00±2.00
AH	125	3.80±0.06	94.30±1.69	9.25±0.49	33.85±0.64	1.05±0.14	59.13±2.26	41.95±0.75	115.50±16.50	43.50±1.30	19.00±0.58
AH	250	3.83±0.10	92.10±1.90	9.58±0.29	141.40±39.30	1.05±0.14	58.60±4.51	41.95±0.75	118.80±19.69	42.50±1.26	19.00±0.71
AH	500	3.73±0.09	94.13±2.31	9.60±0.59	42.27±3.56	0.67±0.17	61.20±2.22	41.23±037	132.19±19.05	42.67±1.33	17.67±0.67
Metformin	100	3.75±0.15	92.10±1.90	10.30±0.50	37.60±3.78	1.05±0.25	56.05±0.48	41.80±1.37	115.50±9.50	49.50±0.50	16.25±0.25

Values are mean ± S.E.M. (n = 5). p>0.05 (One-way ANOVA). AH - *A. hybridus*

Table 8: Effect of the hydroethanolic leaf extract of *A. hybridus* on lipid profile in the subchronic treatment study.

Treatment	Dose (mg/kg)	TG (mg/dL)	CHOL (mg/dL)	HDL (mg/dL)	LDL (mg/Ll)
Normal control	-	159.00±8.80	196.30±5.18	52.1±1.91	112.40±3.80
Diabetic control	-	126.80±5.97	196.00±4.14	51.97±2.45	112.70±3.95
AH	125	148.10±6.87	202.40±7.90	53.50±0.90	119.50±6.77
AH	250	165.70±4.13	192.00±4.18	49.73±4.36	109.40±5.13
AH	500	143.40±6.66	186.90±5.99	51.83±0.84	106.20±7.44
Metformin	100	149.60±10.70	208.20±1.85	53.50±0.90	124.70±1.20

Values are mean ± S.E.M. (n = 5). p>0.05 (One-way ANOVA). AH - *A. hybridus*

Table 9: Effect of hydroethanolic leaf extract of *A. hybridus* on antioxidant indices and MDA level in the liver of alloxan-nicotinamide induced diabetic rats.

Treatment	Dose (mg/kg)	GSH (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	MDA (U/mg protein)
Normal control	-	62.92±0.87	4.74±0.04	26.15±0.48	2.22±0.06
Diabetic control	-	9.60±0.14[a]	8.47±0.23	31.4±1.11	4.4±0.13
AH	125	13.50±0.18[δ]	9.63±0.23[β]	28.30±1.32	3.51±0.08[δ]
AH	250	29.91±0.27[δ]	9.64±0.14[β]	41.50±0.66	2.92±0.09[δ]
AH	500	35.85±0.20[δ]	4.88±0.06[δ]	14.2±0.41[Y]	2.74±0.06[δ]
Metformin	100	38.80±0.33[δ]	7.11±0.33[δ]	13.1±06[δ]	2.55±0.42

Values are mean ± S.E.M. (n=5). [a]p<0.0001 vs. normal control; [α]p<0.05, [β]p<0.01, [ϒ]p<0.001, [ő]p<0.0001 vs. diabetic control (One-way ANOVA followed by Tukey's multiple comparison test). AH - *A. hybridus*

Table 10: Effect of hydroethanolic leaf of *Amaranthus hybridus* on antioxidant indices and MDA level in the kidney of alloxan-induced diabetic rats.

Treatment	Dose (mg/kg)	GSH (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	MDA (U/mg protein)
Normal control	-	45.06±0.27	4.55±0.08	20.47±0.43	3.15±0.03
Diabetic control	-	26.76±0.35[b]	5.51±0.14	17.1±0.45[a]	3.91±0.06
AH	125	42.50±0.25[ő]	5.30±0.05	27.6±0.51[ő]	2.70±0.05[β]
AH	250	44.51±0.26[ő]	4.74±0.13[ϒ]	16.9±0.11	3.03±0.04[α]
AH	500	47.61±0.29[ő]	3.68±0.04[ő]	16.9±0.22	2.54±0.04[β]
Metformin	100	49.19±0.32[ő]	4.26±0.05[ő]	7.81±0.19[ő]	2.50±042[ő]

Values are mean ± S.E.M. (n=5). [a]p<0.05, [b]p<0.01 vs. normal control; [α]p<0.05, [β]p<0.01, [ϒ]p<0.001, [ő]p<0.0001 vs. diabetic control (One-Way ANOVA followed by Tukey's multiple comparison test). AH - *A. hybridus*

Table 11: Effect of the hydroethanolic leaf extract of *A.hybridus* on antioxidant indices and MDA level in the heart of alloxan- nicotinamide induced diabetic rats.

Treatment	Dose (mg/kg)	GSH (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	MDA (U/mg protein)
Normal control	-	38.72±0.28	4.28±0.03	14.95±0.12	1.93±0.07
Diabetic control	-	17.12±0.23[b]	5.05±0.08	17.9±0.46	3.74±0.07[a]
AH	125	32.47±0.32[α]	3.64±0.04[α]	12.7±0.33[α]	3.06±0.07[α]
AH	250	34.16±0.26[α]	5.97±0.07[α]	28.00±0.41[α]	2.76±0.05[α]
AH	500	36.72±0.34[α]	4.97±0.09	19.00±0.19	2.75±0.07[α]
Metformin	100	41.12±0.24[α]	4.34±0.04[α]	10.7±0.20[α]	2.40±0.07[α]

Values are mean ± S.E.M. (n=5). [a]p<0.05, [b]p<0.01 vs. normal control; [α]p<0.0001 vs. diabetic control (One-way ANOVA followed by Tukey's multiple comparison test). AH - *A. hybridus*

Table 12: Effect of the hydroethanolic leaf extract of *A.hybridus* on antioxidant indices and MDA level in the pancreas of alloxan-nicotinamide induced diabetic rats.

Treatment	Dose (mg/kg)	GSH (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	MDA (U/mg protein)
Normal control	-	34.00±0.17	4.83±0.07	20.98±0.49	2.56±0.09
Diabetic control	-	8.93±0.28[c]	3.87±0.03	17.3±0.62[a]	4.89±0.06[b]
AH	125	20.97±0.28[α]	4.18±0.04[a]	22.9±0.39[α]	3.86±0.09
AH	250	44.55±0.35[α]	3.87±0.05	10.30±0.25[α]	3.59±0.05
AH	500	46.71±0.28[α]	3.89±0.02	24.30±0.28[α]	3.41±0.05
Metformin	100	50.68±0.26[α]	3.91±0.03	13.10±0.19[α]	3.17±0.06

Values are mean ± S.E.M. (n=5). [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.0001$ vs. normal control; [α]$p<0.0001$ vs. diabetic control (One-way ANOVA followed by Tukey's multiple comparison test). AH - *A. hybridus*

Table 13: Effect of hydroethanolic leaf extract of *A. hybridus* on vital organs weight in alloxan-nicotinamide induced diabetic rats.

Treatment	Dose (mg/kg)	Liver (g)	Kidney (g)	Heart (g)	Pancreas (g)
Normal control	-	5.81±0.50	0.95±0.09	0.58±0.05	0.43±0.04
Diabetic control	-	3.84±0.15^{a}	1.03±0.09	0.63±0.05	0.36±0.02
AH	125	7.85±0.12$^{a,\beta}$	1.20±0.01	0.79±0.04^{a}	0.51±0.06
AH	250	5.29±0.26	1.16±0.04	0.52±0.03	0.88±0.04$^{b,\alpha}$
AH	500	5.10±0.59	0.83±0.03	0.49±0.02	0.63±0.13
Metformin	100	5.15±0.62	1.45±0.35	0.58±0.01	0.61±0.08

Values are mean ± S.E.M. (n = 5). $^{a}p<0.05$, $^{b}p<0.01$ vs. normal control; $^{\alpha}p<0.01$, $^{\beta}p<0.001$ vs. diabetic control (One-way ANOVA followed by Tukey's multiple comparison). AH - *A. hybridus*

Table 14: Effect of the hydroethanolic leaf extract of *A. hybridus* on body weight in alloxan-nicotinamide induced diabetic rats.

Treatment	Dose (mg/kg)	Day 7 (g)	Day 14 (g)	Day 21 (g)	Day 28 (g)	Day 35 (g)
Normal Control	-	117.3±3.85	119.8±3.40	126.5±2.81	132.3±2.38^{a}	143.3±3.99$^{c,\Upsilon,*}$
Diabetic Control	-	116.0±3.97	115.4±4.97	108.5±4.66	102.0±4.64	90.33±5.49$^{a,\alpha}$
AH	125	125.7±2.49	123.3±2.39	128.8±2.43	135.3±3.09	160.0±10.16$^{c,\Upsilon,**}$
AH	250	115.3±3.77	116.5±3.50	120.5±3.44	129.0±4.38	142.3±7.19$^{b,\beta,*}$
AH	500	119.7±2.17	120.5±2.19	124.5±3.30	133.0±3.00^{a}	152.0±3.61$^{c,\delta,***}$
Metformin	100	112.8±6.33	108.0±6.27	107.8±3.99	118.8±4.27	138.3±7.50$^{\alpha,*}$

Values are mean ± S.E.M. (n = 5). $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ vs. day 7; $^{\alpha}p<0.05$, $^{\beta}p<0.01$, $^{\Upsilon}p<0.001$, $^{\delta}p<0.0001$ vs. day 14; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ vs. day 21. AH - *A. hybridus*

Histopathological evaluation of vital organs of alloxan-nicotinamide induced diabetic rats administered hydroethanolic leaf extract of *A. hybridus*

The summary of histologic sections of the heart in all the treatment groups show interlacing fascicles of cardiac myocytes/myocardial cells with no abnormalities seen (Figure 2). The histologic sections of the kidneys in all the treatment groups show normocellular glomerular tufts disposed on a background containing renal tubules with no abnormalities seen (Figure 3). The summary of the histologic sections of the pancreas shows smudgy pink masses that do not contain viable nuclei in all the study groups. This indicates pancreatic necrosis (Figure 4). Histologic sections of the liver in all the treatment groups show radial plates of hepatocytes with no cytoplasmic fat vacuoles or areas of necrosis seen (Figure 5).

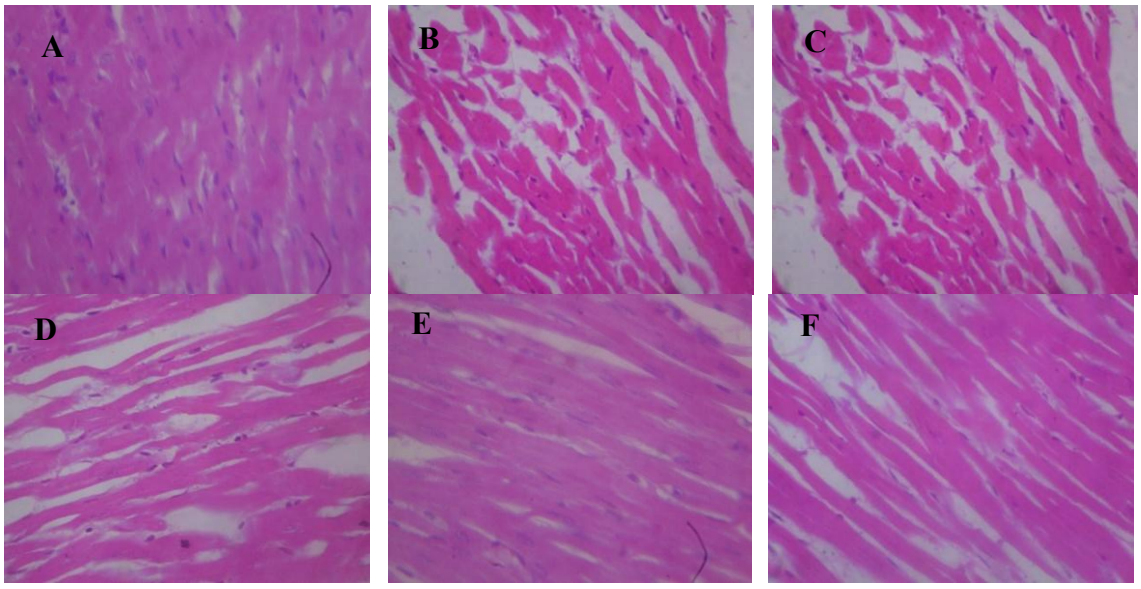

Figure 2: Photomicrograph of heart tissue of rats of normal control (A), diabetic control (B), AH 125 mg/kg (C), AH 250 mg/kg (D), AH 500 mg/kg (E), and metformin 100 mg/kg (F). H&E stain (×400).

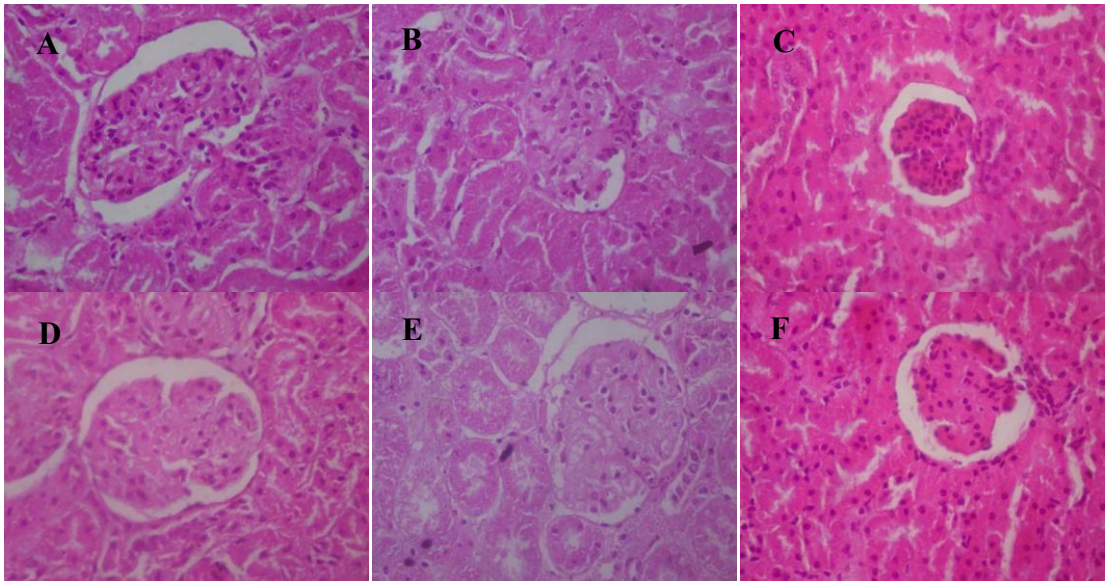

Figure 3: Photomicrograph of kidney tissue of rats of normal control (A), diabetic control (B), AH 125 mg/kg (C), AH 250 mg/kg (D), AH 500 mg/kg (E), and metformin 100 mg/kg (F). H&E stain (×400).

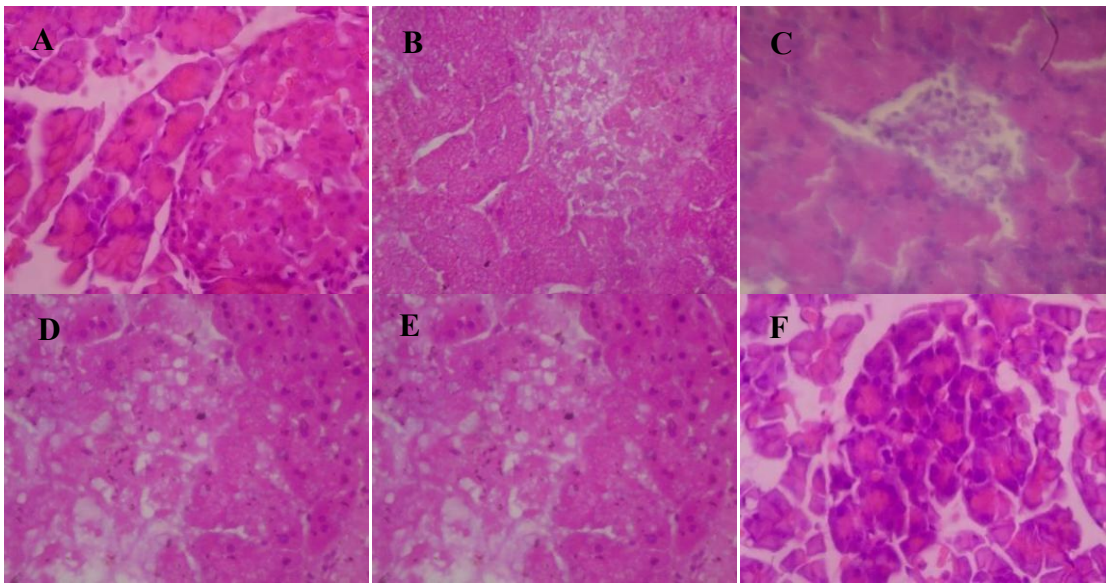

Figure 4: Photomicrograph of pancreatic tissue of rats of normal control (A), diabetic control (B), AH 125 mg/kg (C), AH 250 mg/kg (D), AH 500 mg/kg (E), and metformin 100 mg/kg (F). H&E stain (×400).

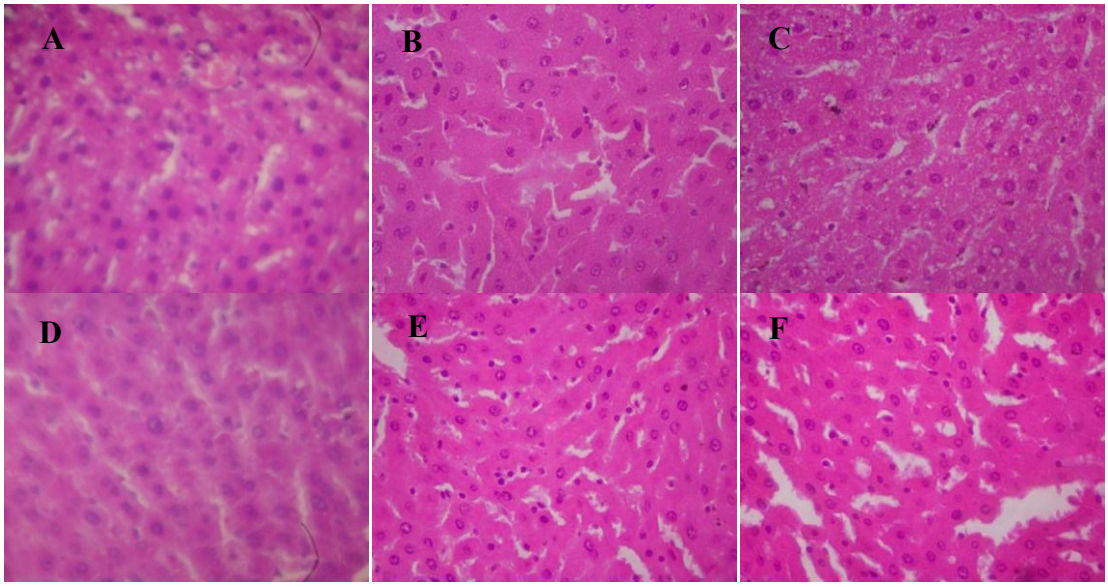

Figure 5: Photomicrograph of liver tissue of rats of normal control (A), diabetic control (B), AH 125 mg/kg (C), AH 250 mg/kg (D), AH 500 mg/kg (E), and metformin 100 mg/kg (F). H&E stain (×400).

DISCUSSION

Diabetes is a worldwide epidemic with increasing prevalence due to changing lifestyles, increasing obesity and age of the population.[1] As reported by Antar *et al.*,[5] it is a complex metabolic disorder that affects multiple organ systems and leads to various complications. These complications, as established and reported, include kidney disease, cardiovascular disease, immune dysfunction, retinopathy, and neuropathy. The pharmacotherapeutic treatment of diabetes targets associated pathologic pathways, including inflammation, oxidative stress, and metabolic memory with underlying epigenetic and cellular changes.[5] Effective management of diabetes will ameliorate associated symptoms, including increased appetite, polydipsia, dysuria, weight loss, increased appetite, and vision problems etc. The various classes of conventional antidiabetic drugs are linked to worrisome side-effects, including muscle pain, liver enzyme abnormalities, gastrointestinal symptoms, weight gain, oedema, risk of fractures, vitamin B12 deficiency, lactic acidosis (rarely), urinary tract infections, genital yeast infections, increased urination, and hypoglycemia.[5-10] These side-effects warrant the search for newer antidiabetic agents with better efficacy and safety profile, especially from natural sources. According to Schmidt *et al.*,[27] the use of herbs is a core part of all systems of traditional medicine which has gained widespread patronage, especially in rural and semi-urban areas of developing countries, due to inadequacy or absence of orthodox medicine facility. It has been estimated that about 25% of the drugs prescribed worldwide are derived from plants, and 121 such active compounds are in use.[11,28] This study was conducted to evaluate the effects of *Amaranthus hybridus* hydroethanol leaf extract in alloxan-nicotinamide induced Type 2 diabetes.

In respect of effect on normal and glucose-loaded rats, the extract at the various doses and Metformin (standard drug) caused reductions in glucose level pre- and post-glucose loading. At their respective peaks, the effect of the extract was slightly higher than that of Metformin. The acute treatment of diabetic rats with the extract and Metformin, separately, did not elicit any significant change in blood glucose level. However, the subchronic treatment of diabetic rats with the extract and Metformin, separately, elicited significant reductions in blood glucose level from days 21-35. The peak effects of the extract on days 28 and 35 were lower compared with those of Metformin. Balasubramanian *et al.*[29] had previously reported that the ethanol leaf extract of *A. hybridus* significantly reduced hyperglycemia-associated oxidative damage in a Type 1 (Streptozotocin-induced) diabetes model. Balasubramanian and Karthikeyan[16] also reported significant nephron-protective effect against oxidative damage in Type 1 diabetic rats.

As mentioned earlier, the symptoms of diabetes include increased appetite, polydipsia, dysuria, weight loss, increased appetite, and vision problems etc. The diabetic control rats in this study had initial higher food take but experienced

reduction in food intake in the later stages. At the later stages, the extract and Metformin increased food intake of the diabetic rats in a comparable manner. The trend of effect observed in diabetic rats, extract and Metformin interventions on water intake of the rats followed the same pattern as that of the food intake. Summarily, the extract at the various doses and Metformin reversed the impact of diabetes on food and water intake in this study.

Oxidative stress has been implicated in the pathophysiology of diabetes. It has also been reported to be the main driver of diabetic complications and the targeting of pathways involved in reactive oxygen species (ROS) production and enhancing endogenous antioxidant defense mechanisms offers immense benefits in the treatment of diabetes.[5] Oxidative stress results in diminution of *in-vivo* antioxidants, including reduced glutathione, superoxide dismutase and catalase, while increasing the level of malondialdehyde (MDA; marker of lipid peroxidation). In this study, the extract and Metformin enhanced *in-vivo* antioxidants while reducing MDA level in liver, kidneys, heart and pancreas in the animal model of Type 2 diabetes. As mentioned earlier, it has been previously reported that the ethanol leaf extract of *A. hybridus* significantly reduced hyperglycemia-associated oxidative damage and offered significant nephron-protective effect against oxidative damage in Type 1 (Streptozotocin-induced) diabetic rats.[16,29]

It has been established that reductions in body weight gain and internal organ weights are simple and sensitive indices of toxicity after exposure to toxic substances.[30,31] As mentioned earlier, weight loss is one of the symptoms of diabetes. In respect of the liver and pancreas, the extract and Metformin significantly reversed the diabetes induced reduction in weight of these vital organs. The extract and Metformin also significantly increased the body weight of rats in comparison with the significant body weight reductions observed in the diabetic control rats. These finding on vital organs and body weight gives credence to the beneficial effect of the extract in the Type 2 model of diabetes in this study.

Except for the pancreas, histologic assessment of the liver, kidneys and heart showed no significant deleterious presentations. The hydroethanolic leaf extract of *A. hybridus* did not produce any mortality when administered orally at the dose of 2000 mg/kg and no visible delayed toxicity or mortality was observed when animals were monitored for further 14 days. This is a pointer to the safety of the extract as agents with acute oral LD_{50} values greater 2000 mg/kg has been reported to be practically non-toxic.[32] GC-MS analysis of the extract revealed the presence of Phytol; Octadecadienoic acid, ethyl ester; Ethyl oleate; 9-Octadecenoic acid (Z)-,2,3-dihydroxypropyl ester; 9,12-Octadecadienoic acid; Octadecadienoic acid, 2-hydroxy-1,3-propane; 12-Methyl-E,E-2,13-octadecadien-1-ol; 2-Methyl-Z,Z-3,13-octadecadienol; and Beta carotene as the most abundant phytochemical principles. One or a combination of these chemical principles may be responsible for the antidiabetic effects of the extract established in the model of Type 2 diabetes in this study.

CONCLUSION

The results obtained in this study suggest that the hydroethanol leaf extract of *Amaranthus hybridus* possess antidiabetic effect in Type 2 animal model possibly due to the enhancement of *in-vivo* antioxidants and consequent amelioration of oxidative stress. It may therefore be useful as nutraceutical agent in the management of diabetes.

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