



Evaluation of Anti-diabetics and Cardiovascular Effects of *Parinari curatellifolia* Seed Extract and *Anthoclista vogelli* Root Extract Individually and Combined on Postprandial and Alloxan-Induced Diabetic Albino Rats

**S. O. Ogonnia¹, G. O. Mbaka^{2*}, E. N. Anyika³, O. Ladiju¹,
H. N. Igbokwe⁴, J. E. Emordi⁵ and N. Nwakakwa⁶**

¹Department of Pharmacognosy, Faculty of Pharmacy, Idi-Araba,
University of Lagos, Nigeria.

²Department of Anatomy, College of Medicine, Lagos State University, Ikeja,
Lagos, Nigeria.

³Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy,
University of Lagos, Idi-Araba, Lagos, Nigeria.

⁴Department of Pharm-Technology and Pharm-Microbiology, Faculty of Pharmacy,
University of Lagos, Idi-Araba, Lagos Nigeria

⁵Department of Pharmacology, College of Medicine, Ambrose Alli University,
Ekpoma, Edo State, Nigeria.

⁶Federal College of Complementary and Alternative Medicine, Lagos, Nigeria.

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ABSTRACT

Objective: To study toxicity, anti-diabetic and cardiovascular effects of hydro-ethanolic extracts of *Parinari curatellifolia* seed extract and *Aristolochia vogelii* roots extract and (1:1) mixture of the above two extracts.

Materials and Methods: Twenty Wister strain albino rats were randomly assigned to four groups; A, B, C and D with each consisting of five animals received extracts as follows: Group I, *P. curatellifolia* and *A. vogelii* mixture (1:1) (500 mg/kg bwt); Group II, A.

vogelli (500 mg/kg bwt); Group III, *P. curatellifolia* seed extract (500 mg/kg bwt); Group IV, 0.5 ml (2% w/v) acacia solution and served as control. After 30 min, the animals were each administered orally with 40% (w/v) glucose at a dose of 1ml /100 g bwt. Blood glucose levels were then monitored at 30, 60, and 120 min. intervals and reported as the average glucose level of each group. Another set of twenty five rats (diabetic rats) were randomly distributed into five groups of five animals each while the additional sixth group was the positive control consisting of five normal rats. Treatments were as follows: Group I, diabetic treated with *A. vogelli* at a dose of 500 mg/kg bwt; Group II, diabetic treated with *P. curatellifolia* at a dose of 500 mg/kg bwt; Group III, diabetic treated with glibenclamide 600µg /kg bwt; Group IV, diabetic treated with mixture of *Parinari curatellifolia* and *A. vogelli* (1:1) (500 mg/kg bwt); Group V, diabetic untreated (control negative) while group VI was the positive control.

Results: A significant reduction in postprandial sugar level was observed after 30 min in all treatments. The extracts individually and in combined form also showed effective decrease in plasma glucose levels on the diabetic rats. There were significant reductions ($p<0.05$) in low density lipoprotein (LDL)-cholesterol levels and significant increase ($p<0.05$) in high density lipoprotein (HDL)-cholesterol in the treated diabetic group compared to the negative control. Furthermore, significant reductions in aspartate aminotransferases (AST) and alanine aminotransferases (ALT) levels were observed in the treated diabetic animals compared to the untreated. Also significant reduction in the creatinine and increase in the protein levels respectively were observed in the treated diabetic groups.

Conclusion: The results showed that the respective extracts and the extract mixture had both good hypoglycaemic activity and beneficial effects on cardiovascular risk factors.

Keywords: Acute-toxicity; postprandial; *Parinari curatellifolia*; *Aristolochia vogelii*; diabetes;

1. INTRODUCTION

Diabetes mellitus (DM) is a major degenerative disease in the world today afflicting many lives both in the developed and developing countries. It has been succinctly described as the common metabolic disorder of carbohydrate and fat metabolism, which is due to absolute or relative lack of insulin and is characterized by hyperglycaemia (Sharon and Marvin, 1975; Walter, 1977). Diabetes is a multiple physiological disease. It has been defined as “a state of premature cardiovascular death that is associated with chronic hyperglycaemia and also associated with blindness and renal failure” (Fisher and Shaw, 2001). This assertion was to draw attention and to encourage multiple clinical approaches that would altogether help reduce cardiovascular risk factors in diabetic patients. It is evident that many deaths associated with diabetes are attributable to cardiovascular and vascular diseases. Two main types of diabetes based on their clinical manifestations were identified as type I diabetes-known as juvenile onset or insulin-dependent diabetes mellitus, IDDM and type II diabetes or non insulin dependent diabetes mellitus (NIDDM) (Gale, 2001; Ogbornia et al., 2010). Type II diabetes is the more prevalent form and has its underlying metabolic causes with combined effects of impairment in the insulin mediated glucose disposal and defective secretion of insulin by the β -cells of the pancreas (Grundy et al., 1999).

The major goals in the treatment of diabetes has been to keep both short-term and long-term glucose levels within acceptable limits, thereby reducing the risk of long term

complications (Park et al., 2009). This could be achieved by optimizing both fasting blood glucose and postprandial glucose levels which has been found to be very important in achieving near normal glucose levels. Postprandial glucose levels have been reported to serve as a better maker of glycaemic control than fasting blood sugar levels (Park et al., 2009). Some drugs, therefore, have been developed to improve postprandial hyperglycaemia by inhibiting α -glucosidase activity.

Diabetes has been conventionally treated with orthodox medicines which function as hypoglycaemic agents, or insulin production modulators and/or lipoprotein lowering agents (Ogbonnia *et al.*, 2008). Sulfonylurea and metformin are valuable in the treatment for hyperglycaemia especially in NIDDM but they are often unable to lower glucose concentrations to within the normal range, or to reinstate a normal pattern of glucose homeostasis (Senthilvel et al., 2006; Ogbonnia et al., 2010). Conventionally oral hypoglycaemic agents such as sulphonylureas and biguanides have hypoglycaemic activities or insulin production modulators and/or lipoprotein lowering activity. They have been commonly employed in the management of diabetes especially in type II diabetes but are associated with serious side effects (Bunyapraphatsara et al., 1996, Ogbonnia et al., 2008). Sulphonylureas are the most widely used oral hypoglycaemic agents but may have some adverse effects such as exacerbating hyperinsulinaemia and causing weight gain in patients (Egwim, 2005) while biguanides are only weak hypoglycaemics agents and have limited clinical use.

Even when effective glycaemic control is achieved, the use of these drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanied undesirable effects (El.Nagar et al., 2005). In addition they are not suitable for use during pregnancy (Senthilvel et al., 2006; Sushruta et al., 2006).

For these reasons, there is a great need for a search for an acceptable, cheap and safe glucose lowering agents that would be effective in the treatment of diabetes and devoid of serious side effects that is associated with currently used oral hypoglycaemic agents. To overcome the problems associated with the oral hypoglycaemic agents, a compelling case is made for more effective modalities to prevent and treat diabetes and to meet this demand an increasing number of people are turning to alternative therapies (Vuksan et al., 2001). Herbs and marine sources are considered the best option and a large number of plants and their products are now recognized to be effective as antidiabetics and are being screened scientifically for such activity. Also the use of various plant parts in herbal remedies is a common practice with traditional herbalists as they believe that the combination of many plant materials (compound recipe) in a preparation gives a better result owing to their additive or synergistic activity.

P. curatellifolia seeds are widely employed in traditional medicine in the treatment of various diseases and the antidiabetic activity of the seeds has been evaluated (Ogbonnia et al., 2008). Also the toxicity effects in rats have been reported (Ogbonnia et al., 2008). *Aristolochia vogelii* has been widely employed in traditional medicine by the Iggede ethnic group in Nigeria in the treatment of fever, as laxative and as anti bacterial agent in the treatment of gonorrhoea and syphilis (Igoli et al., 2005). Its stem bark and leaves decoctions are ethno-botanically employed by some regions in Cameroon in the treatment of various diseases (Jiofack et al., 2009).

Therefore, the objective of this paper is to study the toxicity, anti-diabetic and cardiovascular effects of hydro-ethanolic extracts of *Parinari curatellifolia* seed extract and *Aristolochia vogelii* roots extract and (1:1) mixture of the above two extracts.

2. MATERIALS AND METHODS

2.1 COLLECTION OF PLANT MATERIALS

The seeds of *Parinari curatellifolia* and *A. vogelii* were authenticated by a taxonomist, Dr A.A. Adekunle of Department of Botany, University of Lagos. Their voucher specimens with numbers LUH 3440 and LUH 2914 respectively were deposited with the Department of Botany herbarium, University of Lagos, Akoka.

2.1.1 Preparation of the plant materials for extraction

The *Parinari curatellifolia* seeds, with their coats removed, were dried in an oven at 45 °C for five days (Ogbonnia et al., 2010). *A. vogelii* roots were washed with copious tap water to remove foreign matters, chopped into small pieces and dried in an oven at 40 °C for a week. The dried plant materials were milled to coarse particles respectively and extracted as follows:

- (a) 500 g each coarse powder of *P. curatellifolia* seed and *A. vogelii* were respectively defatted with 1750 ml petroleum ether (60^o - 80°C) for 2 – 3 hrs in a Soxhlet apparatus, the resulting marc was dried and subsequently extracted with 1.5 L of aqueous ethanol (80%) in Soxhlet apparatus for 5 hrs . The extracts were then concentrated in a rotary evaporator and dried in an oven at 40°C which yielded 34.5 g and 42.0 g dry masses respectively (Ogbonnia et al., 2008).

2.2 LABORATORY ANIMALS

Healthy Wistar strain albino rats weighing 160 ± 20 g were obtained from the Laboratory Animal Center of the College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria. The rats were housed in clean cages and kept in a well ventilated room and allowed to acclimatize to the laboratory condition for one week before the experiment. They were fed with standard animal pellet (Pfizer Feeds Plc., Nigeria) and water *ad libitum*. The use and care of the animals, and the experimental protocol were in strict compliance with the Institute of Laboratory Animal Research (ILAR) guidelines on the use and care of animals, in experimental studies (ILAR, 1996).

The animals were distributed randomly into four groups of five animals each for postprandial study and into six groups of five rats each for the alloxan-induced diabetic experiment.

2.3 ACUTE TOXICITY STUDY

The toxicity study was carried out using thirtyfive (35) male and female Swiss albino mice weighing 22.5±2.5g. The animals were randomly distributed into one control group and six treated groups, containing five animals per group. After overnight fasting, the control group received 0.3mL Acacia (2%) suspension orally. The doses, 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0g /kg of body weight were respectively administered orally to the treated groups from 80 % (w/v) of the mixture (1:1) of the extracts in Acacia (2%) suspension. The animals were observed continuously for the first 4 hours and then for each hour for the next 12 hours,

followed by 6 hourly intervals for the next 56 hours giving a total of 72 hrs observations (Shah et al., 1997; Bürger et al., 2005). Death or changes in general behaviour and other physiological activities were noted. The LD₅₀ of the extract would be determined by plotting a graph of probit on the Y-axis against the log dose on the X-axis.

2.4 POSTPRANDIAL TEST

This measures the body's ability to metabolize carbohydrates and produce insulin. Twenty albino rats were randomly assigned to four groups; A, B, C and D with each consisting of five animals. They were fasted for about 18 hrs with access to only water (Egwim, 2005). Glucometer (ACCU-CHEK, Roche Diagnostics) was used to estimate their initial blood sugar level. The extracts suspension (15%w/v) was respectively prepared by dispersing 3.750 g of the extract and in case of the mixture 1.8750 g of each extract combined, in 25mL acacia (2%w/v) solution.

The animals were treated as follows:

- Group I received *P. curatellifolia* and *A. vogelli* mixture (1:1) (500 mg/kg bwt)
- Group II received *A. vogelli* (500 mg/kg bwt)
- Group III received *P. curatellifolia* seed extract (500 mg/kg bwt)
- Group IV received 0.5 ml (2% w/v) acacia solution and served as control.

After 30 min, each animal was administered orally with 40 % (w/v) glucose at a dose of 1ml /100 g bwt. Blood glucose levels were monitored at 30, 60, and 120 min. intervals and reported as the average glucose level of each group.

2.5 ALLOXAN-INDUCED DIABETIC EXPERIMENT

Diabetes was induced by intraperitoneal (ip) injection of alloxan monohydrate (150 mg/kg bwt) dissolved in normal saline. After 72 hrs, blood was withdrawn for blood glucose estimation monitored with a glucometer (ACCU-CHEK, Roche Diagnostics). The animals with blood glucose level ≥ 200 mg/dl were considered diabetic and included in the experiment.

The diabetic animals were randomly distributed into five groups of five animals each while the last group, the positive control, had five normal rats.

Treatments were as follows:

- Group I: Alloxan diabetic treated with *A. vogelli* at a dose of 500 mg/kg bwt.
- Group II: Alloxan diabetic treated with *P. curatellifolia* at a dose of 500 mg/kg bwt.
- Group III: Alloxan diabetic treated with Glibenclamide 600 μ g /kg bwt (Mahadi et al., 2003; Ogbonnia et al., 2010).
- Group IV: Alloxan diabetic treated with mixture of *Parinari curatellifolia* and *A. vogelli* (500 mg/kg bwt).
- Group V: Alloxan diabetic untreated (control negative).
- Group VI: Normal rats (positive control).

2.6 EVALUATION OF LIPID PROFILE

The initial weights of the animals were recorded thereafter, weights were taken at seven days intervals from the beginning of the treatment. On the 31st day, after overnight fast, the animals were sacrificed under mild diethyl ether anaesthesia and blood was obtained via cardiac puncture into fluoride oxalate, heparinized and EDTA containers. The blood collected with fluoride oxalate tube was centrifuged within 5 min of collection at 4000 g for 10 min and plasma obtained was used to determine the blood glucose level. The total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL-cholesterol) levels and other biochemical parameters were estimated with heparinized blood using precipitation and modified enzymatic procedures from Sigma Diagnostics (Wasan *et al.*, 2001). Low density lipoprotein (LDL-cholesterol) level was calculated using Friedwald equation (Crook, 2006). Plasma was analyzed for alanine amino transferase (ALT) and aspartate amino transferase (AST) and creatinine by standard enzymatic assay method. The plasma protein content was determined using enzymatic spectroscopic methods (Hussain and Eshrat, 2002). The haematological parameters and other blood chemistries were determined using blood collected with EDTA containers.

2.7 EVALUATION OF HAEMATOLOGICAL PARAMETERS

Diethyl ether was used to anaesthetize the animals before blood samples were collected through heart puncture into EDTA tubes for analysis of haematological parameters. The blood samples were analyzed for red blood cells (RBC) by haemocytometric method (Dacie and Lewis, 1984); the haemoglobin (Hb) content was by Cyanmethaemoglobin (Drabkin) method (Dacie and Lewis, 1984); packed cell volume (PCV) was according to Ekaidem *et al.*, (2006) while white blood cells (WBC) and its differentials (neutrophil, eosinophil, basophil, lymphocyte and monocyte) were determined as described by Dacie and Lewis (1984).

2.8 TISSUE HISTOLOGY

The hepatic tissue and pancreas were fixed in Bouin's fluid for seven days before embedding in paraffin wax. Each organ tissue was sectioned at 5µm. The hepatic tissue was stained with Haematoxylin and Eosin (H and E) stain while the pancreas tissue was stained with Aldehyde fuchsin. The slide specimens were examined under light microscope at high power magnification for changes in organ architecture and photomicrographs were taken.

2.9 STATISTICAL ANALYSIS

Student's t- test was used and differences were considered significant at $p < 0.05$ or $p < 0.01$. All data have been expressed as mean \pm standard error of the mean.

3. RESULTS AND DISCUSSION

3.1 ACUTE TOXICITY

The acute toxicity study result (Table 1), showed that three out of the five animals that received 20.0 g/kg bwt of the extract died within 4 hr (60 % death) while the animals that received 10 g/kg body weight survived beyond 24 hr. The LD₅₀ of the drug was therefore calculated to be 8.18g/kg bwt. The macroscopic examinations of the animals' organs treated with glibenclamide, the respective extracts and the mixture of the extracts did not show any

changes in colour while the organs of untreated diabetic animals showed some changes compared to the normal control.

Table 1. Acute toxicity of the mixture (1:1) of *A. vogelli* root and *P. curatellifolia* extract in mice

Groups	Dose (g/kg)	Log dose	24 hr. motility	% Motility	Probit	Probit (Approx)
I	1.0	3.00	0/5	0.0	0.000	0.0
II	2.5	3.397	0/5	0.0	0.000	0.0
III	5.0	3.699	0/5	0.0	0.000	0.0
IV	10.0	4.000	1/5	2.0	4.1534	4.1
V	15.0	4.176	1/5	2.0	4.1534	4.1
VI	20.0	4.301	3/5	60.0	5.2533	5.2

Control group received 0.3ml each of acacia (2%) solution, Group I: 25mg, Group II: 62.5mg, Group III: 125 mg, Group IV: 250mg, Group V: 375 mg, Group VI: 500mg

3.2 POSTPRANDIAL TEST

The postprandial test result (Figure 1) showed a significant decrease ($p \leq 0.05$) in blood glucose levels in all treated groups after 30 min of oral glucose administration compared to the control. The extract mixture showed more effective blood glucose controlling capacity than single extract recipes.

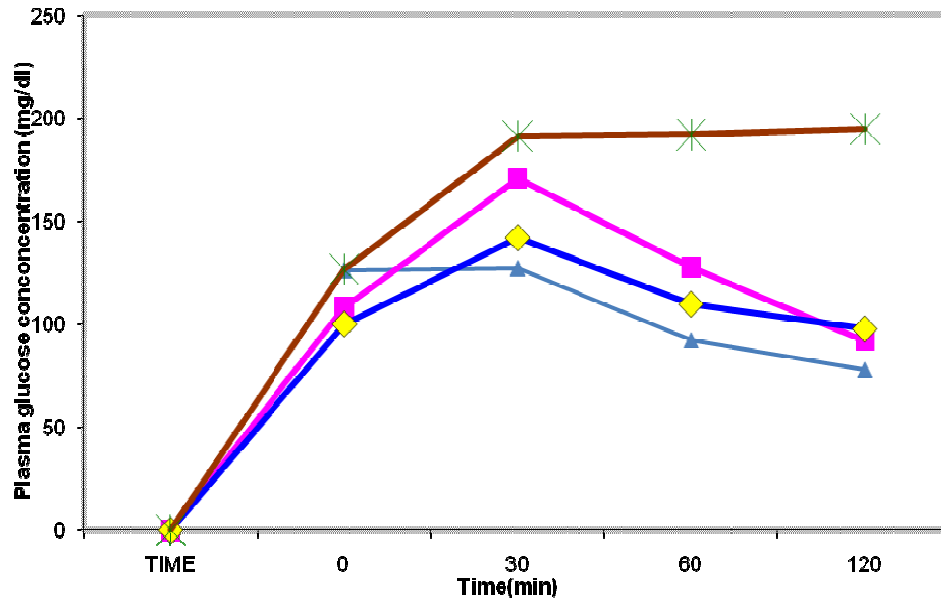


Fig. 1. Evaluation of *A. vogelli* and *P. curatefolia* extracts mixture (1:1), the respective extracts and control effects on prostrandial glucose level in rats

✱ control administered 0.5ml 2% acacia suspension; ▲ *A. vogelli* and *P. curatellifolia* extracts mixture (1:1) extract 500mg/kg body weight; ■ *A. vogelli* extract 500 mg/kg body weight; ◆ *P. curatellifolia* extract 500 mg/kg body weight;

3.3 ALLOXAN-INDUCED STUDY

Figure 2 summarized the results of body weight changes of diabetic animals treated with the different extracts, the mixture and glibenclamide. There was no significant increase in body weight of the diabetic animals treated with the mixture, *A. vogelii* and glibenclamide while a significant weight gain was observed in the group treated with *P. curatellelifolia* compared to the initial body weight. On the other hand, significant decrease ($p<0.05$) in body weight was observed in the untreated diabetic animals compared to the initial body weight.

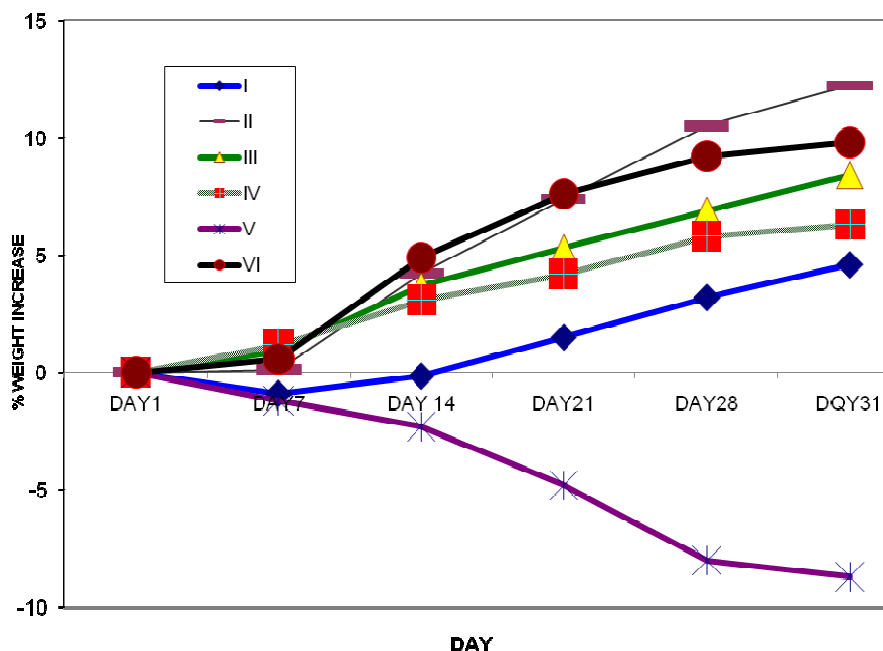


Fig. 2. Percentage increase/decrease in weight of the diabetic rats treated with the extracts, their mixture (1:1), the reference drug (glibenclamide) and induced not treated

♦ Group I: Diabetic rats treated with *A. vogelii*, 500mg extract/kg bwt; - Group II: Diabetic rats treated with *P. curatellelifolia* 500mg extract/kg bwt; ▲ Group III: diabetic rats treated with Glibenclamide 600µg /bwt; + Group IV: Diabetic rats treated with mixture (1:1) of the extracts 500mg /kg bwt; ✕ Group V: Diabetic not treated; ● Group VI Control rats received 0.5ml 2% Acacia suspension

Table 2 summarized the results of the extracts and glibenclamide effects on the biochemical parameters. There was glycaemic decrease in diabetic animals treated with the extracts separately, the extract mixture and glibenclamide. In contrast, plasma glucose level in the untreated animals showed progressive increase. There was equally significant decrease ($p<0.05$) in the plasma AST and ALT levels in the animals treated with the extracts separately, the mixture and glibenclamide compared to the diabetic control.

There was significant decrease ($p<0.05$) in TC in all the treated groups while significant decrease in TG occurred only in the group that received the extract mixture. There was however, marginal decrease in TG level in the other groups that received treatment compared to the diabetic control. LDL-cholesterol also exhibited marginal decrease in all the treated groups. Significant increase ($p<0.05$) in HDL-cholesterol occurred in the group that

received the extract mixture while the animals treated with the extracts separately and glibenclamide showed slight increase. There was no significant change in the levels of total protein and albumin in the groups treated with the extracts and glibenclamide compared to the normal control. A significant increase in creatinine level was observed in treatment with *P. curatellelifolia* compared to the normal control. There were however, significant decrease ($p < 0.05$) in the total bilirubin in the groups that were administered with the mixture and *A. vogelii* while significant increase ($p < 0.05$) occurred in the group that received *P. curatellelifolia* and diabetic untreated compared to normal control. Also a significant increase in urea was observed in the groups that were administered with *A. vogelii*, *P. curatellelifolia* and diabetic untreated compared to normal control.

The effect of the extracts on haematological values was summarized in Table 3. There was significant ($p < 0.01$) increase in the Hb contents in the treated groups with the exception of the group that received the extract mixture compared to the normal control. Similarly, significant increase was observed in PCV and WBC of all the treated groups compared to the normal control whereas in diabetic control, marked decrease occurred in Hb, PCV and WBC. No changes were observed in MCV, MCH and MCHC in all the groups.

The histology of pancreatic tissue of diabetic animals treated with the extracts separately, the mixture, glibenclamide, diabetic control and normal control is shown in Figure 3. The photomicrograph of normal pancreatic tissue (Fig. 3a) demonstrated normal morphological features of beta cells. The compact islet organization showed intact cellular arrangement *in situ*. The photomicrograph of pancreatic tissue of diabetic animals treated with *A. vogelii* (Fig. 3b) showed significant lesion on the beta cells. In fig. 3c, the pancreas of alloxan induced animals treated with *P. curatellelifolia* showed marked necrotic changes on the beta cells with few survivor cells interspacing. The daonil treated (Fig. 3d) showed spots of necrotic changes otherwise it had predominantly viable beta cells. The pancreatic tissue of diabetic animals treated with the extract mixture (Fig. 3e) equally showed marked necrotic changes but with more survivor beta cells compared to treatment with single extract recipe. The photomicrograph of diabetic untreated animals (Fig. 3f) showed more severe necrosis of beta cell forming an aggregation of amorphous eosinophilia.

The cyto-architecture of normal hepatic tissue (Fig. 4a), showed hepatocytes radially arranged from the lobular margins towards the centre vein with each column interspaced by sinusoids. The hepatic tissue of *A. vogelii* treated (Fig. 4b) showed portal trait dilatation however, hepatic tissue pathology was not observed. The hepatic tissue of daonil treated (Fig. 4c) showed mild pyknotic changes coupled with early periportal inflammation. The photomicrograph of the group treated with the extract mixture (Fig. 4d) showed early edematous changes around the hepatic parenchyma and the portal trait. The hepatic tissue of *P. curatellelifolia* (Fig. 4e) treated showed mild edematous changes. The liver tissues of the diabetic control animals (Fig. 4f) showed no evidence of pathological changes.

Table 2. Plasma glucose level and other biochemical profiles of diabetic rats treated with the extracts, mixtures (1:1), glibenclamide respectively, untreated diabetic rats and the normal (control)

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI
Glucose (mmol/L)	2.7±0.0 *	2.2±0.1*	1.6± 0.0*	1.8±0.1*	20.9± 4.5	4.9±0.2
TG (mmol/L)	0.6± 0.0	0.7±0.0	0.6±0.0	0.3±0.0*	1.1±0.3	0.4±0.0
T.CHOL (mmol/L)	1.2± 0.3*	1.3±0.2*	1.4±0.1*	1.4±0.0*	1.8±0.1*	1.6±0.1
HDL (mmol/L)	1.3±0.1	1.4±0.2	1.2±0.1	1.8±0.2*	0.8±0.4	1.5±0.1
LDL (mmol/L)	0.2±0.0	0.2±0.3	0.3±0.1	0.3±0.1	0.4±0.1	0.2±0.1
T. PROT. (g/l)	8.8±2.0	7.6±1.5	9.0±1.6	8.3± 2.1	8.1±2.0	8.2± 2.0
ALB (g/l)	34.5±2.1	34.5±2.1	37.0±3.0	30.3±1.2	35.9±2.5	36.3±2.7
AST (U/L)	15.8±0.5*	14.4±0.2*	16.2±1.2*	14.6±0.7*	21.0±2.4	18.0±0.1
ALT (U/L)	6.5±0.3*	9.7±1.2*	8.1±0.5*	6.5±0.5*	11.9± 1.5	8.9± 2.9
Creat. (µmol/l)	79.1±3.1	66.3±2.7*	88.4±3.3	76.5±2.6	80.4±2.7	73.4±3.5
T.BIL (µmol/l)	90.3±5.2*	112.4±4.5*	100.8±2.7*	80.5±5.2*	198.4±2.8*	100.6±4.6
ALPU/L	320.3±3.5	457.2±4.5	243.2±3.8	297.0±5.2	186.8±3.0	186.8±3.0
Urea(µmol/l)	11.5±0.7*	17.3±1.3*	4.2±0.1	7.5±0.1	12.3±0.1*	8.2±0.2

Mean ± sem, (n=5) *p<0.05; ** p<0.01 vs. control group. .

Group I: Diabetic rats treated with 500mg extract/kg bwt *A. vogelii*; Group II: Diabetic rats treated with 500mg extract/kg bwt *P. curatellifolia*; Group III: diabetic rats treated with Glibenclamide 600µg bwt; Group IV: Diabetic rats treated with 500mg/kg bwt of mixture (1:1); Group V: Diabetic not treated; Group VI Control rats received 0.5ml 2% Acacia suspension

Table 3. Haematological values of control and diabetic rats treated with individual extracts, the mixture (1:1), the reference drug glibenclamide and the control for 30 days in subchronic study

PARAMETER	GP I	GPII	GPIII	GPIV	GPV	GPVI
RBC×10 ⁶	7.5±0.0	6.5± 0.03	7.7±0.4	6.5±0.5	4.2±0.1*	7.1±0.6
Hb(g/dl)	14.1±1.5**	14.0±1.0**	14.2±0.7**	12.6±1.5	7.7±0.5*	13.3±0.8
PCV(%)	26.0±2.2*	30.0 ± 4.5*	41.0± 3.2*	40.5±1.8*	15.6± 0.4	20.0± 2.7
WBC×10 ³	6.5±0.5*	6.3 ± 0.2 *	9.2 ± 0.2*	5.3±0.0*	4.1± 0.0	4.3±0.1
MCV(FL)	54.9±2.5	51.9 ±3.0	52.3± 1.7	54.2±3.5	51.1±2.0	57.4± 3.0
MCH(pg)	18.9±1.6	17.9±2.1	18.3± 0.5	18.9±1.0	18.2± 1.2	18.8± 1.5
MCHC(g/dl)	34.4±1.2	32.4±0.7	35.1±0.5	34.9±0.1	35.6± 3.5	32.8± 3.5

MCH: mean cell haemoglobin; MCHC: mean cell haemoglobin concentration, and MCV: mean cell volume

*Mean ± sem, (n=5) *p<0.05; ** p<0.01 vs control group. .*

Group I: Diabetic rats treated with 500mg extract/kg bwt A. vogelii; Group II: Diabetic rats treated with 500mg extract/kg bwt P. curatellifolia; Group III: diabetic rats treated with Glibenclamide 600µg bwt; Group IV: Diabetic rats treated with 500mg/kg bwt of mixture (1:1); Group V: Diabetic not treated; Group VI Control rats received 0.5ml 2% Acacia

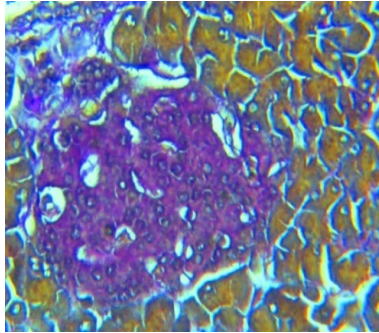


Fig. 3a. Normal morphology of beta cells stained with aldehyde fuchsin. X 400

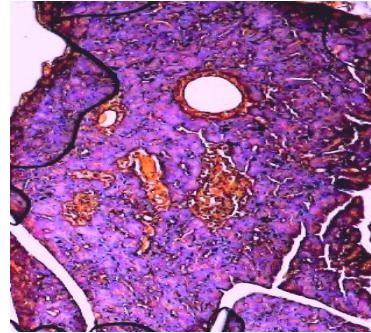


Fig. 3b. *A. vogelii* treated showed severe lesion of beta cells. X 200

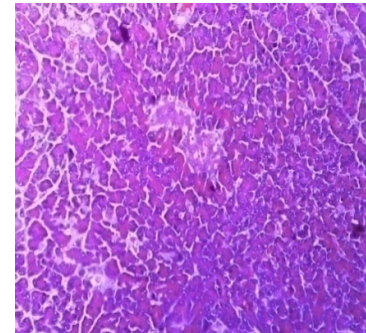


Fig. 3c. *P. curatellefolia* treated showed extensive necrosis of beta cells. X 200

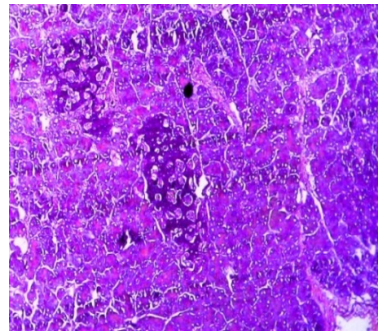


Fig. 3d. (Daonil treated) showed mild necrotic changes. X 200

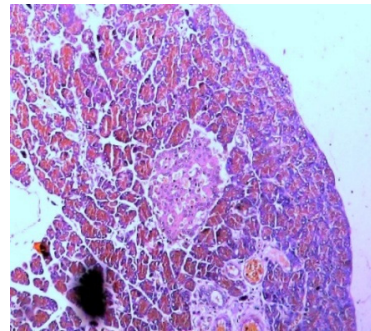


Fig. 3e. (Mixture) indicated partial necrotic changes of beta cells. X 200

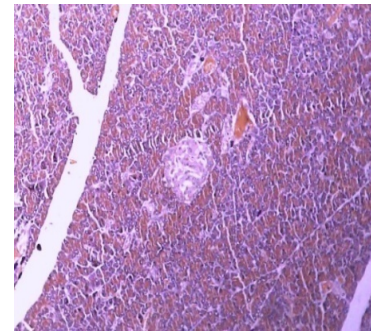


Fig. 3f. (Diabetic) showed severe beta cell necrosis. X 200

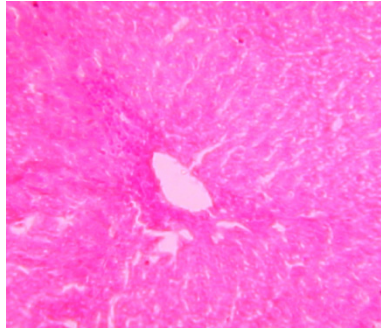


Fig. 4a. Photomicrograph of normal hepatic tissue showed the arrangement of hepatic parenchyma stained with haematoxylin and eosin (H and E). X 200

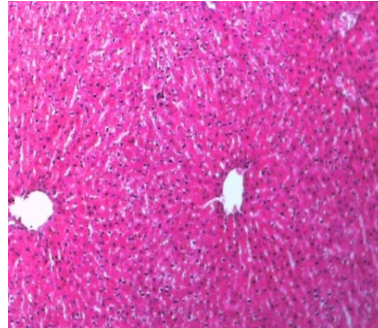


Fig. 4b. Photomicrograph of *A. vogelii* extract treated showed dilatation of portal tract (H and E) X 200

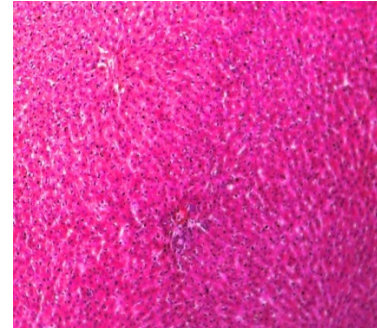


Fig. 4c. Photomicrograph of daonil treated showed mild pyknotic changes coupled with early periportal inflammation (H and E) X 200

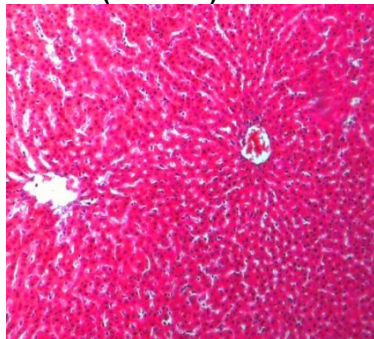


Fig. 4d. Photomicrograph of extract mixture treated showed early edematous changes around the hepatic parenchyma and the portal tract (H and E) X200

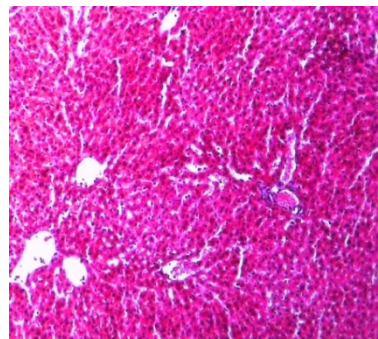


Fig. 4e. Photomicrograph of *Parinari curatellelifolia* extract treated showed mild edematous changes on the hepatic tissue (H and E) X200

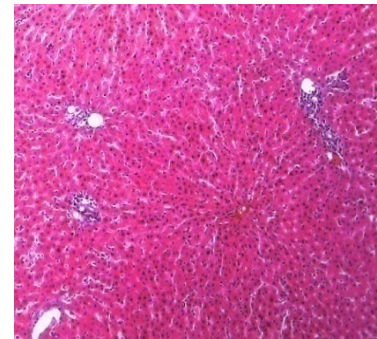


Fig. 4f. Photomicrograph of alloxan diabetic animal showed no pathological changes on the hepatic tissue (H and E) X200

4. DISCUSSION

The median acute toxicity value (LD₅₀) of the extract mixture (1:1) was determined to be 8.18 g/kg bwt. According to Ghosh (1984) and Klaasen *et al.*, (1995), the mixture can be classified as being slightly toxic, since the LD₅₀ by oral route was found to be between 5 - 15 g/kg which was also much higher than WHO toxicity index of 2 g/kg. The extract mixture treated animals showed increased appetite for food intake and water consumption but with no significant weight gain. It is therefore possible that the extract might disfavour fat accumulation that could lead to obesity which is one of the undesirable side effects normally encountered in the treatment of diabetes with sulphonylureas. There were also no changes observed in the macroscopic examinations of the organs of the diabetic animals treated with the extract separately, the extract mixture or glibenclamide. The mixture and the respective extracts demonstrated good postprandial lowering effect on plasma glucose level after 30 min of glucose load indicating that the extracts separately and in the combined form possess α -glucosidase inhibitory activities. The extracts separately and in combined form also exerted effective decrease in plasma glucose levels on the diabetic rats thus suggesting that the few survivor beta cells may have potentiated insulin release as demonstrated by some other plants (Mbaka *et al.*, 2009). By decreasing blood glycaemia the attendant complications associated with diabetes such as cardiovascular risk factor, reduces appreciably.

The hepatic and cardiac tissues release AST and ALT and the elevation of plasma concentrations of these enzymes is an indicator of hepatic and cardiac damage (Crook, 2006). The decrease in ALT and AST levels in the diabetic animals treated with the extracts separately and the extract mixture respectively implied that at the doses used no harmful effects were produced on either the cardiac or the hepatic tissues. In diabetic control, the two enzymes showed considerable increase compared to the normal which suggested increased tendency for problems associated to liver and heart.

The lowering of plasma TC, TG and LDL-cholesterol levels and significant increase in HDL-cholesterol level in the treated animals clearly demonstrated the presence hypolipidaemic agents in the plant extracts. The ability of the extracts and the mixture to manage dyslipidaemia is a potential beneficial effect on cardiovascular risk factors which is a major cause of death in DM (Valli and Giardina, 2002; Zhou *et al.*, 2006). There was no significant increase in plasma creatinine and protein levels in *A.vogelii* root and the extract mixture which suggested that renal dysfunction associated with diabetic condition may have been prevented by the activity of the extracts (Tietz, 1982).

Appreciable recovery in RBC, PCV and Hb levels were recorded in the diabetic animals treated with the extracts and the mixture while WBC count showed a slight increase which could have been in defense to toxic environment (Robins, 1974). The observed increase in the haemoglobin levels in all the treated groups could be due to the increase in iron absorption. There were no significant changes in MCHC in the treated animals compared to the control. Increase in MCHC value has been associated with anemic condition (Agbor *et al.*, 1999). There were also no significant changes in MCV signifying that the respective extracts and the extract mixture did not cause regenerative anemia.

5. CONCLUSION

The high LD50 value (16.8 g/kg) obtained was a clear indication that the poly-herbal preparations were safe for consumption. The study showed that the separate herbal extracts

and the extract mixture had good postprandial glucose lowering effect with hypoglycaemic and hypolipidaemic activities and good reducing effects on the cardiovascular factors. The study also revealed that the drug at doses investigated did not provoke toxic effects to the animals' heart and liver.

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