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Effects of *Parinari Polyandra* Seed Extract on Blood Glucose Level and Biochemical indices in Wistar Rats

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Abstract In light of traditional anti-diabetic claim, therapeutic effect of coconut water extract of *Parinari polyandra* seeds on fasting blood sugar level and serum biochemical indices in alloxan-induced diabetic rats was investigated. Ten normoglycemic and forty diabetic rats (randomly assigned to four groups, n=10) (mean weight of 222±13.01g) were used for the study. The normoglycemic rats (group1) served as normal control and a group of diabetic rats (group II) served as diabetic control. Other groups of diabetic rats (Group III, IV and V) were treated differently with (1) coconut water extract of *Parinari polyandra* seeds (2ml), coconut water alone (2ml) and (3) Glibenclamide, a standard antidiabetic drug (5mg/Kg of body weight). The treatments were done orally for seven days. Blood glucose level, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, total cholesterol, total triglyceride(TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) were examined. The results obtained revealed that rats treated with *Parinari polyandra* seeds extract expressed significant ($p \leq 0.05$) decrease in the levels of glucose, ALT, AST, TAG, total cholesterol, LDL with slight increase in HDL as compared with diabetic control rats. Collectively, the data of the current study indicates that coconut water extract of *Parinari polyandra* has antidiabetic, anti-hyperlipidemia and anticholesterolemia potentials. Hence, its local use in the management of diabetes should be encouraged.

Keywords *Parinari Polyandra,* Anti-Diabetic, Coconut Water, Lipoproteins, Amino Transferases, Blood Glucose, Alloxan

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder which either results from deficiency in insulin production by the pancreas or inability of the insulin produced to bind effectively to its receptor on the cell surface. Either of these conditions leads to accumulation of glucose in the blood (hyperglycaemia) and often impacts negatively on a number of organs in the body, especially the blood vessels. Micro vascular and macro vascular complications are readily seen in diabetes[4, 18]. The complications associated with diabetes are not likely unconnected to oxidative stress induced by hyperglycaemia which overcomes the body natural's antioxidant system.

In the later stage of diabetes, lipid metabolism is affected and seen as hyperlipidemia and hypercholesterolemia which is risk factor in atherosclerosis[15, 17, 11].There is also the possibility of liver damage due to increased gluconeogenesis and ketogenesis[7]. The incidence of diabetes is still on the rise and is estimated to be over 150 million worldwide[21]. There is yet no effective cure for diabetes and the available drugs and insulin currently used in managing the disease are associated with several undesirable side effects[23, 8].

The use of oral anti-diabetic drugs is limited due to their adverse side effects including the haematological, cutaneous and gastrointestinal reactions, hypoglycaemic coma and impairment of liver and kidney functions. In addition, they are not suitable for use during pregnancy[2]. Besides, they are not affordable by low income earners. All these factors have led to the need for plants with hypoglycaemic properties and their employment in the management of diabetes[5, 22].

In Nigeria, several plant species have been claimed to possess medicinal properties and employed in treatment of many ailments. Studies have shown that phytochemicals isolated from plant sources have been used for the prevention and treatment of cancer, heart disease, diabetes mellitus, and high blood pressure[20]. Several species of medicinal plants used in traditional treatment and management of diabetes worldwide have been evaluated[3, 9]. Generally, plants rich in alkaloids and flavonoids have been observed to possess hypoglycaemic properties.

Parinari polyandra benth is a tropical plant which belongs to the family, chrysobalanaceae previously classified as Rosaceae, and widely used to enhance fertility and relief painful and inflammatory conditions[19]. Some species of *parinari* have being claimed to be effective in the treatment

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of veneral diseases and erectile dysfunctions[12]. Phytochemical screening of the plant extracts reveals the presence of flavonoids, tannins, saponin and glycosides[1, 19]. In south west of Nigeria, coconut water extract of *parinari polyandra benth* seeds is used in the treatment of diabetes and acclaimed to have strong anti-diabetic effect. Hence, the present study sought to scientifically investigate the anti-diabetic properties of coconut water extract of *parinari polyandra benth* seeds, and its effects on selected biochemical indices.

2. Materials and Methods

2.1. Collection, Processsing and Extraction of *Parinari Polyandria Seeds*

The seeds of *Parinari polyandra* were bought from a local market in Ibadan, Oyo State, Nigeria; identified, sorted, air-dried and milled into a coarse powdered form. One hundred and sixty five grams (165g) of the powdered seeds was soaked in 2L of coconut water. The mixture was allowed to stand for 72hrs and stirred intermittently to facilitate extraction. The filtrate (extract) was obtained by filtering the mixture with a muslin cloth into a clean, sterilised air-tight container and stored in the fridge at 4^{o}C.

2.2. Experimental Design and Treatment

The animal procedure was carried out according to the standard protocols approved by Animal Research Ethics Committee of Faculty of Information Technology and Applied Sciences, Lead City University, Ibadan, Nigeria, for the use of experimental animals in research. Forty male Wistar rats (mean weight of 222g) were used for the study; and were purchased from a local breeder at Oje, Ibadan,Oyo state, Southwestern part of Nigeria.

The animals were handled humanely, kept in metallic suspended cages in a well ventilated and hygienic rat house under standard conditions of temperature and humidity. They were allowed to acclimatize for two weeks and maintained on normal laboratory chow (Ladokun feeds) and with water *ad libitum*. The animals were also subjected to natural photoperiod of 12 h light/12 h dark cycle.

Diabetes Mellitus was induced in a batch of normoglycaemic rats starved for 12h by injecting intraperitoneally with a single dose of 150mg/Kg body weight (bwt) of alloxan monohydrate dissolved in physiological saline (0.9%)[10]. 72 h after alloxan injection, blood glucose oxidase strip was used to confirm the diabetic level of rats; animals with 12 mmol/L and above were considered as diabetic and included in the study. The rats were randomly assigned to four (4) groups.

Group I*: (Control): Normoglycemic rats given only distilled water.*

Group II: *Untreated diabetic rats*

Group III: *Diabetic rats treated with 2.0ml Parinari polyandra-coconut water extract by gastric intubation*

Group IV: *Diabetic rats treated orally with 2.0ml of coconut water by gastric intubation*.

All the rats were allowed equal access to normal laboratory chow and water *ad libitum*. The treatment was done for seven days.

2.3. Blood Glucose Level Determination

Blood samples were collected by cutting the tail-tip of the rats, and glucose level determination was done at 24 hours interval prior to treatment for 7 days. This was done by using the "ONE-TOUCH Basic" (lifescan, milipitas, CA) instrument and results were recorded in mmol/L. Rats were feed-fasted but allowed access to water for 12h before each determination.

2.4. Tissue Preparation for Biochemical Analysis

After the last glucose level determination, the animals were fasted overnight and weighed. Blood sample was collected from the retro orbital sinus of the eye by ocular puncture into non-heparinised bottles for biochemical analyses. The rats were then sacrificed by cervical dislocation.

2.5. Determination of Biochemical Indices

The activity of Alanine amino transferase (ALT: EC. 2.61.2.1) formerly known as glutamate pyruvate transaminase (SGPT) and aspertate amino transferase (AST: EC. 2.6 1.1) formerly known as glutathione-oxaloacetate transaminase (SGOT) were estimated by the use of end point colorimetric diagnostic kit (Randox Laboratories Limited, England) according to the method of Reitman and Frankel, 1957[14]. Total cholesterol and triglycerides, low density lipoprotein (LDL) and high density lipoprotein (HDL) were determined using test kits (Linear chemicals)

2.6. Statistical Analysis of Data

The data obtained were statistically analysed using the Statistical Package for Social Sciences (SPSS) version 17. Hypothesis testing was by one-way analysis of variance (ANOVA) followed by least significant difference test. P-values of less than 0.05 were considered statistically significant. Results are presented as mean ± SD (n= 10).

3. Result

3.1. Changes in Body Weight of Rats

Changes in body weight of normal and alloxan-induced diabetic albino rats before and after the different treatments are presented in (Table 1). There was a decrease in body weight of rats in all the groups except in the normal control rats which showed a slight increase in body weight (by 0.98%).

Alloxan-induced diabetes (untreated) caused a significant weight loss in rats (by 12.5%) as compared to the treated groups (2.2%) (Table1). Weight loss in diabetic rats was

significantly ($p \leq 0.05$) minimized in animals treated with *Parinari polyhandra* seed extract and glinbeclamide, a standard anti-diabetic drug) (Table 1).

3.2. Blood Glucose Level

Blood glucose levels of normal and alloxan-induced (150mg/kg i.p) diabetic albino rats on the third and seventh day of the experiment are presented in Table 2. Throughout the time of the experiment, the glucose level of the normal control rats remained within normal range (2.5-5.0mmo/l) (Table 2).

In the diabetic control group, an increase in the blood glucose level on the third day and further increase on the seventh day relative to the control group was observed (Table 2). Animals treated with coconut water extract of *Parinari polyandra* seed showed a slight increase in blood glucose level at third day of treatment but later decreased by 29.6% at the seventh day of treatment. Glibenclamide, a standard antidiabetic drug elicited a higher reduction in blood glucose level (42.7%) at the seventh day of treatment as compared with the *parinari* extract (Table 2).

Rats treated with coconut water only showed a very minimal decrease in blood glucose level at the seventh day of treatment (Table 2).

3.3. Biochemical Indices in Control and Alloxan-Induced Diabetic Rats (Treated and Untreated)

The levels of biochemical indices in normal control and diabetic control and treated diabetic rats over a period of seven days are presented in Table 3.

In the diabetic control rats, there were elevated levels of ALT, AST, and TAG, Total cholesterol, LDL but a decrease in HDL relative to the normal control group (Table 3). Rats treated with *Parinari polyandra* seed extract showed decrease in levels of ALT, AST, TAG, Total cholesterol, LDL with slight increase in HDL as compared with the diabetic control rats (Table 3.) Rats treated with coconut water alone also showed the same pattern but the changes in the biochemical indices in respect to the diabetic control group were less pronounced compared to rats treated with *Parinari polyandra* seed extract (Table 3). Treatment with Glibenclamide (a standard antidiabetic drug), caused more decrease in levels of AST, ALT, TAG, Total cholesterol, LDL and higher increase HDL of rats compared to other treatments

Table 1. Body weight Changes in normal and alloxan-induced diabetic albino rats (treated and untreated) before and after the experiment

Group (Treatment)	Before Experiment	After Experiment (g)	% Change in weight
Normal control (2ml saline)	215.0 ± 1.13	217.1 ± 0.67	0.98^{c}
Diabetic control (untreated)	219.2 ± 1.00	191.8 ± 2.06	-12.5^{b}
Coconut water extract of *P.polyandria* seed (2ml)	221.3 ± 3.17	216.6 ± 3.08	-2.2^{a}
Coconut water only (2ml)	225.9 ± 1.12	223.3 ± 1.09	-1.2^{a}
Glibenclamide (5mgkg^{-1} b.wt)	230.4 ± 4.52	224.8 ± 3.67	-2.4^{a}

Values are expressed as mean ± SD. n = 10. Different superscripts on the sane column are statistically significant (*P<0.05) n= number of animals per group, SD=Standard deviation

Table 2. Blood glucose levels of normal and alloxan-induced (150mg/kg i.p.) diabetic albino rats (treated and untreated) on the 3rd and 7th day of the experiment

Group Treatment	Pre-Treatment Day 0	Blood Glucose (mmol/L) Post Treatment		
		Day 3	Day 7	%Δ
Normal control (2ml saline)	3.82±0.18	3.80±0.21	3.81±0.12	-0.26
Diabetic control (untreated)	29.46±1.03	33.21±1.1	38.11±1.3	+29.4*
Coconut water extract of *P.polyandra* seed (2ml)	30.3 ± 0.81	32.09 ±0.56	21.32±0.76	-29.6**
Coconut water only (2ml)	29.11±0.43	29.24±0.12	28.62±0.27	-2.1*
Glibenclamide (5mgkg^{-1})	32.06±0.89	22.06±0.54	18.35±0.31	-42.7**

Values are expressed as mean ± SD (Unit: mmol/L). n = 5. *P<0.05 against normal control. **P<0.05 against diabetic control. %Δ= percentage change in blood glucose level (Normal range for the blood glucose: 2.5 to 5.0 mmol/L). +=increase. - = decrease

Table 3. Effect of treatment with coconut water and Parinari polyandra seed extract for 7 Days on biochemical indices in control and alloxan-induced diabetic rats

Group (Treatment)	ALT (IU/L)	AST (IU/L)	Total triglycerides (mg/dl)	Total Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Normal control (2ml saline)	8.2±0.14	11.42±0.19	82.7±2.01	108.2±2.01	89.8±2.01	43.4±2.01
Diabetic control (untreated)	21.3±2.01*	33.4±2.04*	135.4±1.31*	154.1±0.98*	143.4±2.04*	33.6±0.86*
P.polyandra seed extract (2ml)	18.3±1.23	29.1±1.31	101.4±1.01**	137.4±2.11**	139.4±0.31	32.2±0.35
Coconut water only (2ml)	19.8±0.51	30.1±0.81	133.4±1.71	149.1 ±2.21	142.1±0.81	34.2±0.45
Glibenclamide	15.2±1.20**	19.6±1.23**	91.45±2.23**	129.3±3.23**	141.1±4.23	32.4±0.45
			5mgkg^{-1}			

Values are expressed as mean ± SD. n = 5. *P<0.05 against normal control. **P<0.05 against diabetic control. n= number of animals per group, SD=Standard deviation

4. Discussion

In view of the data obtained, the present study suggests that oral administration of *Parinari polyandra* seed extract elicited anti diabetic properties by significantly lowering blood glucose level in alloxan-inuced diabetic rats as compared to the diabetic control animals. This observation lends scientific credence to the traditional use of *Parinari polyandra* seed extract in treatment of diabetes. The finding is also consistent with the published report of Emeka *et al*., (2011) that informed that ethanolic extracts of *Parinari polyandra* and *Spondias mombin* collectively reduced blood glucose level in Wister rats[6]. Salihu *et al.,* (2009) also reported a similar observation with coconut water extract of *Picrilima nitida* seeds[16].

The mechanism by which coconut water extract of *Parinari polyandra* reduced blood glucose level in the diabetic rats was not elucidated in this study. However, it is probably by increasing the pancreatic secretion of insulin, possibly via regeneration of the beta cells. Also, the possibility of facilitating the binding of insulin to its receptors cannot be undermined. These mechanisms of action are suggested for other plants that have been studied[2].

Alhough diabetes is characterized by high level of glucose in the blood, the body cells are still starved of this essential source of energy. In response, the metabolism of lipids is severely affected and this results in hyperlipidemia (high levels of lipids, particularly TG in the blood) and hypercholesterolemia (high cholesterol level). The anti-hyperlipidemia and anti-hypercholesterolemia demonstrated by *P.polyandra* extract in the current study is likely connected to its hypoglycaemic effect. It may also be attributed to the ability of the plant extract to facilitate the mobilization of cholesterol and triglycerides from the blood into tissues. This may have probably occurred through the induction or suppression of enzymes critical to the metabolism of these lipids.

There is also the possibility of liver damage due to increased gluconeogenesis and ketogenesis in diabetic animals[7]. Aspartate transaminase (AST) and alanine transaminase (ALT) assay are important in the diagnosis of liver damage caused by drug toxicity or harmful chemicals[13]. In this study, alloxan induced diabetes was characterized by elevated levels of AST and ALT in rats. This observation is consistent with previous reports[6, 7]. Treatment of diabetic rats with *Parinari polyandra* seed extract lowered the level of these enzymes, the difference between the diabetic control rats and the extract treated rats was however not statistically significant. The non significant changes in the liver enzymes in groups treated with *Parinari polyandra* suggests no apparent toxicity to the liver and may have expressed the non hepatotoxic property of the plant. This observation lends support to its use in ameliorating diabetes.

The results of this study also indicate that coconut water elicited some degree of glucose lowering effects, although very minimal. This explains is use as a common extraction solvent for anti-diabetic plants. Further studies are in progress to identify the active compounds in *Parinari. polyandra* benth seeds and elucidate its exact anti-diabetic mechanism of action.

5. Conclusions

In comparison with the standard anti-diabetic drug (glibenclamide) used in this study, coconut water extract of *Parinari polyandra* demonstrated appreciable anti-diabetic property. Hence, its local use in the treatment of diabetes should be encouraged. The active compounds responsible for the anti-diabetic property in *Parinari polyandra* benth seeds when identified, isolated and purified will surely elicit a stronger anti-diabetic effect. Further studies are therefore in progress to identify the active compounds in *Parinari polyandra* benth seeds and elucidate its exact anti-diabetic mechanism of action

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