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Amelioration of histological changes and associated metabolic abnormalities by a combination of *Morinda lucida* and metformin in diabetic rats

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ABSTRACT

This work investigates the ability of *Morinda lucida* and co-administration of *Morinda lucida*/metformin in the control of biochemical and histological changes in alloxan-induced diabetic rats. Alloxan diabetic rats were treated with 200 mg/Kg body weight of *Morinda lucida* leaves extract, 1 mg/Kg BW of metformin or a combination of the two treatments for 28 days. Results of the studies revealed that *Morinda lucida* leaves extract significantly improved lipid profile and kidney function in diabetic rats. These positive outcomes were enhanced by combined treated with *Morinda lucida* leaves extract and metformin. Furthermore, the calculated atherogenic index of treated animals were close to those of normal rats as opposed to diabetic rats. Similarly, histological studies showed that *Morinda lucida* leaves extract and metformin administered together or singly, ameliorated damages in pancreas and kidneys from alloxan diabetic rats. It can therefore be inferred that combined treatment with *Morinda lucida* leaves extract and merformin could improve the potency of *Morinda lucida* leaves used in the management of diabetic complications.

Keywords: Diabetes, *Morinda lucida*, Metformin, atherogenic index.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterised by hyperglycemia [1, 2]. It results from the in-ability to produce sufficient amounts of insulin or insensitivity to available insulin. Diabetes mellitus is believed to be a multifactorial disease resulting from genetics and lifestyle [3]. Over 300 million people suffer diabetes mellitus around the world; this number is expected to double within 20 years [1]. The estimated number of persons with new cases of diabetes mellitus yearly stands at seven (7) million. These statistics encompasses the two main forms of diabetes namely: Type 1 which is due to insufficient amounts of insulin and Type 2 due to insensitivity to available insulin [1]. Diabetes mellitus is associated with numerous health complications prominent amongst which are nephropathy, hyperglycemia, oxidative damage, neuropathy, dyslipidemia and weight loss [1, 3, 4].

Orthodox drugs abound for the treatment of diabetes. These drugs effect their actions through a variety of biochemical mechanisms such as: enhancing secretion of insulin, decrease glucose release from the liver, reduce gastrointestinal absorption of sugars [5]. However, these drugs are expensive and still have many side effects [3, 6]. Therefore, there is need to adopt new treatment protocols aimed at ameliorating side effects from anti-diabetic drugs. Such treatment regimes may also improve the efficacy of drug action.

Combination therapy is a widely accepted treatment regime which offer advantages of reduced side effects as well as improve efficacy of drugs [7]. Current used measures include, 'drug-drug' and 'drug-herb' combinations. The approach of 'drug-herb' combination is very popular in some parts of the world. Consequently, in this work, we sought to study what advantage(s) a commonly used anti-diabetic drug, metformin and plant with anti-diabetic property *Morinda lucida* could offer when used together.

Morinda lucida has been reported to have several properties which include: anti-malarial activity, anti-leishmanial, anti-oxidant, anti-trypanosomal, anti-diabetic to mention but a few [8-10]. However, to the best of our knowledge, no study have investigated the beneficial effects or otherwise of combined treatment of *Morinda lucida* with any anti-diabetic drug.

Chemicals and drugs

Diagnostic kits for creatinine, urea, total cholesterol (TC), high density lipoprotein (HDL) and

triglyceride (TG) were purchased from Randox Diagnostics (UK). Alloxan was purchased from Sigma (St. Louis, Germany). Accu-check Glucometer was a product of Roche Diagnostics (Germany). Metformin and other chemicals used in this work were of analytical grade obtained locally.

Experimental animals

Male albino rats (average weight, 160 g) were used in this study. The animals were housed in the Animal Care Facility of the Department of Biochemistry, Kogi State University, Nigeria. Acclimatization of the animals was done for one (1) week prior to experiments. The animals were fed standard, commercial rat feed and water *ad libitum* throughout the acclimatization experimental period.

Identification and preparation of methanol extract of *Morinda lucida*

Morinda lucida leaves were obtained locally from Anyigba, Nigeria and its identity was authenticated by Prof S.S. Usman of the Department of Biological Sciences, Kogi State University, Anyigba, Nigeria. Leaves of *Morinda lucida* were washed, air dried and pulverized to powder with a blender. A portion of the powdered leaves was suspended in Methanol at a concentration of 200 g/L for 24 hours. After which the suspension was filtered and the filtrate was concentrated in a rotary evaporator. The yield of the extract was calculated according to the formula below:

$$\text{Percentage of extract yield} = \frac{Weight\ of\ extract\ (g)}{Weight\ of\ plant\ material\ (g)}\ x\ \frac{100}{1}$$

Phytochemical screening

Qualitative phytochemical screening was carried out on the methanol extract of *Morinda lucida* according to the protocols of Sofowora [11, 12]; Trease and Evans [13] and Harborne [14].

Induction of diabetes

Diabetes was induced by single intraperitoneal injection of freshly prepared alloxan [150 mg/kg body weight (BW)] to overnight fasted rats. Fasting blood glucose concentration of the rats was determined two (2) days after induction and animals with blood glucose concentration greater than or equal to 250 mg/dL were considered diabetic.

Experimental design

Survivors of alloxan-diabetes induction were allocated to five (5) groups of eight (8) rats each as follows:

- Group 1, Normal control: Fed rat chow and water
- Group 2, Diabetic control: Untreated diabetic rats
- Group 3, Metformin group: Treated with 1 mg/Kg BW/day of Metformin
- Group 4, *Morinda lucida* group: Treated with 200 mg/Kg BW/day of *Morinda lucida*
- Group 5, Metformin + *Morinda lucida*: Co-administered with 1 mg/Kg BW/day Metformin + 200 mg/Kg BW/day *Morinda lucida*

Treatment of animals lasted 28 days and on the 29th day animals were sacrificed. Blood glucose concentration, however, was monitored weekly during the 28-day treatment by the glucose oxidase method using a one touch Accu check glucometer.

Biochemical assays

Biochemical assays for creatinine, urea, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and triglyceride (TG) levels in plasma were carried out using Randox Diagnostic kits according to manufacturer's instructions. LDL and atherogenic index was calculated according to the formulae below:

$$\text{LDL} = \text{TC} - \text{HDL} - \frac{\text{TG}}{5}$$

$$\text{Atherogenic index} = \frac{\text{TC} - \text{HDL}}{\text{HDL}}$$

Histological studies

On the 29th day animals were sacrificed, pancreas and kidneys were removed and immersed in 10% formalin for histopathological examination. Thin sections of the pancreas were cut and stained with hematoxylin-eosin for histological examination.

Statistical analysis

Statistical analyses (Mean, Standard Deviation and Analysis of Variance) were performed with the Microsoft Excel (Version 2010) and SPSS 16.0.

RESULTS AND DISCUSSION

In the present study, fasting blood glucose (FBG) was used for weekly assessment of our 'drug formulations'. It was found that treatment with *Morinda lucida* and metformin restored the FBG of alloxan-diabetic rats to normal within 28 days (results not shown). Hence, the rationale to conduct this experiment for only 28 days. Animals were sacrificed on the 29th day, serum biochemistry and histology was performed to access efficacy of the drug administered. Induction of animals with diabetes resulted in significant elevaton ($p<0.05$) in plasma total cholesterol (TC), low density lipoproteins (LDL) and triglyceride (TG) and a concomitant decrease in the concentration of high density lipoprotein (HDL) (Table 1). This pattern of dyslipidemia is not uncommon, infact, it is consistent with other experiments which utilised the rat-alloxan model of diabetes [15-17]. Elevation in concentration of TC, LDL and TG is an established has been reported to be consistent marker of hyperglycemic conditions responsible for non-alcoholic liver disease in diabetic patients [18]. Destruction of β-cells of the pancreas by alloxan results in insulin deficiency responsible for the loss of 3-hydroxy-3-methyl-methylglutarylcoenzyme-A reductase (HMG-CoA reductase) inhibition. HMG-CoA reductase is a key enzyme in cholesterol synthesis. In the absence of sufficient insulin, cholesterol synthesis is enhanced leading to cardiovascular risks and a variety of metabolic derangements such as diabetic retinopathy [18, 19]. The elevated levels of TG, TC and LDL indicates atherosclerosis. This is reflected in the high atherogenic index 54.96 which is 27-fold higher than in normal control rats (Table 1). From the results, *Morinda lucida* and metformin proved to be effective in managing the dyslipidemia potentiated by alloxan. This is evident in reduction of TG, LDL and TC of rats treated with *Morinda lucida* or metformin. Interestingly, the concentrations of HDL were elevated by administration of this

extract. This result is further mirrored by the reduced atherogenic index treated rats. The reduced atherogenic index is an indication that the treatments effectively manage cardiovascular diseases that accompanies diabetes. It is noteworthy that co-administration of *Morinda lucida* and metformin was more effective than the treatments administered singly (Table 1). This results may be attributed to the presence of flavonoids, tannins, saponins and phenolic compounds in *Morinda lucida* reported by previous studies and confirmed by this work [20, 21]. Reports have shown that plants rich in flavonoids are effective in managing diabetes and its complications [2, 22]. Flavonoids elicit their protective properties through a variety of pathways include but not limited to inhibition of HMG-CoA reductase, suppression of acyl CoA: cholesterol acyl transferase (ACAT) activities, reduced gene expression of glucose-6-phosphatase (G6Pase) and fatty acid binding protein 4 (FABP4) and increased expression of GLUT-4 [23]. These phytochemicals present in *Morinda lucida* posses antioxidant which are capable to neutralising oxidant species resulting from alloxan intoxication [24, 25].

Nephropathy resulting from diabetes investigated in the present work. Insulin deficiency causes inability to metabolise carbohydrates efficiently for the production of ATP leading to increased breakdown of protein to form urea and creatinine. These end products overload the kidney leading glomerular dysfunction [18]. Table 1 reveals significant increase in the concentration of urea and creatinine indicative of renal dysfunction, degradation of nitrogenous metabolites and muscle respectively. Accordingly, we observed loss of weight in diabetic animals. Treatment with *Morinda lucida* and metformin significantly remedied the dysfunction of the kidney. As with the lipid profile, co-administration of *Morinda lucida* and metformin was better at restoring the kidney function than the treatments administered singly.

The findings of this work are supported by histological evaluation of the kidneys and pancreas of the various groups in the study design. Figures 1 and 2 shows the microscopic architecture of the pancreas and kidneys respectively. Taking together, alloxan caused distorted the cellular architecture of the pancreas and kidneys characterised by severe pycnosis, vacoulation and infiltration of immune. These indicators of cellular damage by alloxan was effectively countered by treatment with *Morinda lucida* and metformin especially in the experimental group where both regimens were co-administered.

Table 1: Biochemical indices of normal and diabetic rats treated with *Morinda lucida*

	Biochemical indices (mg/dL)						
Group	**TC**	**HDL**	**LDL**	**TG**	**UREA**	**CREATININE**	**AI**
Normal control	105.42±3.79	34.39±4.09	14.09±7.38	39.82±3.09	33.46±13.69	0.50±0.27	2.07
Diabetic control (untreated)	338.73±5.43 $^{\alpha}$	6.04±1.20 $^{\alpha}$	128.44±2.10 $^{\alpha}$	362.09±5.33 $^{\alpha}$	67.02±4.92 $^{\alpha}$	1.87±0.05 $^{\alpha}$	54.96
Metformin	187.95±4.47 $^{\alpha\beta}$	22.84±1.12 $^{\beta}$	34.79±1.14 $^{\alpha\beta}$	167.94±14.25 $^{\alpha\beta}$	56.62±5.32 $^{\alpha}$	1.62±0.03 $^{\alpha}$	7.23
Morinda lucida extract	128.31±14.87 $^{\beta}$	25.41±1.69 $^{\beta}$	46.56±4.05 $^{\alpha\beta}$	163.23±16.67 $^{\alpha\beta}$	36.87±6.72 $^{\alpha\beta}$	1.43±0.43 $^{\alpha\beta}$	4.05
Metformin + *Morinda lucida* extract	111.30±10.68 $^{\beta}$	26.25±0.54 $^{\beta}$	41.82±5.32 $^{\alpha\beta}$	154.06±20.09 $^{\alpha\beta}$	29.07±3.59 $^{\alpha\beta}$	1.32±0.36 $^{\alpha\beta}$	3.24

α-Statistically significantly different from normal control ($p<0.05$); β-Statistically significantly different from diabetic untreated rats ($p<0.05$)

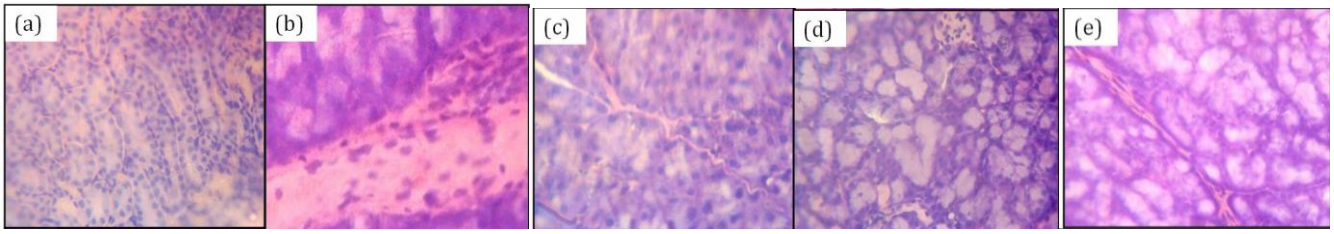

Figure 1: Histological examination of pancreas from normal and alloxan-diabetic rats treated with Metformin and *Morinda lucida*. (a) Pancreas from normal rat showing un-distorted, normal architecture of the islet of the langerhans and the acini with granulated β-cells appearing intact. (b) Pancreas from diabetic rat revealing cellular infiltration (lymphocyte and neutrophil) in the endocrine region, vacoulation of cells of the islet of langerhan and endocrine cells are reduced, with severe pycnosis. (c) Pancreas from Metformin treated diabetic rat showing evidence of normal histological arrangement of endocrine with mildly disordered exocrine, with pycnosis. Regeneration of cells is also observed. (d) Pancreas from diabetic rat treated with *Morinda lucida* showing signs of cell regeneration observed in the islet of langerhan, with mild pycnosis (e) Pancreas from diabetic rat treated with both Metformin and *Morinda lucida* shows moderate hypocellularity of the islet of langerhan, a fast regeneration of cells in the islet of langerhans is observed and minimal pycnosis in both endocrine and exocrine regions. (Magnification x 40, H&E stain)

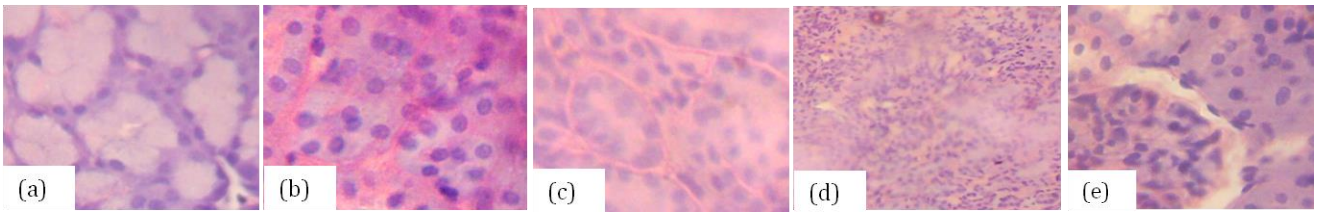

Figure 2: Microphotographs of histology of the kidneys from different groups post-administration of *Morinda lucida* (Eosin-Haematoxylin staining, 40x). The micrographs show kidneys from (a) normal control rats regular glomeruli. The kidney showed impressive and normal histological architecture of both distal and proximal convoluted tubule. (b) Untreated diabetic rats showing evidence enlargement, vacuolation and cast formation in the renal tubules, thickening of the media in the lobular arteries, hyperplasia and thickening of the collecting ducts. (c) Kidney from metformin treated rats showing intact cellular border, faint appearance of cells with low regenerative arrangement of cells along the tubule (dista and proximal). However, is a great cell pycnosis or necrosis at the glomeruli tissues. (d) Kidney from extract treated animals. This kidney showed a mild pycnosis at the cortex junction. The kidney of this group showed gradual cells regeneration in the glomeruli and few necrotic cells with obvious tubular border rebuild. (e) Combined treatment with *Morinda lucida* and metformin showing evidence of regeneration/restoration of cellular integrity. Although mild but isolated sections of tissue necrosis was observed.

CONCLUSION

Combination therapy is common practice in, but not limited to the management of diabetes. In this study we have been able to show for the first time that co-administration of *Morinda lucida* and metformin is beneficial in the management of experimental diabetes and its complications in rats

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