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Original Research Article

Ocimum Gratissimum and *Moringa Oleifera* Ameliorates Diabetic Nephropathy in a Synergistic Manner Similar to Insulin

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ABSTRACT

The study investigated the synergistic effects of combined administration of *Ocimum gratissimum* and *Moringa oleifera* in diabetic rats. Seven groups of 6 rats each were used. Groups 1 and 2 representing Normal and Diabetic Control groups (NC and DC respectively) received 0.5ml Dimethyl sulphoxide (DMSO). Groups 3 received 5UI/Kg b.w insulin (intraperitoneally) and group 4 received 5mg/Kg b.w glibenclamide (orally); while groups 5 and 6 received 500mg/Kg b.w of *Moringa oleifera* (MO) and *Ocimum gratissimum* (OG) extracts respectively. Group 7 MO + OG (M/O) treated group received 250 mg/ Kg b.w of each extract. There was reduction in electrolyte levels for the extract treated groups when compared with DC. The combined group compared well with NC and INS groups. Bilirubin increased for the treated groups, while urea and creatinine concentrations decreased for the treated groups. Triglyceride (TG), Total Cholesterol (TC), Very Low Density Lipoprotein (VLDL) and Low Density Lipoprotein (LDL) levels reduced in a synergistic manner when compared with DC. High Density Lipoprotein (HDL) increased when compared with DC. These results show the efficacy of the combined extracts at better managing diabetes induced nephropathy in a synergistic pattern when compared with the single extracts and standard drugs insulin and glibenclamide.

Keywords: Diabetes mellitus; electrolyte; kidney; *Moringa oleifera*; *Ocimum gratissimum*; cholesterol

INTRODUCTION

Diabetes mellitus (DM), both insulin-dependent DM (IDDM) and non-insulin dependent DM (NIDDM) is a common and serious metabolic disorder throughout the world. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycaemia and disturbances of carbohydrate, lipid and protein. As a consequence of metabolic derangement in diabetes, various complications developed, including both

macro and micro vascular dysfunctions metabolism [1]. Human bodies possess enzymatic and non-enzymatic antioxidative mechanisms which minimize the generation of reactive oxygen species, responsible for many degenerative diseases including diabetes [2].

The kidneys are important target organs of diabetes and kidney failure often leads to death in diabetes. It is believed that uncontrolled high blood sugar leads to the development of kidney damage, especially when high blood pressure is also present. Diabetes causes glomerular lesions, atherosclerosis of renal veins, pyelonephritis and nephropathy [3]. Increased urine volume and creatinine clearance can also be observed in diabetes [4]. Diabetic ketoacidosis can also affect the levels of potassium and sodium in the body. Hyponatremia, or low sodium, results from an increased flow of fluid out of the cells into the blood stream, diluting the sodium level. Low potassium can also occur during diabetic ketoacidosis [5].

Electrolyte imbalance in diabetes is primarily a result of elevated blood glucose. With hyperglycemia, the body tries to rid itself of the excess blood glucose by increasing urinary output. Increased urination produces water and electrolyte loss, which then upsets the body's balance of electrolytes. The balance is especially disturbed between sodium and potassium. Hyperglycaemia is the principal factor responsible for structural alterations at the renal level. Diabetic Control and Complication Trial Research Group (DCCTRG) has elucidated that hyperglycaemia is directly linked to diabetic microvascular impairment particularly in the kidney [6]. Kidney disease is secondary to diabetes and comprises the fastest-growing subgroup of Chronic Kidney Disease (CKD) and end-stage kidney disease (ESKD) in the United States [7].

Chronic renal disease is accompanied by characteristic abnormalities of lipid metabolism, which appears as a consequence of nephritic syndrome or renal insufficiency and is reflected in an elevated plasma lipid level [8]. The concentration of total TC, VLDL, LDL and TG rises with increasing albumin excretion rate in patients with type 1 diabetes while HDL levels also tend to be reduced [9]. Studies in a variety of animal studies have shown that hypercholesterolemia accelerates the rates of progression of kidney disease [10]. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and glinides. Many of them have a number of serious adverse effects; therefore, the search for more effective and safer hypoglycemic agents is one of the important areas of investigation [11].

Traditional plant treatments have been used throughout the world for the therapy of diabetes mellitus, dyslipideamia and nephrotic conditions [12 and 13]. Among many medications and other alternative medicines, several herbs have been known for the control of diabetes and treatment of several kidney diseases. The hypoglycemic effect of several plants used as antidiabetic remedies has been confirmed, and the mechanisms of hypoglycemic activity of these plants are being studied. Traditional medicines from readily available medicinal plants offer great potential for the discovery of anti-diabetic drugs. Thus, the rationale of the study is to ascertain the probable synergistic impact of *Ocimum gratissimum* and *Moringa oleifera* at ameliorating and reversing diabetes induced nephropathy.

MATERIALS AND METHODS

Collection of Plant Materials

The fresh leaves (500g) of the shrubs were collected from the Endocrine Research farm, University of Calabar, Calabar.

Chemicals used

All chemicals and drugs used were obtained commercially and of analytical grade.

Preparation of extract

Fresh leaves of *M. oleifera and O. gratissimum* were collected, macerated and allowed to stand in 80% alcohol at room temperature for 48 hours. The filtrate was evaporated in a rotary evaporator and allowed to concentrate in a water bath at 36^{o}C. A greenish paste was obtained. The extraction of *M. oleifera* and *O. gratissimum* leaves was done in the Department of Biochemistry, University of Calabar. The obtained leaf extracts were stored at 4^{o}C.

Experimental animals

Forty two rats weighing between 120- 180g, were obtained from the department of biochemistry animal house, University of Calabar divided into seven groups of 6 rats each. Before the experiment, the rats were allowed to acclimatize to the animal house for 7 days. Standard environmental conditions such as temperature (26 $\pm$ 2^{o}C), relative humidity (45-55%) and 12 hrs dark/light cycle were maintained. All the animals were fed with standard rat chow and water was allowed *ad- libitum* under strict hygienic conditions.

Acute toxicity test (LD_{50})

The oral acute toxicity of the ethanol extract (EE) was determined in mice as described by [14].

Induction of diabetes

STZ was prepared in citrate buffer (0.1 M, pH 4.5). STZ solution was injected intraperitoneally at a concentration of 40mg/kg of body weight in a volume of 0.5ml/rat. Diabetic condition (type I) was confirmed in fasting rats from blood glucose level more than 150mg/100ml determined after 72hours after day of injection.

Experimental Design

42 adult male and female wistar albino rats weighing 120- 180g were grouped into seven (7) as shown in table 1.

Table 1. Animal Grouping

Groups	Treatment administered	No. of animals	Dose
1	NC	6	0.5ml DMSO
2	DC	6	0.5ml DMSO
3	INS	6	5IU/kg b.w
4	GB	6	5mg/kg b.w.
5	M. O.	6	500mg/kg b.w
6	O.G.	6	500mg/kg b.w
7	MO/OG	6	250mg/kg b.w. of each

Treatment was administered twice daily (12 hourly) for 28 days

Statistical analysis

Blood glucose levels were expressed in mg/dL as Mean±SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group by Dunnett's method. The values of $p<0.05$ were taken as significant.

RESULTS

Effects of Treatment on Serum Bilirubin, Creatinine and Urea

Bilirubin concentration reduced for DC when compared with NC, but increased in the herbal treated groups. Urea and creatinine concentration was increased in DC when compared with NC. 28 days treatment of diabetic rats resulted in a reduction of both parameters when compared with DC and was comparable with that of the standard drugs. There was no synergistic effect observed for the double extract groups. A more significant reduction in urea was observed for MO, while a more significant reduction in creatinine was observed for OG.

Effects of treatment on Na concentration

Na concentration reduced for DC when compared with NC. Observed was a reduction in Na concentration for the treated groups, although the double extract group showed a synergistic effect.

Effects of treatment on K concentration

A reduction was observed in K concentration for DC when compared with NC. K concentration increased for MO when compared with DC. Although K concentration reduced for OG, but when in combination with MO recorded an increase which was comparable with INS.

Effects of treatment on Cl concentration

Cl concentration reduced for DC. There was no significant change in Cl concentration for MO when compared with DC. OG concentration increased when compared with DC but reduced when in combination with MO.

Effects of treatment on Ca concentration

Ca concentration reduced for DC when compared with NC, but increased in all treated groups when compared with DC. Concentration of Ca for MO treated group increased and was comparable with NC.

DISCUSSION

Diabetes mellitus has been reported to produce serious cardiovascular, renal, neurologic and retinal complications [15]. Impaired function of the kidneys related to diabetes mellitus results from structural alterations at the renal level which is directly linked to diabetic microvascular impairment. The results of the present study showed that the ethanolic extracts of *Moringa oleifera* and *Ocimum gratissimum* leaves have protective effects on the diabetic impaired kidneys as seen in results in Table 2, where there was an increase in bilirubin concentration for the treated groups when compared with the diabetic control. It has been recognized that bilirubin may exert cytoprotective effects due to its antioxidant potential [16]. From table 2, there was also a reduction in the concentrations of urea and creatinine when compared with the diabetic control and agrees with finding by [17]. Although there wasn't a synergistic effect of the combined groups for each of the parameters, the result compared well with the standard drug insulin and normal control group.

High serum glucose enhances the movement of potassium from extracellular fluid into the cells [18]. From Fig 1- 4, the role of glucose in K^+ metabolism was evidenced by lower serum K^+ in the diabetic control group. Although there was a significant reduction in Na^+ for OG and increase in MO treated groups, their combination resulted in an increase in Na^+. The pattern of result for Na^+ was similar with that obtained for K^+ and Ca^+. The lower value of chloride for the diabetic group gave the same pattern as the level of Na^+ in the diabetic group because Na^+ is always (in most cases) in association with chloride. However, there was no significant change in the concentration of Cl^- for the treated groups when compared with the diabetic group. A synergistic effect was observed in electrolytes. These results agree with earlier reports by [15].

Results from table 3 shows that the lipid fractions (VLDL, LDL, TC and TG) were significantly elevated ($p<0.05$) in the diabetic control and was the reverse for HDL. There was a reversal of these results for the treated groups. The combined group recorded a more significant reduction ($P<0.05$) in the lipid fractions when compared with the standard drug insulin and the single extracts. While a more significant increase ($P<0.05$) in HDL was noticed for the combined group when compared to other treated groups. Dwivedi J et al [19] reported similar results for lipid profile in diabetes.

Table 2. Effects of Treatment on Serum Bilirubin, Creatinine and Urea

Groups	Bilirubin(mg/l)	Urea (mg/dl)	Creatinine(mg/l)
NC	3.91±0.76	87.84±8.40	0.90±0.06
DC	2.11±0.51	209.16±1.02*	1.10±0.03
INS	5.22±0.16	141.96±17.51*, a	1.04±0.10
GB	1.90±0.64*,a,b	164.13±27.33*	0.78±0.06
MO	4.59±0.00 a	84.95±6.40 a	0.99±0.10
OG	3.34±0.50a,b	110.20±11.34 a	0.84±0.02
MO/OG	4.57±0.33	136.23±11.98 a	1.07±0.07

Values are expressed as mean ± SEM.
**significantly different from NC at p<0.05*
a = significantly different from DC at p<0.05
b = significantly different from INS at p<0.05

Table 3. Effect of treatment on lipid profile

Groups	TC(mg/dl)	TG(mg/dl)	VLDL(mg/dl)	LDL(mg/dl)	HDL(mg/dl)
NC	146.55±5.24	98.72±8.35	19.74±1.67	88.1±6.46	88.71±1.64
DC	153.67±3.13*	140.51±7.49*	28.04±1.45*	93.97±5.24	31.66±3.00
INS	147.03±3.79 a	120.45±10.33*,a	24.09±2.07*	84.23±5.39 a	38.71±2.76
GB	133.59±5.12 a	124.37±17.90*, a	24.87±3.58a,b	74.95±5.52 a	33.77±3.00
MO	148.12±1.50 a	134.48±21.61*	26.89±4.32*	82.19±15.57 a	39.04±12.81
OG	140.59±5.06*,a	98.44±18.19a,b	15.23±0.00a	90.07±0.00 a	35.29±0.50
MO/OG	152.09±16.44*	76.63±3.23 a	15.33±0.64 a	65.08±6.30*,a	71.68±28.20*,a,b

Values are expressed as mean ± SEM.
**significantly different from NC at p<0.05*
a = significantly different from DC at p<0.05
b = significantly different from INS at p<0.05

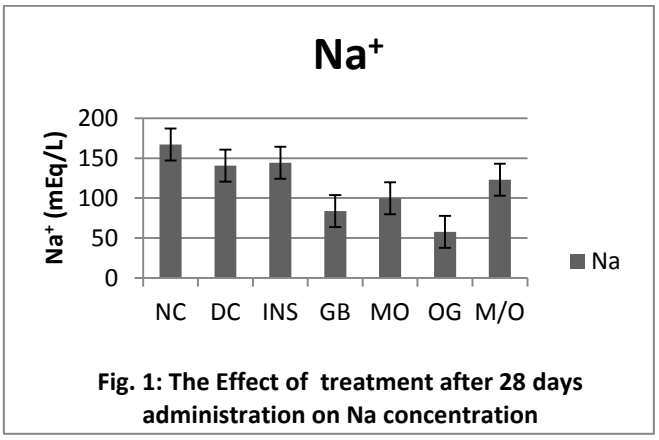

Fig. 1: The Effect of treatment after 28 days administration on Na concentration

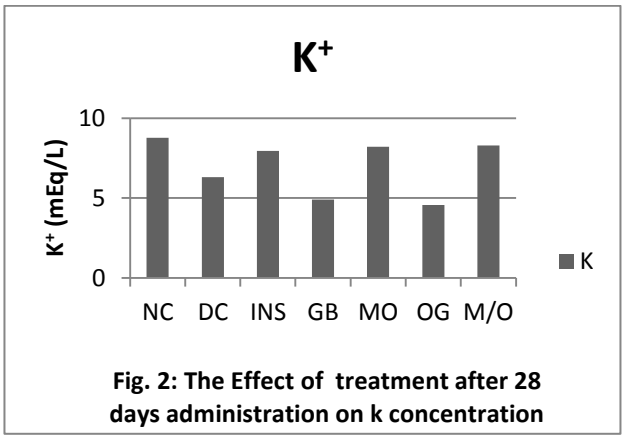

Fig. 2: The Effect of treatment after 28 days administration on k concentration

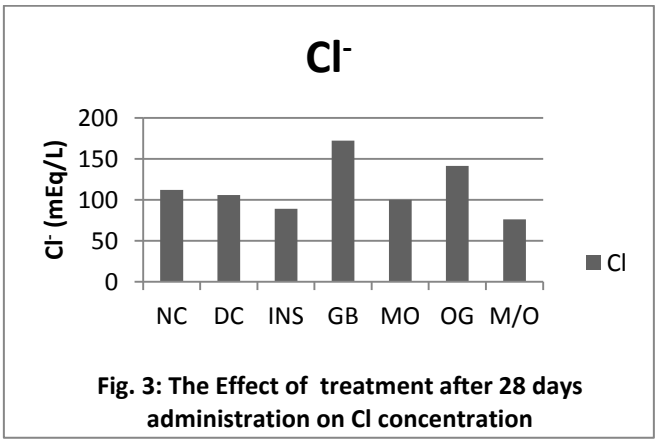

Fig. 3: The Effect of treatment after 28 days administration on Cl concentration

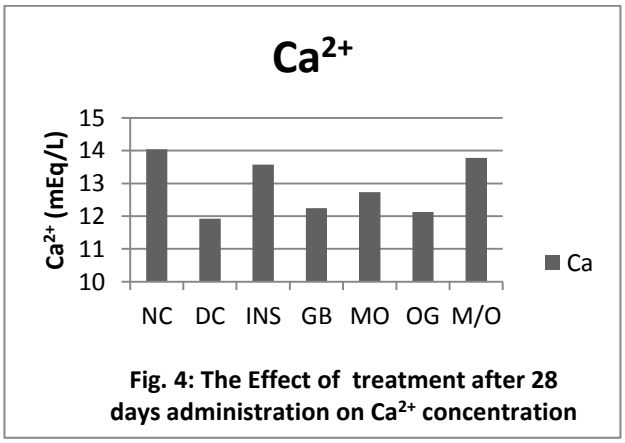

Fig. 4: The Effect of treatment after 28 days administration on Ca^{2+} concentration

Although, combined extracts did not show synergistic effects for bilirubin, urea and creatinine but compared well with insulin and the normal control group, it can be proposed that continual administration of the extracts could result in a synergistic effect of the extracts for the said parameters. From the results from other parameters exhibiting strong synergistic effects, it is can be concluded that the plant extracts are more potent in the management of diabetes induced nephropathy when combined. The active principles of the plants as well as the different points of action of the extracts must be probable reasons for the synergistic effects observed in the combined group. Therefore, the combination therapy should be considered as an excellent candidate for future studies in diabetes mellitus. In addition, further comprehensive pharmacological investigations, including experimental chronic studies, should be carried out.

CONCLUSION

The data from this work shows that the *Ocimum gratissimum* and *Moringa oleifera* extracts was found to improve kidney function in diabetes induced rats. Furthermore, the effect of the combined extracts of *Ocimum gratissimum* and *Moringa oleifera* ameliorated diabetic nephropathy more effectively than the single extracts in a synergistic manner similar to insulin.

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