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Research Article

Evaluation of the Nephroprotective Effects of Combined Extracts of *Vernonia amygdalina* and *Moringa oleifera* in Diabetes Induced Kidney Injury in Albino Wistar Rats

Iwara, I. A*, Otu, E. A, Efiong, E. E, Igile, G. O, Mgbeje, B. A. I, Ebong, P. E
Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, P.M.B 1115, Calabar, Nigeria

***Corresponding author**
Iwara, Iwara Arikpo
Email: iwaraarik@yahoo.com, iwaraiwara83@gmail.com

Abstract: The study is aim at investigating effect of combine extracts of *Vernonia amygdalina* (V.A) and *Moringa oleifera* (M.O) on STZ induced kidney damage in experimental rat models. The dried leaves of each plant species were extracted in 80% (V/V) ethanol. Thirty-six male and female rats of Wister strain weighing about 140-180g and divided into 6 groups, each group consisting of 6 rats were used for the investigation. Group 1and 2 represented diabetic control, received placebo. Group 3, 4, 5 and 6 serves as diabetic treated groups and received insulin (5IU/b.w), M.O (500mg/kg), V.A(500mg/kg) and combine M.O/V.A (250mg/kg each) respectively. At the end of the 21days, animals were euthanized under chloroform vapor and sacrificed. Whole blood was collected from them via cardiac puncture. Significant increase ($P<0.05$) in K+ ,Na+, CL- and Urea concentration in groups treated with VA, MO, and MO/VA compared to DC and closely related to NC and Insulin groups was obsevered. Serum glucose levels showed a significant decrease compared with the DC groups and closely related to NC. In conclusion, Result suggests the synergistic effect of the plants in amelioration of nephrotoxicity associated with diabetes mellitus.
Keywords: Hepatotoxicity, Amelioration, *Vernonia amygdalina, Moringa oliefera* and Synergistic

INTRODUCTION

Plants and herbs are mines of large number of bioactive phytochemicals that might serve as lead for the development of effective, safe, cheap novel drugs [1]. Herbal formulation alone or in combination with oral hypoglycaemic agents sometimes produces good therapeutic responses in some resistant cases where modern medicines alone have failed [2]. Several medicinal plants are widely used for the treatment of diabetes mellitus in the traditional medicine of many countries.

Kidneys are one of the important organs in the body, with pivotal roles in the excretion of toxin that are pathologically harmful to the human body. Therefore damage to the kidneys by nephrotoxic agents is of grave consequences.

Diabetes mellitus has been described as a metabolic disease marked by an elevated blood glucose concentration and the excretion of excess glucose in the urine [3]. The disease occurs either because of lack of insulin or the presence of factors that opposes the actions of insulin [2]. However, complete cure of the disease has been eluding physician for centuries and the quest for the development of more effective antidiabetic/hypoglycaemic agents is being pursued relentlessly [4-6].

Vernonia amygdalina and *Moringa oleifera* are among medicinal plants reported to be used in traditional settings for the management of ailments. Report by Atangwho [7] showed *Vernonia amygdalina* to restore the damage previously done to the β cells of the pancreas i.e. protective ability of the extracts on the pancreas, as the probable mechanism of action in exerting anti-diabetic action. *Moringa oleifera* has great use medicinally both as preventative and treatment. Its bark, sap, roots, leaves, seeds, oil, and flowers are used in traditional medicine in several countries. A folk remedy for stomach complaints, catarrh, cancer [8] , gastric ulcers, skin diseases, lowering blood sugar, increasing bone density, nervous conditions, diabetes, fatigue, increase lactation, hay fever, impotence, edema, cramps, hemorrhoids, headaches, sore gums; to strengthen the eyes and the brain, liver [9]. Tende [10] observed the effect of ethanolic extract of M.O on the blood glucose level in STZ-induce diabetic and non-non diabetic rats and further suggested that due to the closely related reduction of blood glucose by this plant to insulin, the hypoglycemic effect of the plant may appear to be probably exerted via a mechanism that may be similar to that of insulin. It is on this basis that this present study is design to investigate the nephroprotective effect of combined extracts of *Vernonia amygdalina* and *Moringa oliefera* in diabetic induced kidney injury in albino wistar rats.

MATERIALS AND METHOD

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.

Animals

Thirty-six male and female rats of wistar strain weighing about 140-180g were obtained from the Animal House of the Department of Zoology and Environmental Biology, University of Calabar, Calabar. The animals were allowed to acclimatize for 14 days in the animal house of Biochemistry Department, University of Calabar, Calabar. The animals were housed in well ventilated cages (wooden bottom and wire mesh top) and kept under controlled environmental conditions of temperature (25±5^{0}C) relative humidity (50±5%) and 12 hour light/dark cycle. The animals were fed with standard pellet and water *ad libitum*. Animals were divided into 6 groups, each group consisting of 6 rats were used for the investigation. Group 1and 2 represented diabetic control, received placebo. Group 3,4,5 and 6 serves as diabetic treated groups and received insulin (5IU/b.w), M.O (500mg/kg), V.A(500mg/kg) and combine M.O/V.A (250mg/kg each) respectively. At the end of the 21days, were euthanized under chloroform vapour and sacrificed. Whole blood was collected from them via cardiac puncture using sterile syringes and needles, and emptied into plain tubes and allowed to clot for about two hours. The clotted blood was thereafter centrifuged at 3,000 rpm for 10 minutes to recover serum from clotted cells. Serum was separated with sterile syringes and needles and stored frozen until they are required for biochemical analyses.

The principle of laboratory animal care was followed and the research has been determined exempt from review by the university animal research.

Experimental design

Thirty six adult male and female wistar albino rats weighing 140 - 180g were grouped into six (6) as follow and Treatment was administered twice daily (12 hourly) for 21 days.

Animal Grouping

Group	treatment	Number of animal	Dose administered
1	NC	6	0.5ml DMSO
2	DC	6	0.5ml DMSO
3	Insulin	6	5IU/kg b.w
4	M.O	6	500mg/kg b.w
5	VA	6	500mg/kg b.w
6	M./V	6	250mg/kg b.w each

NC= Normal control. DC=diabetic control, M.O=*Moringa oleifera*, V.A= *Vernonia amygdalina*, M/V= *Moriga oleifera*/*Vernonia amygdalina*, DMSO=Dimethylsulphoxide

Acute toxicity test

The oral acute toxicity study of *Moringa oliefera* and *Ocimum gratissimum* was determined in mice as described by lorke [11].

Induction of diabetes

Diabetes was induced in a 12hrs fasted rats with streptozotocin (40mg/kg b.w) dissolved in citrate buffer (0.1 M, pH 4.5) and injected intraperitoneally in a volume of 0.5ml citrate buffer/rat. After 48hrs of injection, diabetes was confirmed with a fasting blood sugar (FBS) concentration ≥ 200mg/dl. This was estimated using One Touch ® Glucometer (Lifescan, Inc. 1996 Milpas, California, U.S.A) with blood obtained from the tail vein of the rats.

Determination of Electrolyte Profile

The concentration of electrolyte viz: sodium (Na^{+}), chloride (Cl^{-}), Potassium (K^{+}) and Urea were assay using reported standard kit methods.

Statistical Analysis

Results obtained were express as mean± S.E.M; differences in mean values were estimated by the use of ANOVA followed by Dunnet's post hoc test. The levels of significance was setup at p=.05.

RESULTS AND DISCUSSION

Acute toxicity test

Death was recorded in 3000mg/kg dose therefore a geometric mean of the dose where death occurred and the preceding the recorded death was calculated and the LD_{50} was found to be 2500mg/kg (2.5g/kg). The behavioral change noted in these animal following extracts administration was dullness for M.O with no behavioral change with O.G at the onset of extract administration. The animal treated with M.O later became active after some hours of extract administration. At doses above this levels however, the animal exhibited some toxicity.

Effect of extract on serum electrolyte and glucose level

Changes in concentration of serum electrolyte and serum glucose levels of diabetic control groups compared with the normal control indicating hyperglycemic condition with associating kidney damage was observed in (fig 1-4). On administration of the extracts, significant increase (P=.05) in K^+ concentration in groups treated with VA, MO, and MO/VA compared to DC and closely related to NC and Insulin groups. Also Na^+ concentration showed a significant increase (P=.05) in groups treated with VA and MO/VA compared with DC and closely related to insulin and NC. A significant increase (P=.05) was observed in Cl^- levels of DC, VA, MO and MO/VA groups when compared to both NC and Insulin groups. Urea concentration showed a significant (P=.05) increase when compared with NC. Also from (fig 5) serum glucose levels showed a significant decrease compared with the DC groups with close relations with the insulin treated group.

Diabetes mellitus is one of the most common chronic global diseases affecting children and adolescents in both the developed and developing nations. Sustained hyperglycaemia is a common result of uncontrolled diabetes and, over time, can damage the heart, eyes, kidneys and nerves, mainly through deteriorating blood vessels supplying the organs. Therefore, emphasis on diabetes care and management is on optimal blood glucose control to avert these adverse outcomes (Musabayane, 2012).

Nephrotoxicity is one of the most common kidney problems and occurs when your body is exposed to a drug or toxin that causes damage to your kidneys. When kidney damage occurs, you are unable to rid your body of excess urine, and wastes. Your blood electrolytes (such as potassium and magnesium) will all become elevated.

Sustained hyperglycaemia is the main cause of the changes in kidney function in diabetes mellitus. Hyperglycaemia leads to the increased formation of advanced glycation end-products (AGEs), oxidative stress, and activation of the polyol pathway and hexosamine flux, causing inflammation and renal demage [12]. The accumulation of AGEs can be prevented by antioxidants such as flavonoids or by preventing the glucose-dependent formation of intermediate products [13].

Ethnobotanical plants have traditionally been used for the management of diabetes and its complications [6, 14-16}. Current pre-clinical and clinical studies have demonstrated that many have beneficial effect on some processes associated with reduced renal function in experimental animal [17]. The active phytochemicals responsible for their activities have been identified [18].

From this investigation on nephroprotective effect of combined extracts of *Vernonia amygdalina* and *Moringa oliefera* in diabetes induced kidney injury in albino wistar rats, the result showed Significant increase in K^+ ,Na^+, CL^- and Urea concentration in groups treated with VA, MO, and MO/VA compared to DC and closely related to NC and Insulin groups. Serum glucose levels showed a significant decrease compared with the DC groups and closely related to NC. This observation may be attributed to the reported presence of bioactive component that are present in this plants and consistent with findings of Musabayane, [12] and Mapanga [17], which shows that combined leaf extracts of *Vernonia amygdalina* and *Moringa oliefera* which possess hypoglycemic effect, has the ability to excrete electrolyte in STZ diabetes mellitus, suggesting that this plant may be beneficial in the management of renal dysfunction associated with diabetes mellitus.

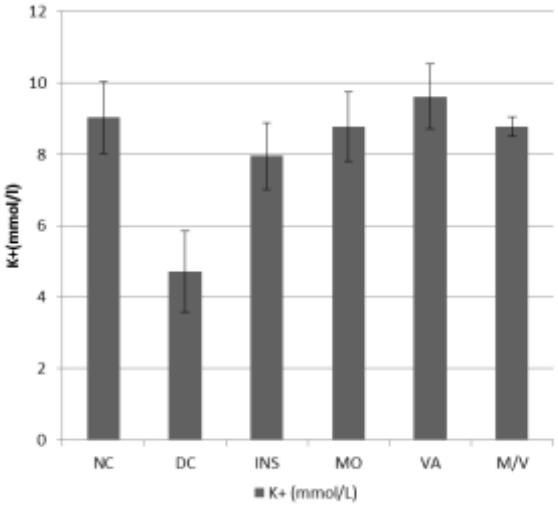

Fig. 1: Effect of 21 day administration combined extracts of VA and MO on serum electrolytes and glucose concentrations in STZ induced diabetic rats

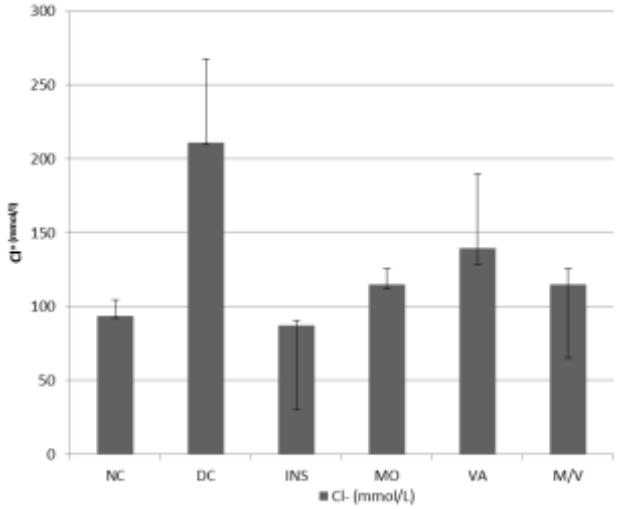

Fig. 3: Effect of 21 day administraton combined extracts of VA and MO on Cl^+ concentrations in STZ induced diabetic rats

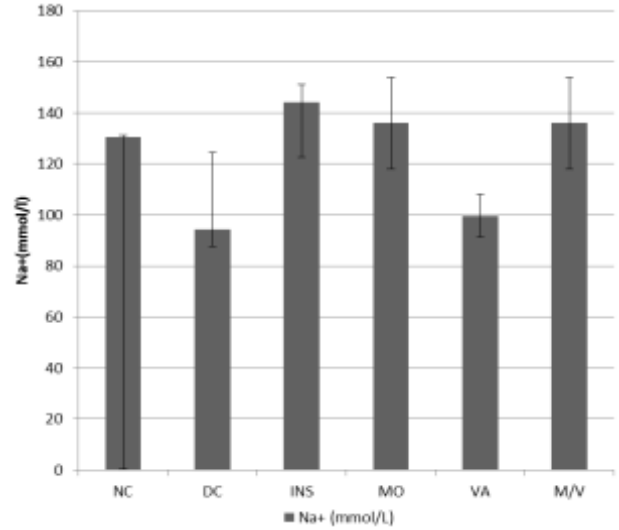

Fig. 2: Effect of 21 day administration combined extracts of VA and MO on Na^+ concentrations in STZinduced diabetic rats

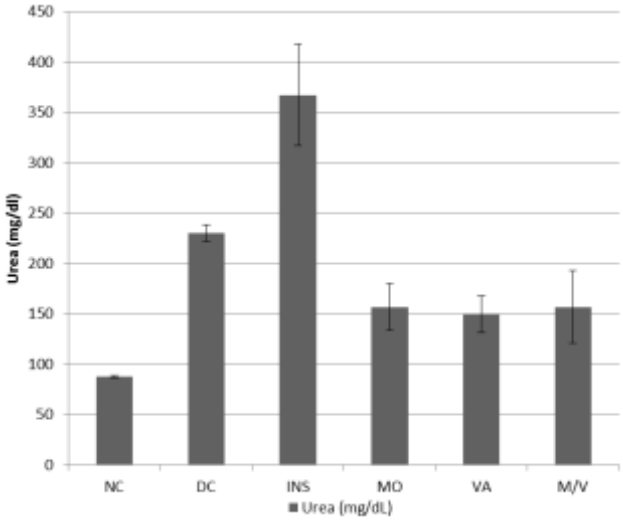

Fig. 4: Effect of day 21 day administration combined extracts of VA and MO on urea concentrations in STZ induced diabetic rats

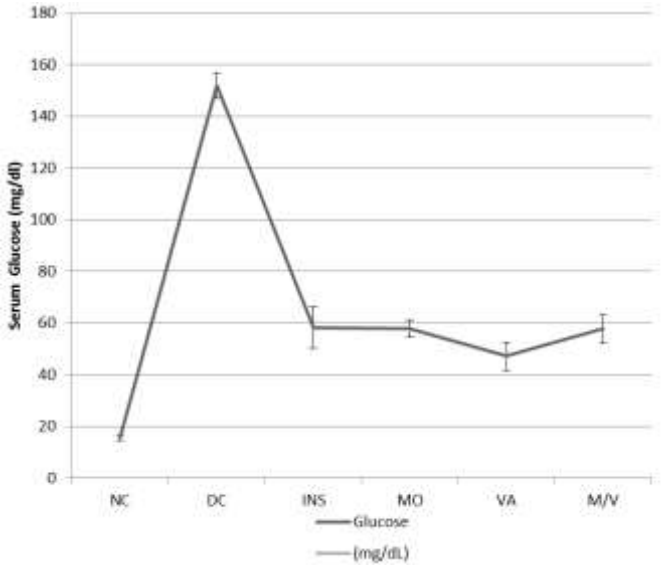

Fig. 5: Effect of 21 day administration combined extracts of VA and MO on serum Glucose concentrations in STZ induced diabetic rats

CONCLUSION

The result thus suggests the synergistic effect of the plants in amelioration of nephrotoxicity associated with diabetes mellitus and a possible therapeutic remedy for the management of diabetes.

AUTHORS' CONTRIBUTIONS

The research was carried out in collaboration between all authors. Author EPE project concept and design, coordination and interpretation of data. Author IIA experimentation and acquisition of data; preparation of final manuscript, graphics, analysis, interpretation of data and coordination. Author OEA experimentation and acquisition of data; preparation of draft manuscript. Author EEI experimentation and acquisition of data; preparation of draft manuscript. Author IGO experimentation and acquisition of data; preparation of final manuscript, graphics, analysis, interpretation of data and coordination. Author MBAT experimentation and acquisition of data; preparation of final manuscript, graphics, analysis, interpretation of data and coordination.

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