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Original Article

Metformin Potentiates the Antidiabetic Properties of *Annona muricata* and *Tapinanthus globiferus* Leaf Extracts in Diabetic Rats

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ABSTRACT

Background: There is paucity of experimental evidence on the complementary use of standard anti-diabetic drugs with herbal formulations. **Materials and Methods:** In this study, extracts of *Annona muricata* (AME) and *Tapinanthus globiferus* (TGE) were administered with metformin to diabetic rats in order to study the potential complementary effects. Diabetes was induced by a single intraperitoneal (i.p) injection of alloxan (150 mg/kg BW). AME and TGE (200 mg/kg BW each) and Metformin (100 mg/kg BW) was administered to diabetic rats orally for 21 days. At the end of the study, rats were sacrificed; blood was collected for assessment of lipid profile and kidney function. **Results:** Treatment of diabetic rats with AME and TGE caused a significant decrease ($p < 0.05$) in the concentrations of total cholesterol (TC), triglyceride (TG) and Coronary Risk Index (CRI) with a concomitant increase in High Density Lipoprotein (HDL). Similarly, urea and creatinine concentration decreased in diabetic rats administered plant extracts. Co-administration of the plant extracts with metformin significantly improved lipid profiles and kidney function relative to rats administered metformin alone. Co-administration of AME and TGE extracts with metformin produced significant improvement in biochemical indices of diabetic rats. **Conclusion:** The results suggest potential synergistic interaction between the plant extracts and metformin. Furthermore, this work provides scientific support for the concomitant use of the plants used in the study with orthodox drugs for the management of diabetes.

Key words: *Annona muricata*, Diabetes, Drug interaction, *Tapinanthus globiferus*.

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INTRODUCTION

Diabetes, a chronic metabolic disease is one of the leading causes of death globally. In the most recent publication by WHO, 1.6 million deaths were directly caused by diabetes and another 2.2 million deaths were attributable to hyperglycemia in 2016. In 1980, the WHO reported that 108 million people were diabetic and by 2014, this number had increased four (4) folds (422 million).[1] In fact, studies by Guariguata and co-workers suggests that by 2035, 592 million people will be diabetic.[2] This alarming statistics necessitate research to negate the trend. There are two major types of diabetes namely: type 1 also known as Insulin Dependent Diabetes characterised by insufficient production of insulin and type 2 which is characterised by insensitivity of insulin receptors to insulin.[3,4] Both types of diabetes have common metabolic hallmarks which include: hyperglycemia, dyslipidemia, disturbances in protein metabolism and oxidative stress.[5,6,7,8] Current strategies for the management of diabetes include life style changes e.g. diet control, exercise and medical interventions e.g. insulin injection, use of hypoglycemic drugs. Commonly prescribed drugs are poorly tolerated by most users hence, have numerous side effects such as heart and kidney disorders.[9] Herbal formulations on the other hand are believed to be safe for consumption due to their 'food likeness' and richness in antioxidants.[10] As a result, some diabetics take modern anti-diabetic drugs with herbal formulations without prescription.[11,12] Co-administration of modern drugs with herbal formulations has its roots from the believe that herbs are harmless even though there is evidence to show that some drugs derived from plants have narrow therapeutic index.[11] Concomitant administration of herbs and modern drug can alter the pharmacodynamics and pharmacokinetics of the former through herb-drug interaction.[12] This interaction of plants extracts with drugs can be additive, synergistic or antagonistic changing drug efficacy.[9,13,14] In fact, it is a known fact that herbal drugs can have beneficial effects in terms of reducing side effects, increasing efficacy and reducing dosage of modern drugs.[9]

Recently, there have been intensified efforts to check the efficacy and safety of herbal drug combinations with anti-diabetic drugs through pre-clinical studies.[14] The present work represents one of such efforts. In the present work the effects of concurrent administration of two plants *Annona muricata* and *Tapinanthus globiferus* (mistletoe) with metformin is evaluated.

MATERIALS AND METHODS

Chemicals and drugs

Methanol and Alloxan monohydrate were products of Sigma (St. Louis, Germany). Diagnostic kits for Total Cholesterol (TC), High Density Lipoprotein (HDL) and Triglyceride (TG), creatinine, urea, were manufactured by Randox Diagnostics (UK).

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Metformin (Glucophage 500 mg) was purchased from a local pharmacy in Anyigba, Kogi State, Nigeria. Other chemicals used were of analytical grade obtained from reputable vendors.

Ethical permission

The study was granted approval for use of plants and animal experiments by Department of Biochemistry, Kogi State University, Nigeria. The study was conducted according to Nigerian Community regulations in conformity with international standards regarding the protection of experimental animals.

Experimental animals

Male Wistar albino rats weighing 180 – 200 g were procured from the Animal Facility of the Department of Biochemistry at Kogi State University, Anyigba. The animals were housed in controlled environment (12 hr light/dark cycle and temperature of ≃ 25 °C) and fed standard, commercial rat feed and water *ad libitum*.

Plant collection and extract preparation

Leaves from *Annona muricata* and *Tapinanthus globiferus* plants were collected during rainy season, May 2018 from Anyigba town, Kogi State, Nigeria. The plants were identified by a botanist. Leaves of the plants were air dried at room temperature, and then pulverized using an electric grinder. Portions of the pulverized leaves were suspended in Methanol at a ratio of 1:15 (w/v) for 48 hr followed by filtration and concentration of the filtrate in a rotary evaporator.

Induction and assessment of diabetes

Diabetes was induced in overnight fasted rats by single intraperitoneal injection of freshly prepared alloxan [150 mg/kg body weight (BW)] according to published protocols.[7,15] Effective induction of diabetes was confirmed 48 hrs after alloxan injection by measuring Fasting blood glucose (FBG) concentration of the rats. Rats with FBG levels greater than or equal to 250 mg/dL were considered diabetic and used for the study.

Experimental design

Forty eight (48) rats allocated to eight (8) groups were used for this study. The groups were as follows: Group 1 served as normal control and was fed rat chow and water *ad libitum*. All other groups were induced with alloxan and treated as follow: Group 2 served as diabetic control group; Group 3 served as reference drug control and received 100 mg/kg BW Metformin. Groups 4 & 5 received 200 mg/kg BW of AME and TGE each in a ratio of 1:1, respectively. Groups 6 & 7 received co-administration of Metformin/AME (100 mg kg^{-1} BW/ 200 mg kg^{-1} BW) and Metformin/TGE (100 mg kg^{-1} BW/ 200 mg kg^{-1} BW), respectively. Group 8 received a combination of Metformin/AME/TGE (100 mg kg^{-1} BW/ 200 mg kg^{-1} BW/ 200 mg kg^{-1} respectively). All treatments lasted 21 days. Doses of plant extracts used for this study was based on published results of therapeutically effective, no toxic doses for AME and TGE as well as Metformin.[16-18]

Biochemical estimations

Fasting Blood Glucose (FBG) concentration was determined by glucose oxidase method using a one-touch AccuCheck glucometer. Serum was separated from whole blood for the analysis of lipid profile and kidney function. Analysis of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), urea and creatinine levels in serum were carried out using Randox Diagnostic kits according to manufacturer's instructions. Coronary Risk Index (CRI) was calculated according to the method described by Mohammed et al. [19] as follows:

Coronary Risk Index = TC /HDL-c

Statistical analysis

Results were expressed as Mean ± SD of six (6) determinations. Differences among experimental groups was analyzed by Analysis of Variance (ANOVA) using SPSS 16.0. Differences among groups was considered significant when $p < 0.05$.

RESULTS

Effect of AME and TGE on lipid profile

The data for the effect of AME and TGE on lipid profile are shown in Table 1 and 2. Significant increase ($p<0.05$) in serum TC and TG with a concomitant significant decrease in HDL was observed in alloxan diabetic rats when compared with normal rats. Separate treatments with AME and TGE caused a reduction in TC and TG compared to diabetic untreated rats. This reduction was more significant with AME than with TGE. However, insignificant fluctuactions in TC and TG were observed when AME and TGE or both was combined with metformin ($p<0.05$).

The decrease in serum HDL caused by alloxan was significantly reversed by treatment with AME alone or TGE alone. Co-administration of these plant extracts with metformin further elevated the serum concentration of HDL. However, administration of AME + TGE + metformin did not seem to offer benefits with respect to increasing the HDL levels when treated with AME alone or TGE alone.

Coronary risk index

The calculated coronary risk index (CRI) is presented in Table 1. CRI increased significantly ($p<0.05$) in diabetic rats 11 folds thereby complementing the TC and TG levels of diabetic rats. However, treatment with the plant extracts significantly attenuated CRI. This positive effect of the plant extracts was improved by co-administration with metformin.

Kidney function markers

Elevated serum concentrations of urea and creatinine were observed in diabetic rats when compared with normal control rats as shown in Table 2. Treatment with extracts of AME alone or TGE alone did not significantly influence the levels of serum urea and creatinine relative to diabetic rats ($p>0.05$). However, co-administration of either AME or TGE alone with metformin significantly decreased ($p<0.05$) urea and creatinine serum concentrations when compared with groups that received plant extract alone. The group that was administered AME + TGE+ metformin also experienced a significant reduction in the urea and creatinine concentrations when compared with diabetic untreated control and the group administered with extracts alone.

DISCUSSION

The effects of most diabetogenic agents including alloxan are realized through dysregulation of glucose metabolism in the liver, muscle and adipose tissue.[14] Alloxan causes glucose dysregulation by destroying pancreatic β-cells through an oxidative stress mediated mechanism which leads to insulin deficiency.[20] Many biochemical pathways are sensitive to deficiency of insulin which accounts for concordant dysregulation of carbohydrate, lipid and protein metabolism in diabetics.[15,20] Metformin tops the list among other modern hypoglycemic drugs commonly prescribed for the management of diabetes.[14,21] Therefore, this study evaluated the benefits or otherwise of combinations of two anti-diabetic plants (*Annona muricata* and *Tapinanthus globiferus*) with the standard anti-diabetic drug, metformin. A rat model of alloxan-induced diabetes was employed; and, in the present study, alloxan was intended to induce indices of hyperglycemia, hyperlipidemia and elevation in levels of kidney

Table 1: Blood lipid profile of diabetic rats administered *Annona muricata*, *Tapinanthus globiferus* and metformin.

Groups	Blood lipid profile			
	TC (mmol/L)	HDL (mmol/L)	TG (mmol/L)	Coronary artery risk index (CRI)
Normal untreated	1.81 ± 0.20	1.22 ± 0.23	1.54 ± 0.03	1.50 ± 0.12
Diabetic untreated	5.53 ± 0.15$^{\alpha\gamma\delta\varepsilon}$	0.36 ± 0.07$^{\alpha\gamma\delta\varepsilon}$	5.76 ± 1.14$^{\alpha\gamma\delta\varepsilon}$	16.64 ± 2.58$^{\alpha\gamma\delta\varepsilon}$
Metformin 100 mg/kg BW	2.69 ± 0.09$^{\beta}$	0.88 ± 0.35$^{\beta}$	1.93 ± 0.09$^{\beta}$	3.40 ± 1.36$^{\beta}$
AME 200 mg/kg BW	3.35 ± 0.08$^{\beta\varepsilon}$	0.69 ± 0.08$^{\beta}$	2.74 ± 0.18$^{\beta\varepsilon}$	4.90 ± 0.45$^{\beta\varepsilon}$
TGE 200 mg/kg BW	3.99 ± 0.18$^{\beta\varepsilon}$	0.66 ± 0.26$^{\beta}$	2.39 ± 0.18$^{\beta}$	6.76 ± 2.59$^{\beta\varepsilon}$
AME 200mg/kg BW + Metformin 100 mg/kg BW	2.60 ± 0.10$^{\beta\gamma}$	0.96 ± 0.10$^{\beta\gamma}$	2.13 ± 0.22$^{\beta\gamma}$	2.72 ± 0.19$^{\beta\gamma}$
TGE 200mg/kg BW + Metformin 100 mg/kg BW	3.06 ± 0.14$^{\beta\delta}$	0.95 ± 0.07$^{\beta\delta}$	2.14 ± 0.07$^{\beta}$	3.23 ± 0.09$^{\beta\delta}$
AME 200 mg/kg BW + TGE 200mg/kg BW + Metformin 100 mg/kg BW	2.44 ± 0.12$^{\beta\gamma\delta}$	0.75 ± 0.15$^{\beta}$	2.14 ± 0.07$^{\beta\delta}$	3.37 ± 0.50$^{\beta\gamma\delta}$

Data is presented as mean ± SD of six (6) animals. Superscript α-ε indicate significantly different group $p<0.05$.
$p<0.05$ compared to normal untreated rats$^{\alpha}$
$p<0.05$ compared to diabetic untreated group$^{\beta}$
$p<0.05$ compared to AME 200 mg/kg BW group$^{\gamma}$
$p<0.05$ compared to TGE 200 mg/kg BW group$^{\delta}$
$p<0.05$ compared to the AME + TGE+ Metformin group$^{\varepsilon}$

Table 2: Kidney function markers of diabetic rats administered *Annona muricata*, *Tapinanthus globiferus* and metformin.

Groups	Kidney function markers	
	Serum Urea (mg/dl)	Serum Creatinine (mg/dl)
Normal untreated	26.07 ± 2.10	0.85 ± 0.12
Diabetic untreated	135.50 ± 4.38$^{\alpha\varepsilon}$	2.63 ± 0.11$^{\alpha\varepsilon}$
Metformin 100 mg/kg BW	82.16 ± 2.76$^{\beta}$	1.70 ± 0.06$^{\beta}$
AME 200 mg/kg BW	114.10 ± 11.59$^{\beta\varepsilon}$	2.24 ± 0.11$^{\beta\varepsilon}$
TGE 200 mg/kg BW	130.20 ± 2.16$^{\beta\varepsilon}$	3.14 ± 0.02$^{\beta\varepsilon}$
AME 200mg/kg BW + Metformin 100 mg/kg BW	73.45 ± 1.98$^{\beta\gamma}$	1.85 ± 0.05$^{\beta\gamma\varepsilon}$
TGE 200mg/kg BW + Metformin 100 mg/kg BW	78.32 ± 5.47$^{\beta\delta}$	1.48 ± 0.02$^{\beta\delta}$
AME 200 mg/kg BW + TGE 200mg/kg BW + Metformin 100 mg/kg BW	69.25 ± 1.32$^{\beta\gamma\delta}$	1.52 ± 0.08$^{\beta\gamma\delta}$

Data is presented as mean ± SD of six (6) animals. Superscript α-ε indicate significantly different group $p<0.05$.
$p<0.05$ compared to normal untreated rats$^{\alpha}$
$p<0.05$ compared to diabetic untreated group$^{\beta}$
$p<0.05$ compared to AME 200 mg/kg BW group$^{\gamma}$
$p<0.05$ compared to TGE 200 mg/kg BW group$^{\delta}$
$p<0.05$ compared to the AME + TGE+ Metformin group$^{\varepsilon}$

function markers consistent with previous studies.[22,23] These indices have previously been reported to be suppressed by aqueous extract of *A. muricata* and aqueous-ethanol extract of *T. globiferus* leaves.[22,24] The use of the drug metformin has added benefits such as improvement of oxidative stress, renal function and lipid profiles, hence justifying the choice of the drug for this study.[14,25] Dyslipidemia in diabetic rats was quantitatively observed as increase in serum levels of TC, TG and reduction in the levels of HDL-c. Blood lipids were reduced when animals were treated with the plant extracts. Combination of the plant extracts with metformin also enhanced the anti-hyperlipidemic activity of the extracts. Interestingly administration of AME+TGE+Metformin caused a small but significant improvement in the levels of serum lipids in diabetic rats when compared with diabetic rats administered metformin with AME or TGE separately. Observation of the outcomes of treating diabetic rats with the combinations reveals synergistic effect of the components of the formulations. Metformin is known to interact with many enzymes and exert its beneficial effects through multiple biochemical pathways e.g phosphatidylinositol-3-kinase/protein kinase B, PI-3-kinase/AKT, 5-AMP-activated protein kinase/acetyl-CoA carboxylase and mitogen activated protein kinase pathways.[26] The plant extracts used in this study might be mimicking the same pathways thereby enhancing the anti-hyperlipidemic properties of metformin. Activation of these pathways improves secretion and sensitivity to insulin and restoration of dyslipidemia through increased hepatic and adipose tissue uptake of serum lipids or decreased lipolysis of fatty acids for β-oxidation and reduction of available acetyl-CoA for cholesterol synthesis.[25,26,27]

Disturbance of serum lipid profile expressed as Coronary Risk Index (CRI) is an established cardiovascular risk indicator.[19,21] A high CRI portrays a high likelihood of developing a cardiovascular complication and vice versa. In the present study, induction of diabetes caused a significant increase in CRI which was attenuated by the drug combinations used in this study to treat the animals. This result points to the fact that although metformin administered alone is cardioprotective, concomitant administration with the plant extracts enhances its activity.

Nephropathy is a common consequence of hyperglycemia in diabetics causing a significant decline in the physiological roles of the kidney.[28] Diabetic patients suffer from difficulty in filtration of solutes which may be partly due to hyperglycemia potentiated oxidative stress on the kidney.[29] In the present study, kidney damage was assessed by measuring the serum levels of urea and creatinine. Alloxan accentuated the serum levels of urea and creatinine signifying nephropathy. However, treatments containing metformin formulation s significantly decreased the serum levels of urea and creatinine to near normal ($p<0.05$). This portrays the effectiveness of the drug formulations in combating diabetic nephropathy in the alloxan model used for this study.[30] Combination of the plant extracts with metformin significantly improved the indices of kidney function when compared with treatment with plant extracts alone. Again, this demonstrates positive synergism between the plant extracts and metformin.

Although the mechanism behind the overall positive outcomes recorded with co-administration of AME and TGE with metformin is not clear, however, it could be attributed to toe phytochemical constituents of the extracts. As evidenced in published works, these plant extracts contain flavonoids, tannins, alkaloids, saponins, anthraquinones, glycosides and steroids.[31,32] The roles of these phytochemicals in the treatment of many human diseases have been established through scientific research. In fact, many of the modern drugs used for treatment of human diseases belongs the family of these phytochemicals.

In conclusion, the results of the study indicate that AME and TGE when co-administered with metformin possess higher anti-hyperlipidemic and nephroprotective activity than the AME or TGE alone. These potentials can be explained by the presence of therapeutic phytochemicals in the plant extracts which enhance the drug properties of metformin.

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COMPETING INTERESTS

Authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

The research idea was conceived by FOA. OJA, FOA and OBI were involved in the experiments which was supervised by FOA. Development of the manuscript, data analysis was done by FOA, OEY and OJA.

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ABBREVIATIONS

AME: Annona muricata extract; CRI: Coronary Risk Index; TGE: Tapinanthus globiferus extract.

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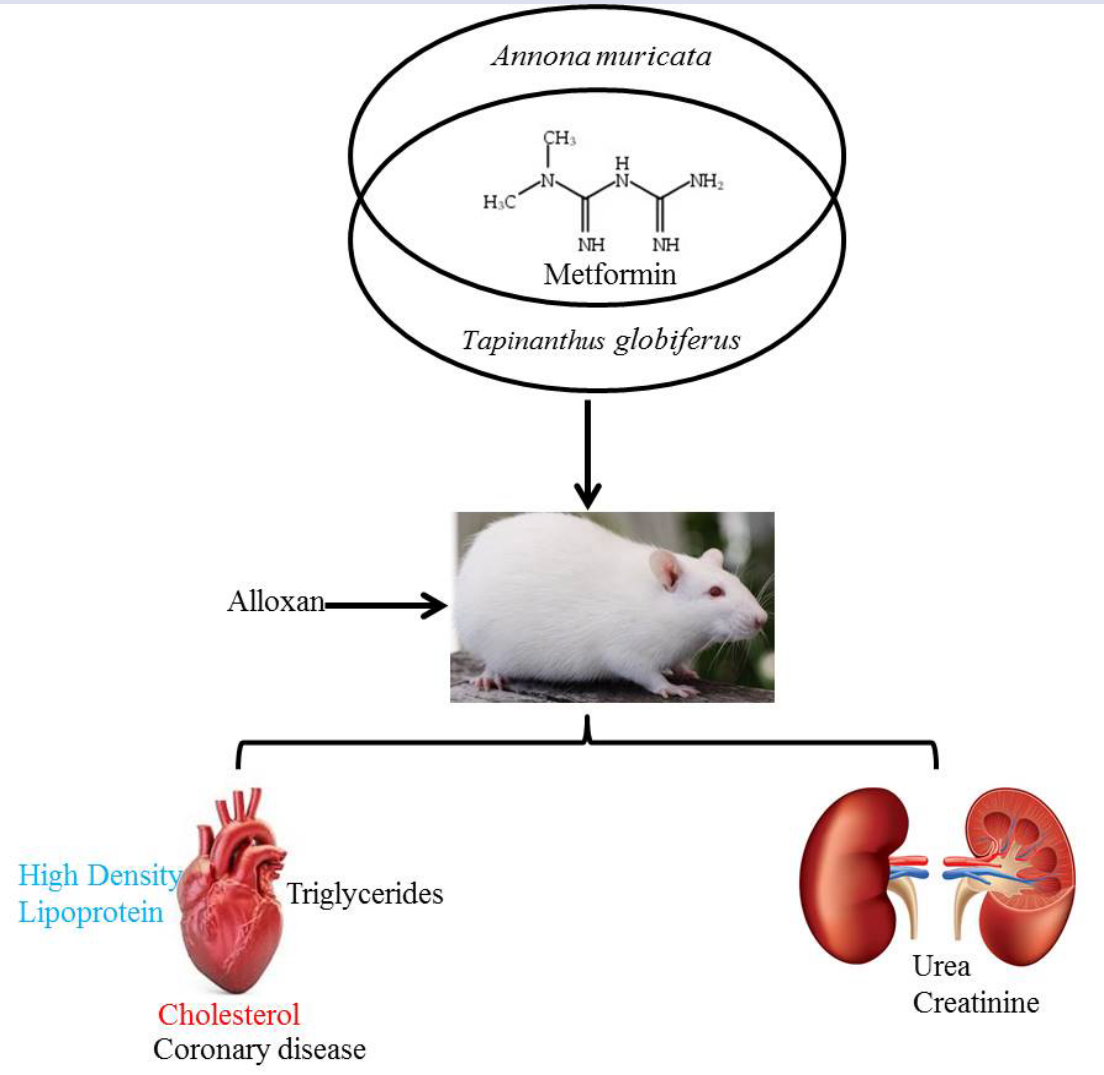
GRAPHICAL ABSTRACT



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