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Phytochemical, Antihyperglycaemic and Lipid Profile Effects of Methanol Extract Fraction of *Ricinus comminus* Seeds in Alloxan Induced Diabetic Male Wistar Albino

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ABSTRACT

The study investigated phytochemical, antihyperglycaemic and lipid profile effects of methanol extract fraction of *Ricinus comminus* seeds in alloxan induced diabetic male Wistar albino rats alloxan. Eighteen mice were used for the lethal dose (LD_{50}) study while 54 Wistar albino male rats were used for the antihyperglycaemic and lipid profile, respectively. The result of the phytochemical analysis showed the richness of the extract in important phytochemicals that could confer some health benefits. The methanol extract fraction had LD_{50}>5000 mg kg^{-1} b.wt., which indicate that the extract was safe to larger extent. The baseline blood glucose concentration showed non-diabetic glucose level 105.10±4.61 mg dL^{-1}. Diabetes was induced with alloxan monohydrate. Significant decrease ($p<0.05$) in blood glucose levels in the test animals were observed when compared with positive control. The extract had significant effect on the blood glucose levels and lipid profile of the treated rats closer to that of the standard drug (metformin) with the serum total cholesterol and triacylglycerol levels decreasing significantly ($p<0.05$) in all test groups relative to positive control. However, serum HDL showed significant increase ($p<0.05$) in group 4 and 5 when compared with positive control. The results of the study showed that the methanol extract fraction of *Ricinus communis* seeds exhibited significant antihyperglycaemic activities against alloxan induced diabetes in Wistar albino rats. It suggests that the methanol extract fraction possess bioactive compounds that when properly harnessed could help in improving the health state of diabetic patients.

Key words: *Ricinus communis,* antihyperglycaemia, phytochemicals, alloxan induced diabetic rats, serum lipid profile

INTRODUCTION

In the past decades, research have been focused on scientific evaluation of traditional drugs of plant seed origin and screening of more effective and safe hypoglycemic agents has continued to gain medicinal importance (Ndenecho, 2009). The medicinal values of many of these seeds cannot be over emphasized in the light of oral traditions and folklores from the distant past that have continued to extol the healing virtues of these seeds and their extracts (Yadav *et al*., 2014). Generally, the active principles found in *Ricinus comminus* can be extracted and used in different forms which include infusion, syrups, concoctions, decoctions, infused oils, essential oils, ointments

and creams (Sofowora, 1993) in the treatment/management and prevention of some diseases (WHO., 2003). Plant seeds contain bioactive components such as flavonoids, glycosides, saponins and tannins,which possess medicinal properties that are harnessed for the treatment of different diseases (Feher and Schmidt, 2003). The active ingredients for a vast number of pharmaceutically derived medications contain components originating from phytochemicals in seeds. Dietary plant seeds with proven antioxidant properties may function as a direct anti-radical chain breaker of free radical propagation, interaction with transition metals and inhibition of Reactive Oxygen Species (ROS) generating enzymes (Ilavarasan *et al*., 2006). *Ricinus communis* is one of such medicinal seeds whose medicinal values have stood the test of time.

Ricinus communis seeds are widely used in African folk medicine for the treatment of malaria, fever, stomach-ache, coughs, sexually transmitted diseases, tooth ache, breast cancer and constipation (Iwu, 1983). The seed has been found to be effective in the treatment of haemorrhoids, jaundice, ulcer, headache, sores, epilepsy, rheumatism and sciatica (Saini *et al*., 2010). It has also been found useful for ear ache, sore feet, chest pain, epilepsy and children's convulsion. The leaves and seeds have been used for febrifuge, wound dressing and stomach ache (Iwu, 2000). The anti-fertility activity of methanol extract of the seed (Sani and Sule, 2007; Sandhyakumary *et al*., 2003) has been reported in literature while the anti-inflammatory (Ilavarasan *et al*., 2006) and anti-malarial (Gbeassor *et al*., 1990) activities of the root extract have been documented. The seed is used traditionally for the treatment of diabetes (Nath *et al*., 2011), diarrhoea, dysentery (Yadav *et al*., 2014). Recent study has shown that the methanol seed extract of the plant has antidiabetic activity (Jena and Gupta, 2012). Scientific reports on the phytochemical constituents of the seed reveal the presence of alkaloids, saponins, flavonoids and tannins on ethyl acetate extract; alkaloids, flavonoids, tannins on ethanol extract; alkaloids, saponins, resins, tannins and flavonoids on aqueous seed extract (Obumselu *et al*., 2011). The juice of the fresh seeds of *Ricinus communis* is used effectively for the treatment of jaundice in Bundelkhand region of India (Padmapriya *et al*., 2012). This study was aimed at evaluating the phytochemical constituents, effect of methanol extract fraction of *Ricinus communis* seed on the blood glucose and lipid profile of alloxan induced diabetic Wistar albino rats.

MATERIALS AND METHODS

Plant materials: *Ricinus communis* seeds were used for this study. They were obtained from NkwoIbagwa-Aka Market, Igbo-Eze South LGA, Enugu State, Nigeria. They were identified by Mr. Alfred Ozioko of Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State, Nigeria.

Animals: Fifty four adult male Wistar albino rats were used for the antihyperglycaemic and lipid profile studies while 18 albino mice were used for the acute toxicity study. All the animals used were obtained from the Animal House of the Department of Zoology, University of Nigeria Nsukka. The animals were acclimatized to laboratory conditions for one week under standard conditions of 12 h light and dark cycles. The animals were fed with standard Grower's Mash rats' pellets (Grand Cereal Ltd., Enugu) and water.

Preparation of plant material: The *Ricinus communis* seeds were cleaned and sorted to remove broken ones and contaminants. The shells were removed manually and the kernelswere stored in a clean dry plastic container for the extraction (Akande *et al*., 2012).

Extraction of plant material: One thousand seven hundred and 55 g (1755 g) of the prepared seeds of *Ricinus communis* were soaked in 1.9 L of chloroform and methanol in 2:1 ratio with occasional shaking for 24 h after which they were filtered using muslin sieve and Whatman number 4 filter paper. The two resultant layers were separated using separating funnel and the respective filtrate was later evaporated to dryness using rotary evaporator at a temperature of 40°C. The methanol extract fraction was used for the study while the chloroform extract fraction was discarded.

Experimental design: Fifty four adult male Wistar albino rats weighing 70-150 g were used for the study while 18 adult male albino mice were used for the acute toxicity (LD_{50}) study. After acclimatization, the mice were divided randomly into 6 group of 3 mice each and three groups of mice each were used for the phase I and II of the acute toxicity study, respectively. The male Wistar albino rats were randomly distributed into 6 groups of 9 rats each. The methanol extract fraction of *Ricinus communis* seeds were administered orally daily after alloxaninduction of diabetes for 21 days, the blood glucose level and lipid profiles were monitored.

Group 1: Normal rats treated with normal saline (control)
Group 2: Diabetic rats, no treatment (positive control)
Group 3: Diabetic rats treated with standard drug: Metformin (5 mg kg^{-1} b.wt.)
Group 4: Diabetic rats treated with first dose of extract (100 mg kg^{-1} b.wt.)
Group 5: Diabetic rats treated with second dose of extract (200 mg kg^{-1} b.wt.)
Group 6: Diabetic rats treated with third dose of extract (400 mg kg^{-1} b.wt.)

Qualitative phytochemical screening of methanol seeds extract of *Ricinus communis*: Qualitative phytochemical analysis of methanol extract fraction of *R. communis* seeds were carried out according to the method of Harborne (1989) and Trease and Evans (1989) to identify its active constituents.

Quantitative determination phytochemical constituents of methanol seeds extract of *Ricinus communis*: The steroid, saponin, tannin, alkaloid, flavonoid and glycoside were determined by the methods described by Edeoga *et al.* (2005), Brunner (1984), Swain (1979), Harborne (1989), Oyedemi *et al.* (2010) and Usman and Osuji (2007), respectively.

Acute toxicity study of the methanol extract fraction: The median lethal dose (LD_{50}) of the methanol extract fraction was determined by the method of Lorke (1983). Eighteen albino 18 mice were utilized in this study. The test involved two stages. In Phase I, the mice were grouped into three groups of three rats each. They were administered orally 10, 100 and 1000 mg kg^{-1} b.wt., of the extract, respectively. In the Phase II, 1600, 2900 and 5000 mg kg^{-1} b.wt., of the extract were administered orally to the mice. The administration of the methanol extract fraction was done orally. The mice were then observed over a 24 h period for nervousness, dullness, in-coordination or death.

Induction of diabetes with alloxan

Principle of alloxan induction: The administration of alloxan, adiabetogenic drug induces diabetes mellitus by selective destruction of β-cells of the islet of Langerhans. It causes β-cells of

the islet of Langerhans to degranulate and consequently degenerate. Alloxan induces irreversible diabetes mellitus after 24 h following its administration and the condition becomes chronic after seven days (Dunn and McLetchie, 1943).

Reaction principle: The baseline blood glucose levels were determined before the induction of diabetes after 7 days of acclimatization. The rats were fasted overnight prior tointra-peritoneal administration of alloxan120 mg kg^{-1} b.wt. After 3 days, rats showing Random Blood Glucose, (RBC) level ≥200 mg dL^{-1} (11.1 mmol L^{-1}) were considered diabetic and used for the experiment (Frode and Medeiros, 2008). The treatment lasted for 21 days in which blood glucose levels and lipid profile of the rats were determined on day 0, 3, 9, 15 and 21, respectively.

Determination of blood glucose levels: Diabetes status was monitored with blood samples obtained from tail vein puncture using an automated glucose sensor machine Glucometer Analyser (AccuChek Active). This method is based on the reaction of glucose and oxygen in the presence of glucose oxidase (GOD) to yield gluconic acid and hydrogen peroxide (H_2O_2). The hydrogen peroxide formed subsequently reacts under catalysis of peroxidise (POD), with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator. In other words, it oxidizes the dyes in a reaction mediated by peroxidise producing a blue coloured product. The intensity of the colour which is proportional to the glucose concentration in the sample was read from the Accu-chek active glucometer.

Determination of total cholesterol: The total cholesterol level was determined using the method described by Allain *et al.* (1974)

Determination of High Density Lipoprotein (HDL): The high density lipoprotein concentration was determined using the method described by Albers *et al*. (1978).

Determination of Low Density Lipoprotein (LDL): The high density lipoprotein concentration was determined using the method described by Assmann *et al*. (1984).

Determination of triacylglycerol concentration: Triacylglycerol (TAG) concentration was determined using the method described by Tietz (1990).

Statistical analysis: The data obtained were analysed using Statistical Product and Service Solutions (SPSS) version 20 and the results expressed as Mean±standard error of mean. Significant differences of the result were established by one-way analysis of variance (ANOVA) and the acceptance level of significance was $p \leq 0.05$ for all the results.

RESULTS

A quantity, 1755 g of *R. communis* seeds after extraction with methanol and chloroform in 2:1 ratio gave a percentage yield of 1.04% methanol extract fraction.

The qualitative screening of the methanol extract fraction of *R. communis* seeds showed that tannins has the highest presence among all the phytochemicals screened. Steroids, alkaloids, flavonoids and glycosides were moderately present. However, hydrogen cyanide was present in low concentrations, whereas resin was not detected as shown in Table 1.

Table 1: Results of the qualitative phytochemical constituents of methanol extract fraction of *Ricinus communis* seeds

Phytochemicals	Methanol extract fraction	Interpretation
Hydrogen cyanides	+	Present in low concentration
Steroids	++	Present in moderate concentration
Saponins	+	Present in low concentration
Tannins	+++	Present in high concentration
Alkaloids	++	Present in moderate concentration
Flavonoids	++	Present in moderate concentration
Glycosides	++	Present in moderate concentration
Resins	-	Not detected

Table 2: Results of the quantitative phytochemical constituents of methanol extract fraction of *Ricinus communis* seeds

Phytochemicals	Quantity (mg g^{-1})
Hydrogen cyanides	0.02±0.00
Steroids	4.58±0.05
Saponins	1.36±0.04
Tannins	5.74±0.03
Alkaloids	3.57±0.04
Flavonoids	3.63±0.06
Glycosides	2.56±0.04

Values are Mean±standard deviation of triplicate determination (n = 3)

Table 3: Phase I and II of the median lethal dose (LD_{50}) of *Ricinus communis*

Groups	Dosage (mg kg^{-1} b.wt.)	Seeds mortality
Phase I		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

Values are Mean±standard deviation (n = 3)

The findings of the quantitative phytochemical analysis of the methanol extract fraction of the *R. communis* seeds indicated that tannins (5.74±0.03) and hydrogen cyanide (0.02±0.00) were the highest and least phytochemical constituents of the extract respectively while other phytochemicals found in the qualitative screening were present in significant amount as shown in the Table 2.

The acute toxicity (LD_{50}) of the methanol extract fraction of *R. communis* seeds showed no death or adverse reaction in the mice administered with various doses of the extract up to 5000 mg kg^{-1} body weight as shown in Table 3.

The baseline blood glucose levels showed that the male Wistar albino rats used for the study were non-diabetic before the alloxan induction, with average blood glucose level of 105.10±4.61 mg dL^{-1}. The day 3 blood glucose levels indicated that all the animals in the test groups with the exception of group 1 were diabetic. A significant decrease ($p<0.05$) was observed in the mean blood glucose values of group 3, 4, 5 and 6 after day 9, 15 and 21 of the treatment when compared with positive control group 2 (Fig. 1).

There was a significant ($p<0.05$) decrease in the total serum cholesterol in group 3, 4, 5 and 6 after alloxan induction of diabetes when compared with group 1 (normal control) and 2, (diabetic untreated). However, significant ($p<0.05$) increase was observed in the total serum cholesterol of groups 2 relative to group 1 (Fig. 2).

The result indicated that the methanol extract fraction caused significant ($p<0.05$) increases in the serum HDL concentration in group 4 and 5 when compared with group 2, however, no

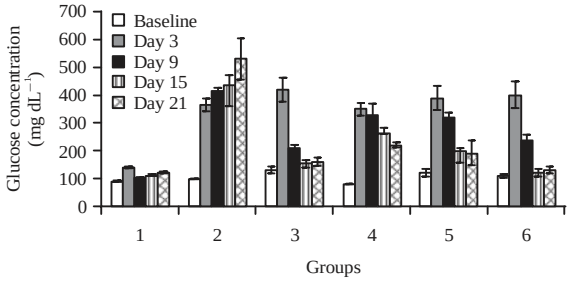

Fig. 1: Effect of methanol extract fraction of *Ricinus communis* seeds on blood glucose levels in alloxan induced diabetic male wistar albino rats

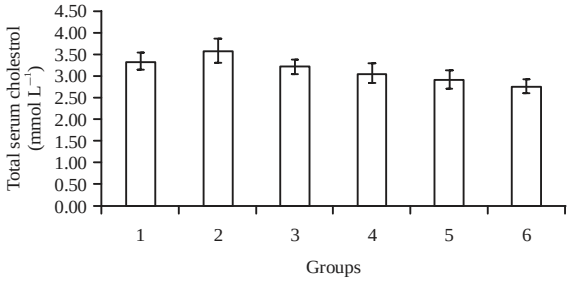

Fig. 2: Effect of methanol extract fraction of *Ricinus communis* seeds on serum total cholesterol levels in alloxan induced diabetic male wistar albino rats

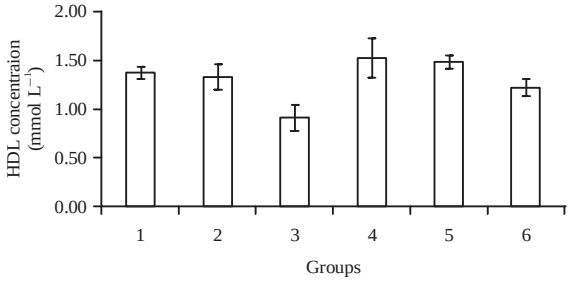

Fig. 3: Effect of methanol extract fraction of *Ricinus communis* seeds on HDL levels in alloxan induced diabetic male wistar albino rats

significant difference ($p>0.05$) in serum HDL concentrations were observed between group 6 and group 2. A significant ($p<0.05$) decrease in serum HDL concentration was observed in group 3 when compared with group 2 as shown in Fig. 3.

A significant ($p<0.05$) reduction in serum LDL concentrations in the groups 4, 5 and 6 rats treated with the methanol extract fraction when compared with group 2 (diabetic untreated rats). Also, no significant difference ($p>0.05$) in the serum LDL concentrations was observed in the group 3 when compared with group 2 as shown in Fig. 4.

There was no significant ($p>0.05$) increase in the serum triacylglycerol concentration observed in the test groups when compared with group 2. However, TAGs in all the test groups including group 2 showed significant ($p>0.05$) increase when compared with group 1 Fig. 5.

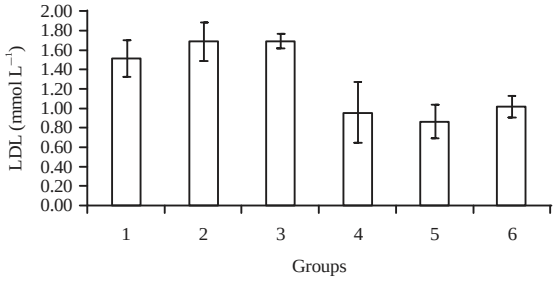

Fig. 4: Effect of methanol extract fraction of *Ricinus communis* seeds on LDL levels in alloxan induced diabetic male wistar albino rats

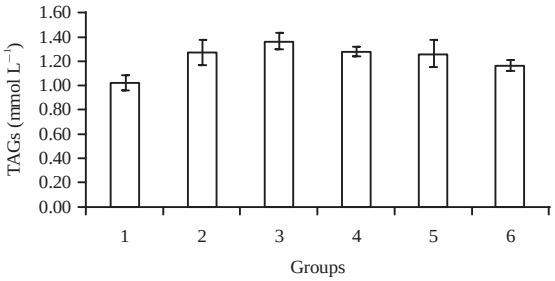

Fig. 5: Effect of methanol extract fraction of *Ricinus communis* seeds on TAGs concentrations in alloxan induced diabetic male Wistar albino rats

DISCUSSION

Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency, insensitivity of insulin receptors to insulin, resulting in abnormally high blood glucose level and leads to acute and chronic complications. Diabetes mellitus is on the increase most especially in developing countries necessitating sourcing alternative ways of managing it and if possible cure.

The high phytochemical constituents observed in the methanol extracts fraction of *R. communis* from qualitative phytochemical analyses were indication of its richness in important phytochemicals which could offer useful health benefit to human when consumed in right proportion at right time. The quantitative photochemical analysis of the methanol extract fraction indicated the abundance phytochemical constituents such as steroids, tannins, alkaloids and flavonoid in the methanol extract fraction which validated the result of qualitative phytochemical analysis. The flavonoids, tannins and alkaloids possess vital medicinal properties needed to improve human health when present in required concentrations. Consequently, high concentrations, these phytochemicals could be toxic to man and may impair body metabolism (Oyedemi *et al*., 2010). The presence of these biologically active compounds suggests that the plant could serve as a potential source of drugs and could exert some positive biological activities when taken by animals.

The non-toxicity of the methanol extract fraction of *R. communis* seeds up to 5000 mg kg^{-1} b.wt., suggests that the extract is safe for human and animal consumption and complements earlier finding by Nath *et al*. (2011), who also reported that the extract is safe.

The significant reduction of the blood glucose level of the diabetic rats treated with the extract in a dose dependent manner could be attributed to the anti-hyperglycemic effect of the extract

which is in agreement with earlier studies on plants as alternative source of antihyperglycaemic drugs (Latha and Pari, 2003; Badole *et al*., 2006). The anti-diabetic effect of the extract is probably due to the presence of flavonoids in the extract as reported by Trivedi *et al*. (2004) that flavonoids constituted the active biological principles of most medicinal plants with hypoglycemic and antidiabetic activities.

In diabetic conditions, there is increase in blood glucose level which is usually accompanied by an increase in plasma cholesterol, TAGs, LDL and decrease in HDL (Mitra *et al*., 1995). Under normal conditions insulin activates the enzyme lipoprotein lipase, which hydrolyzes triglycerides. However in diabetic state, lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridaemia. Alteration of serum lipid profile is known to occur in diabetes and this is likely to increase the risk of coronary heart disease (Al-Shamaony *et al*., 1994). Oral administration of methanol extract fraction of *R. communis* seeds produced significant beneficial effects in the lipid profile of the treated diabetic rats, significantly reducing total cholesterol, low density lipoprotein and triacylglycerol at higher dose whereas high density lipoprotein was significantly increased. The findings is close to the finding of Yusufoglu *et al*. (2015) that oral administration of *F. duranii* reversed the changes in plasma lipoproteins of diabetic rats and significantly improved their values towards near normal levels. These results suggest that the extracts might be considered as a substitute for drugs to reduce complications associated with diabetic conditions. Thus an excess fatty acid in the plasma produced by alloxan induced diabetes promotes the conversion of excess fatty acids into phospholipids and cholesterol in the liver. These two substances along with excess TAGs formed in the liver may be discharged from the liver in the form of lipoproteins. The HDL is an anti-atherogenic lipoprotein which transports cholesterol from peripheral tissues into the liver, thereby acting as a protective factor against coronary heart disease (Shirwaikar *et al*., 2004). It is well known that LDL plays an important role in arteriosclerosis and hypercholesterolemia. The decrease in cholesterol and LDL levels achieved by the administration of methanol extract fraction of *R. communis* seeds demonstrated a possible protection against hypercholesterolemia.

CONCLUSION

The findings of this study have demonstrated that methanol extract fraction of *R. communis* seeds possess anti-hyperglycemic effect close to that of the standard drug (metformin) and effectively maintained the lipid profile within acceptable level. This suggests that the extract could be efficiently used to treat diabetes mellitus and prevent most of the health complications associated with the condition.

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