Research Article

Effect of Methanol Extract of *Ricinus communis* (RC) Seeds on Blood Glucose Levels, Antioxidant Enzymes and Hematological Parameters of Alloxan Induced Male Wistar Albino Rats

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Abstract

The study investigated the effect of methanol extract of *R. communis* seeds on blood glucose levels, antioxidant enzymes and hematological parameters of alloxan induced male Wistar albino rats. The methanol extract had LD<sub>50</sub> value above 5000 mg kg<sup>-1</sup> b.wt. After alloxan induction of diabetes, a significant (p<0.05) decrease in blood glucose levels were observed in all the test groups treated with the extract, when compared with positive control (group 2). The extract treated groups showed significant (p<0.05) increase in PCV, Hb, RBC and significant decrease (p<0.05) in WBC counts, when compared with positive control, however, no significant (p<0.05) difference was observed in total serum protein. With exception of group 4, malondialdehyde concentration decreased significantly (p<0.05) in test groups relative to positive control. A concentration dependent increase in glutathione activity in extract treated groups close to metformin treated group was observed when compared with normal and positive controls respectively. At higher concentrations of the extract, superoxide dismutase activity increased significantly (p<0.05) when compared with positive control. At lower concentrations of the extract, catalase activity increased significantly (p<0.05) close to metformin treated group but decreased significantly (p<0.05) with increasing concentrations of the extract close to positive control. The results of the study showed that the methanol extract of RC possess antihyperglycaemic properties and ability to modulate activities of antioxidant enzymes. It also suggests that the extract has positive effect on the hematological parameters.

Key words: *Ricinus communis*, antioxidant enzymes, antihyperglycaemic, hematological parameters, alloxan

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Data Availability: All relevant data are within the paper and its supporting information files.
INTRODUCTION

The practice of traditional medicine is as old as the origin of man (Okafor, 1983). The use of plants in traditional medicine referred to as herbalism or simply botanical medicine (Edeoga et al., 2005) falls outside the mainstream of the Western or Orthodox medicine. It has been estimated that two third of the world population (mainly in the developing countries) rely on traditional medicine as their primary form of health care (Caceres et al., 1990). The use of traditional medicine cannot fade out in the treatment and management of diseases in African continent and this could be attributed to socio-cultural and socio-economic life styles, lack of basic health care and qualified medical personnel (Elujjoba et al., 2005). Plant seeds contain active components such as flavonoids, alkaloids, 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 pharmacologically derived medications contain components originating from phytochemicals in seeds. These substances that contain the healing property are known as the active principles and are found to differ from seed to seed. Among these seeds are the pumpkins, whose parts are eaten as supporting food or main dishes and which could be aromatic, bitter or tasteless (Galm and Shen, 2007).

Worldwide, more than 171 million people suffer from diabetes, making this one of the most common non-communicable diseases and the number of affected individuals with diabetes is expected to double by 2025 (Wild et al., 2004). The countries with the largest number of diabetics are India, China and United States (American Diabetes Association, 2007). In the past decades, research has been focused on scientific evaluation of traditional drugs of plant seed origin and screening of more effective and safe hypoglycemic agents has continued to be a quarrying domain (Ndenecho, 2009). A very large area of Nigeria ecological zones is populated with many seed species which have found their usefulness either directly or indirectly for humans (Oliver-Bever, 1986). The medicinal values of many of these seeds cannot be over emphasized in the light of oral traditions and folklore from the distant past that have continued to extol the healing virtues of these seeds and their extracts. Generally, the active principles found in Ricinus communis 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). Despite the effectiveness of chemically synthesize medicine, screening for seed drugs will continue for the development of new pharmaceuticals to resolve both old and new health problems (Iwu, 1983).

The plant kingdom offers a wide range of natural antioxidants and medicinal values (Lewis and Manony, 1977). Dietary plant seeds with proven antioxidant properties may function as a direct antiradical chain breaker of free radical propagation, interaction with transition metals and inhibition of Reactive Oxygen Species (ROS) generating enzymes. Ricinus communis is one of such medicinal seeds whose medicinal values have stood the test of time. Although the antidiabetic activity of the seed extract has been reported by Nath et al. (2011), a comparative study of the seed methanol extracts on some biochemical and hematological parameters has not been considered. Hence, this present study was designed to study the effect of methanol extract of Ricinus communis (RC) seeds on blood glucose levels, antioxidant enzymes and hematological parameters of alloxan induced diabetic Wistar albino rats.

MATERIALS AND METHODS

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

Animals: All the animals used were obtained from the Animal House of the Department of Zoology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria. 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. The guide for the care and use of laboratory animals procedures were followed in this study (Indian Council of Medical Research, 2001).

Preparation of plant material: The RC seeds were cleaned and sorted to remove broken ones and contaminants. They were dehulled manually by breaking between two hard surfaces and the chaffs were separated, the kernels were stored in clean dry plastic container for extraction (Akande et al., 2012).

Extraction of plant material: A quantity, (1755 g) of the RC seeds 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 No. 4 filter paper. The filtrate was later evaporated to dryness using rotary evaporator at a temperature of 40 and yield calculated.

**Experimental design:** A total of 18 male albino mice were used for the acute toxicity (LD_{so}) while, study 54 male Wistar albino rats were used for the main study. After seven days acclimatization, the mice were divided into six groups of 3 mice each and used for the phase I and II of the acute toxicity study while, the Wistar albino rats were randomly distributed into 6 groups of 9 rats each for the main study. Treatment with seed extract started after induction of diabetes, the blood glucose level, lipid peroxidation, total serum protein, antioxidant enzymes and hematological parameters were determined as:

- **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.)

**Acute toxicity test:** The method of Lorke (1983) was used for the acute toxicity test. Eighteen albino mice were utilized in this study. The test involved two stages. In stage one; the animals were grouped into three groups of three rats each. They were administered 10, 100 and 1000 mg kg⁻¹ b.wt., of the extract, respectively and in the second stage, 1600, 2900 and 5000 mg kg⁻¹ b.wt., of the extract were administered to the animals. The administration of the extract was done orally. The animals were then observed over 24 h period for nervousness, dullness, in-coordination or death.

**Induction of diabetes and blood glucose determination**

**Reaction principle:** The baseline blood glucose levels were determined before the induction of diabetes on the seventh day of acclimatization. The rats were fasted overnight prior to injection of alloxan monohydrate dissolved iniced cold normal saline at a dose of 120 mg kg⁻¹ b.wt. and the route of administration was intra-peritoneal. After 3 days, rats showing Random Blood Glucose (RBG), 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 of the rats were determined on day 0, 3, 9, 15 and 21.

**Determination of blood glucose:** 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 to yield gluconic acid and hydrogen peroxide (H₂O₂). The hydrogen peroxide formed subsequently reacts under catalysis of peroxidase with phenol and 4-aminoazobenzeno to form a red-violet quinoneimine dye as indicator. In other words, it oxidizes the dye in a reaction mediated by peroxidase producing a blue coloured product. The intensity of the colour which is proportional to the glucose concentration in the sample was read from the AccuChek active glucometer.

**Determination of Hematological parameters:** All the Hematological indices were assayed by the method outlined by Dacie and Lewis (2001).

**Determination of packed cell volume**

**Principle:** When whole blood sample is subjected to a centrifugal force for maximum RBC packing, the space occupied by the RBCs is measured and expressed as percentage of the whole blood volume.

**Determination of hemoglobin (Hb) concentration**

**Principle:** When whole blood is added to Drabkin’s reagent: A solution containing KCN and K₄Fe(CN)₆. The KCN converts Hb-Fe^{2+} (ferrous) to Hb-Fe^{3+} (ferric) state to form methaemoglobin which then combines with KCN to form a stable pigment, cyanmethaemoglobin complex. The colour intensity of this mixture is measured in a spectrophotometer at a wavelength of 540 nm. The optical density of the solution is proportional to the hemoglobin concentration. All forms of Hb (Hb-C, Hb-O, etc.) except Hb-S are measured with this cyanmet-method.

**Determination of red blood cells (RBCs)**

**Principle:** When whole blood is diluted with an isotonic fluid, it prevents lysis and facilitates counting of the red cells. Some isotonic solutions in use include Hayem’s solution, Gower’s solution or 0.85% NaCl solutions.

**Determination of white blood cells (WBCs)**

**Principle:** Whole blood is diluted by 1 in 20 in an acid reagent (WBC diluting fluid, a weak acid solution to which gentian
violet is added to stain the nucleus of white cells) which haemolysis the red cells, leaving the white cells to be counted. White Blood Cells (WBCs) were then counted microscopically using an improved Neubauer ruled counting chamber (haemocytometer) and the number of WBCs per liter of blood calculated. The EDTA anticoagulated blood or capillary blood was used for counting white blood cells.

**Determination of serum total protein:** Determination of serum protein was carried out according to the method of Gornall et al. (1949).

**Principle:** The protein in the sample reacted with copper II ion in alkaline medium forming a coloured complex that was measured spectrophotometrically.

**Determination of lipid peroxidation concentration:** The concentration of lipid peroxidation product (MDA) was determined by the method described by Wallin et al. (1993).

**Principle:** The principle for the estimation is based on the fact that thiobarbituric acid (TBARS) reacts with malondialdehyde (MDA) to give a red or pink colour, which absorbs maximally at 532 nm.

**Assay of catalase (CAT) activities:** The assay of catalase (CAT) activities was determined by the method described by Aebi (1984).

**Principle:** The ultraviolet absorption of hydrogen peroxide can be easily measured at 240 nm. On the decomposition of hydrogen peroxide with catalase, the absorption decrease with time and from this decrease catalase activity can be measured.

**Assay of glutathione peroxidase (GPX) activities (Randox Commercial Kit):** The assay of glutathione peroxidase activities (GPX) was determined by the methods described by Paglia and Valentine (1967).

**Principle:** Glutathione peroxidase (GPX) catalyzes the oxidation of glutathione (GSH) by cumenehydroperoxide. This method is based on that of Paglia and Valentine (1967). In the presence of Glutathione Reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺.

**Assay of superoxide dismutase (SOD) activities:** The assay of superoxide dismutase (SOD) activities was determined by the method described by Fridovich (1983).

**Principle:** The ability of superoxide dismutase to inhibit the autoxidation of adrenaline is the basis of the SOD assay. Superoxide generated by the xanthine oxidase reaction is shown to cause the oxidation of adrenaline to adrenochrome. The yield of adrenochrome produced per superoxide introduced increases with pH and also with increasing concentration of adrenaline which proceeds by at least two distinct pathways, one of which is a free radical chain reaction involving superoxide radical and hence could be inhibited by SOD.

**Statistical analysis:** The data obtained were analyzed using Statistical Product and Service Solutions (SPSS) version 20 and the results expressed as mean±standard error of mean. Significant differences in the mean of the results were established using one-way analysis of variance (ANOVA) and the acceptance level of significance was p≤0.05 for all the results.

**RESULTS**

The extraction of 1755 g finely ground sample of RC seeds with methanol and chloroform in 2:1 ratio gave a percentage yield of 1.04% (18.2 g) and 2.36% (41.5 g) in methanol and chloroform fractions, respectively.

The acute toxicity test of the of methanol extracts of RC seeds (Table 1) showed that no deaths were recorded in the
mice up to 5000 mg kg$^{-1}$ b.wt., of the extract and the animals showed no signs of toxicity at this concentration of the extract within 24 h of constant observation.

The mean blood glucose levels of rats treated with methanol extract of RC seeds showed baseline bars for nondiabetic rats with glucose level of 105.10±4.61 mg dL$^{-1}$. The day 3 bars indicated that all the animals in the test groups with the exception of group 1 were all diabetic. A significant (p<0.05) decrease in the mean blood glucose values of group 3, 4, 5 and 6 were observed after day 9, 15 and 21 of treatment, when compared with that of the positive control (group 2) as shown in the Fig. 1.

Figure 2 showed that there as significant (p<0.05) increase in the packed cell volume level of treated rats, when compared with untreated group (group 2). The group 3 that receive metformin drug and group 6 that higher dose of the extract had significant (p<0.05) increase in PCV levels compared with group 2 (diabetic rats untreated).

Figure 3 showed the effect of the extract on hemoglobin (Hb) concentration of alloxan induced Wistar male albino rats. The methanol extract of RC seeds significantly (p<0.05) increased hemoglobin concentration of the treated groups when compared with the diabetic untreated rats.
Figure 4 showed the effect of methanol extract of RC seeds on red blood cell counts of alloxan induced diabetic rats. There was significant (p<0.05) increase in the red blood cell count of the treated groups when compared with that of the diabetic untreated group.

Figure 5 showed that the diabetic untreated group (group 2) had the highest WBC count. The methanol extract treated groups showed significant (p<0.05) concentration dependent decrease in WBC count with increasing concentrations of the methanol extract. Both metformin and methanol extract treated groups showed significant (p<0.05) decrease in WBC count relative to group 2 diabetic untreated, however, WBC count of group 3 that received metformin and group 4 that received low dose of the methanol extract showed significant (p<0.05) increase in WBC compared with group, the normal control.

There was no significant (p>0.05) observed between the serum total protein of the normal control (group 1) and diabetic untreated (group 2). Group 4 shows no significant (p>0.05) decrease in the serum total protein when compared to the normal control and diabetic untreated. However, groups 3, 5 and 6 showed no significant (p>0.05) increase in the serum total proteins when compared with the controls as shown in Fig. 6.

There was no significant difference observed in the MDA concentration of group 4 that received low of the methanol extract when compared with group 2 untreated diabetic rats but significantly (p<0.05) higher than the normal control (group 1). At higher concentration, the extract had significant (p<0.05) reduction of MDA concentration as observed in groups 5 and 6 which is equivalent to that of group 3 that
received standard drug (metformin) when compared with group 2 untreated diabetic rats as shown in Fig. 7.

Figure 8 revealed that the extracts increase the catalase activity of the diabetic rats treated with the methanol extracts of *Ricinus communis* seeds. A significant (p<0.05) increase in catalase activity was observed in the test groups when compared with group 2, which had low level of catalase activity. However, there was no significant (p>0.05) difference in the catalase activity of rats in group 6 when compared with the positive control.

Figure 9 revealed that the extracts stimulated superoxide dismutase (SOD) activity in diabetic rats treated with the methanol extracts of RC seeds. A significant (p<0.05) increase was observed in the SOD activity of rats in the test groups when compared with the positive control.

Figure 10 revealed that the extracts increased the glutathione peroxidase activity of diabetic rats treated with the methanol extracts of RC seeds. A significant (p<0.05) increase was observed in the GSH activity of rats in the test groups. However, no significant difference (p>0.05) was observed in the GSH activity of rats in group 4 when compared to the positive control.

**DISCUSSION**

The study investigated the effect of methanol extract of *Ricinus communis* (RC) seeds on blood glucose levels, total serum protein, antioxidant enzymes and hematological parameters of alloxan induced Wistar albino rats. The acute toxicity (LD<sub>50</sub>) study of the methanol extract of RC seeds shows
that the seeds extract was not toxic up to 5000 mg kg$^{-1}$ b.wt. This showed that methanol extract of RC seeds is safe for human and animal consumption to a larger extent which is agreement with the findings of Nath et al. (2011), who had earlier reported similar result.

The reduction of blood glucose levels in the diabetic male Wistar albino rats treated with varied doses of methanol extract of RC seeds showed that the extract possessed antihyperglycaemic properties which could be attributed to the presence of abundant antioxidant compounds such as flavonoids, vitamins E and C as reported by Trivedi et al. (2004), who asserted that flavonoids constituted active biological principles of most medicinal plants with hypoglycemic activities. The study showed that oral administration of methanol extract of RC seeds for 21 days effectively controlled hyperglycemia. This is in line with the findings of Rana et al. (2012), who earlier reported that ethanolic extract of roots of *R. communis* significantly decreased fasting blood glucose levels of diabetic rats from an initial level of 386±41-79±16 mg dL$^{-1}$ on 20th day. Contrary to his findings, the methanol extract of *Ricinus communis* seeds in this study did not reduce the glucose levels in normal rather, it did reduced blood glucose in diabetic rats in a dose dependent manner. This agrees with the findings of Shokeen et al. (2008), who also reported that *Ricinus communis* is a potent phytomedicine for diabetes.

Packed Cell Volume (PCV) can be used as a screening tool for anaemia and can also indicate the degree of fluid loss during dehydration. The treatment of alloxan induced diabetic rats with methanol extract of RC seeds increased the PCV levels, which suggested that the extract was rich in blood boosting bioactive components or that the extract caused dehydration of fluid in the blood of the treated rats and led to no significant increase in PCV observed.

Hemoglobin is an oxygen carrying pigment in the red cells. The increased hemoglobin concentration observed in the treated groups could suggest that the extract was rich in iron and other precursor needed for haem and eventually hemoglobin production, thereby maintaining high concentration of hemoglobin in the blood. The reduction in hemoglobin concentration in diabetic untreated rats (group 2) might be due to glycation of the red blood cells which led to destruction of hemoglobin faster than its synthesis.

The increased red blood cell count observed in the alloxan induced male Wistar albino rats treated with graded doses of methanol extract of RC seeds when compared with the group 2 (alloxan induced untreated rats) may be attributed blood boosting potentials of the extract which stimulated production of more red blood cells to ensure efficient transport of oxygen. Protection against the increased red blood cells may be due to protection of erythrocyte cell damage by increased glutathione peroxidase activity, which has antioxidant effects against organic peroxides through glutathione oxidation (Day, 2009).

The White Blood Cell (WBC) count determines the body’s ability to fight infections. The significant reduction of WBC count in alloxan induced male Wistar albino rats treated with methanol extract of RC seeds showed that the ability of the extract to prevent hypersensitivity associated with excessive circulating white blood cell count. The significant reduction of the WBC count was an indication that at very high concentration, the extract could compromise the innate immune system which may be detrimental to the survival of an individual, who consumed it. The haematopoietic system is a very sensitive target for most toxic compounds which has made it mandatory to study every alteration resulting from substance with toxicity potentials (Olson et al, 2000). Positive effects of the methanol extract of *Ricinus communis* seeds on Hematological indices on treated groups obtained in this study further showed that the extract was not toxic to the rats.

The significant increase in the total serum protein may be attributed to the extract having no destructive effect on the liver. This increase could also be due to dehydration which could be in the form of excessive water loss by urination associated with diabetes mellitus or due to diabetic acidosis or as a result of increased proteins synthesis by the liver. While,
Lipid peroxidation is one of the characteristic features of chronic diabetes (Satheesh and Pari, 2004). In this study, a marked increase in the concentration of malondialdehyde (MDA) the product of lipid peroxidation was observed in the untreated diabetic rats while, a significant reduction (p<0.05) of MDA was observed in the diabetic rats treated with methanol extract of RC seeds. The treatment of diabetic rats with methanol extract of RC seeds might have caused regeneration of beta (β) cells of the pancreas and leading to secretion of more insulin from regenerated β cells. The increase in insulin secretion and the consequent decrease in blood glucose level may lead to inhibition of lipid peroxidation and control of lipolytic enzymes (Odetola et al., 2006).

The increased lipid peroxidation during diabetes as found in the present study may be due to the inefficient antioxidant system prevalent in diabetes. The status of lipid peroxidation as well as altered levels of certain endogenous radical scavenger is taken as direct evidence for oxidative stress (Rajeshkumar et al., 2013). Free radical scavenging enzymes like superoxide dismutase (SOD) and catalase (CAT) protect the biological system from oxidative stress (Del Rio et al., 2005). The decrease in the activity of the enzymes in the present study could be attributed to the excessive utilization of these enzymes in attenuating the free radical generated during the metabolism of alloxan. Previous reports have shown an elevation in the status of lipid peroxidation in the liver after alloxan induction (Szkudelski et al., 1998), which was in accordance with the findings of this study. The activities of antioxidant enzymes observed in this study could have prevented reactive oxygen species from causing further damage to membrane lipids. Increase in glutathione peroxidase level in turn contributed to the recycling of other antioxidants such as vitamin C and E (Exner et al., 2000). The increase the activity of glutathione peroxidase could be attributed other antioxidant compound such as flavonoids, vitamin E and C, which may be present in the methanol extract as Ashokkumar and Sudhandiran (2008) reported that flavonoids in addition to inhibiting the action of xanthine oxidase, which catalyzes the oxidation of hypoxanthine to xanthine and generates superoxide anion during the process. The reduced production of xanthine, together with flavonoids, vitamin A and C have the potential of scavenging superoxide, leading to a reduction in the concentration of the free radical superoxide (Chaabane et al., 2012), which is dismutated by SOD (Fridovich, 1995) to form hydrogen peroxide which increase activity of glutathione peroxidase, which metabolizes H2O2, reduces the concentration of this reactive species required for the activity of catalase. This may suggest that the extract maintains glutathione peroxidase homeostasis thereby protecting the cells from further oxidative damage.

CONCLUSION

The study investigated the effect of methanol extract of *Ricinus communis* seeds on blood glucose levels, antioxidant enzymes, total serum protein and hematological parameters of alloxan induced Wistar albino rats. The results of the present study have shown that the methanol extract of RC seeds possesses anti hyperglycaemic activity, ability to modulate antioxidant enzymes activities, positive effect against lipid peroxidation and hematological parameters, thus could be useful in the treatment of diabetes mellitus.

REFERENCES


