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## Anti-inflammatory Evaluation of the anti-diabetic effect of *Rauvolfia vomitoria* and *Citrus aurantium* decoction on streptozotocin-induced diabetic Wistar rats

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### ABSTRACT

**Background:** Diabetes mellitus is a relatively common metabolic disorder which leads to complications that affects the heart, liver, kidney, and other vital organs. This investigation examines the biochemical effects of *Rauvolfia vomitoria* and *Citrus aurantium* decoction in the treatment of streptozotocin-induced diabetes in rats. **Materials and Methods:** Twenty-Eight (28) healthy Wistar rats, with average weights of  $100 \pm 20$ g, were divided into four groups, each consisting of five rats. Group I served as the normal control whereas diabetes was induced with 50 mg/kg body weight of streptozotocin in Groups II – IV. While rats in Groups III and IV were treated with 2 mL of plant decoction and metformin, respectively, rats in Group II served as untreated control. The effect of the decoction on the total protein, glucose, creatinine, lipid profile, ALT, SOD and CAT levels were determined. **Results:** Treatment of diabetic rats with the decoction resulted in a significant reduction in the blood glucose, total protein, creatinine as well as catalase levels compared with the untreated control ( $p < 0.05$ ). Furthermore, administration of the decoction to diabetic rats caused a significant decrease ( $p < 0.05$ ) in the lipid profile parameters compared with the untreated control (Group II). These effects were similar to those observed with metformin treatment. **Conclusion:** These results show the hypoglycaemic potential of the plant decoction and presents a cheaper and readily available remedy for the management of diabetes in low-income countries.

**Keywords:** *Rauvolfia vomitoria*, *Citrus aurantium*, Streptozotocin, Diabetes.

### INTRODUCTION

Diabetes is a chronic disease, characterized by a persistent hyperglycemia as a result of defects in insulin secretion, insulin action or a combination of both [1]. Several factors including the increasing proportion of the aging population, intake of calorie-dense diets, weight problems and sedentary lifestyles, have been directly implicated in the increased number of diabetics globally [2]. In 2021, an estimated 10.5 % (536.6 million) of the global population of 20 –79-year-olds were found to be diabetic and this number is projected to increase to 12.2 % (783.2 million) in 2045 [3]. Unfortunately, there exists a huge economic burden due to its management costs and the complications are skyrocketing [4]. Presently, treatment options that rely on insulin secretion and insulin sensitization result in undesired side effects in patients. Furthermore, the use of gene therapies as well as induced  $\beta$ -cells regeneration are not widely embraced in Nigeria as most patients cannot afford the cost associated with such treatments. The result is that a sizeable number of households in Nigeria depend exclusively on medicinal herbs for the management of diabetic disease conditions. Hyperglycemia, which is the main symptom of diabetes mellitus, generates reactive oxidative species (ROS) which causes lipid peroxidation and membrane damage. Plants containing natural antioxidants can preserve  $\beta$ -cell function by inhibiting the peroxidation chain reaction [5].

*Citrus aurantium*, a sour or bitter orange belonging to the family “Rutaceae”, is an antiseptic, anti-bilious and hemostatic juice. Its extracted juice is used for gastrointestinal disorders including ulcer in the intestine, diarrhea, constipation, among other uses. It is also used in lowering blood sugar levels in diabetic patients, regulation of fat levels in blood, stimulating heart circulation, blood purification, disorders of liver and gall bladder [6]. Active substances such as flavonoids and phenolic contents in *Citrus aurantium* may account for both its antidiabetic and anti-hypercholesterolemia effects [7]. *Rauvolfia vomitoria*, a shrub rich in alkaloids, saponins and tannins, has been used extensively for lowering blood pressure, as an antimalarial, antipyretic and analgesic [8]. In this study, we investigated the antidiabetic effect of the decoction of *Rauvolfia vomitoria* leaves and *Citrus aurantium* fruit in the management of streptozotocin-induced diabetes in Wistar rats.

## MATERIAL AND METHODS

### Collection and identification of plant samples

The leaves of *Rauvolfia vomitoria* was harvested from the Vice Chancellor Office (VCO) area of University of Benin, where it is grown as an ornamental plant while *Citrus aurantium* fruits were collected from Ewu in Esan Central Local Government Area of Edo State, Nigeria. The authentication of these plants was done in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, by a Taxonomist, Prof. Akinnibosun.

### Preparation and extraction of the plant samples

The harvested leaves and fruits were first washed to remove dirt. *Rauvolfia vomitoria* leaves (1200 g) were weighed using an electronic weighing balance and was put into a cooking pot. In addition, fifteen (15) pieces of *Citrus aurantium*, weighing 2000 g, were each cut into four parts and added to the contents of pot. Finally, six (6) liters of distilled water was added into the cooking pot. The plant materials were subsequently boiled for two (2) hours after which the decoction was filtered off into a clean pot using a muslin bag. Its residue was discarded while 2 mL of the decoction was orally administered via a gavage 12 hours daily.

### Animals grouping and administration of decoction

Experimental rats were purchased from the Anatomy Department of Obafemi Awolowo University, Ile Ife, Osun State. Twenty diabetic rats were divided into four groups as follows: Group I – Normal control; Group II – Test control (Diabetes was induced with streptozotocin but without treatment); Group III – Diabetic rats treated with *Rauvolfia vomitoria* and *Citrus aurantium* decoction (2 mL) and Group IV – Diabetic rats treated with metformin (standard drug).

The animals were kept in a well-ventilated cage at room temperature ( $28 \pm 2$  °C) on a 12 h light/dark cycle. Ethical approval was obtained from the Department of Plant Biology and Biotechnology, Faculty of Life sciences, University of Benin. The animals were handled according to the Institutional Animal Guidelines (1912/PO/Re/S/16/CPCSEA). Diabetes was induced by administration of streptozotocin (STZ) at a dose of 50 mg/kg body weight of rats. Streptozotocin was administered intraperitoneally in physiologic saline, with pH adjusted with 3.85 mL of 0.1M Na-citrate buffer as previously described [9]. Animals with fasting blood glucose level greater or equal to 150 mg/dL were considered diabetic. Treatment with the decoction commenced immediately diabetes was confirmed and was done daily using a gavage according to the method described previously [10]. The animals were sacrificed at the end of the treatment period (21 days) after an overnight fast. A portion of the blood was collected by cardiac puncture into plain sample bottles for biochemical analyses.

### Biochemical parameters

#### Measurement of blood glucose levels

**Table 1:** Mean values of weight of rats

Groups	Weight before treatment (g)	Weight after treatment (g)	Weight difference (g)
Group I	202.00±8.97	226.25±14.89	24.25±5.98
Group II	138.00±6.06*	104.75±1.93*	-33.25±7.22*
Group III	205.25±3.61*#	168.75±8.78#	-36.50±5.39*
Group IV	141.50±0.29*	137.75±2.18*	-5.75±2.06*#

Data are represented as Mean ± S.E.M (n=4). \*  $P < 0.05$  compared to the control; #  $p < 0.05$  compared to the untreated control

The blood glucose level was measured using ROCHE ACCU CHEK PERFOMA BLOOD GLUCO METER for confirmation of diabetes via collection of blood from the tip of the tail pierced with a lancet and a drop from the blood that oozes out was applied to test strip inserted the glucometer. The concentration of glucose for each rat was displayed on the screen of the glucometer. This took place before the commencement of treatment procedure and thereafter four days after treatment.

### Total Protein Estimation

The total protein level was estimated using a commercial kit obtained from Randox Laboratories Limited, Crumlin, UK. The manufacturer's instructions were strictly adhered to.

### Determination of Serum Creatinine Concentration

The serum creatine level was estimated using the colorimetric method previously described [11] with commercial kit obtained from Randox Laboratories Limited, Crumlin, UK.

### Estimation of Lipid Profile

The procedure for determining the total cholesterol level was based on the colorimetric method as described [12]. The levels of triglycerides, HDL and LDL cholesterol were determined using commercially available diagnostic kits (Randox Lab, UK) following manufacturer's instruction.

### Determination of enzymes activities

Enzyme activity was performed as described [13] while the superoxide dismutase antioxidant capacity was evaluated according to the procedure and principle described previously [14]. In addition, catalase activity was determined as described by Cohen [15].

### Data Analyses

Results are presented as mean ± SEM and were analyzed using one-way ANOVA. Differences between the mean values between groups were compared by the Bonferroni post-hoc test. The data were statistically analyzed by IBM SPSS v23.  $P$ -values less than 0.05 were considered statistically significant.

## RESULTS

### Comparison of the mean values of weight of rats

Induction of diabetes with streptozotocin resulted in a significant reduction in the mean weight of rats compared with the control ( $p < 0.05$ ). Furthermore, treatment with the decoction did not ameliorate the weight loss induced by streptozotocin in the Group III rats. However, treatment with metformin caused a significant increase in weight in streptozotocin-treated rats compared to the diabetic untreated rats ( $p > 0.05$ ; Table 1).

### Comparison of the glucose levels of rats treated with plant decoction

Table 2 shows the mean glucose levels of rats before and after treatment with the decoction of *Rauvolfia vomitoria* and *Citrus aurantium*. The result shows that there was a significant increase in the glucose concentration in the groups induced with streptozotocin

before treatment with the extract compared to the normal control ( $p < 0.05$ ). After treatment however, a significant reduction in the blood glucose levels in the group administered the decoction (Group III) was observed in comparison with the diabetic untreated group ( $p > 0.05$ ). Metformin also reduced the streptozotocin-induced increase in the mean glucose concentration in the Group IV rats ( $p < 0.05$ ).

**Table 2:** Mean values of glucose levels of rats

Groups	Before treatment (mg/dL)	After treatment (mg/dL)	Difference (mg/dL)
Group I	78.50±4.44	65.75±4.27	-12.75±7.44
Group II	354.25±21.72*	371.00±37.93*	16.75±17.88
Group III	333.25±15.88*	259.25±59.74 <sup>#</sup>	-74.63±63.20
Group IV	211.00±4.95 <sup>#</sup>	97.50±10.51 <sup>#</sup>	-113.50±14.96

Data are represented as Mean± S.E.M (n=4). \*  $P < 0.05$  compared to the control; #  $p < 0.05$  compared to the untreated control

### Effect of administration of plant decoction on the lipid profile of diabetic rats

The effect of the extract on the lipid profile is presented in Table 3. Induction of diabetes was lead to a significant increase in the total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol concentrations compared with the normal control ( $p < 0.05$ ). On the

other hand, treatment with the decoction of *Rauvolfia vomitoria* and *Citrus aurantium* caused a significant decrease in all lipid profile parameters compared with the test control (Group II), which was similar to the effect observed with metformin treatment. The decreases in the triglycerides and LDL cholesterol levels after treatment with the plant decoction were not significantly different from the normal control ( $p > 0.05$ ).

**Table 3:** Mean values of lipid profile of rats

Groups	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-Chol (mg/dL)	LDL-Chol (mg/dL)
Group I	195.75±2.56	107.25±6.22	49.00±3.34	125.30±5.23
Group II	330.00±44.81*	151.25±3.86*	70.75±3.97*	227.01±47.25*
Group III	237.50±19.87 <sup>#</sup>	129.75±9.20 <sup>#</sup>	57.00±2.41 <sup>#</sup>	139.55±11.17 <sup>#</sup>
Group IV	193.50±3.66 <sup>#</sup>	124.00±8.67 <sup>#</sup>	51.50±4.70 <sup>#</sup>	117.20±7.14 <sup>#</sup>

Data are represented as Mean± S.E.M (n=4). \*  $P < 0.05$  compared to the control; #  $p < 0.05$  compared to the untreated control

### Effect of treatment on total protein and creatinine levels

As depicted in Table 4, there was a significant increase ( $p < 0.05$ ) in the total protein and creatinine concentrations in the streptozotocin-induced diabetic group (Group II) in comparison with the normal control. However, the increases in these parameters observed in the diabetic rats were reversed in the group treated with the plant decoction ( $p < 0.05$ ). The total protein and creatinine levels were also significantly reduced by the treatment with the standard drug in comparison with the diabetic control ( $p < 0.05$ ).

difference in the SOD levels between the group administered the decoction and the diabetic untreated group ( $p > 0.05$ ). However, there was a significant decrease in the catalase (CAT) levels in the untreated diabetic group in comparison with Group I. This streptozotocin-induced decrease was significantly reversed by the treatment with the plant decoction. The plant decoction was more proficient than

**Table 4:** Mean values of total protein and creatinine levels of rats

Groups/Treatment	Total protein (g/dL)	Creatinine (mg/dL)
Group I	7.24±0.18	1.06±0.03
Group II	8.85±0.13*	2.31±0.23*
Group III	6.55±0.37 <sup>#</sup>	1.04±0.08 <sup>#</sup>
Group IV	6.89±0.78 <sup>#</sup>	0.98±0.03 <sup>#</sup>

Data are represented as Mean± S.E.M (n=4). \*  $P < 0.05$  compared to the control; #  $p < 0.05$  compared to the untreated control

the standard drug in its effect on the catalase levels in diabetic rats (Table 5).

**Table 5:** Mean values of enzyme levels of rats

Groups	ALT (μ/L)	SOD (μ/mg tissue)	CAT (k/min)
Group I	12.39±0.23	0.13±0.02	45.10±2.12
Group II	13.48±0.22	0.08±0.03	36.24±2.25*
Group III	10.97±0.42 <sup>#</sup>	0.09±0.02	48.59±5.84 <sup>#</sup>
Group IV	10.38±0.25 <sup>#</sup>	0.71±0.19 <sup>#</sup>	41.60±1.58 <sup>#</sup>

Data are represented as Mean± S.E.M (n=4). \*  $P < 0.05$  compared to the control; #  $p < 0.05$  compared to the untreated control

### Effect of plant extract on the activity of liver enzymes in streptozotocin-induced diabetic rats

There was a slight, non-significant increase in the ALT levels in the diabetic untreated rats compared with the normal control ( $p > 0.05$ ). Treatment of the rats with a decoction of *Rauvolfia vomitoria* and *Citrus aurantium* resulted in a significant decrease in the ALT levels. This decrease was comparable to the ameliorative effect of metformin on the enzyme marker ( $p < 0.05$ ). Also, induction of diabetes with streptozotocin produced a non-significant decrease in the SOD levels compared with the normal control ( $p > 0.05$ ). There was no significant

### DISCUSSION

Diabetes is a chronic health issue with devastating but preventable consequences. Diabetes mellitus is a group of metabolic disorders characterized by high blood sugar levels and disturbances in insulin production and function. This study assessed the hypoglycemic potential of the decoction of *Rauvolfia vomitoria* and *Citrus aurantium* on some biochemical parameters in streptozotocin-induced diabetic rats. Streptozotocin-induction of diabetes provides an experimental model to determine the effects of plant extracts in ameliorating the disease [16]. In this study, diabetes was induced by the

administration of streptozotocin in albino rats of the Wistar strain. Streptozotocin is transported to the  $\beta$ -cells via the GLUT2 receptors due to its structural similarity to glucose, where it selectively attacks the  $\beta$ -cells of the Islets of Langerhans [17]. In addition, STZ-induced necrosis of the pancreatic  $\beta$ -cells occurs by methylation of the DNA, leading to fragmentation, which subsequently manifests as diabetes mellitus. DNA fragmentation stimulates nuclear poly (ADP-ribose) synthetase, which depletes intracellular NAD<sup>+</sup> and ATP levels, thus, inhibiting the production of proinsulin [18]. Streptozotocin induction of diabetes has been shown to increase the level of carbohydrate, protein, lipids, creatinine and enzymes as well as decrease the SOD, catalase levels and body weight in rats [19].

In this study, the administration of streptozotocin caused a significant amount of weight loss when compared to the control. Reduction in body weight can be ascribed to the breakdown of tissue proteins in diabetic rats [20]. This may be due to the breakdown of tissue proteins (muscular wasting), hypoinsulinemia and reduced adipose tissue mass, which is consistent with previous reports [20, 21].

Treatment with the decoction of *Rauvolfia vomitoria* and *Citrus aurantium* resulted in a decrease in the blood glucose levels. Previous studies have shown that extracts of *Rauvolfia vomitoria* caused a dose-dependent reduction in blood glucose levels in both normoglycemic and hyperglycemic rats [22]. In addition, extract of *Citrus aurantium* is known to significantly reduce blood levels in diabetic rats [23]. Hence, this result proves that the plant decoction is effective in suppressing high blood glucose level of diabetic rats.

Diabetes mellitus leads to fatty liver, hypercholesterolemia and hypertriglyceridemia [24]. The results from table III for lipid profile levels shows a significant difference ( $p > 0.05$ ) between diabetic control rats and normal rats. The serum values of cholesterol, triglyceride, HDL-Cholesterol and LDL-Cholesterol vary significantly among the groups with diabetic control showing hypertriglyceridemia and hypercholesterolemia, high levels of LDL and HDL. The reduction in LDL-cholesterol and cholesterol levels in diabetic rats that received plant decoction compared to diabetic control is suggestive of the potential of the plant in improving coronary heart diseases and atherosclerosis. Though the plant extract was able to reduce the level of lipid profile, its effects is not to be compared with the standard drug, metformin. In addition, the results obtained for total protein and creatinine levels show a significant increase in serum of diabetic control rats compared to normal control rats. Total protein and creatinine are indices of biosynthetic capacity of the liver and clearance capacity of the kidney, respectively. The serum total protein and creatinine levels of STZ-treated group was significantly reduced by administration of the decoction when compared to the normoglycemic rats. This indicates that the biosynthetic function of the liver as well as the clearance capacity of the kidney were improved by treatment with the plant extract.

The liver is a central organ for the detoxification and expulsion of xenobiotics. The activities of superoxide dismutase (SOD) and catalase enzymes constitute an important antioxidant defense against oxidative stress. While SOD catalyzes the breakdown of superoxide anion free radical (O<sub>2</sub><sup>-</sup>) into molecular oxygen and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), catalase and peroxidases convert hydrogen peroxide into water [25]. No significant differences was observed in the ALT and SOD levels in the diabetic untreated groups. The catalase activity was significantly increased in the diabetic untreated group. The levels of the antioxidant enzymes, SOD and Catalase were slightly reduced when compared to normal control rats. This finding is at variance with the report of Henriksen who showed a reduced level of catalase in rats induced with STZ [26]. On the other hand, treatment with the plant decoction significantly reversed the STZ-induced differences in ALT and CAT, consistent with the effect of metformin on the diabetic rats. Previous reports have also revealed that *C. aurantium* leaf extracts exert the most DPPH radical scavenging activity in that study. The levels of SOD and catalase in extract-treated rats is suggestive of the plant's ability to reduce oxidative stress.

## CONCLUSION

The results from this study support the hypoglycemic potential of the plant decoction and presents a cheaper remedy for the management of diabetes in low-income countries.

## Conflict of interest

The authors declared no conflict of interest.

## Financial Support

None declared.

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