

Assessment of Liver and Kidney Antioxidant Enzymes and Lipid Profiles of Type-1 Diabetic Rats Treated with Selected Medicinal Plants

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Abstract

The aim of this study was to evaluate the activity of the liver and kidney antioxidant enzymes as well as the lipid profile of Type-1 diabetic rats treated with methanol leaf extract of cashew (*Anacardium occidentale*), pawpaw (*Carica papaya*) and guava (*Psidium guajava*). Dry Plant leaves were processed into powder. 500 g of the powdered plant sample resulting from each of the plant materials was macerated to form extract. A total of thirty (30) apparently healthy adult male wistar rats were divided into six (6) groups of five (5) rats per group. Fasted rats were induced with Type-1 diabetes by a single intraperitoneal injection of 60 mg/kg b.w of streptozotocin. **Group I:** Normal control; **Group II:** Untreated diabetic rats, **Group: III-V** Diabetic Rats administered with 200 mg/kg b.w of methanol leaf extract of *Carica papaya*, *Psidium guajava*, *Anacardium occidentale* orally respectively **Group VI:** Diabetic rats administered with standard drug (Glibenclamide) orally. Treatment lasted for 28 days. Rats were sacrificed 24hrs after the last treatment. Blood, liver and kidney were harvested and processed. Induction of diabetes significantly elevated the lipid profile and the activity of both liver and kidney antioxidants enzymes. However, administration of 200 mg/kg bw of methanol leaf extract of *C. papaya*, *P. guajava* and *A. occidentale* to rats in groups III-V respectively resulted in a reduction that was not significantly ($P>0.05$) different from the values obtained for group VI treated with the standard drug (Glibenclamide). In conclusion, this research reveals that leaves from the studied plants can be explored to offer a helping hand in the management of diabetes related complications.

Keywords: *Carica papaya*; Glibenclamide; Type-1 diabetes; methanol.

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1. Introduction

Diabetes mellitus is a common disorder of metabolism that results from defect in insulin secretion, activity or both. It is characterized by hyperglycemia often accompanied by glycosuria, polydipsia and polyuria [1]. In accordance with the Diabetes Atlas of 2011, 366 million people are battling with diabetes globally, and the number will attain 552 million by the year 2030 with the largest proportion of the menace in regions dominated by developing economies [2].

Hyperglycemia triggers free radical generating pathways some of which include hexosamine metabolism, protein kinase C activity, glucose autooxidation, oxidative phosphorylation, sorbitol formation and consequently oxidative stress within the hepatocytes and the kidney cells, resulting to a depleted enzymatic antioxidants system of the affected organs [3,4].

Lipid profiles include lipids in the plasma such as triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), and low density lipoprotein (LDL) [5]. Studies have shown that effective control of lipid profiles can adequately prevent complications such as cardiovascular disease, diabetic nephropathy and diabetic retinopathy [6].

World Health Organization (WHO) reports that not less than 90% of the populations of developing countries explore therapeutic plants to tackle many human diseases [7]. The interest in the use of medicinal plants in the treatment of diseases can be attributed to its affordability and effectiveness among other advantages [8].

Carica papaya (Pawpaw) is predominantly cultivated in the tropics. It is a member of the family *Caricaceae* [9]. Research has shown that parts of *C. papaya* are embodiment of compounds with wide therapeutic potentials. Notable instances of such compounds are the anti-oxidants which have been found in the various parts of the plants [10]. Leaf of *C. papaya* has been applied in the treatment of diseases like dyspepsia, hyperacidity, dysentery and constipation etc [10]. *Psidium guajava* (guava) primarily grown for its fruit belongs to the family *myrtaceae*, it is grown in South Africa [11]. The leaf is efficacious in the treatment of numerous human diseases [12]. The fruit which is consumed as food is rich in vitamins, minerals and notable antioxidants. In folk medicine, it is used in the management of certain deleterious diseases owing to the presence of certain compounds of pharmacological significance [13]. *Anacardium occidentale* commonly called cashew is a member of the *Anacardiaceae* family [14]. Although found in tropical countries, it is native to Brazil. Parts of the tree such as the leaf, root, and stem have been used in the treatment of diverse diseases such as bacterial and fungal infections as well as oxidative stress related conditions [14]. Owing to the existing information on the antioxidant property of plants being studied, the need to scientifically validate their possible restorative effect in oxidative stress influence initiated by diabetic condition becomes imperative

2. Material and methods

Collection of plant material

Freshly harvested leaves of pawpaw (*Carica papaya*), cashew (*Anacardium occidentale*) and guava (*Psidium*

guajava) obtained from the ABU Botanical Garden, Samaru Zaria. The leaves were subsequently identified and authenticated at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria, where voucher specimen were deposited and numbers (571, 184, and 3253) assigned.

Preparation of plant extracts

Freshly harvested leaves were dried separately at room temperature, after which they were ground to powder with the aid of an electric grinder. The resulting powder from the various leaves was sieved to obtain fine powder. 500 g of the powdered plant sample was separately suspended in 2 Liters of 70 % methanol for a period of 72 hours and was subsequently stirred intermittently. The extracts were filtered and the filtrates concentrated at 40°C [15].

Animals

Thirty apparently healthy adult male wister rats (150-200 g) procured from the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used for the study. The animals were kept in well ventilated cages and fed with rat feed and water *ad libitum*.

Acute toxicity study

Acute toxicity test was performed in accordance with the method described by Lorke [16]. The first phase of the experiment was characterised by the division of the rats into three groups i.e. groups (I-III) of three rats per group and were orally administered with 10 mg, 100 mg and 1000 mg of the extracts per kg body weight respectively in separate experiments. They were observed for 24 hours for signs of toxicity in the absence of which the second phase was commenced and consisted of 3 rats which were divided into 3 groups of 1 rat per group administered with 1600, 2,900 and 5,000 mg/kg bw of test sample extracts. The LD₅₀ was determined using the results obtained from the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose.

Induction of Diabetes

Diabetes mellitus was induced by single intraperitoneal injection of 60 mg/kg b.w of streptozotocin, (dissolved in 0.1mol fresh cold citrate buffer, (pH 4.5) into 24 hrs fasted rats [17]. Glucose solution (5%) was administered after 6 hours of induction. After 3 days, the blood sugar levels was determined with a glucometer (Acc-cheek Advantage Roche diagnostics GmbH, Germany) and the rats with fasting blood glucose level more than 200 mg/dl (11.1 mmol/L) were considered diabetic hence selected for study.

Experimental design

Thirty adult male wistar rats were divided into four groups of five rats each.

Group I: Normal control was fed with only rat chow and water *ad libitum*.

Group II: Untreated diabetic rats

Group III: Diabetic rats administered with 200 mg/kg b.w of methanol extract of *Carica papaya* (pawpaw) leaf orally.

Group IV: Diabetic rats administered with 200 mg/kg b.w of methanol extract of *Anacardium occidentale* leaf orally.

Group V: Diabetic rats were administered with 200 mg/kg b.w of methanol extract of *Psidium guajava* (guajava) leaf orally.

Group VI: Diabetic rats were administered with standard drug (Glibenclamide) orally.

Treatment lasted for a period of 28 days by gastric intubation after which animals were anaesthetized using chloroform and sacrificed 24 hrs after the last treatment and their liver and kidney harvested into properly labeled containers and homogenized using 50 mM potassium phosphate buffer (pH 7.4). The homogenate was centrifuged at 3,000 x g for 15 minutes. The supernatant was decanted and used for analyses.

Assay of liver and kidney antioxidant enzymes

Catalase assay

Catalase activity was determined using the spectrophotometric approach described by Aebi and his colleagues [18] with minor modifications [19]. The degradation of H₂O₂ was determined at 240 nm. In accordance with this method, the mixture which is made up of 0.01 M phosphate buffer (pH-7.0), 0.02 M H₂O₂ and 20 µL liver homogenate were thoroughly mixed and absorbance taken at 240 nm after every 30 seconds for 3 minutes. One catalase unit was defined as the amount of enzyme that decomposed 1 µmol of H₂O₂ per min at 37°C. The results were expressed as U/mg protein.

Superoxide dismutase (SOD) activity

Superoxide dismutase activity of the liver and kidney was evaluated as described by Misra and Fridovich [20]. The organs were diluted with Tris-HCl pH 7.4 at a proportion of 1/60 (w/v) and 1/40 (w/v), respectively. The results were expressed as U/mg protein.

Lipid peroxidation

Lipid peroxidation was estimated using colorimetric method by measuring TBARS as described by Ohkawa and his colleagues [21] and expressed as nmol MDA/mg protein.

Evaluation of Lipid Profiles

Total cholesterol estimation

This was carried out using the method described by Stein [22]. Exactly 1 ml of cholesterol reagent was added to all the required test tubes. 10 µL of the sample was added to the test sample test tubes, 10 µL of standard reagent was added to the standard test tube and none to blank. It was incubated at room temperature for 20 minutes. The absorbance of the test sample and the standard was read at 505 nm and the concentration of the sample was calculated using the formula:

Absorbance of test × Concentration of Standard

Absorbance of Standard

Determination of serum triacylglycerol

Exactly 1 ml of the sample reagent was introduced into all the required test tubes. 10 µL of the sample was added to the test sample test tube, after which 10 µL of standard reagent was placed in the standard test tube and none to the blank. The samples were subsequently incubated at room temperature for 15 minutes. The absorbance of the test sample and that of the standard was read at 500 nm and the concentration of the sample was calculated using the formula;

Absorbance of test × Concentration of Standard

Absorbance of Standard.

High Density Lipoprotein Cholesterol (HDL-c) Estimation

This was evaluated with using assay kit as described by Wacnic and Albers [23].The value was expressed in the unit of mg/dl. The proteins were precipitated using phosphotungstic acid, in the presence of magnesium and all other cholesterol in the solution. 1 ml the sample reagent was added to the required test tubes, 10 µL of the sample was added to the test sample test tube, and 10 µL of standard reagent was added to the standard test tube and none to blank. It was incubated at room temperature for 15 minutes. The absorbance of the test sample and the standard were read at 500nm and the concentration of the sample was calculated using the formula:

Absorbance of test × Concentration of Standard

Absorbance of Standard

Determination of serum Low Density Lipoprotein- Cholesterol (LDL-C)

The serum level of (LDL-c) was measured according to the method of Friedewald *and his colleagues* [24].

The value was expressed as the unit of mg/dl. LDLC was calculated using the formula:

Statistical Analysis

Results were expressed as mean $\pm$ standard error of mean (SEM). The data was analyzed using analysis of variance (ANOVA). Differences in mean among groups were compared using the Duncan Multiple Range Test. $P < 0.05$ was considered statistically significant.

3. Result

Table 1.0: Lipid profile of diabetic rats treated with selected medicinal plants

Groups	Treatments	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group I	Normal control	86.67 $\pm$ 6.67 ^{ab}	93.33 $\pm$ 6.67 ^{ab}	43.33 $\pm$ 3.33 ^b	44.67 $\pm$ 4.67 ^a
Group II	Diabetic control	160.00 $\pm$ 11.55 ^c	193.33 $\pm$ 12.02 ^a	53.33 $\pm$ 3.33 ^a	104.67 $\pm$ 7.69 ^c
Group III	<i>C. papaya</i>	123.33 $\pm$ 8.82 ^b	123.33 $\pm$ 6.67 ^c	33.33 $\pm$ 6.67 ^{ab}	39.33 $\pm$ 12.72 ^b
Group IV	<i>P. guajava</i>	126.67 $\pm$ 6.67 ^b	166.67 $\pm$ 6.78 ^{bc}	36.67 $\pm$ 3.33 ^{ab}	40.00 $\pm$ 6.11 ^b
Group V	<i>A. occidentale</i>	120.00 $\pm$ 5.77 ^b	96.67 $\pm$ 6.67 ^{ab}	37.72 $\pm$ 3.45 ^{ab}	41.00 $\pm$ 9.87 ^b
Group VI	Glibenclamide	103.33 $\pm$ 8.82 ^a	86.67 $\pm$ 8.81 ^a	40.00 $\pm$ 5.78 ^b	46.00 $\pm$ 15.01 ^{ab}

Values are expressed as mean $\pm$ standard error of mean. Values with same superscript in a column are not significantly ($P < 0.05$) different

Table 2.0: Liver antioxidant enzymes of rats treated with selected medicinal plant

GROUPS	SOD	CAT	MDA (nmol/mg protein)
Group I	1.70 $\pm$ 0.100 ^{bc}	48.67 $\pm$ 4.48 ^b	1.63 $\pm$ 0.09 ^a
Group II	3.97 $\pm$ 0.203 ^a	32.00 $\pm$ 0.58 ^a	2.47 $\pm$ 0.22 ^b
Group III	1.60 $\pm$ 0.100 ^b	44.67 $\pm$ 2.73 ^b	1.77 $\pm$ 0.13 ^a
Group IV	1.60 $\pm$ 0.116 ^{bc}	41.00 $\pm$ 2.31 ^b	1.70 $\pm$ 0.10 ^a
Group V	1.60 $\pm$ 0.578 ^b	49.67 $\pm$ 2.93 ^b	1.70 $\pm$ 0.25 ^a
Group VI	2.00 $\pm$ 0.58 ^c	49.67 $\pm$ 2.73 ^b	1.63 $\pm$ 0.16 ^a

Values are expressed as mean $\pm$ standard error of mean. Values with same superscript in a column are not significantly ($P < 0.05$) different

Table 3.0: Kidney antioxidant enzymes of diabetic rats treated with selected medicinal plants

GROUPS	TREATMENT	SOD	CAT	MDA (nmol/mg protein)
Group I	Normal control	1.53 $\pm$ 0.98 ^b	42.00 $\pm$ 1.55 ^b	1.67 $\pm$ 0.33 ^a
Group II	Diabetic control	2.93 $\pm$ 0.15 ^b	32.67 $\pm$ 0.67 ^a	2.50 $\pm$ 0.46 ^b
Group III	<i>C. papaya</i>	1.63 $\pm$ 0.88 ^b	48.67 $\pm$ 2.40 ^{bc}	1.87 $\pm$ 0.12 ^a
Group IV	<i>P. guajava</i>	1.67 $\pm$ 0.82 ^b	42.00 $\pm$ 2.31 ^b	1.60 $\pm$ 0.17 ^a
Group V	<i>A. occidentale</i>	1.63 $\pm$ 0.98 ^b	46.33 $\pm$ 3.18 ^{bc}	1.60 $\pm$ 0.20 ^a
Group VI	Glibenclamide	2.03 $\pm$ 0.12 ^c	50.67 $\pm$ 2.73 ^c	1.73 $\pm$ 0.36 ^a

Values are expressed as mean $\pm$ standard error of mean. Values with same superscript in a column are not significantly ($P < 0.05$) different

4. Discussion

Table 1.0 shows the lipid profile of Type-1 diabetic rats administered with methanol leaf extract of *C. papaya* (pawpaw), *P. guajava* (guava), and *A. occidentale*. Induction of diabetes elevated the lipid profile of induced rats; TC (160.00 ± 11.55 mg/dl), TG (193.33 ± 12.02 mg/dl), HDL (23.33 ± 3.33 mg/dl) and LDL (104.67 ± 7.69 mg/dl) compared to the non-diabetic normal control TC (86.67 ± 6.67 mg/dl), TG (93.33 ± 6.67 mg/dl), HDL (43.33 ± 3.33 mg/dl) and (24.67 ± 4.67 mg/dl). However, administration of 200 mg/kg bw of methanol leaf extract of *C. papaya*, *P. guajava* and *A. occidentale* to rats in groups III-V respectively resulted in a reduction to level that was not significantly ($P > 0.05$) different from the values obtained for group VI administered with the standard drug. This may be as a result of the possible therapeutic effect of the compounds in these plants. This finding is consistent with the work of Ukwenya and his colleagues [12], which affirms that treatment with *A. occidentale* resulted in reduction in hyperglycemia, similarly, Ukpabi and his colleagues [25], established the anti-hyperlipidemic potential of aqueous extract of *Carica papaya*. Table 2.0-3.0 shows the activity of liver and kidney antioxidant enzymes of Type-1 diabetic rats treated with 200 mg/kg bw of leaf of the aforementioned medicinal plants respectively. Streptozotocin administration caused a significant ($P < 0.05$) increase in the activity of liver antioxidant enzymes; SOD (3.97 ± 0.203 U/mg protein), CAT (32.00 ± 0.58 U/mg protein) and MDA (1.63 ± 0.09 μ mol/mg protein) compared to the control SOD (1.70 ± 0.100 U/mg protein), CAT (48.67 ± 4.48 U/mg protein), and MDA (1.63 ± 0.09 μ mol/mg protein). Meanwhile, administration of 200 mg/kg bw of methanol leaf extract of *C. papaya*, *P. guajava* and *A. occidentale* to rats in groups III-V respectively resulted in levels of reduction that were not significantly ($P > 0.05$) different from the values recorded for group VI which was treated with the standard drug. Induction of Type-1 diabetes caused a significant ($P < 0.05$) increase in the activity of the kidney antioxidant enzymes; SOD (2.93 ± 0.98 U/mg protein), CAT (32.67 ± 0.67 U/mg protein) and MDA (2.50 ± 0.46 μ mol/mg protein) compared to the non-diabetic control group; SOD (1.53 ± 0.33 U/mg protein), CAT (42.00 ± 1.55 U/mg protein), and MDA (1.67 ± 0.33 μ mol/mg protein).

On the other hand, administration of 200 mg/kg bw of methanol leaf extract of *C. papaya*, *P. guajava* and *A. occidentale* to rats in groups III-V respectively caused a non-significant ($P < 0.05$) reduction in the activity of the kidney anti-oxidant enzyme compared to group VI administered with the standard drug. This may be as a result of the presence of phytochemicals e.g. Onuh and his colleagues [26] established a positive correlation between total phenolic content of *A. occidentale* and its antioxidant activities. This study is consistent with the work of Lalita and Soottawat which affirmed that ethanolic leaf extract of *A. occidentale* retarded lipid peroxidation. Similarly, Onuh and his colleagues [27] reported that cashew fruit extract exhibited significantly high percentage of DPPH scavenging activities at all concentration.

5. Conclusion

The outcome of this work is an indication that the leaves of the aforementioned plants wield the potential to ameliorate complications that could result from hepatic and renal dysfunctions. The results also point to the possible presence of certain compounds of medicinal values.

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