Original Research Article

ANTIOXIDANT PROPERTY OF METHANOL CRUDE EXTRACT OF UNRIPE PULP OF *Carica papaya* ON ALLOXAN-INDUCED DIABETIC MALE ALBINO RATS

ABSTRACT

This study investigated the effects of the methanol extract of *Carica papaya* leaves on antioxidants in alloxan-induced diabetic rats. *Carica papaya* leaves were extracted using 80% methanol. 24 healthy rats weighing 110–150 g was grouped into eight (8) groups of three rats each: Group 1 (normal control), Group 2 (negative control), and Group 3 (positive control); Group 4 (200 mg/kg) of extract; Group 5 (400 mg/kg) of extract; Group 6 (600 mg/kg) of extract; Group 7 (800 mg/kg) of extract; and Group 8 (1000 mg/kg) of extract. Diabetes was induced by a single intraperitoneal administration of 120 mg/kg body weight of alloxan. Groups 1-3 served as normal, negative (untreated), and positive (standard drug) controls, respectively; groups 4–8 were treated groups. Administration was done orally for twenty-eight (28) days, and fasting blood glucose levels were obtained at a seven-day interval. After treatments, the rats were anaesthetized, and blood was collected by cardiac puncture for determination of redox status using standard analytical procedures. Diabetic rats treated with *Carica papaya* leaf extract significantly reduced ($p < 0.05$) their glucose level when compared with the positive control and the negative control. The reduction in fasting blood glucose levels of the extract-treated groups was consistently observable in groups 7 and 8, respectively, which were treated with high doses. The serum glutathione peroxidase, reduced glutathione, catalase, and superoxide dismutase catalase activities of rats treated with extracts showed a significant increase ($p<0.05$) when compared with the diabetic untreated (negative) control. There was a significant reduction in malondialdehyde levels in all the groups treated with the extract when compared with the diabetic untreated (negative) control. The non-enzymatic antioxidants (Vitamin C and E) increased significantly in some of the treated groups when compared with the negative control. The present study showed that the methanol extract of *Carica papaya* leaves offered a significant hypoglycemic effect and antioxidant effect in alloxan-induced diabetic rats, which can be a result of the presence of certain phytochemicals responsible for the increase in the antioxidant enzyme activity of the experimental animals.

Keywords: Alloxan monohydrate, *carica papaya* leaves, antioxidants, diabetes mellitus

1.0 INTRODUCTION

Diabetes mellitus is a metabolic disease (disorder) that prevents the body from properly using the energy from carbohydrate, fat, and protein metabolism as a result of defects in insulin hormone production, produced by beta cells of the pancreas [1, 2]. Diabetes mellitus is also a collection of disorders that indicate too much sugar in the blood, called hyperglycemia, which comprises both pre-diabetes and diabetes. For a better understanding of diabetes, it is necessary to understand the

normal physiological processes occurring during and after a meal. The blood vessels and the blood are the highways that transport sugar from where it is either taken in the stomach or manufactured in the liver to the cells, where it is used by muscles or stored. Sugar cannot go into the cells by itself. The pancreas releases insulin into the blood, which serves as a helper or key that lets sugar into the cells for use as energy [1]. The rise in glucose causes insulin to be released from the pancreas so glucose can move inside the cells and be used [3]. It is also associated with hypercholesterolemia, hyperlipidemia, and hepatic steatosis, which are the result of defects in insulin secretion [4]. The increasing nature of the disorder requires constant reassessment of hyperglycemic control in persons with diabetes and proper adjustment of curative regimens. When hyperglycemic control is not managed with a single agent, the addition of a second or third drug is always comparatively more effective than switching to another single agent [5].

Available synthetic anti-diabetic drugs, besides being very costly, produce severe side effects. Apart from currently available therapeutic options, many traditional medicines and herbs are recommended for the treatment and management of diabetes mellitus. Medicinal plants have the greatest advantage of having no side effects [2]. Natural products have a chemical compound or substance that is found in nature, and medicinal plants have played a central role in disease prevention, management, and treatment [6].

Carica papaya (Linnaeus family *Caricaceae*) is a medicinal herb that has been used in traditional medicine practices since prehistoric times because of its numerous phytochemicals with potential or established biological activity identified for disease treatment, management, and prevention. *C. Papaya* is a plant that grows in tropical climates. Its leaf, fruit, seed, and root are used for the treatment of wounds, skin infections, reproductive organ stimulation, cancer, aiding digestion, lowering blood pressure, and improving blood glucose control in people with diabetes [7,8]. It contains vitamins, minerals, and other vital nutrients that help to maintain the body's normal health status [9]. Earlier studies showed the efficacy of effective use of *C. papaya* on wound healing in diabetic rats [10] and an antibacterial effect on common wound microorganisms [11]. Interestingly, the health-enhancing activities of *C. papaya* are also recognized due to its antioxidant activity [12]. *C. Papaya* is widely used for the treatment of diabetes mainly because of its effectiveness, being readily available and cheaper than modern medicines, and its antioxidant property [13, 14]. *C. Papaya* pulp contains 88% water, 11% carbohydrates, and negligible fat and protein. It is rich in nutrients and phytochemicals like cardenolides, saponins, carotenoids, polyphenols, and cyanogenic glycosides, etc. *C. Papaya* is loaded with antioxidants that can reduce inflammation, fight disease, help keep you looking young, and reduce blood glucose levels to normal [15].

Oxidative stress is caused by a difference between how reactive oxygen species (ROS) are made and how well a biological system can get rid of the reactive intermediates [16]. When the normal redox state of the cell is upset, peroxides and free radicals are made that damage lipids, proteins, and DNA. Oxidative stress from oxidative metabolism causes base damage as well as strand breaks in DNA. Base damage is mostly indirect and caused by reactive oxygen species (ROS) generated [17]. Vitamins C and E, β-carotene, flavonoids, tannins, anthocyanin, and other phenolic compounds are plant-derived compounds with antioxidant activities capable of scavenging free radicals and represent a special group of nutritional supplements [18, 19]. Lack of control over the excess production of ROS has been involved in the pathophysiology of many disorders like diabetes mellitus. Hence, this study was set up to evaluate the potency of the leaf

extract on induced diabetic rats. Specifically, the study aimed to investigate the effect of the extract on the antioxidant parameters (CAT, GSH, and MDA) in the experimental rats. This work is important because biochemical changes are major observable clinical and pathological features common with diabetes.

2.0 MATERIALS AND METHODS

2.1 Plant Materials

C. papaya leaves were collected from pawpaw trees in Lodu Ndume, Umuahia North Local Government Area of Abia State, Nigeria. The leaves were identified and authenticated by a plant taxonomist (Dr Ibe K. Ndukwe) from the forestry department, College of Natural Resources and Environment Management (CNREM), and Michael Okpara University of Agriculture Umudike, where voucher specimen (IHF 26123) was deposited in the departmental herbarium. The leaves were collected, washed, and dried under shade at room temperature to a constant weight, then weighed and milled into powder. The powdered leaves were soaked in methanol and distilled water in the ratio of 80:20, respectively, and left to stand for 3 days with occasional shaking. This was filtered using Whatman No. 1 filter paper, and the filtrate was subsequently evaporated to obtain the dry matter using a rotary evaporator under reduced pressure at 40^{o}C.

2.2 Phytochemicals and Toxicity of Methanolic Extract of *Carica papaya*

Phytochemical screening was performed for the presence of alkaloids, carbohydrates, amino acids, glycosides, protein, phenolic compounds, and tannins from respective solvents such as hexane, ethyl acetate, methanol, and ethanol, according to standard procedure [20, 21]. Acute oral toxicity (LD50) was performed by the method of Lorke [22]. Three groups of rats each comprising of three rats each were administered with 500, 800 and 1000 mg/kg of herbal formulation by mouth and examined for mortality within 24 hours. Following the results of mortality in each group, another set of three groups of rats were administered higher doses of the test drug, to achieve the least and most toxic value and LD50 was calculated by geometric mean of the mortality values. LD50was calculated as: LD50 = [M0 + M1] ÷ 2, where M0 = highest dose of test substance that gave no mortality and M1= lowest dose of test substance that gave mortality

2.3 Ethical adherence and Experimental Animals and Design

The study adhered strictly to the ethical guidelines on animal use as stipulated by the National Research Council, NRC, USA (2011). Twenty (24) healthy adult wistar rats (110-150g) procured from College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State was used for the study. All animals were allowed free access to food and water and were housed in aluminum cages maintained under standard laboratory conditions with light and dark cycles of 12 h each and room temperature of 25 ºC. Acclimatized adult male albino rats (110-150g) were randomly allotted to eight groups of 3 rats each. Group 1 rats received normal saline (1 ml). Group 2 (induced with diabetes but not treated), and Group 3 (induced with diabetes and treated with standard drug (Glibenclamide, 2 mg/kg bw)); Group 4 (200 mg/kg) of extract; Group 5 (400 mg/kg) of extract; Group 6 (600 mg/kg) of extract; Group 7 (800 mg/kg) of extract; and Group 8 (1000 mg/kg) of extract. At the end of acclimatization, the animals were allowed to fast and then diabetes was induced by intra-peritoneal (IP) injection of 120mg/kg body weight of alloxan monohydrate solution by the method of Yanardag and Colak [23]. Rats

with elevated blood glucose concentration above 150mg/dl were considered diabetic after 4 days of induction using fasting blood sugar test and were used for the study. Experimental administration was orally by gavage and daily for 28 days. At the end of 28 days experimental period, the animals were sacrificed by cervical dislocation, and blood samples were collected by cardiac puncture into plain bottles (to obtain clotted blood). The blood thus collected was allowed to clot after standing for 10 minutes at ambient temperature. Thereafter, the respective serum was separated by centrifuging the coagulated blood samples at 3000 × g for 15 minutes and used for the determination of fasting blood glucose (FBG), MDA, SOD, CAT levels. The serum indicators level of antioxidant parameters (MDA, SOD, CAT) was respectively determined with Randox commercial Kits.

2.4 Estimation of blood glucose concentration

The blood glucose levels of the animals were determined using a Glucometer Acu-check (Tyson Bio Evolve glucometer, Tyson Bioresearch Inc., Hangzhou, China) and subsequently on a weekly basis at days 0, 7, 14, 21 and 28 throughout the period of treatment with standard drug and extract.

2.5 Determination of antioxidant Activity

2.5.1 Determination of superoxide dismutase (SOD)

Superoxide dismutase was determined using the method Aebi [24]. Adrenaline (10 mg) was dissolved in 17 mL of distilled water to make adrenaline solution. Serum sample (0.1 mL) was added to 2.5 mL of phosphate buffer (pH 7.8). Adrenaline solution (0.3 mL) was added, mixed well and absorbance was read at 450 nm at 30 seconds interval for 5 times

2.5.2 Determination of catalase activity

Determination of catalase activity was according to the method of Obi and Egbuonu [25]

2.5.3 Determination of reduced glutathione

Glutathione concentration was determined according to the method of Ellman [26]

2.5.4 Determination of glutathione peroxidase

The activity of reduced glutathione peroxidase was determined using the method of Paglia and Valentine [27].

2.5.5 Determination of peroxidase activity

The activity of reduced glutathione peroxidase was determined using the method of Paglia and Valentine [27].

2.5.6 Malondialdehyde (MDA) level Determination

Lipid peroxidation was determined spectrophotometrically by measuring the level of lipid peroxidation product, malondialdehyde (MDA), as described by Onkawa et al. [28]. Malondialdehyde reacts with thiobarbituric acid (TBA) to form a red or pink coloured complex that absorbs maximally in acid solution at 532 nm.

2.5.7 Vitamin C Determination

Vitamin C level was determined using the method of Omaye *et al.* [29].

2.5.8 Vitamin E Determination

Vitamin E level was determined using the method of Rosemberg [30]

2.6 Statistical Analysis

Data obtained from the experiments were analyzed using one-way analysis of variance (ANOVA). Statistical package for social sciences (SPSS) version 20.0. The analysis data was reported as mean ± standard error of mean (SEM). Significant difference using Tukey's Post Hoc test was accepted at 95% confidence level of probability i.e., at $p < 0.05$.

3.0 RESULTS

3.1 Glucose Concentration

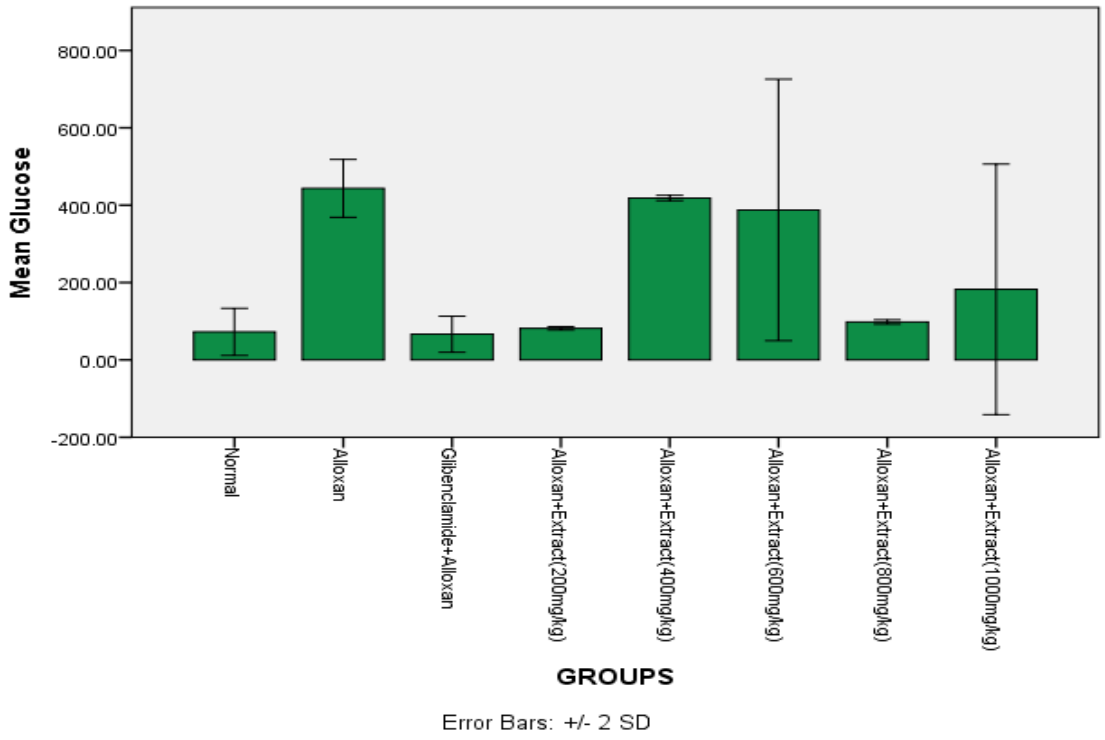

Figure 1: Blood glucose concentration in diabetic rats treated with extracts of C*arica papaya* leaves.

Table 1: Acute toxicity of methanolic extract of *Carica papaya* leaves

Groups	Concentration (mg/kg)	Mortality
Group 1 (Control)	-	Nil
Group 2	400mg/kg	Nil
Group 3	600 mg/kg	Nil
Group 4	800 mg/kg	Nil
Group 5	1000 mg/kg	Nil

The result of the acute toxicity study indicated that the *Carica papaya* leaf extract does not have any acute toxicity since no mortality was recorded at the highest dose of 1000 mg/kg (Table 1).

Table 2: Qualitative analysis of *Carica papaya* leaves extract: Phytochemical screening

S. No.	Sample	Alkaloid	Carbohydrate	Amino acid	Glycoside	Phenols tannin	Proteins	Saponin	Quinine	Oxalate	Anthocyanin
1.	Methanol	+	+	+	+	+	–	+	+	+	–

The result of the phytochemical assay, revealed the presence of important bioactive compounds such as Alkaloid, Carbohydrate, Amino acid, Glycoside, Phenols, tannin, Proteins, Saponin, Quinine, Oxalate, Anthocyanin (Table 2)

Table 3: The effect of treatment with different doses of *Carica papaya* leave extracts for the period 28 days

Antioxidant Enzymes	Group 1 Normal Control	Group 2 Negative Control	Group 3 Positive Control	Group 4 200 mg/kg extract	Group 5 400 mg/kg extract	Group 6 600 mg/kg extract	Group 7 800 mg/kg extract	Group 8 1000 mg/kg extract
GPx (iu/l)	24.27±1.10*	15.50±0.51	27.80±1.00*	28.22 ± 0.14*	30.19±1.21*	31.59±1.38*	32.00±0.05*	35.63±0.32*
GSH (mg/dl)	5.35± 0.06*	3.90±3.09	4.98±0404*	4.75±0.02*	5.00±0.05*	5.56±0.10*	5.42±0.12*	5.59±0.10*
CAT (iu/l)	2.85± 0.05*	2.22±0.11	3.58± 0.52*	3.89±0.14*	4.52±0.12*	5.01±0.07*	5.27±0.00*	5.98±0.80*
SOD (iu/l)	3.09± 0.02*	2.10±0.00	3.12± 0.12*	4.14±0.00*	4.15±0.04*	5.15±0.32*	5.16± 0.02*	5.17±0.00*
MDA	3.01± 0.05	4.97±0.14	3.34±0.04	3.02±0.04	3.18±0.05	3.00±0.02	2.96±0.01	2.63±0.04

Result is expressed as mean ± Standard Deviation (n=5). Mean values (*) are significantly different from the negative control group at $p<0.05$.

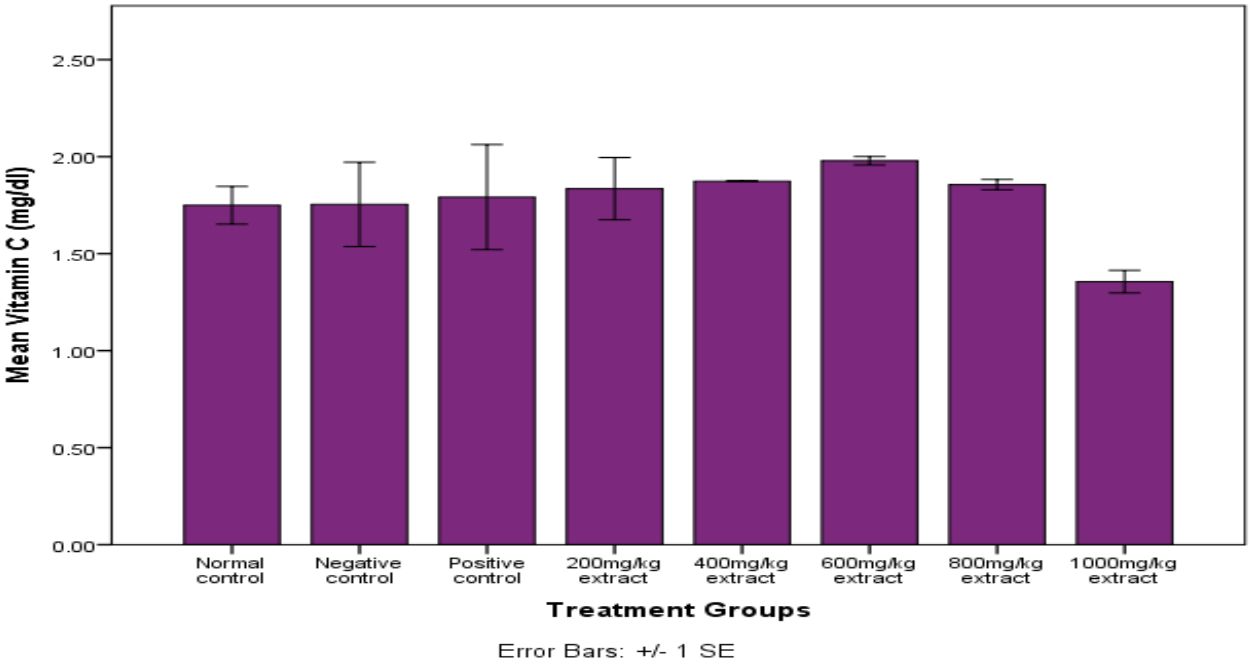

Figure 2: Vitamin C concentration in diabetic rats treated with extracts of C*arica papaya* leaves

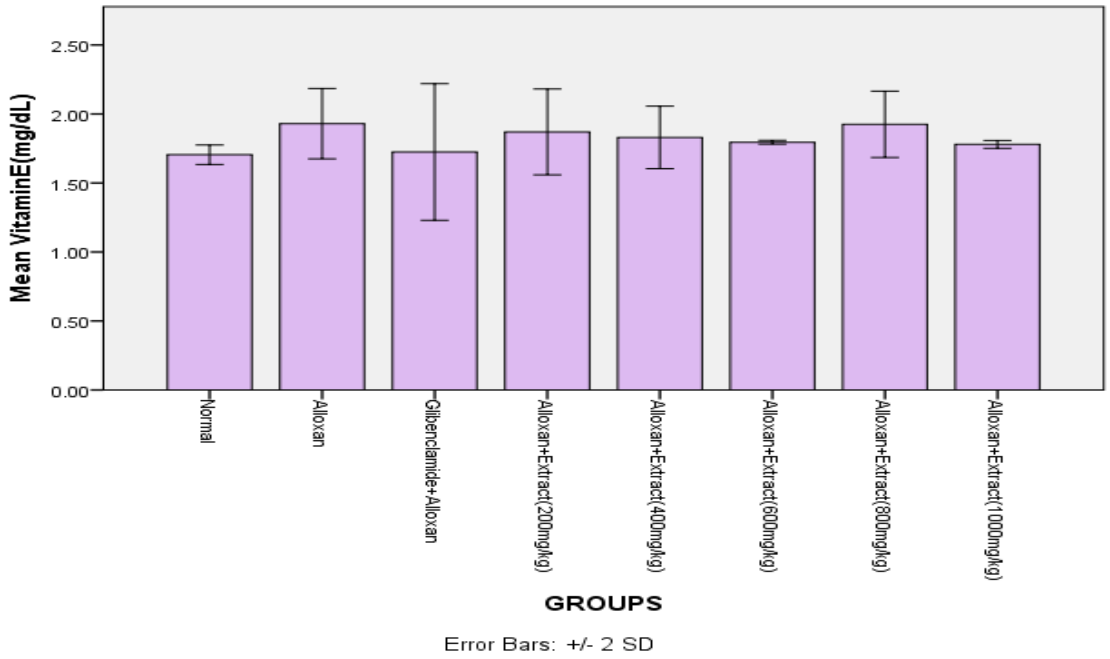

Figure 3: Vitamin E concentration in diabetic rats treated with extracts of C*arica papaya* leaves

DISCUSSION

C. papaya is a tropic fruit that is very rich in antioxidants (Vitamin C, topopherols, phenols and β-carotene) [31]. *C. papaya* is one of the fruits with several application in traditional medicine as anti-hyperlipidemic [32], anti-helminthic and anti-amoebic [33], Hypoglycemic, hypolipidemic [34] among many others.

The results showed significant ($p<0.05$) decrease in blood glucose concentrations in the extract treated groups in comparison to the alloxan induced untreated group (Group 2) and normal control (group 1). The results were in line with the findings of Ezekwe *et al.,* [34], who reported similar low blood sugar (hypoglycemic) effect using *C. papaya* leaf on alloxan induced diabetes in rats. This agrees with the study of Airaodion *et al.,* [35] on the effect of oral intake of African locust bean on fasting blood sugar and lipid profile of albino rats. It also corresponds with another report Airaodion *et al.,* [36] who studied the effect of methanolic extract of Corchorus olitorius leaves on hypoglycemic and hypolipidaemic activities in albino rats. Several other plants and extracts have also been reported to have an antihyperglycemic and an insulin-stimulatory effect [37]. Most of the plants with hypoglycemic properties have been found to contain metabolites such as glycosides, alkaloid and flavonoids [38].

The result of the antioxidant enzyme activities was shown in table 3. The activities of all groups treated with *Carica papaya* extract at varied doses significantly ($p<0.05$) increased when compared with the untreated negative (diabetic group) control. The extract at a dose of 1000mg/kg body weight also showed a significant ($p<0.05$) increase in the activity of some antioxidant enzymes like Glutathione peroxidise, Reduced glutathione, Catalase and superoxide dismutase when compared with the negative control group. There was no significant ($p<0.05$) deference in the mean values of malondialdehyde (MAD) of the treated group when compared with the negative control group. This result was in line with the findings of Ukpabi-Ugo *et al.,* [39] who reported that a significant rise in activity of antioxidant enzymes protect the cell from oxidative damage caused by reactive oxygen species. Also, Raghavan and Krishhankumar, [40] reported that the decrease in the activities of superoxide dismutase enzyme result in the involvement deleterious oxidative changes and also insufficient availability of MAD.

Administration of methanol extract of pawpaw (*Carica papaya*) leaves elicited effective reverse of the alloxan-induced oxidative stress. This antioxidant effect of pawpaw leaves is due to its phytochemical constituents that include polyphenols, vitamins and minerals as reported by Nwangwa and Ekhoye [33] and [34].

Non-enzymatic antioxidants (Vitamin C and E) levels showed no significant difference ($p<0.05$) in the untreated diabetic rats when compared with the normal control. This result was in line with the findings of Ukpabi-Ugo *et al.,* [39] who reported similar no significant difference in vitamin E and D levels in the untreated diabetic rats.

CONCLUSION

The present study showed that the methanol extract of *Carica papaya* leaves offered a significant hypoglycemic effect and antioxidant effect in alloxan induced diabetic rats. These properties of extract may be attributed to its constituents which are manly polyphenolic compounds with its

antioxidant properties. *Carica papaya* leaves can be used in the management of diabetes whose pathogenesis and progression are known to be influenced by oxidant species.

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