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REGULAR ARTICLE

STUDIES ON BIOCHEMICAL EFFECTS OF AQUEOUS EXTRACT OF *CARICA PAPAYA* LEAF ON ALLOXAN-INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Studies on biochemical effects of aqueous extract of *Carica papaya* leaf on alloxan-induced diabetic albino rats were undertaken. One hundred albino rats of Wistar strain were obtained and divided into five groups of twenty rats each and properly treated with the aqueous extract after four out of the five groups were induced with diabetes. The diabetic induced groups were diabetic control, test groups I (treated with 200 mg/kg) and II (treated with 50 mg/kg), and reference (treated with the standard glibenclamide). The diabetic free group was designated as normal control. Results obtained revealed that oral administration of aqueous extract of *C. papaya* leaf on alloxan-induced diabetic albino rats significantly reversed the damage associated with alloxan-induced diabetes revealing its hypoglycemic, liver and renal function integrity effects. The presence of phytochemicals and phytonutrients found in *C. papaya* could explain the observed pharmacological property of the studied extract. This study has revealed the biochemical effects of aqueous extract of *C. papaya* leaf on alloxan-induced diabetic albino rats.

Keywords: Alloaxan, Aqueous extracts, Biochemical effects, *Carica papaya*

INTRODUCTION

Different diseases affect the health of living organisms on this planet Earth. Man through his efforts has deployed different therapies [1] to ameliorate, prevent, salvage or cure some of the diseases. Among the therapies, the use of synthesized or compounded drugs; and plants are the two most accepted ones that have been confirmed effective against disease causing microorganisms [1-5]. It has been reported that most of the synthesized drugs are the chemical analogues of compounds derived from plants [6-9]. Different authors have reported that medicinal plants are those plants that have bioactive constituents with physiological activity against disease causing pathogens [10-14]. These bioactive constituents according to Duru *et al.* [15]; Edeoga *et al.* [16]; Okwu [17]; Okwu and Ekeke [18]; and Duru *et al.* [19], are phytochemicals, vitamins, minerals and proximate contents found in plants. Apart from the phytochemicals, the vitamins, minerals and proximate contents found in plants are classified as phytonutrients [20-22]. Phytochemicals and phytonutrients interact to bring out their known effects against disease causing pathogens that influence the health of other living organisms negatively [23].

Carica papaya is among the known medicinal plants with both phytochemicals and phytonutrients that interact to improve the health of diseased living organisms. *C. papaya* is commonly known as pawpaw and belongs to the family Caricaceae. It is believed to have originated in the tropics of the Americans. Duru *et al.* [24] noted that *C. papaya* is an important plant containing many medicinally active principles. The usefulness of different parts of *C. papaya* has been reported by different authors. Unripe fruit sap can be used against many infections [25] and crude juice extract has medicinal values [26] like malaria [27]. The antihypertensive property [28]; phytochemicals and nutrients evaluation [29] of *C. papaya* leaves have been reported. Hasimuna *et al.* [30] reported the analgetic activity of papaya (*Carica papaya* L.) leaves extract. Other compounds such as benzylglucosinolate, phenylpropanoids as well as enzymes in *C. papaya* have also been reported [31-34]. Locally, the Igbos and the Yorubas address *C. papaya* as okpurukwa" and "Ibepe" respectively. Amadi *et*

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al. [35] noted that though the leaves of *C. papaya* are not among the known conventional vegetables but they are sometimes eaten as prescribed by herbalist to remedy some disease conditions that are prevalent in Nigeria.

Diabetes mellitus is among the prevalent disease conditions in many parts of the world [36-39]. In recent years, progress has been made with use of synthetic drugs in the treatment of diabetes. However, due to limitations of the available medications, there is need to move towards phytomedicine for herbal formulation against the disease diabetes.

The present study looked into this area, and investigated the biochemical effects of aqueous extract of *C. papaya* leaf on alloxan-induced diabetic albino rats.

MATERIALS AND METHODS

Source and preparation of plant material

Leaves of C. papaya were collected from Imo State University school farm, Owerri, Nigeria. The collected leaves were identified by Prof. F. N. Mbagwu of Plant Science and Biotechnology Department, Imo State University as C. papaya leaves. The leaves were air dried and powdered.

Preparation of extract

Two (2) kilograms of the powder sample were soaked in 2.0 L of distilled water for 48 h at room temperature. Removal of the solvent from the extract took place under reduced pressure through filtration, followed by evaporation to dryness at 36 °C, to yielded 200g (10%) of a dark green residue as filtrate.

Experimental animals, alloxan induction, and experimental design

A total of one hundred male albino rats of Wistar strain of the same age, weighing between 180-220 g were purchased from the animal colony of Imo State University school farm, and were transported to the animal colony of Department of Medical Biochemistry of the same citadel of learning. There, the rats were placed in standard cages for 7 d to acclimatize to their new environment. Pelletized commercial rat feed (Vital feed growers, a subsidiary of Nigeria PLC), and potable water were given to the rats *ad libitum* within this period. The study was approved by the Animal Welfare and Ethics Committee of Imo State University, Nigeria, with the reference number (IMO/DEC/MEDBCM/020). All conditions of animal use were adhered to as obtained from National Institute of Health (NIH), Guide for Care and Use of Laboratory Animals [40]. The limit doses test of up and down procedures according to OECD [41] and Dixon [42] were followed for acute oral toxicity studies (LD_{50}) and the toxic dose for the extract was found to be well above 1000 mg/kg. After acclimatization period, the rats were allocated to groups of twenty rats each. Alloxan 150 mg/ml and diabetes was induced by intraperitoneal administration of aqueous alloxan monohydrate (150 mg/kg b. w) to some of the groups. The rats with blood glucose level greater than 200 mg/dl, three days post-induction, were considered diabetic.

The treatments given to the animals are designated as follows

Normal control: No alloxan induced and no aqueous extracts of *C. papaya*

Diabetic control: Alloxan induced diabetic rats with no aqueous extract of *C. papaya* leaf

Group I: Alloxan induced diabetic rats given aqueous extract of *C. papaya* leaf (200 mg/kg).

Group II: Alloxan induced diabetic rats given aqueous extract of *C. papaya* leaf (50 mg/kg).

Reference: Alloxan induced diabetic rats treated with glibenclamide (group used as standard).

The treatment of the rats lasted for twenty-one days, and five rats from each group were sacrificed on day zero (day 0; three days post-induction), after seven days (day 7), after fourteen days (day 14), and after twenty-one days (day 21).

Blood sample collection

Five rats from each group were reweighed, anaesthetized with chloroform vapour and dissected during each set of rats` sacrifice. Blood was collected by cardiac puncture into clean tubes for biochemical assays.

Biochemical assay

Serum aspartate aminotransferase (AST), and alanine aminotransferase (ALT) [43], Alkaline phosphatase (ALP) [44], Creatinine and urea [45,46] were analysed. Levels of sodium, potassium, chloride, bicarbonate, direct and conjugated bilirubin; and blood glucose were estimated spectrophotometrically by using standard ready to use kits from Randox Laboratory Ltd.

Statistical analysis

Results were presented as means and standard deviations of five determinations. Fig. were used to present the results for better and clear understanding, while the test groups were compared to the diabetic control, normal control and reference group using Anova at $p<.0.05$.

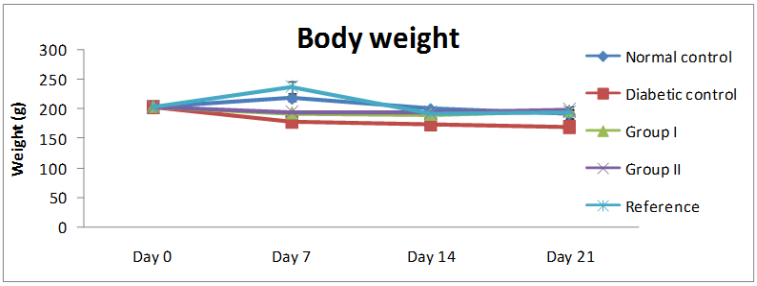

Fig. 1: Weight of rats for the number of days

RESULTS AND DISCUSSION

The use of plant to treat disease conditions is as old as mankind on this planet Earth [39, 47]. It has been noted that diabetes is a disease associated with weight reduction [48]. Such observation was clearly seen in diabetic control group as represented in fig. 1. Diabetic control group had progressive reduction in weight (fig. 1), while the weight change observed in groups I and II, compared favorably with those of normal control and reference (fig. 1). *C. papaya* leaf extracts used in this study may have possessed poor appetite and digestion stimulation properties which influence the efficiency in utilization of feed, resulting in poor growth. This speculation is not in line with the reports of Mahmood *et al.* [49], and Tanko *et al.* [50]. The weight reduction observed in diabetic condition has been linked to insufficient insulin, which prevents the body from getting glucose from the blood into the cells to use as energy. Hence, the body reverts to the burning of fats of the body and muscle for energy, which results in the overall weight loss of body weight.

Diabetes is known to bring about the structural and functional changes in intestinal glucose absorption [51]. Glucose increase is paramount in diabetic condition [52]. The glucose level of normal control group was the least and ranged from 71.54±2.57 mg/dl on first day (day 0) to 69.19±1.54 mg/dl; those of groups I (202.18±1.05–160.75±1.25 mg/dl) and II (207.17±0.89-172±1.23 mg/dl) progressively decreased after day 7, and were significant ($p<0.05$) when compared to diabetic control group, but compared favorably with those of the reference group (fig. 2). Glucose level of diabetic control group was progressively on the increase with days as against those of groups I and II; and reference (fig. 2). This result may project *C. papaya* leaves as possible anti-hyperglycemic agent. Hence, confirming previous reports on the plant and some species of the family [52]. Several studies have noted that the biological activities of *C. papaya* could be due to the presence of phytochemicals such as flavonoids, alkaloids, steroids and quinones found in the plant [53-54]. Interactions between the phytochemicals and phytonutrients earlier reported on *C. papaya* leaves by Agomuo *et al.* [55], could be behind the ability of the studied leaf extracts to bring about the reduction effect on glucose level of diabetic rats as observed in the present study. The hypoglycemic activity of *C. papaya* leaves in the present study is in line with previous report on the fruit and leaves by Aruoma *et al.* [56]; and that of Danese *et al.* [57] on fermented papaya preparation.

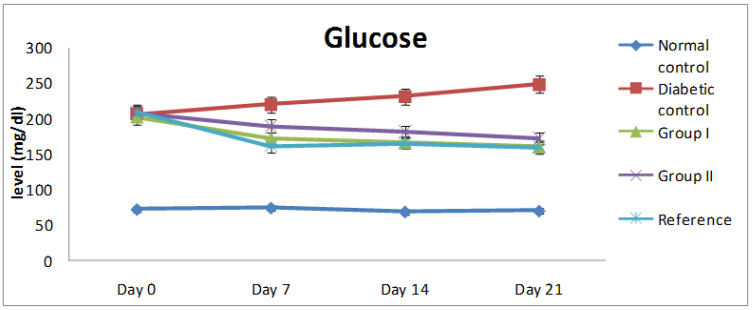

Fig. 2: Glucose levels in rats for the number of days

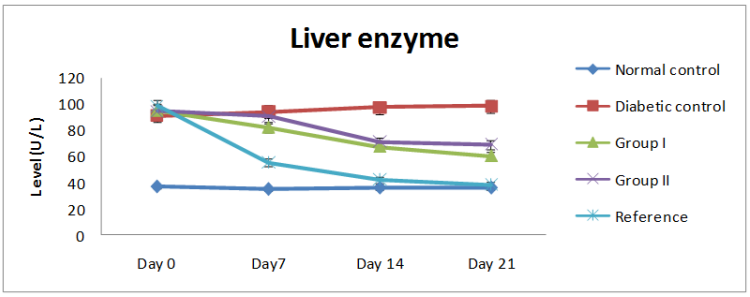

Fig. 3: AST levels in rats for the days

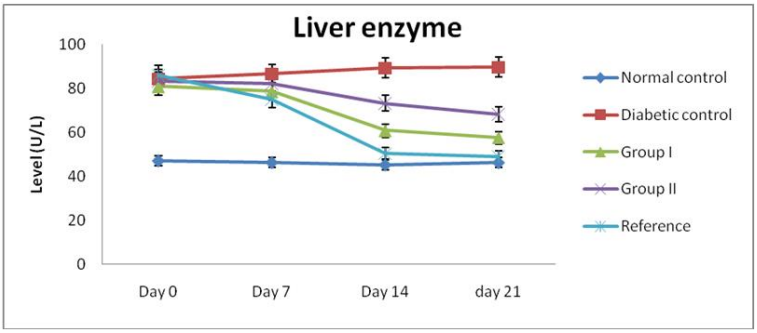

Fig. 4: ALT levels in rats for the days

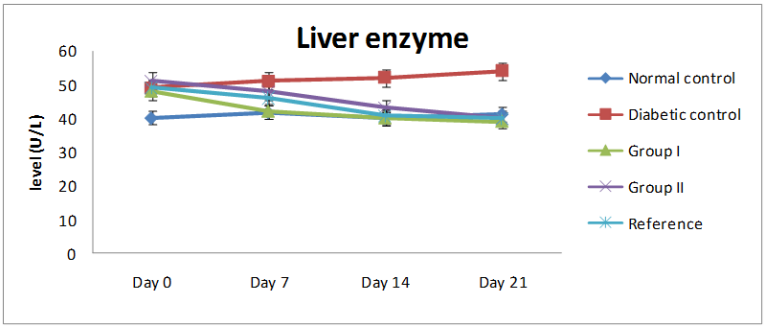

Fig. 5: ALP levels in rats for the days

Liver balances the glucose levels [58] and abnormal liver function may lead to type 2 diabetes [59]. AST and ALT liver enzymes are considered as markers of hepatocellular health [60-65]. The observed levels of AST, an ALT of groups I and II significantly ($p<0.05$) reduced against diabetic control group after day 7, but compares favorably with the reference group (fig. 3 and 4). The observation of the present study showed that impairment in the liver function caused by the induction of diabetes with alloxan, could be restored by administration of *C. papaya* leaf extracts. This could be attributed to the bioactive constituents present in the leaf extracts [52]. ALP levels in groups I and II also followed the same order as in AST and ALT levels in the present study (fig. 5).

According to Nwogo *et al.* [39], levels of renal markers such urea and creatinine are known to increase in diabetic condition. The progressive increase in urea (fig. 6) and creatinine (fig. 7) of diabetic control in the present study is in line with the observation of Nwaogo *et al.* [39]. A notable elevation in the level of urea during diabetes has been associated with increased catabolism of both the liver and plasma proteins that accompany glyconeogenesis [66]. *C. papaya* leaf extracts used in this study exhibited potent anti–diabetic activity with the significant reduction of urea and creatinine levels of groups I and II in this study against diabetic control group but compares favourably with the reference group (fig. 6 and 7 respectively).

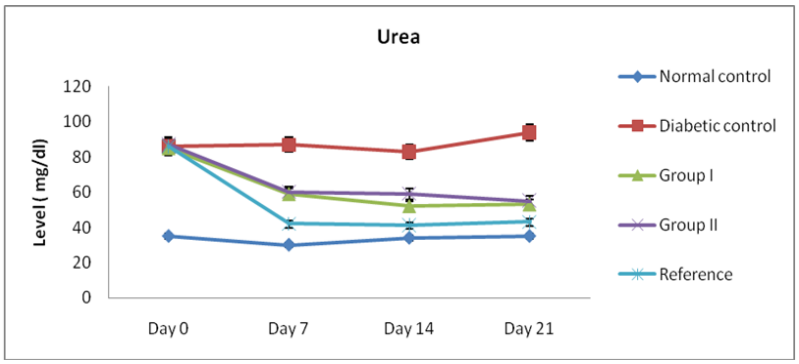

Fig. 6: Urea levels in rats for the days

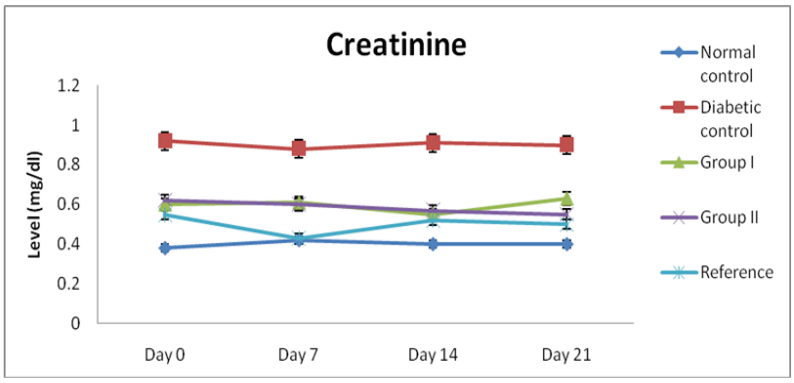

Fig. 7: Creatinine levels in rats for the days

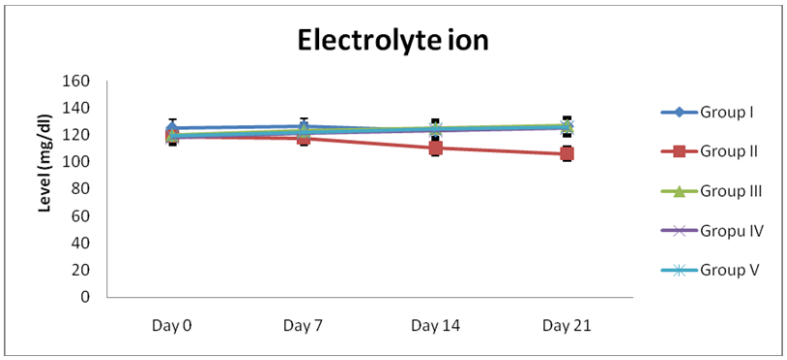

Fig. 8: Na^+levels in rats for the days

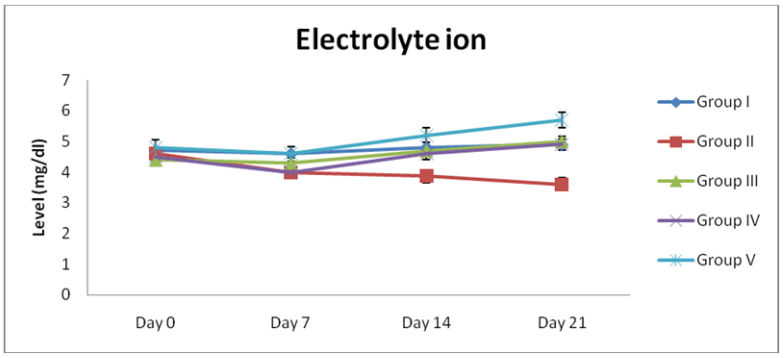

Fig. 9: K+levels in rats for the days

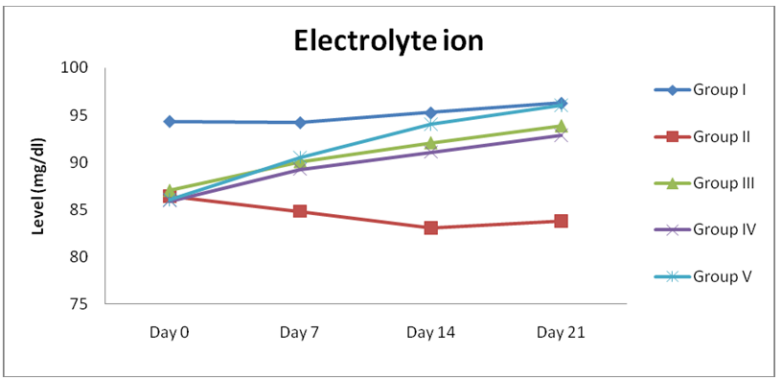

Fig. 10: Cl-levels I rats for the days

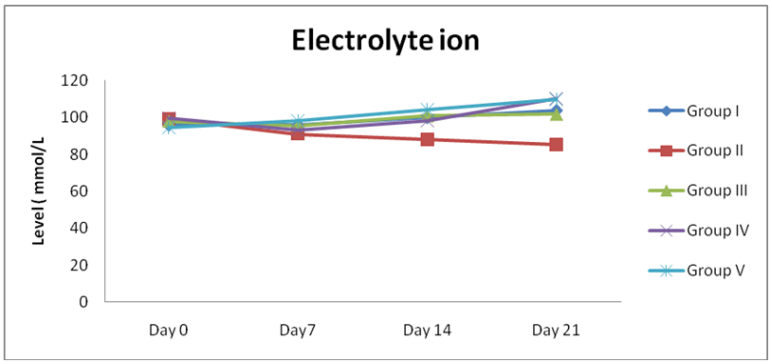

Fig. 11: HCO_3^-levels I rats for the days

The kidney regulates fluid and ion balance of the plasma by excretion of urine [67-69]. Though the reabsorption processes depend on mineralocorticoid aldosterone, which is monitored by the kidney; and ion pumps in the body [70]. There was a significant retention of Na^+, K^+, $Cl^-$and HCO_3^-electrolytes after day 7, in diabetic control group against those of groups I and II, normal control and reference group. Excretion of electrolyte ions in groups I and II favorably compared to those of normal control and reference group in this study (fig. 8-11). The observed retention of the electrolyte ions in diabetic control rats when compared to those of test groups I and II, normal control and reference is in line with the observations of Khalid *et al.* [71] and Al-Jameil [72] on hyponatraemia; partially in line with Wang *et al.* [73], who reported that only 0.6% of diabetes had hypokalemia and 1.2% of diabetes subjects had hyperkalemia; and agree with the observation of Al-Jameil [72] and Bayejid *et al.* [71] on chloride levels in diabetic condition. However, the observed electrolytes in groups I and II, compared favourably with those of normal control and reference group as presented in fig. 8-11 respectively.

CONCLUSION

Conclusively, the administration of aqueous extract of *C. papaya* leaf on alloxan-induced diabetic albino rats significantly reversed the damage associated with alloxan-induced diabetes revealing its hypoglycemic, liver and renal function integrity. The presence phytochemicals and phytonutrients found in *C. papaya* could explain the observed pharmacological property of the studied extract. This study has revealed the biochemical effects of aqueous extract of *C. papaya* leaf on alloxan-induced diabetic albino rats.

CONFLICT OF INTERESTS

The authors declare no conflict of interests over this article. The arrangement of the authors was properly agreed by the all the authors involved.

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