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Changes in histological and liver function assessment of unripe pulp of *Carica papaya* using diabetic albino rat model

Ahamefula Sunday Ezekwe[1], Ify Elekwe[2], Kelechi Uzoma Osuocha[2*]

[1]*Department of Medical Biochemistry, College of Medicine, Imo State University Owerri, Nigeria*
[2]*Department of Biochemistry, Faculty of Biological and Physical Sciences, Abia State University, Uturu, Nigeria*

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Abstract

The present study evaluated the effect of unripe pulp of *Carica papaya* on alloxan induced diabetic albino rats. Animals weighing (160-200g) were divided into 3 (three) groups of 10 animals each; Group 1(normal control), Group 2(diabetic control) and Group 3(test control). Animals in groups 1 and 2 received normal rat feeds while animals in group 3 were fed with unripe pulp of *Carica papaya* for a period of 28 days. Body weight and glucose levels were measured on days 0, 7, 14, 21, and day 28. Animals were sacrificed on day 28. Histological and Liver enzyme assessment were carried out using standard analytical procedures. Improvement in histopathological features was noticed also intake of unripe pulp of *Carica papaya* reduced significantly serum liver enzymes of the test groups when compared to control ($P< 0.05$). However, unripe pulp of *Carica papaya* offers promising antidiabetic, hypolipidemic , organ and cell protective and regenerative effects which may be attributed to some potent bioactive constituents of the pulp.

* **Corresponding Author:** Kelechi Uzoma Osuocha ✉ osuochak@gmail.com

Introduction

From time immemorial, man has depended on plants either directly or indirectly for existence and the resource will continue to serve as primary providers of human needs. In order to have a healthy population that can promote development, the relationship between food, nutrition and health must be reinforced (Nidhi *et al.*,2013). Gardner *et al*, (2007) reported that there is a worldwide belief that herbal remedies are safer and less damaging to human than synthetic drugs. Oduola *et al.*,(2007) reported that from ancient times, the healing power of herbs has been recognized and botanic medicine has been one of the oldest practiced professions by mankind.

The pathogenesis of diabetes mellitus and the possibility of its management by the oral administration of hypoglycemic agents have stimulated a greater interest in recent years. In spite of the presence of known anti diabetics in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease (Bhattaram *et al,* 2002). Treatment imposes economic burden and documented incidence is quite ambiguous(Okewumi, 2012). Herbal remedies from medicinal plants have been used traditionally in many parts of the world where access to formal healthcare is limited, especially in tropical Africa as several bioactive constituents of plants have been reported (Okeniyi *et al,* 2007).Herbal remedies may have recognizable therapeutic and less toxic side effects (Thakkar and Petal, 2010). Scientific evidences have shown that *Carica. papaya* has the following activities: anti-diabetes (Gbolade, 2009; Robert *et al.*, 2008), diuretic (Spripanidkulchai, 2001), antihyperlipidemic (Banerjee *et al.*, 2006), antihelminthic, anti-amoebic (Okeniyi *et al.*, 2007), contraceptive in mice rats (Verma *et al.*, 2006; Lohiya *et al.,* 2006; Chinoy *et al.*, 1985), hypoglycemic (Adeneye and Olagunju, 2009; Ezekwe *et al.*,2014), nephroprotective (Olagunju *et al.*, 2009), bactericidal (Emeruwa, 1982) wound and burn healing (Nayak *et al.*, 2007; Hewitt *et al.*, 2000), anti-oxidant (Majdi and Luciana, 2010), anti-nociceptive, anti-inflammatory (Anaga and Onehi, 2010) and anti-ulcer (Ezike *et al.*, 2009; Indran *et al.*, 2008).Management of diabetes is mostly directed at glycemic control with little attention to lifestyle modification and screening for other risk factors. This has contributed to the increased morbidity and mortality associated with diabetes. A more holistic approach to diabetes management is therefore required with emphasis on both pharmacological therapy, lifestyle modification and the introduction of effective alternative therapies using medicinal plants like *Carica papaya*. The study is aimed at evaluating the activities of liver enzymes , histological analysis of the pancreas and liver on alloxan induced diabetic rats.

Materials and methods

Laboratory Animals and Experimental Design

Thirty (30) weaned male albino rats (about 8-9 weeks) were obtained from the animal farm of the university of Nigeria ,Nsukka. The animals were taken to the laboratory where they were housed in a plastic cage and placed on commercial feeds[growers marsh/rat chow] bought from the local market as produced by , Vital feed Nigeria Ltd and allowed to drink water freely [*ad libitum*] till the end of the acclimatization for two weeks, the animals weighed between 160 - 200g.

Grouping of Animals

The animals were divided into three (3) groups of ten(10) rats each.

Group I and Group II Were fed with normal rat chow and allowed free access to water *ad libitum* while Group III were fed with grated unripe pulp of *carica papaya* and allowed free access to water *ad libitum*. Diabetes was induced into rats in Groups II and III.

Plant Materials

Unripe pulp of *Carica papaya* was obtained from a local farm in Owerri North Local Government Area of Imo State and was authenticated by a botanist before use.

Induction of Diabetes

Diabetes mellitus was induced by a single intraperitoneal injection of 120mg/kg body weight of alloxan monohydrate Alloxan was dissolved in 0.9% normal saline as vehicle .

Estimation of alkaline phosphatase(ALP) activity

Exactly 0.5 ml of working buffered substrate was added in clean tubes. 1.5 ml of purified water was added in all the tubes. They were mixed well and incubated at 37 °C for 3 min. 0.05 ml of serum was added in test (T), 0.05 ml of Phenol standard, 10 mg% was added in standard (S) and 0.05 ml of purified water was added in blank (B) tubes. All the tubes were mixed well and incubated at 37 °C for 15 min. 1 ml of Chromogen reagent was added in all the tubes. 0.05 ml of serum was added in control (C) and O.D. was measured at 510 nm.

Estimation of glutamate oxaloacetate transaminase(AST) activity

Serum glutamate oxaloacetate transaminase(AST) was estimated by the method of using GOT test kit. Exactly 0.25 ml of Buffered Aspartate α-KG substrate, pH 7.4 was added in clean test tubes and incubated at 37°C for 5 minutes. 0.05 ml of serum was added in the test, 0.05 ml Working Pyruvate Standard, 2 mM was added in standard and 0.05 ml distilled water was added in the blank. They were mixed well and incubated at 37 °C for 60 minutes. Thereafter, 0.25 ml of DNPH colour reagent was added to all the tubes, mixed well and allowed to stand at room temperature (15-30 °C) for 20 minutes. Then 2.5 ml of Solution I was added to all the tubes, mixed well and allowed to stand at room temperature (15-30 °C) for 10 min. The absorbance of blank, standard and test were read at 505 nm.

Estimation of Glutamate Pyruvate Transaminase(ALT) Activity

Serum glutamate pyruvate transaminase(ALT) was estimated using GPT test kit. Exactly 0.25 ml of Buffered Alanine α-KG substrate, pH 7.4 was added in clean test tubes and incubated at 37 °C for 5 minutes. 0.05 ml of serum was added in the test, 0.05ml of Working Pyruvate Standard, 2 mM was added in the standard and 0.05 ml distilled water was added in the blank. They were mixed well and incubated at 37 °C for 30 minutes. Thereafter, 0.25 ml of DNPH colour reagent was added to all the tubes, mixed well and allowed to stand at room temperature (15-30 °C) for 20 minutes. Then 2.5 ml of Dilute 1 ml of Sodium Hydroxide, 4 N I up to 10 ml of D/W was added to all the tubes, mixed well and allowed it to stand at room temperature (15-30 °C) for 10 min. The absorbance of blank, standard and test were read at 505 nm.

Methods for Histopathological Studies

The organs (Liver and pancreas) were transferred to 4% formaline solution for fixation and later on processed for histopathological studies following the standard procedure . The microtome sections were cut processed and stained with haematoxylin and eosin. The section thus obtained was scanned in Trinocular Carl-Zeiss microscope under different magnifications.

Statistical Analysis

Results were expressed as mean ± SEM [standard error of mean]. Statistical analysis were performed using One Way Analysis of Variance [ANOVA]. And values of $P< 0.05$ at 95% level of significance was used to assess significant difference between control and treated groups.

Results and discussion

PLATES/HISTOLOGIC MICROGRAPHS OF THE PANCREASE AND THE LIVER (DAY 28) Magnification x400

PHOTOMICROGRAPH/HISTOLOGIC MICROGRAPH OF THE PANCREASE

Discussion

Effects of unripe pulp of *Carica papaya* on the weight of pancrease and liver and the relative organ weight of these organs were evaluated. It shows that the organs; liver and pancreas had weight loss in the test induced group(Group 3) when compared with diabetic control group 2 at $P<0.05$, but no significant

difference between Groups 2(diabetic control) and Groups 1(normal control). For the relative body weights; there was significant difference at P<0.05 of the pancreas between Groups 3(test control) and Groups 1(normal control), but no significant difference between Groups 2(diabetic control) and Groups 1(normal control).For the relative weight of the liver , there is significant difference at P<0.05 between Groups 3(test group) and Groups 1(normal control), but no significant difference between Groups 2(diabetic control) and Groups 1(normal control) and Groups 2(diabetic control) and Groups 3(test control). This followed the weight loss of the animals feed on papaya (Group 3) which could exemplify the weight reducing effect of papaya. Effect of unripe pulp showed significant decrease of elevated liver enzymes of the test induced group (Group 3) when compared to the induced diabetic control group (Group 2) and normal control group (Group 1) at a significant level of P<0.05. These enzymes AST, ALT and ALP (liver enzymes) are markers of cellular damage following the induction of diabetes and complications associated with the disease state. This study presents a significant decrease in the liver enzymes viz; ALT, AST and ALP of the treated group when compared with the induced control(diabetic group) and the normal control groups at (P<0.05). During diabetes, an increase in serum alkaline phosphatase (ALP) has been observed and this has been reported to be the soluble form of intestinal ALP leached from intestine and translocated to the circulation (Unakami ,1990) . The effect of *Carica papaya* unripe pulp on AST, ALT and ALP observed in the present study, is in accordance to a study on the effect of S-allyl cysteine sulphoxide isolated from garlic on alloxan diabetic rats (Sheela and Agusti, 1992). Accelerated gluconeogenesis, negative nitrogen balance and muscle wasting are among the hallmarks of uncontrolled diabetes ,resulting in increased ALT and AST. There is a catabolism of branched amino acids and alanine release by skeletal muscle . Glutamate is an obligate precursor of alanine and glutamine production by muscles. The later two amino acids comprise more than 50% of all the amino acids released by the muscle, alanine being the preferred amino acid precursor of gluconeogenesis in the liver and glutamine in the kidney (Odessey *et al* ,1997). The improved histological nature of the organs studied also collaborates the effectiveness of *C. papaya* in preventing organ damage secondary to severe complication of uncontrolled diabetes.

Table 1. Effects of unripe pulp of *Carica papaya* on organ weights (g) and relative organ weight on normal and alloxan induced diabetes in male albino rats(DAY 28).

	PANCREAS	LIVER	RELATIVE WEIGHTOF PANCREAS	RELATIVE WEIGHT OF LIVER
GROUP 1	7.01±0.47^{a}	56.91±1.03^{a}	3.19±0.18^{a}	25.98±0.58^{b}
GROUP 2	6.45±0.10^{a}	52.85±0.42^{a}	3.42±0.04^{a}	28.08±0.37^{b,a}
GROUP 3	2.61±0.26^{b}	40.90±3.24^{b}	1.94±0.18^{b}	30.43±1.95^{a}

Data are expressed as mean±standard error of mean(SEM).n=10 and statistically significant by analysis of varience (ANOVA) at p<0.05. Means with the same letter in the same column are not significantly different.

Table 2. Effects of unripe pulp of *Carica papaya* on liver enzymes (IU/L) of normal and alloxan induced diabetes in male albino rats (DAY 28).

	ALT	AST	ALP
GROUP 1	3.99±1.24^{c}	14.72±1.96^{c}	204.17±0.37^{b}
GROUP 2	14.61±0.21^{a}	28.40±0.21^{a}	225.37±9.25^{a}
GROUP 3	8.72±0.65^{b}	22.24±0.16^{b}	159.76±2.71^{c}

Data are expressed as mean±standard error of mean(SEM).n=10 and statistically significant by analysis of varience(ANOVA) at p<0.05. Means with the same letter in the same column are not significantly different.

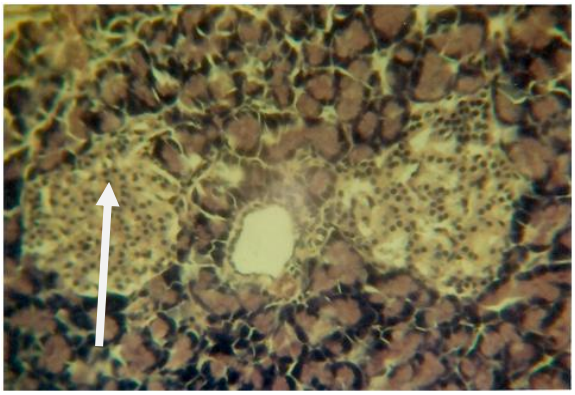

Fig. 1. The pancreatic tissues of control shows normal pancreatic acini and islets of Langerhans cells with absence of damage to islet cell and absence of hyperplasia.

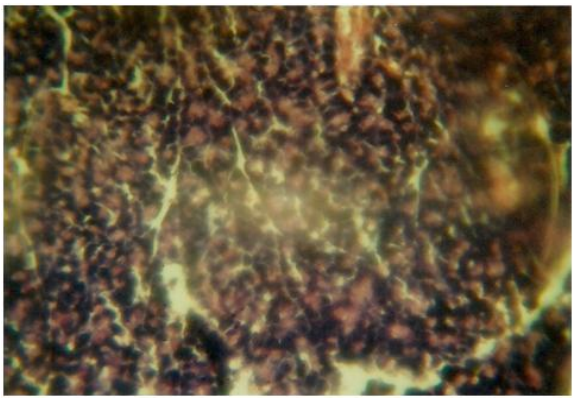

Fig. 2. The pancreatic tissues of diabetic rats shows severe congestion, decreased cell number with extensive damage and cell death of pancreatic acini and islets of Langerhans cells and presence of hyperplasia.

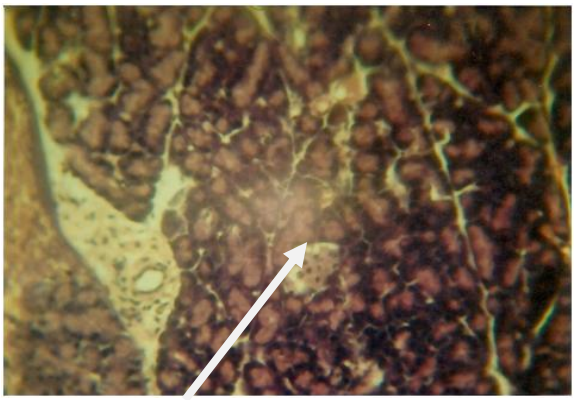

Fig. 3. The pancreatic tissues of diabetic rats treated with unripe pulp of *Carica papaya* shows reduced injuries with moderate congestion and decreased cell numbers of pancreatic acini and islets of Langerhans cells and moderate hyperplasia when compared with diabetic control group.

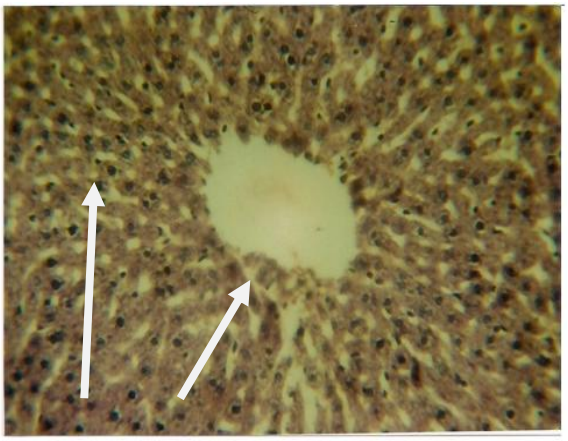

Fig. 4. Showing normal cords of the hepatocytes(normal hepatic architecture).

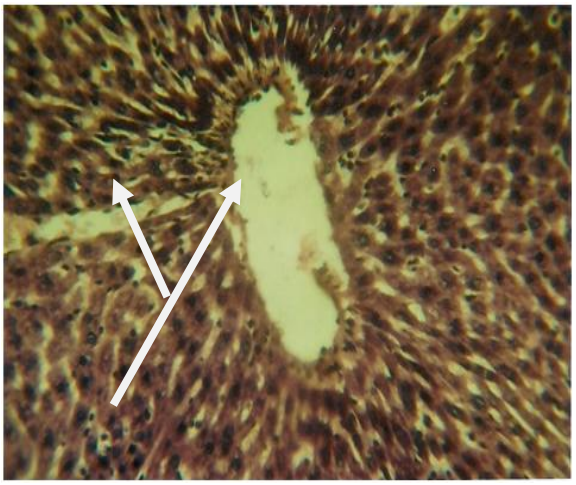

Fig. 5. Showing (arrows) severe fatty changes and congestion with sinusoidal dilatation.

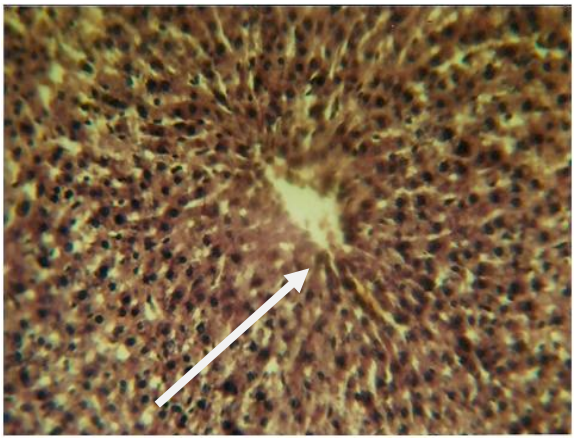

Fig. 6. Showing mild fatty changes and mild sinusoidal dilatation and congestion. It there fore shows that the animals fed with unripe pulp of *Carica papaya* demonstrated some regenerative and healing effects on the organs of the pancrease and liver respectively.

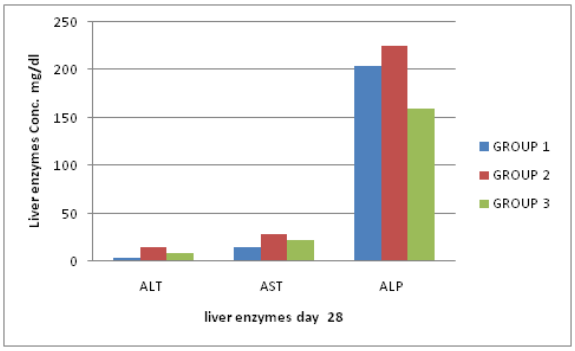

Fig. 7. shows the effect of unripe pulp of *Carica papaya* on liver enzymes.

Conclusion

This study demonstrated regenerative and enhanced improvement in the pathological states of the liver and pancreas of male albino rats caused by alloxan induced diabetes mellitus and reduction in serum liver enzymes by feeding them with unripe pulp of *Carica papaya*. Therefore, unripe pulp of *Carica papaya* may contain active substances that ameliorates the toxic nature of the drug and the untowards complications in organ damage caused by diabetic states.

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