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RESEARCH PAPER

THE INCIDENCE OF HEPATIC INFARCTION IN RATS FED WITH GRADED DOSES OF CARICA PAPAYA SEEDS

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ABSTRACT

This study investigates the sub-acute and chronic effects of graded doses of *carica papaya* seed-treatment on the liver of rats. The study involved 15 Sprague Dawley rats (95.0 ± 10.0 grams) within the ages of 7 ± 1weeks. They were divided into three groups: A (control; n = 5), B= (Test 1; n = 5) and C= (Test 2; n = 5). Test Groups B and C were fed with same doses of powdered *Carica papaya* seed (6 grams) but for 3 weeks (group B; sub-acute test duration) and 6 weeks (group C; chronic test duration) respectively. The histological observations revealed that there were dosage-duration-dependent distortions in the parenchyma of the liver, especially the histological signs of hepatic infarction/hemorrhage, exudations, pyknosis and parenchymal erosions. These findings suggest therefore, that *Carica papaya* seeds have the capacity to induce liver damage and considering the increasing usage of herbal medications amongst the population, its clinical consequences can only be better imagined.

Keywords: Carica papaya, Hepatic infarction, Herbs, Nigeria.

INTRODUCTION

It has been noted that some substances ingested as drugs may subject the liver to a variety of disorders (Fernandez-Checa and Kaplowitz, 2005). In fact, drug induced hepatotoxicity and acute liver disease is the most common reason for withdrawal of an approved drug from the market (Friedman, 2003). Judging by the assertion that this era is characterised by a rise in the use of herbal products (Maclannan *et al*., 2006; Ness *et al*., 2005; Cheung *et al*., 2007), it becomes realistic to hypothesize that there might be associated cases of organ damage amongst consumers of certain herbal products.

Of interest in this study are the scientific claims that *carica papaya* seeds have medicinal properties (Adeneyea and Olagunju, 2009; Gill, 1992; Okeniyi *et al*., 2007). Phytochemically, *carica papaya* is known to be rich in carpine, chymopapain and papain, a bactericidal aglycone of glucotropaeolin, benzyl isothiocyanate, nicotine a glycoside sinigrin, the enzyme myrosin (Akah et al., 1997; Jackwheeler 2003; Eno et al., 2000, Wilson et al., 2002; Seigler et al., 2002). Udoh and Udoh, (2005) in their analysis of the phytochemical constituents of *Carica papaya* seeds reported that glycosides and polyphenols were present in excess, among other compounds such as alkaloids, saponin, flavanoids and quinnones. Additionally, they contain active ingredients such as caricacin, an enzyme carpasemine and oleanolic glycoside (Emeruwa, 1982).

Unfortunately some of the phytocomponents of *Carica papaya* like saponins and Benzyl isothiocyanate have been reported to be poisonous particularly in high doses (Foerster, 2006, Wilson *et al*., 2002). Similarly, Flavonoids has been reported to induce mutation, morphological transformation, chromosomal aberration and sister chromatid

exchanges (Maruta *et al*., 1979; Umezawa *et al*., 1977; Yoshida *et al*., 1990; Elliott *et al*., 2000). Additionally, Papain an active ingredient of *carica papaya* has been shown to significantly decrease the absolute and relative weights of the liver in rabbits, (Bitto and Gemade, 2001).

Furthermore, Udoh and Udoh, (2005) has shown that *Carica papaya* seeds induced mild to severe metaplasia of hepatocytes in a dose dependent manner; as well as proliferation of kupffer cells and hepatic cell cirrhosis, elevation of serum levels of acid phosphatase, alkaline phosphatase and aspartate amino transferase. There is evidence also that *carica papaya* induces cellular disarrangement in the liver as well as hepatic necrosis in fish (Ezekiel and Benedict, 2008).

Considering the rising use of herbal products containing *Carica papaya* for therapeutic purposes and the functional significance of the liver as regards ingested substance-metabolism, this study therefore, investigates the acute and chronic effect of graded doses of *carica papaya* treatment on the liver of rats.

MATERIALS AND METHODS

Experimental Animals: 15 Sprague Dawley rats of 7 ± 1 week old and weights ranging from 95.0 g to 105.0g and comparable sizes were procured from the animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Nigeria. They were moved to the site of the experiment at No. 23 St. Mary Street, Ekpoma, where they were allowed 2 weeks of acclimatization. The animals were weighed on the first day of the acclimatization period and fed 50 grams of feed with water giving *ad libitum*. They were housed in well ventilated labelled wooden cages at the site of the experiment. The cages were designed to secure the animals properly especially from wild animals/insects and cleaned daily.

Substance of Study: Unripe *Carica papaya* was collected from the premises of the animal house, College of Medicine, Ambrose Alli University, Ekpoma, and authenticated by a botanist in the Department of Botany, Faculty of Natural Science, Ambrose Alli University, Ekpoma.

Substance Preparation: The outer peel of unripe *Carica papaya* was removed and the seeds obtained and sun dried. The dried seeds were then crushed into fine powder using electric blender. The fine powder was measured using Electric Balance (Denver Company, USA, 200398. IREV.CXP-3000) and packaged in small plastic envelopes for storage pending usage.

The feeds (grower mesh) produced by Grand Cereals Ltd, a subsidiary of UAC of Nigeria Plc, Jos, Plateau State, were weighed using a goat scale weighing balance (China).

For the purpose of this study, pellets were prepared by adding measured quantities of *Carica papaya* to the feed as described by Nwaopara *et al*. (2011).

Study Duration: The preliminary studies, animal acclimatization, ingredients procurement (Carica papaya preparation and production), actual animal experiment, histological processing and microscopy and evaluation of results, lasted from October, 2011 to February, 2012. However, the actual administration of *Carica Papaya* to the test animals lasted for 6 weeks (subacute: 3 weeks; chronic: 6 weeks).

Phase 1 administration (3 weeks): Group A (control group) received 50.0g of feed and distilled water alone. Test Group B received 44.0g feed, distilled water plus 6g of *Carica papaya*.

Phase 2 administrations (6 weeks): The study animals involved in this phase of the study (C) received similar ratios per day except that the duration was six weeks unlike the 3 weeks for phase 1.

Sample Collection and Analysis: Weight was measured before and after acclimatization, similar weighted measurements were done at the end of the subacute and chronic treatment periods and the average recorded accordingly.

The growth performance and feed utilization of the rats were determined at the end of the experiment as described by Dada and Ikuerowo (2009).

The liver of each rats were obtained at the end of each stage under chloroform anaesthesia and fixed in 10% formalin for histological processing. The obtained data were then subjected to statistical analysis using SPSS (version 17). The test groups' values were compared with the values of the control group using ANOVA (LSD) at 95% level of confidence.

Histological Analysis: The tissues were processed using automatic tissue processor according to the processing schedule used in Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-ife, Osun State, Nigeria. The fixed plastic tissue cassettes in 10% formalin were automatically processed by passing them through different grades of alcohol. After the last grade, the tissues were removed from their plastic cassettes and placed at the centre of the metallic tissue mould and then filled with molten paraffin wax. They were also left to solidify after which they were placed in the refrigerator at 5^{o}C for 15 minutes. The blocks were then removed from the metallic case using a knife and trimmed.

The blocks were then sectioned using 3nm on a rotary microtome. The sections were floated in water bath at 55^{o}C and picked up by the use of a clean frosted end slides. The frosted end slides were now placed on the hot plate for 40 minutes for adequate attachment of the sections on the slides after which the sections were de-waxed, hydrated, air dried and stored in a slide box ready for staining with Haematoxylin and Eosin.

RESULTS

Liver micrographs from group A (control) presented normal hepatic features (figure 1). However, those from group B (treated with 6g of *carica papaya* seeds for three weeks) showed distortions in cellular architecture with histological signs of infarction and hemorrhage (figure 2); severe infarction with parenchymal erosion (figure 3 and 4). Micrographs from group C (treated with 6g of *carica papaya* seeds for six weeks) also presented severe infarction, (figure 5), and parenchyma erosion, (figure 6 and 7).

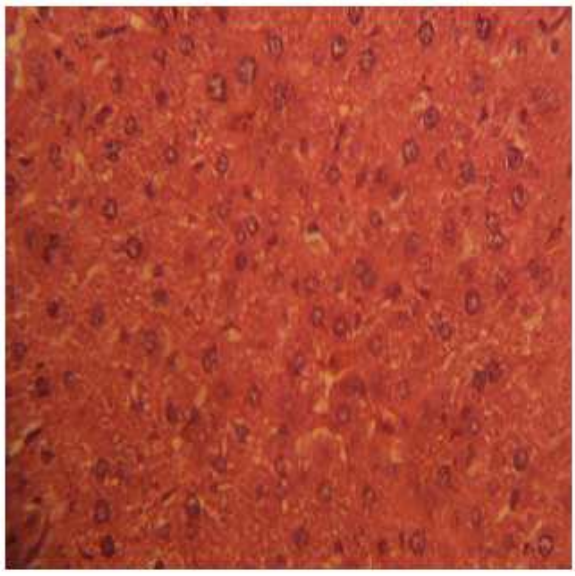

Figure 1: Liver Section A–Control (H&E; X400) showing normal hepatic architecture

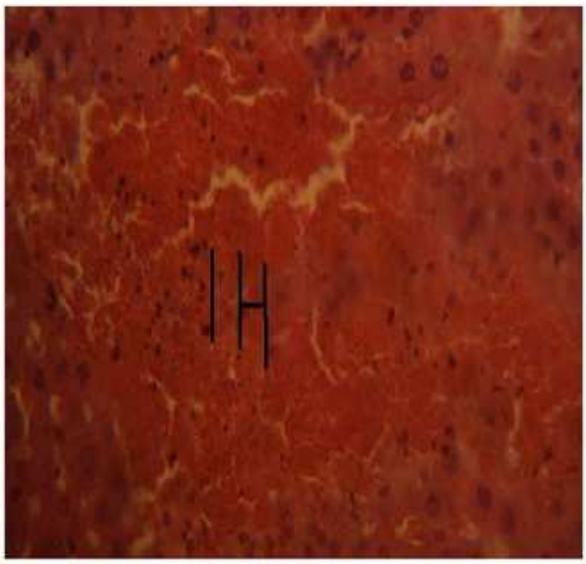

Figure 2: Liver Section B (H&E; X400) showing distortion in cellular architecture with histological signs of infarction with hemorrhage (IH)

DISCUSSION

The result of this study suggests that *carica papaya* seeds have the capacity to induce hepatic infarction in rats. Earlier studies on the liver by Pass (1935), Losner *et al*., (1950), and Parker (1955), indicated that infarction of the liver can be caused by thrombosis or ligation of the main hepatic artery between the origin of the right gastric artery and the hilum of the liver, but the more common causes are infected emboli or polyarteritis nodosa.

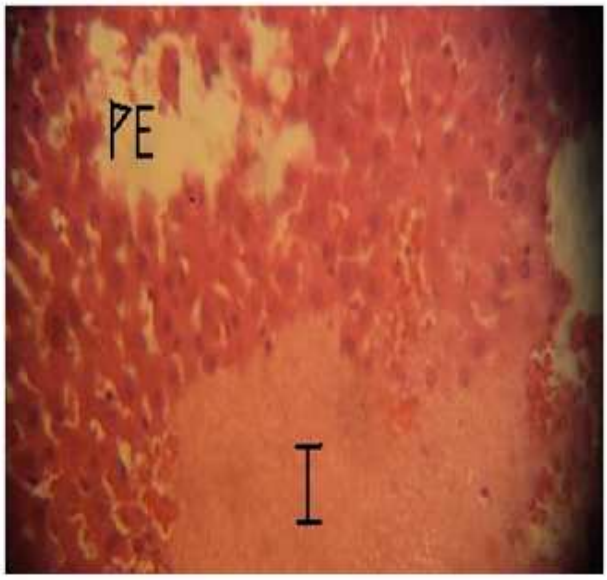

Figure 3: Liver Section B (H&E; X400) showing severe infarction (I) with parenchyma erosion (PE)

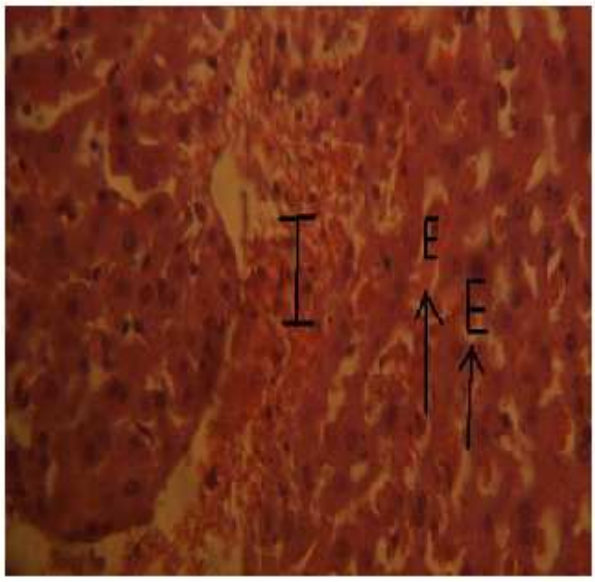

Figure 4: Liver Section B (H&E; X400) showing severe infarction (I) with eosinophilic cells (E)

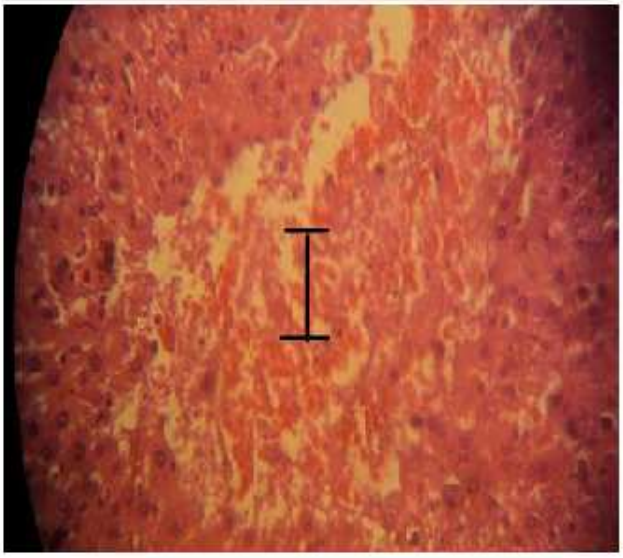

Figure 5: Liver Section C (H&E; X400) showing severe infarction

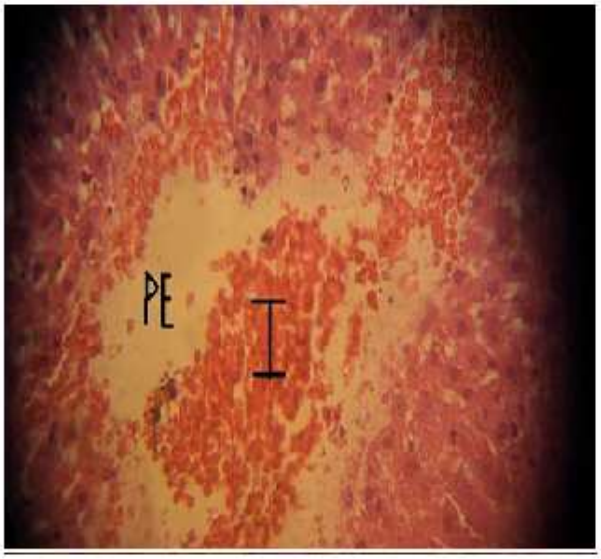

Figure 6: Liver Section C (H&E; X400) showing severe infarction with parenchyma erosion

In fact, several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. Many chemicals damage mitochondria, an intracellular organelle that produces energy. Its dysfunction releases excessive amount of oxidants which, in turn, injure hepatic cells. Activation of some enzymes in the cytochrome P-450 system such as CYP2E1 also leads to oxidative stress (Jaeschke *et al*., 2002). Injury to hepatocyte and bile duct cells lead to accumulation of bile acid inside the liver. This promotes further liver damage (Patel *et al*., 1998) Non-parenchymal cells such as Kupffer cells, fat storing stellate cells, and leukocytes (i.e. neutrophil and monocyte) also have a role in the mechanism.

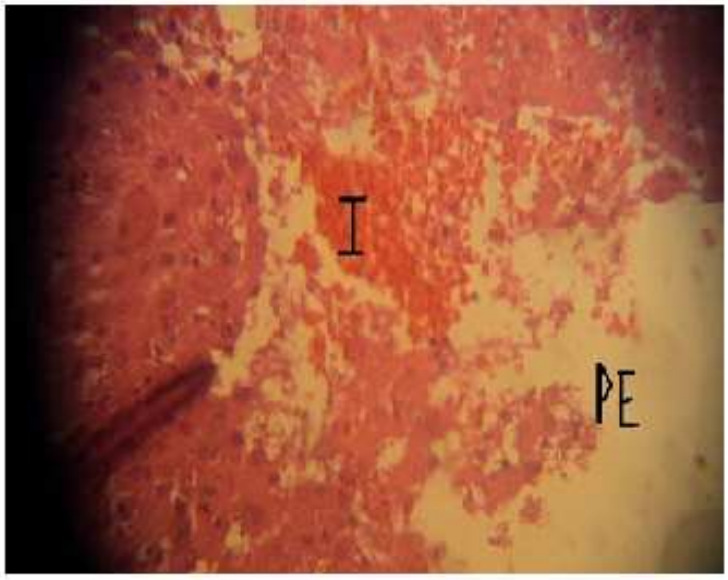

Figure 7: Liver Section C (H&E; X400) showing severe infarction and parenchyma erosion

Of interest are the reports from previous studies that some of the phytocomponents in *Carica papaya* can elicit a wide range of biological activities. Specifically, pyrrolizidine alkaloids are highly toxic (Roeder, 1995**;** Stegelmeier *et al*., 1999**;** Roeder, 2000; Smith and Culvenor, 1981**)** and it is now well recognized that a large variety of animal species are susceptible to pyrrolizidine alkaloid toxicity (IARC, 1976; Mattocks, 1986).

Moreover, chemicals produce a wide variety of clinical and pathological hepatic injury. Biochemical markers (e.g. alanine transferase, alkaline phosphatase and bilirubin) are often used to indicate liver damage. Liver injury is defined as a rise in either (a) ALT level more than three times of upper limit of normal (ULN), (b) ALP level more than twice ULN, or (c) total bilirubin level more than twice ULN when associated with increased ALT or ALP (Mumoli *et al*., 2006; Bénichou, 1990). Liver damage is further characterized into hepatocellular (predominantly initial Alanine transferase elevation) and cholestatic (initial alkaline phosphatase rise) types.

These facts implicate the phytocomponents of carica papaya as indicated by its toxicity potentials (Roeder, 1995; Foerster, 2006; Wilson *et al*., 2002). It is our candid opinion therefore, that there is an urgent need to regulate the inclusions of certain herbal products like carica papaya seeds in herbal preparations in order to check the rising cases of organ failures hitherto considered as idiopathic. Most importantly, the involvement of the liver is particularly challenging considering the peculiar problems associated with organ donation and compatibility issues, as well as the huge cost of transplant surgery.

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AUTHORS' CONTRIBUTIONS

Bankole J.K. supervised this study. Dikibo E. and Okpidu EE. actively took charge of the daily experimental animal care, substance administration (to test animals) and data collection; while Idenhen C, was involved in necessary histological assistance. All the authors contributed towards the successful presentation of this manuscript.