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Hydro-Methanol Extract of Ripe Carica Papaya Seed is Not Friendly with Histology of Albino Wistar Rats' Liver

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Abstract: Carica papaya (Pawpaw) is a large herb that grows up to 6-9m in height, the seeds are small, black, ovoid and corrugated. Papain and chymopapain are among its biologically active compounds. The aim of the study was to determine the histologic effects of hydromethanol seed extracts of ripe carica papaya (HSEC) on wistar rats' liver. The objectives were to determine: (a) the LD50 of HSEC. (b) The histologic effect(s) of HSEC. (c) If the effect is dose- dependent and/or time- dependent. (d) If the effect is reversible. HSEC was prepared from blended ripe pawpaw seeds using Hydromethanol in a Rotary evaporator. The LD50 was determined using thirteen albino wistar rats. Thirty-five (35) albino wistar rats were acclimatized divided into three (3) groups (A, B and C) of ten rats and a control group of five. Groups A-C ingested 6, 30 and 60 mg/kg of extract, respectively daily for three weeks. A wash-out period was allowed. Two rats were harvested from; (a) each experimental group on 8th,15th and 22nd days.(b) control group on 22nd (c) each experimental and control group on 50th day. The liver harvested were processed histological and viewed under the light microscope. Photomicrographs were produced for permanent comparative studies. The LD50 'ip' of HSEC was found to be 299 mg/kg. Ballooning necrosis of hepatocytes was seen at higher doses in the second and third weeks. This significant hepatotoxicity increased with increased time and dosage may be attributed to presence of alkaloids. The wash-out study revealed hepatic histology similar to the control means that the withdrawal overcame the hepatotoxicity. The LD50 of HSEC is 299mg/kg. HSEC is hepatotoxic in a time and dose-dependent manner. The hepatotoxicity of HSEC is reversible.

Keywords: Ballooning, hepatocyte, necrosis, photomicrograph

INTRODUCTION

Carica papaya is the sole species in the genus carica of the plant family caricaceae. Originally from southern Mexico is cultivated in most countries with tropical climate like Nigeria (Akinloye and Morayo, 2010). It is a large herb that grows up to 6-9m in height, with a hollow green or deep-purple stem becoming 30-40 cm or more thick at the base and roughened by leaf scars. The fruit is melon-like, oval to nearly round, somewhat elongated club-shaped 15-50 cm long and 10-20 cm thick, weighting up to 20 lb. When the fruit is green and hard, it is rich in white latex. As it ripens, it becomes light or deep yellow externally and the thick wall of succulent flesh becomes aromatic yellow, orange or various shades of Salmon or red. Attached lightly to the wall by soft white, fibrous tissue are usually numerous small, black, ovoid, corrugated, peppery seeds about 5cm long each coated with a transparent gelatinous aril (Maton *et al*., 1993).

Carica papaya contains many biologically active compounds. The important compounds are papain and chymopopain, the proteolytic enzyms (Brocklehurt and Salih, 1985). The latter is most abundant but papain is twice as potent. These compounds aids in digestion. The level of the compounds varies in the fruit, latex, leaves and roots. In addition, plants parts from male and female trees differ in the quantity of the compounds. For example phenolic compounds tend to be higher in male trees than in female trees. The quantity of fresh papaya latex and dry latex (crude papain) also vary with the sex of the trees and age of the tree. Female and hermaphrodite trees yield cruder papain than male trees and older fruit yield more than younger fruit. However, the activity of the papain is higher in the extracts from the young fruit than the older fruits (Maton *et al*., 1993).

Collaborating chemists in Italy and Somalia identified 18 amino acids in papaya seeds, principally in descending order of abundance; glutamic acid, arginine, proline and aspartic acid in the endosperm and proline, tyrosine, lysine, aspartic acid and glutamic and in the sarcotesta (Maton *et al*., 1993). A yellow to brown, faintly scented oil was extracted from the sun

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dried, powdered seeds of unripe papaya at the central food technological research institute, mysore India. White seed yielded 16.1% and black seeds 26.8% and it was suggested that the oil might have edible and industrial uses. Papaya seeds are sometimes found as an adulterant of whole black pepper in India. Extracts of ripe and unripe papaya fruit and seeds are active against gram-positive bacteria. Strong doses are effective against gram-negative bacteria. The fresh crushed seeds yield Benzy Isothiocyanate (BITC) which is bacteriostatic, bactericidal and fungicidal (Tayeb *et al*., 1974).

In a study on rat ovaries and uterus using aqueous seed extract, the fertility test was hundred percent negative and oestrous cycle irregular with a predominance of dioestrous. Uterine contractility was increased. The extract manifested anti-fertility, anti-implantation and abortifacient effect. The withdrawal however overcame the adverse effect (Chinoy *et al*., 1995).In reproduction, various extracts of carica papaya seed have been shown to have antifertility activity in male (Chinoy and Padman, 1996) and female rats (Chinoy *et al*., 1997). Treatment with the benzene chromatographic fraction of the chloroform extract of papaya seed at a dose of 10mg/rat/dry for 150days showed total inhibition of motility, reduced sperm count and infertility (Manivannan *et al*., 2004). Igiri *et al*. (2003) studied the daily ingestion of unripepapaya seed extracts by guinea pigs for seven weeks and reported that the 200mg group showed reduction in interstitial and in number of spermatozoa in the lumen. At a dose of 440mg, there was degeneration of the basement membrane of the tubules suggesting a cytotoxic effect.

Carica papaya has been known to be used in the treatment of malaria (Titanji *et al*., 2008).Satrija *et al*. (1994) studied the efficacy of papaya latex against Ascaris suum in sixteen pigs and concluded that papaya latex is effective against Ascardia galli in chickens.Latex extracts also showed yeast inhibition (Osato *et al*., 1993). Studies have shown that carica papaya have a hepatoprotective effect on the liver (Nwangwa, 2012). Papaya latex and root extract have also shown anti-microbial activity as non-aqueous extract have shown inhibition of candida albicicans (Giordani and Lafon, 1993).The papaya latex, which is probably of most interest to livestock producers is an antihelmitic (Kumar *et al*., 1991). The plant parts have been used as tenderizer due to the presence of proteoltyic enzymes in the sap, as a haemostat and antidote against venoms and rabies and treatment of diabetes (Burkill, 1985).

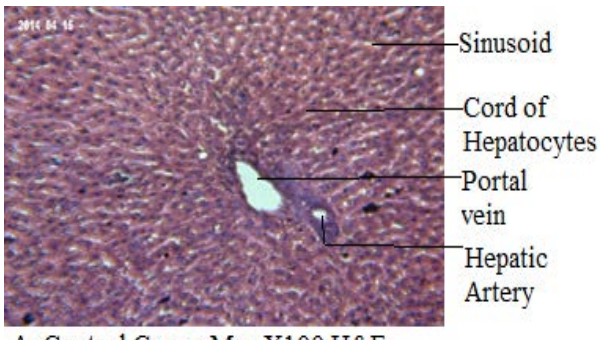
The aim of the study was to determine the histologic effects of hydromethanol seed extracts of ripe carica papaya on wistar rats' liver. The objectives were to determine: (a) the LD_{50} of hydromethanol seed extract of carica papaya. (b) The histologic effect(s) of Hydromethanol leaf extract of pawpaw. (c) If the effect is dose- dependent/or time-dependent. (d) If the effect is reversible or not.

MATERIALS AND METHODS

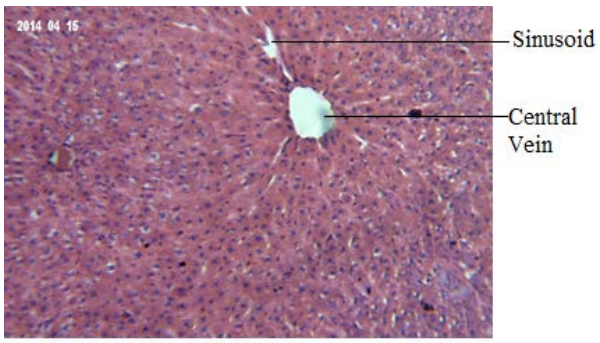
Ripe pawpaw seeds were obtained, washed and dried at room temperature before blending into fine powder. The extract was prepared from the powder using hydromethanol in a rotary evaporator.

Acute toxicity test: The acute toxicity test was divided into two stages and each stage lasted for 24 h. Thirteen wistar rats were used.

Experiment proper: Thirty-five albino wistar rats weighing 130-280 g were acclimatized for a period of two weeks. They were divided into three experimental groups (A, B and C) of ten rats and a control group of five. These rats were fed with pelleted animal feed and Distilled water daily. Experimental groups A-C ingested 6, 30 and 60 mg/kg of extract respectively for three weeks. The wash-out period lasted for a period of four weeks. Two rats were harvested from; (a) each experimental group on 8^{th}, 15^{th} and 22^{nd} days. (b) Control group on 22^{nd} (c) each experimental and control groups on 50^{th} day. The liver removed were processed histological for paraffin wax embedding, sectioned, and mounted on slides, stained and viewed under the light microscope. Photomicrographs were produced for permanent comparative studies.



A: Control Group Mag.X100 H&E



B: Group A Mag.x100 H&E

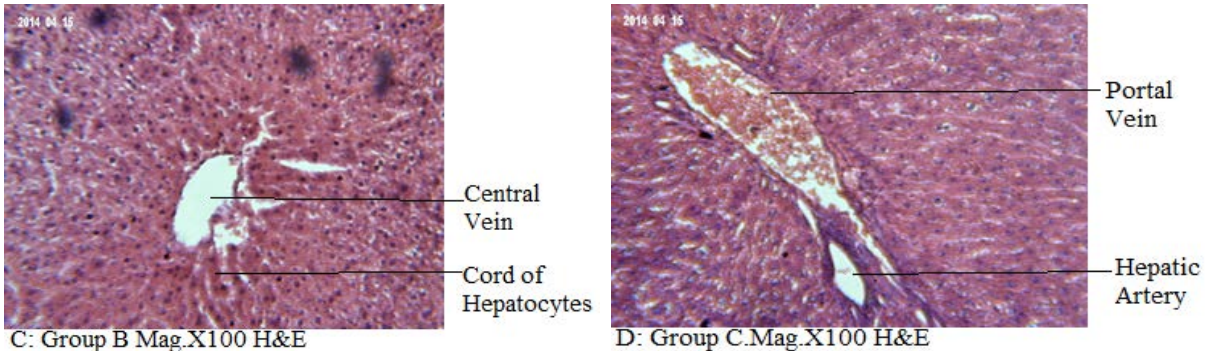


Fig. 1: Photomicrographs of albino wistar rats liver at the end of week one

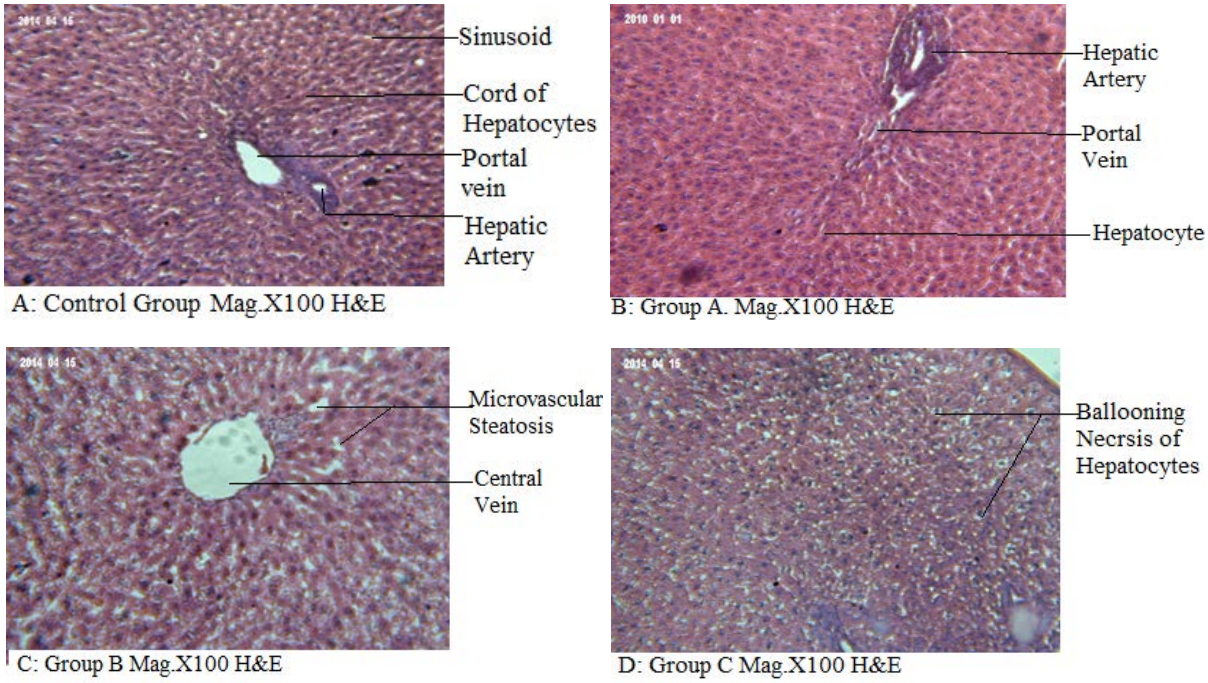


Fig. 2: Photomicrograph of albino wistar rats liver at the end of week two

RESULTS

The LD_{50}'ip' of Hydromethanol leaf extract of Carica papaya was found to be 299 mg/kg. Figure 1 to 4 showed the photomicrograph of the different groups throughout the experimental and wash-out periods.

Micrographs: Figure 1 showed albino wistar rats' liver at the end of the first week; Experimental groups A (Fig. 1B), B (Fig. 1C) and C (Fig. 1D) have similar features to the control group (Fig. 1A). Figure 2 showed albino wistar rats liver at the end of the second week; Groups A (Fig. 2B) and B (Fig. 2C) are similar to the control group. However, Group C (Fig. 2D) showed mild ballooning necrosis of hepatocytes. Figure 3 showed albino wistar rats liver at the end of third week; Group A (Fig. 3B) showed enlarged central veins, while Groups B (Fig. 3C) and C (Fig. 3d) showed marked generalized ballooning necrosis of hepatocytes. Figure 4C showed the findings at the end of the wash-out period and the features are similar to the control group.

DISCUSSION

All the micrographs from the week-one study were similar to the control group; the implication is that Hydromethanol seed extract of carica papaya does not cause significant histologic effect when administered for a short duration. Week-two study revealed mild ballooning necrosis of hepatocytes at higher doses. Week-three study showed generalized ballooning necrosis of hepatocytes. This significant destruction of hepatocyte that increased with increased time and dosage may be due the presence alkaloids (like carpaine). This is in agreement a suggested cytotoxic

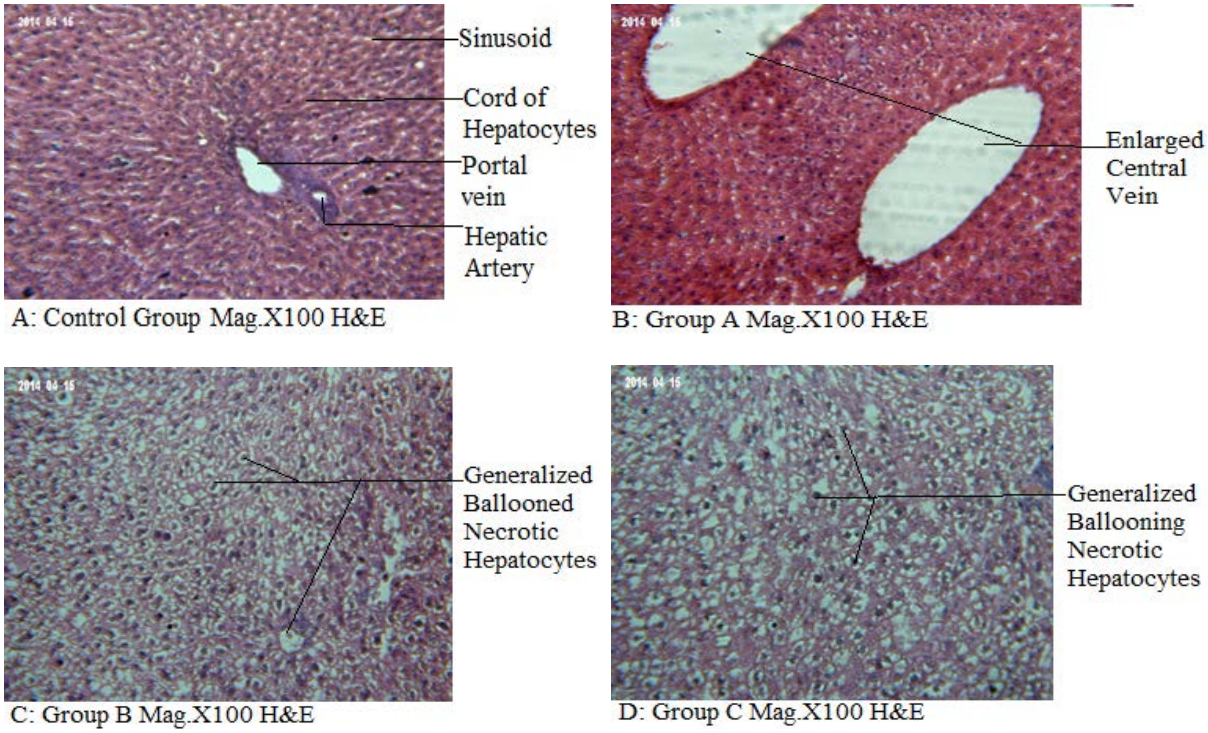


Fig. 3: Photomicrograph of albino wistar rats' liver at the end of week three

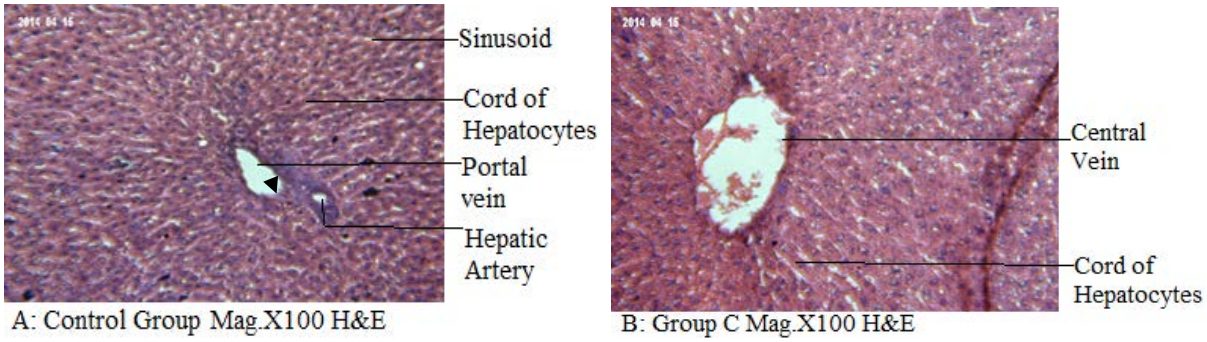


Fig. 4: Photomicrograph of albino wistar rats' liver at the end of wash-out period

effect associated with carica papaya seed extract (Igiri *et al*., 2003). Since Carica papaya seeds contain benzy isothiocyanate which is bacteriostatic, bacteriocidal and fungicidal (Tayeb *et al*., 1974), as a drug its detoxification in the liver may account for the hepatotoxic effect seen. This contradicts its already established hepatoprotective effect (Nwangwa, 2012). The wash-out study revealed hepatic histology similar to the control means that the withdrawal overcame the adverse effect (Chinoy *et al*., 1995).

CONCLUSION

The LD_{50} of hydromethanol seed extract of carica papaya (HSEC) is 299mg/kg. HSEC is hepatotoxic in a time and dose-dependent manner. The hepatotoxicity of HSEC is reversible.

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