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RESEARCH ARTICLE

TOXICITY EFFECT OF CALCIUM CARBIDE COERCED RIPENED PAWPAW ON THE LIVER MASS OF THE WISTAR RATS

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ABSTRACT

The liver, which is one of the largest organs of the body with varying degree of functions is prone to diverse ailments. This study is aimed at evaluating the effect of Calcium Carbide coerced ripened Pawpaw on the Liver mass of the Wistar rats. Mature unripe Pawpaw's were plucked off from the parent plant and were dividing into two groups; The first group was kept to rip at normal room temperature while the second group was induced with Calcium Carbide to rip. 10gram of Calcium carbide was placed in a bowl containing 5ml of water for dissolution in a closed metal bucket containing 1kg of the fruit (pawpaw) rapped with black nylon and was allowed for two days. After ripening, sampled fruits were washed and juiced. 600g of both the naturally ripened and calcium carbide ripened pawpaw were peeled separately and blended in an electric blender with 350ml/1L of deionized water. The juice was filtered and poured into clean bottles labeled (CaC2 induced ripened juice and naturally ripened pawpaw juice). A total of 21 adult Wister rats of both sexes weighing between 126.9- 214.3g were used. The Wistar rats were divided into three groups based on the body weight and then different concentrations of naturally ripened and calcium carbide induced ripened Pawpaw were administered orally. LD50 was carried out using Lorke, 1983 method. Group 1: Normal control group of 4 rats (2 males and 2 females) receive normal water and feeds only as placebo. Group 2 : Treatment Group (1) of 4 rats (2 males and 2 females). Group 3: Treatment Group (2) of 4 Wistar rats (2 males and 2 females) received Calcium Carbide ripened pawpaw for 4 weeks. 5ml/kg for both the natural fruit and the CaC2 ripened fruits were administered against each body weight of the adult Wistar rats. The Wistar rats were weighed and one Wistar rat was sacrificed weekly in groups. Blood and organ(liver) were collected from the three groups for histopathological analysis. The results showed Hepatocyte Hypertrophy, focal inflammation, Sinusoidal Dilation and amongst other. In conclusion, the consumption of fruits ripened with Calcium carbide pose detrimental effect on the liver, which could lower its functionality and integrity.

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INTRODUCTION

The liver is the most largest organ in the human body has diverse functions such as Bile production, Absorbing and metabolism of bilirubin, Supporting blood clots, Vitamin and mineral storage, Helps metabolize proteins, Filters the blood, Immunological function, Production of albumin, Synthesis of angiotensinogen, fat and carbohydrate metabolism and amongst others. The consumption of fruits ripened with calcium carbide could lower the body's potential to resist infection by weakening the immune system, affect hormonal balance which could lead to infertility (1). Findings have shown that oral supplementation with vitamin B12 can protect mice against CaC2-mediated toxicity, inflammation and oxidative stress. The findings provide vital tools for forensic and diagnostic indicators for harmful CaC2 exposure; while providing useful insights into how vitamin B12 can be explored further as an adjunct therapy for CaC2 toxicity (2). To evaluate the effects of Calcium carbide (CaC2) in biological system, an in vivo study was carried out on Long Evans rats.

Histopathological analysis of liver, heart, spleen, kidney and lungs were performed to observe any change due to the administration of CaC2. Remarkable changes were observed during the histopathological study of lungs and kidney only. The histopathological analysis of kidney showed the thickening of the lining of collecting tubules with changes in cell structure while lungs were found to be increased moderately in weight, with focal areas of consolidation that was found red-brown to red (3). Cell differentiation is associated with changes in metabolism and function. Understanding these changes during differentiation is important in the context of stem cell research, cancer, and neurodegenerative diseases (4). The consumption of fruits ripened with Calcium Carbide pose devastating effect on the bone marrow, deleterious effect on the circulating blood, heart and brain, that will evenly lead to myocardiac infarction, Eosinophilia, Anemia, thrombocythopenia, paralysis, stroke, seizure and mortality may eventually arise (5). The use of chemicals for fruit ripening is constantly on the increase and inadvertently, such chemicals are consumed unperceived (6). A study is to check nutritional programming on the second filial generation pups of the

Calcium Carbide coerced orange juice fed Wistar rats was carried out and the results showed significant increase in PCV, hemoglobin, Total RBC, lymphocytes and reduction in Total WBC, Platelet and Monocytes in the second filial generation pups from the Wistar rats fed with Calcium carbide coerced orange juice. Haematological Indices are biomarkers that indicates functionality of the blood cells with regards to low, normal or high range. There is evidence of nutritional programming in the second filial generation pups as seen in this results (7).A research was carried to assessing calcium carbide and natural ripened pawpaw (Carica papaya) fruit on the biochemical parameters of the Wistar rats. Results showed elevated levels of creatinine, total cholesterol and lactate dehydrogenase may result to kidney injury, cardiovascular and heart diseases. There is therefore need for institutional and legislative strengthening as well as enforcement to prevent the use of calcium carbide in the ripening of pawpaw and other fruits (8). Hematological and histopathological analysis of calcium carbide forced ripened pawpaw on the kidney of the Wistar rats showed increase in Creatinine, Urea, and Albumin of the treated groups in contrast to the control ($p<0.05$). Also there is moderate to severe renal tubular degeneration and tubular necrosis of the Calcium Carbide treated group as against the control. In conclusion Calcium carbide causes complete renal failure and subsequently death may arise (9).

MATERIALS AND METHODS

Materials

Materials used for this study include Wistar rats, Calcium carbide, Water, Pawpaw , Syringes and Needles, Hand Gloves, Incubator, stop watch, Oven, centrifuge Model 800, cotton wool, Chloroform, 40% formaldehyde, Desiccator, Methylated spirit, EDTA bottles, normal sample bottles, Animal weighing balance, Water bath, and amongst others.

METHOD

Study Design: This is an experimental study of adult Wistar rats fed with naturally ripened and Calcium Carbide induced ripened fruits (pawpaw).

Fruit Collection: Mature unripe pawpaw's were plucked off from the parent plant and were dividing into two groups; one group was kept and allowed to rip at normal room temperature at the Histology Laboratory, Bayelsa Medical University, Yenagoa, Bayelsa State. The other group was induced with Calcium Carbide to ripe.

Calcium Carbide Application: Calcium carbide was bought at Swali Market, Yenagoa, Bayelsa State. 10gram of Calcium carbide was placed in a bowl containing 5ml of water for dissolution in a closed metal bucket containing 1kg of the fruit (pawpaw) rapped with black nylon and was allowed for two days(48 hours) for ripening. After ripening, sampled fruits were washed and juiced.

Sample preparation: 600g of both the naturally ripened and calcium carbide ripened fruits (pawpaw) were peeled separately and blended in an electric blender with 350ml/1L of deionized water. The juice was filtered with a clean fine sieve and was poured into clean bottles labeled (CaC2 induced ripened juice and naturally ripened pawpaw juice).

Experimental Animals: A total of 21 adult Wistar rats of both sexes weighing between 126.9- 214.3g were used. The animals were purchased and kept in standard environmental condition, given standard rodent food (formulated) and water ad libitum. The rats were divided into three groups for each sex, based on the body weight and then different concentrations of naturally ripened and calcium carbide induced ripened Pawpaw were administered orally. Animals were allowed to acclimatize for two (2) weeks and was fed with standard grower mash with clean water before the commencement of treatment following the protocols of (9).

Sample Administration: LD50 was carried out using (10) method for administration of samples. A total of nine (9) Wistar rats were used for this section grouped into three (3) each group containing three (3) rats. 12 Wistar rats were used for the main experiment. Group 1: Normal control group of 4 rats (2 males and 2 females) receive normal water and feeds only as placebo. Group 2.

Treatment Group (1) of 4 rats (2 males and 2 females) received 5ml/kg naturally ripened ripened fruits (pawpaw juice) . Group 3: Treatment Group (2) of 4 Wistar rats (2 males and 2 females) received Calcium Carbide ripened fruits (pawpaw) for 4 weeks (A month). 5ml/kg for both the natural fruit and the CaC2 ripened fruits were administered against each body weight of the adult Wistar rats.

Organ Collection: The Wistar rats were weighed each week and one Wistart rat was sacrificed in each groups, and samples were collected for histopathological assay.

RESULTS

Histological slides of the liver were prepared and analyzed from the various groups of the Wistar rats (Control, Natural and CaC2 treated groups) for two (2) and four (4) weeks and the results are shown below.

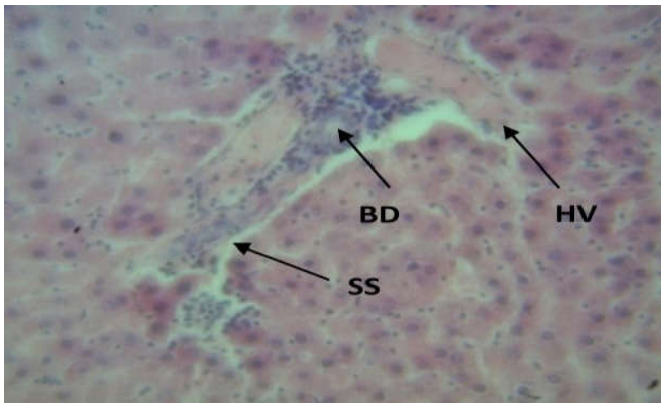

Figure 1. Photomicrograph (H&E x400) of the liver of a male Wistar rat fed with normal grower mesh (Control) showing a normal structure of the periportal area: there is hepatic (HV), blood sinusoids (SS) and bile duct (BD).

Diagnostic Lesion: Normal Architecture

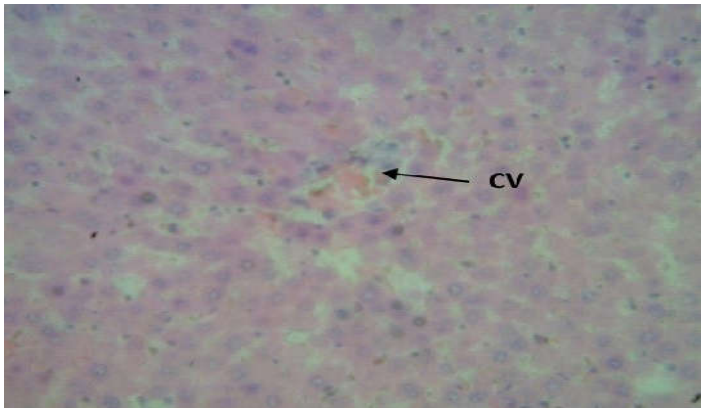

Figure 2. Photomicrograph (H&E x400) of the liver of a female Wistar rat fed with normal grower mesh (Control).showing normal architecture of the centrilobular area with central vein (CV) and sinusoids between the cord like arrangements of hepatocytes.

Lesion Diagnosis: Normal Architecture

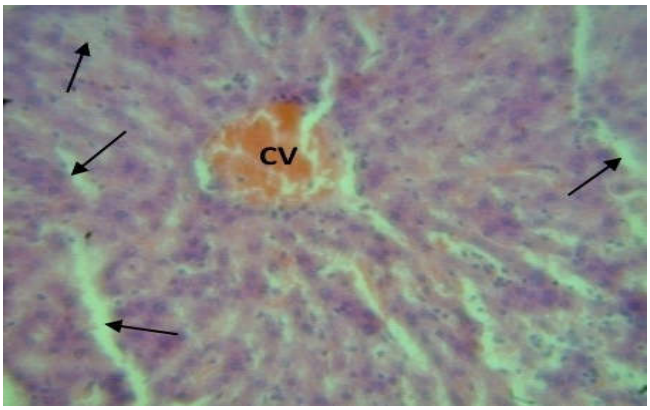

Figure 3: Photomicrograph (H&E x400) of the liver from male Wistar fed with CaC2 for (2 weeks) showing a narrow centrilobular area with an engorged central vein (CV). There is diffuse hepatocyte hypertrophy with finely granular eosinophilic cytoplasm; hence the cytologic alteration responsible for the hepatocyte hypertrophy was considered hyaline change. There is an associated mild sinusoidal dilation.

Lesion Diagnosis: Hepatocyte Hypertrophy.

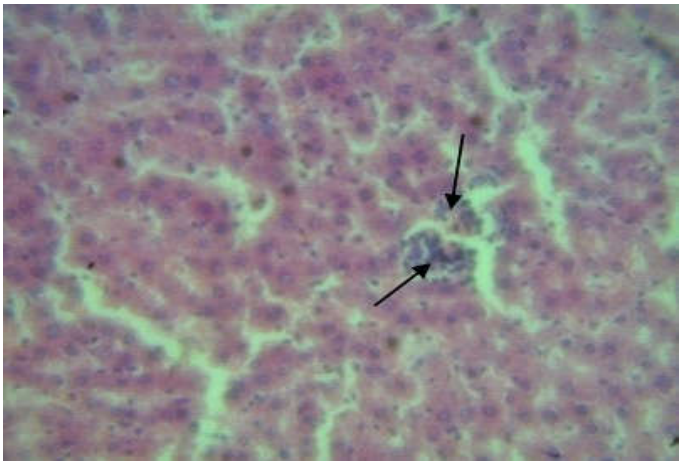

Figure 4: Photomicrograph (H&E x400) of the liver from a female Wistar fed with CaC2 for (2 weeks) showing multi-focal inflammation (arrows). The lesion is also associated with mild sinusoidal dilation and architectural distortion.

Lesion Diagnosis: Multi-focal inflammation.

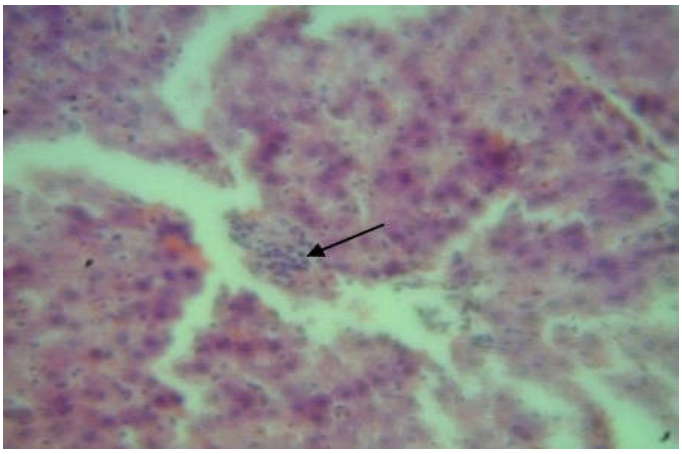

Figure 5: Photomicrograph (H&E x400) of the liver from male Wistar fed with natural fruit for (2 weeks) showing focal inflammation (arrow). The lesion is also associated with moderate sinusoidal dilation and architectural distortion

Lesion Diagnosis: focal inflammation

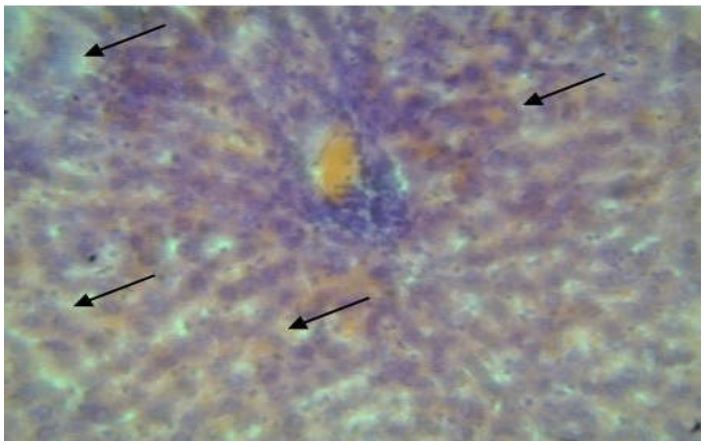

Figure 6. Photomicrograph (H&E x400) of the liver from male Wistar fed with natural fruit for (2 weeks) showing panlobular mild sinusoidal dilation (arrows). The lesion is also associated with moderate extracellular pigmentation.

Diagnostic Lesion: Mild sinusoidal Dilation

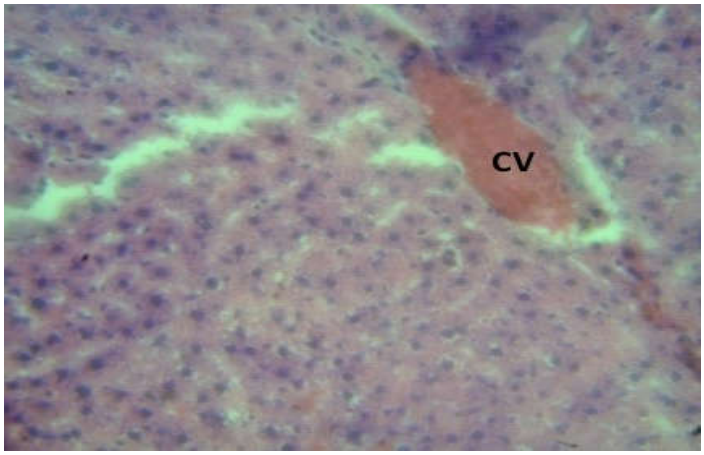

Figure 7: Photomicrograph (H&E x400) of the liver from a male Wistar fed with CaC2 for (4 weeks) showing a normal architecture of the centrilobular area: The central venin (CV) is in view

Diagnostic Lesion: Normal Architecture

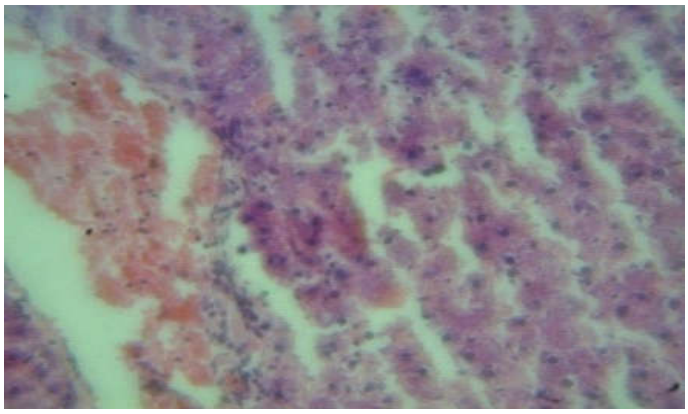

Figure 8: Photomicrograph (H&E x400) of the liver from a female Wistar fed with CaC2 for (4 weeks) showing mild centrilobular sinusoidal dilation with associated inflammatory cell infiltration

Diagnostic Lesion: Moderate Sinusoidal Dilation

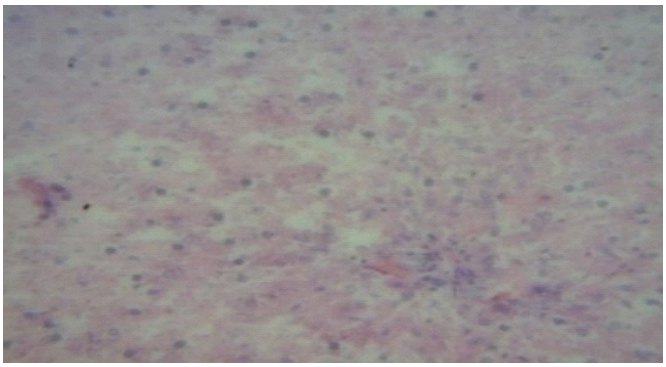

Figure 9: Photomicrograph (H&E x400) of the liver from male Wistar fed with natural fruit for (4 weeks) There is moderate panlobular hepatocyte necrosis (nuclear pyknosis and karyolysis) accompanied by hepatocyte degeneration (cytoplasmic vacuolation) and microvesicular fatty change, and inflammatory cell infiltration.

Diagnostic Lesion: Submassive panlobular hepatocyte necrosis

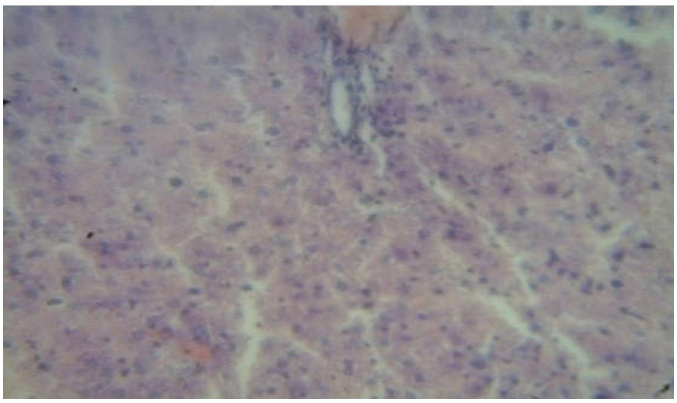

Figure 10. Photomicrograph (H&E x400) of the liver from a female Wistar fed with natural fruit for (4 weeks). There in mild cellular infiltration with normal liver architecture.

Diagnostic Lesion: Cellular Infiltration.

DISCUSSION

The liver is unique in that it receives blood from two sources: the hepatic artery and the portal vein. As these vessels enters the liver, their terminal branches run alongside and branches of the bile ducts and course together throughout the liver parenchyma within periportal area, which consists of four vessels: hepatic artery, portal vein, bile ductile and lymphatic vessel, forming the portal area. The periportal blood vessels supply's the liver parenchyma by flowing through the liver sinusoids to the central vein, which lies in the centre of the centrilobular area.The blood supply to the liver parenchyma flows from the portal area to the central veins. Accordingly, the hepatic parenchyma of classical liver lobule is divided into 3 zones (11):

- **Zone 1** or the periportal (peripheral) area is closest to the arterial and portal blood supply and hence bears the brunt of all forms of toxic injury.
- **Zone 2** is the intermediate midzonal area.
- **Zone 3** or the centrilobular area surrounds the central vein and is most remote from the blood supply and thus suffers from the effects of hypoxic injury.

The sinusoids are flanked by hepatocytes. Hepatocytes are the chief functional cells of the liver and performs astonishing number of metabolic, endocrine and secretory functions. Roughly 80% of the mass of the liver is contributed by hepatocytes. In three dimensions, hepatocytes are arranged in plates that anastomose with one another. The cells are polygonal in shape and their sides can be in contact either with sinusoids (sinusoidal face) or neighbouring hepatocytes (lateral faces). A portion of the lateral faces of hepatocytes is modified to form bile canaliculi. Microvilli are present abundantly on the sinusoidal face and project sparsely into bile canaliculi. From the results the Wistar rats fed with Cac2 for two (2) weeks for both male and female showed Hepatocyte hypertrophy and Multi-focal inflammation. Hepatocyte hypertrophy is a form of cytologic alteration that is diagnosed based on observable increased size of the hepatocytes when compared with concurrent control liver. It is most readily apparent when it has the commonly occurring centrilobular distribution pattern; when it is panlobular, comparison with concurrent controls can provide diagnostic confirmation (11, 12). Hepatocyte Hyalinization is a process of conversion of stromal connective tissue into a homogeneous, acellular translucent material which accumulation as excess normal protein droplets inside cells (intracellular hyaline) or outside the cell (extracellular hyaline). When stained with H&E the cells appear homogeneous, glassy eosinophilic. Normal homogeneous, glassy eosinophilic protein is also called hyaline. Nevertheless, hyalinization could provide insights into the biologic behaviour and prognosis of pathological lesions (11). Focal Inflammation is the term used to denote single or multiple, focal, randomly distributed aggregates of inflammatory cells that are seen in the liver as a background lesion. However, these foci of inflammatory cells can occur spontaneously in livers in prechronic studies. The infiltrating cells are predominantly lymphocytes but may include fewer numbers of neutrophils and/or macrophages (12).

The results the Wistar rats fed with natural fruit for two (2) weeks for both male and female showed mild Focal Inflammation and Sinusoidal dilatation. Sinusoidal dilatation (SD) is characteristically an enlarged hepatic sinusoids as a predominant histopathological feature. Sinusoidal dilatation may actually be a nonspecific feature of impaired portal venous blood inflow, and it is usually caused by or a feature of severe systemic inflammatory reaction syndrome (SIRS) – Inflammatory diseases (e.g. Crohn's disease), infections, vascular diseases (e.g. idiopathic noncirrhotic portal hypertension (INCPH)), and hepatic metastasis. Pigment Accumulation is a degenerative lesion. Both endogenous and exogenous pigment can occur in hepatocytes, but pigmentation occurs more often in Kupffer cells. Pigment may be prominent in portal areas. Identification of hepatic pigment typically requires multiple special stains. Different pigments frequently contain some iron and will thus have variable positivity with Prussian blue stain. Inflammatory cell infiltration is a potent antimicrobial effector response that usually is recruited by an innate immune intermediary to induce the full weight of their response. Neutrophils is the most abundant circulating phagocytes in the human host, are recruited to sites of infection and inflammation where they are activated to degranulate, phagocytize, and release neutrophil extracellular traps (NETs), to kill microorganisms. In response to tissue infection, circulating neutrophils adhere to adjacent vascular endothelium, extravasate across it, and migrate to the site. Circulating neutrophils are short-lived (approximately 24 hours), and about 1011 cells die each day. This constant stream of neutrophil death would be potently inflammatory without the extraordinarily efficient uptake and processing of apoptotic neutrophils by macrophages to prevent release of toxic constituents, a process called efferocytosis.

This process can lead to tissue resolution and healing. The liver of the male Wistar rats fed with CaC2 for (4 weeks) showed normal architecture while the liver of the female Wistar rats fed with CaC2 for (4 weeks showed moderate sinusoid dilation. The liver of the male Wistar rats fed with natural pawpaw for (4 weeks) showed Submassive panlobular hepatocyte necrosis. Hepatocytes Necrosis: Hepatic necrosis is defined as death of hepatocytes, which maybe single cell, multiple cells in piecemeal, focal, multifocal, submassive or massive. Submassive hepatic necrosis is defined as necrosis involving 26%-75% of the parenchymal volume, while massive necrosis involves more than 75% (13). The extent, pattern, and morphologic features of hepatocellular necrosis depend on the degree of metabolic activation of hepatotoxic xenobiotics, host response to the toxicant, dose and duration of xenobiotic exposure, and timing of

liver sample evaluation after dosing. Classical coagulation necrosis is typically caused by ischemia or infarction, and tissue architecture is somewhat maintained because lysosomal enzymes responsible for proteolysis are denatured. Another form of necrosis, liquefaction necrosis, may result in cellular dissolution and loss of cytologic architecture. Changes that may accompany necrosis include hemorrhage, fatty change, cytoplasmic vacuolization, cytologic degeneration, and inflammatory cell infiltration (11,12). While the liver of the female Wistar rats fed with natural pawpaw for (4 weeks) showed cellular infiltrate. The term "cellular infiltrate" has been used to describe the presence of inflammatory cells without other evidence of an inflammatory process (e.g., oedema, necrosis or degeneration of cells, or evidence of vascular injury – m haemorrhage). Such infiltrates of inflammatory cells are generally quite small and may not warrant diagnosis. If significant enough to warrant documentation, accumulations of leukocytes should be diagnosed as "inflammation." Occasionally, infiltrates of nonleukocytic cells such as mast cells are seen in the liver, these should be diagnosed as "cellular infiltrate. (12).

CONCLUSION

The liver as a major organ saddled with diverse responsibilities is constantly open to a plethora of assaults from consumed foods and fruits. It is evident from this study , that the consumption of fruits ripened with Calcium carbide pose detrimental effect on the liver, which could lower its functionality and integrity.

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