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EFFECTS OF CHEMICAL RIPPENING AGENT ON MATERNAL AND FOETAL LIVER OF WISTAR RAT

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ABSTRACT

The aim of this study is to investigate the effects of calcium carbide ripened banana on the histological parameters of maternal and foetal liver in Wistar rats. Unripe bananas were subdivided into four groups; with three artificially ripened, using calcium carbide and one ripened via non artificial means. The pulp of these fruits were mixed with pelletized feeds and orally administrated to the Wistar rats for three weeks during and before pregnancy. Histological analysis was carried out on two groups of female wistar rats receiving different doses of calcium. Results show an infiltration of inflammatory cells, moderate congestion of hepatic vessels, distortion of hepatic tissues and architecture, before and during pregnancy. While further examination during pregnancy revealed moderate congestion of hepatic vessels, distortion of hepatic tissues and architecture, etc. Findings from this study suggest that consumption of calcium carbide ripened bananas is causing changes in the histological profiles of pregnant women. This may eventually result in severe health hazards and liver damage

Keywords: Histological, Maternal and foetal liver, Calcium carbide, Ripening, Banana, Wistar rats

INTRODUCTION

Banana (*Musa spp)* is a climacteric fruit that is often consumed as a complimentary food or as a whole meal (Adekalu *et al*., 2011; Vaidiya *et al*., 2016). It is the 4th most important global commodity with an annual production of 88 million tonnes (Nura *et al*., 2018). Banana is highly nutritious as it provides Vitamins (A and C), carbohydrates and minerals such as phosphorus, potassium, magnesium, sodium and iron to the human body.

It has a short-shelf life resulting in significant post-harvest losses due to poor transportation and storage facilities. This high-post harvest losses forces farmers to sell the fruit when green but mature; the harvested fruits are then ripened with artificial agents to avoid financial losses (Fattah and Ali, 2010; Sogo-temi *et al*., 2014). There are different types of agents used for ripening such as ethylene gas, etephon, ethreal and calcium carbide (Hussain *et al*., 2015).

Calcium carbide is banned in most countries under the prevention of food adulteration act; however it is freely available in developing countries like Nigeria because it is cheap and once dissolved in water produces acetylene gas (Pataore *et al*., 2007; Iyare *et al*., 2020).

Hussain *et al*., (2015) reported that most calcium carbide utilized by fruit sellers are of commercial grade and are often purchased from unauthorized sources. This commercial grade is impure as it contains traces of arsenic and phosphorus. Several studies have shown that arsenic exposure leads to adverse health effects such as diabetes, neurotoxicity and toxic effects on liver, kidney, spleen and cardiovascular system (Chen *et al*., 2009; Al-forkan *et al*., 2015).

In addition, the ingestion of arsenic during gestation can move through the placenta and permeate into the foetal system which confirms genotypic and phenotypic changes to arsenic as a plausible exposure route (Waalkes *et al*., 2004). It is on this basis that this study investigated the exposure of female wistar rats to carbide ripened banana and its histological effect on the maternal and foetal liver.

MATERIAL AND METHODS

Plant Material: Unripe matured bananas were purchased from Margaret Umahi International Market Abakaliki, Ebonyi State. The fruits were exposed to 500g of calcium carbide usingthe method of Reena *et al*., (2018).

Banana Treatment with Calcium Carbide: The fruits were divided into four groups labeled S1, S2, S3, and S4 respectively. Each fruit was subjected to three levels of calcium carbide treatments as follows: 2g, 4g and 6g calcium carbide per kg of fruit to induce ripening; while S1 is the controls (without calcium carbide). Calcium carbide was crushed into small pieces and weighed using analytical weighing balance PW 184 Adam model. The reported method (Rao Sudhakar, 2012), was used in this study to quicken the ripening process of the banana fruits as described.

In this method the fruits were kept in small perforated plastic container and exposed to acetylene gas released from

ca lcium carbide. After 24 hours of exposure, the fruits were left to complete the ripening process at room temperature. Room temperature and humidity readings were recorded daily. Fruit ripening was monitored. Sample S1 (5kg) of the banana was soaked in normal water to form the control group. Sample S2 (5kg) of the unripe banana was dipped in 1% CaC2 solution (50g/5litre). Sample S3 (5kg) of the unripe banana was dipped in 2% CaC2 solution (100g/5litre).A market sample treatment, S4 5kg ripened banana fruit was procured from a local market Margret Umahi International market Abakaliki,Ebonyi State.

Experimental Animals: 36 matured healthy Nulliparous females adult wistar rats weighing between 150- 200g were purchased from animal house,department of anatomy, Faculty ofMedicine, Ebonyi State University, Abakaliki. The rats were housed in special clear sided cages with a 12-12hour light: dark cycles and was allowed free access to drinking water and standard rat pellet feed in accordance with the US National Institutes of Health Guidelines for the care and use of laboratory animals.

All findings on animal experimentation were in accordance with international acceptable guideline for laboratory animal use and care by National Institute of Health, 1985; publication No. 8523.

Experimental Design: Group 1 consisted of four (4) sub groups with 4 nulliparous albino wistar rats. Treatment lasted for three weeks after which males were introduced into the cages for mating to occur. After successful mating and confirmation of pregnancy, the male albino wistar rats were removed and treatment continues till the 19th day of pregnancy, then it was sacrificed.

Group 2: This group consists of four (4) subgroups also. There was no treatment prior to pregnancy. Treatment commenced immediately pregnancy was confirmed. They were sacrificed also on day 19 of pregnancy.

The animals were fed with palletized mash and the pulp from the previously ripened banana fruits by CaC2 kept in the refrigerator. The blended bananas were mixed with the rat feed according to the method by Igbinaduwa (2016). At pregnancy day 19, the mother was sacrificed; blood sample was collected first through ocular puncture. Then the maternal and fetal liver was harvested and both maternal and fetal weight noted too. Blood sample was collected in a plain bottle which was allowed to cloth before its spinning in other to separate the serum from blood cells, after which the liver parameters were tested. The maternal and fetal liver was harvested and its tissue fixed for histological studies. They were passed through normal histological processes for the slide to be produced.

Histological Processing Techniques: Excised liver tissues were fixed in 10% formal saline for one week. On dehydration, the tissues were immersed in ascending order of absolute for 45 mins each and embedded in paraffin. Embedded tissues were sectioned to form ribbons of 3um thickusing a rotary microtune machine and rehydration of sections followed by passing the sections through descending grades of alcohol in two changes for 1minute eachand after

which they were rinsed in running tap water for 5minutes.

Sections were then stained with haematoxylene for 15minutes and blued in running tap water for 15minutes before counter staining with eosine for 1minute. Sections were dehydrated in ascending grades of alcohol for a minute and then absolute alcohol for 1minute. From the absolute alcohol, the sections were transferred to xylene in two changes each for 2minutes before mounting in DPX and covered with cover slips each. Sections were then dried in the oven.

RESULTS

The histology performed to observe changes in the liver tissues within two groups (Group 1 and 2) of pregnant wistar rat receiving different doses of calcium carbide ripened banana(1%, 2% and Market sample) with respect to control group are as follows;

Group 1(Administration Done before and During Pregnancy): For Group 1B an exposure to 1% of Cac2 showed mild congestion and proper perfusion of the hepatocytes for the adult pregnant wistar rat as seen in plate 4.7.2, while that of the neonatal liver section showed mild congestion of hepatocytes, clustering of hepatocytes and good perfusion of hepatocytes as seen in plate 4.7.6.

In Group 1C, an increased percentage (2%) of CaC2 ripened banana showed a moderate congestion of the hepatic vessels, distortion of hepatic tissues and mild infiltration of inflammatory cells as seen in plate 4.7.3, while that of the neonatal section of the liver showed distortion of hepatic architecture with extra hepatic hemorrhage as seen in plate 4.7.7.

Group 1D which was exposed to the unregulated and unquantified market sample revealed an appropriate perfusion with normal hepatic structures, indistinctive boarders of the hepatic cells and congestion of the hepatic vessels as seen in plate 4.7.4, while that of the neonatal liver section showed mild to moderate distortion, extravasation of blood within the hepatocytes as seen in plate 4.7.8.Group 2 (Administration was doneduring pregnancy)

Histological examination of the adult pregnant and neonatal liver section of wistar rat in the case of group 2B showed; mild infiltration of inflammatory cells as seen in plate 4.8.2, and that of the foetal liver section showed good perfusion, mild congestion of the hepatic vessels as seen in 4.8.6.

Group 2C showed; increased perfusion with indistinctive boarders and focal hepatic architectural distortion as seen in plate 4.8.3, while that of the foetal liver section showed; focal hepatic disruption of the foetal liver as seen in plate 4.8.7. In Group 2D congestion of the hepatic vessels, increased perfusion, mild infiltration of inflammatory cells and focal hepatic distortion was seen in plate 4.8.4, whereas that of the foetal liver section showed; hepatic parlour (no blood seen) and generalized distortion of hepatic architectureas seen in plate 4.8.8.

4.7 HistologicalEffects of the Treatments to the Liver

Treatment was done before pregnancy and was continuous after pregnancy was confirmed

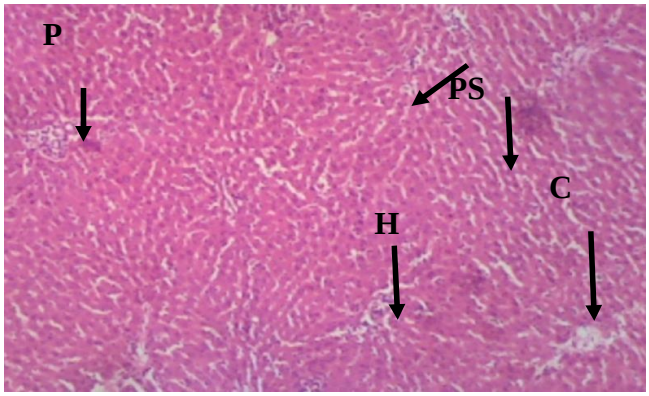

Plate 4.7.1: Photomicrograph of Wistar Liver (1A control group) treated with normal feed and distilled water showing Normal Hepatocytes (HC), Central Vein (CV), Peri-Sinusoid Spaces (PSS) and Portal Triads (PT). Stain used: Haematoxylin and eosin. Original magnification: X60

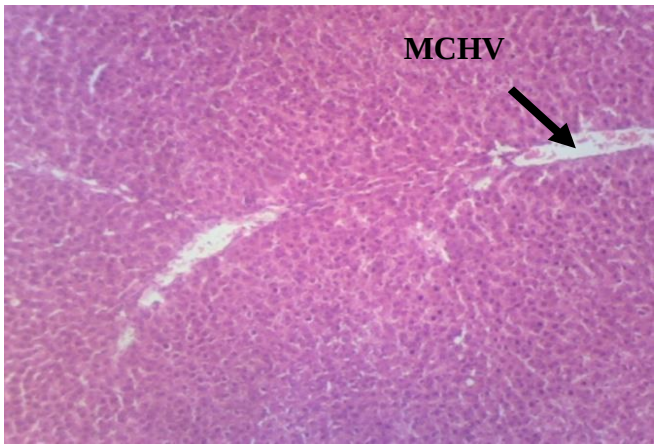

Plate 4.7.2: Photomicrograph of wistar liver (1B) treated with 1% CaC_2 ripened banana showing Mild Congestion of the Hepatic Vessels (MCHV) and Proper Perfusion of the Hepatocytes. Stain used: Haematoxylin and Eosin. Original Magnification: X60

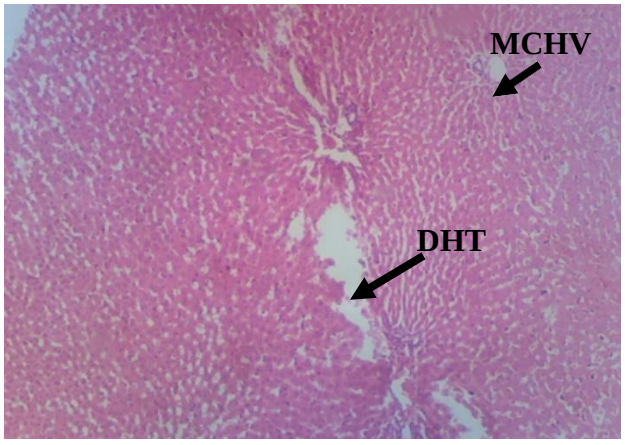

Plate 4.7.3: Photomicrograph of wistar liver (1C) treated with 2% of CaC_2 ripened banana showing Moderate Congestion of the Hepatic Vessels (MCHV), Distortion of Hepatic Tissues (DHT) and Mild Infiltration of Inflammatory Cells. Stain used: Haematoxylin and eosin. Original magnification: X60

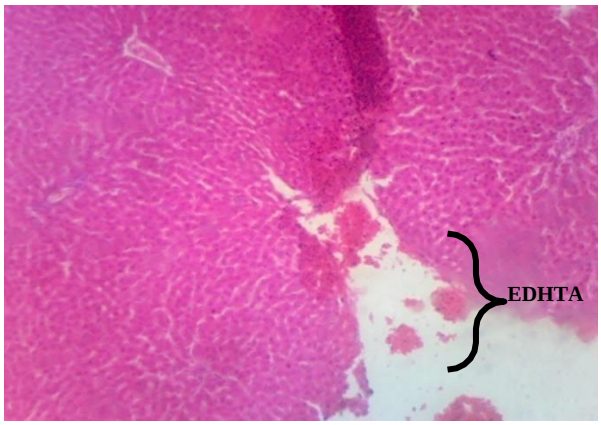

Plate 4.7.4: Photomicrograph of wistar liver (1D) treated with market sample banana showing Extensive Distortion of Hepatic Tissues Architecture (EDHTA). Stain used: Haematoxylin and Eosin. Original magnification: X60 Foetus Liver

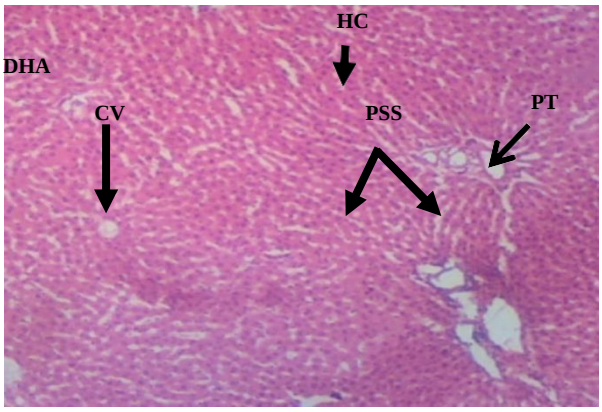

Plate 4.7.5: Photomicrograph of wistar liver (1A control group) treated with normal feed and distilled water showingNormal Hepatocytes (HC), Central Vein (CV), Peri-SinusoidSpaces (PSS) and Portal Triads (PT). Stain used: Haematoxylin and Eosin. Original magnification: X60

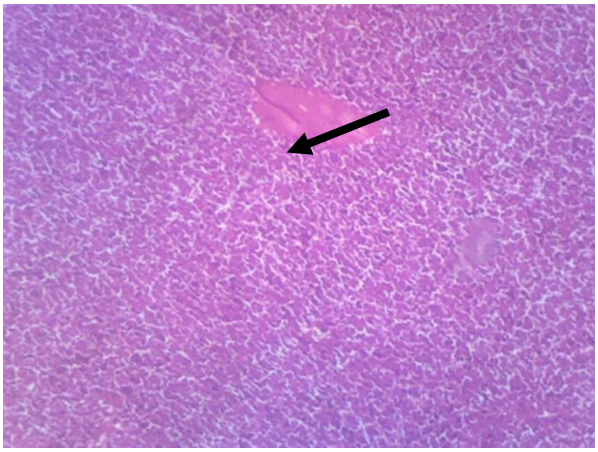

Plate 4.7.6: Photomicrograph of wistar liver (1B) treated with 1% of CaC_2 ripened banana showing Mild Congestion of Hepatocytes (MCH), Clumping of Hepatocytes and GoodPerfusion of Hepatocytes. Stain used: Haematoxylin and Eosin. Original magnification: X60

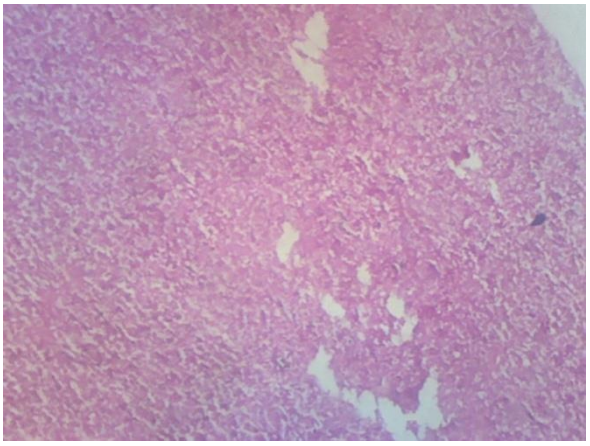

Plate 4.7.7: Photomicrograph of wistar liver (1C) treated with 2% CaC_2 ripened banana showing Distortion of Hepatic Architecture (DHA) with ExtraHepatic Hemorrhage. Stain used: Haematoxylin and Eosin. Original magnification: X60

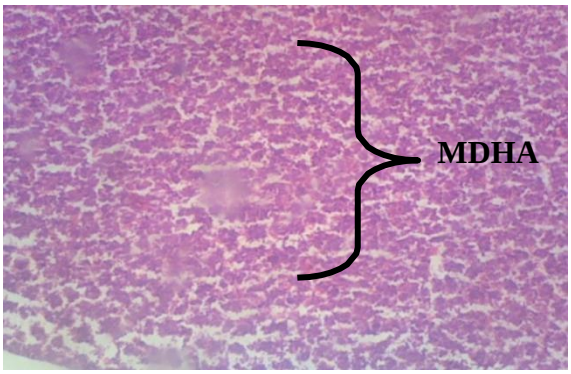

Plate 4.7.8: Photomicrograph of wistar liver (1D) treated withbanana from market sample showing Mild-to-Moderate Distortion of Hepatic Architecture (DHA) with Mild Infiltration of Inflammatory Cells. Stain used: Haematoxylin and Eosin. Original magnification: X60

Group 2: Treatment commenced during pregnancy

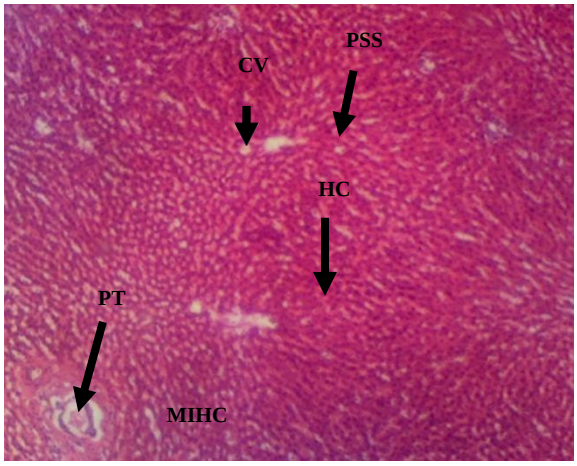

Plate 4.8.1: Photomicrograph of Wistar Liver (2A control group) treated with normal feed and distilled water showing Normal Hepatocytes (HC), Central Vein (CV), Peri-Sinusoid Spaces (PSS) and Portal Triads (PT). Stain used: Haematoxylin and eosin. Original magnification: X60

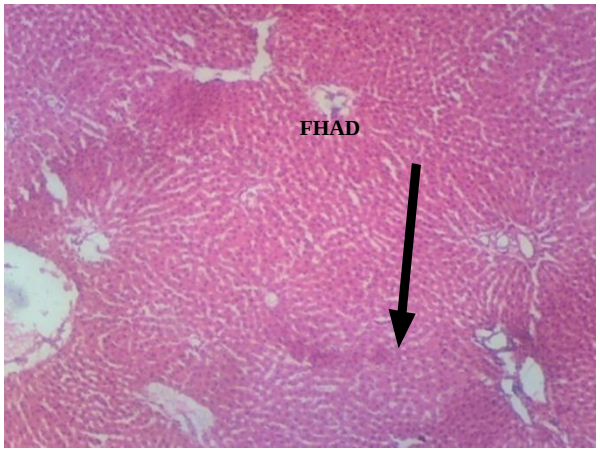

Plate 4.8.2: Photomicrograph of wistar liver (2B) treated with 1% of CaC_2 Ripened Banana showing Mild Infiltration of Hepatic Cells (MIHC). Stain used: Haematoxylin and Eosin. Original magnification: X60

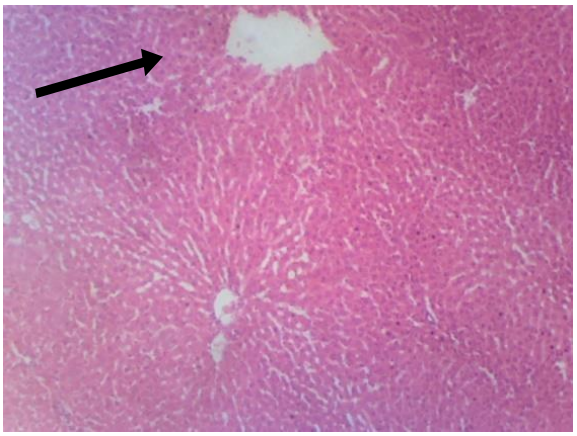

Plate 4.8.3: Photomicrograph of wistar liver (2C) treated with 2% CaC_2 Ripened banana showing Focal Hepatic Architectural Distortion (FHAD) and Increased Perfusion with Indistinctive Borders. Stain used: Haematoxylin and eosin. Original magnification: X60

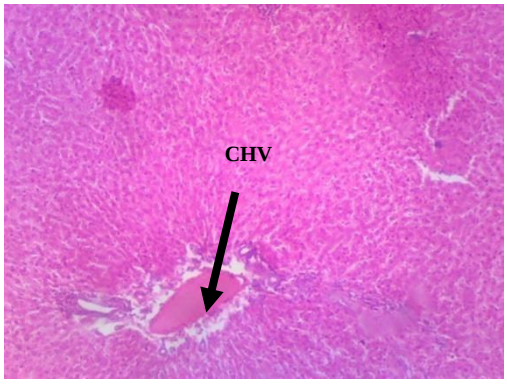

Plate 4.8.4: Photomicrograph of wistar liver (2D) treated with banana from market sample showing Congestion of the Hepatic Vessels (CV), Increased Perfusion and Focal Hepatic Distortion. Stain used: Haematoxylin and Eosin. Original magnification: X60

Group 2: Foetus Liver

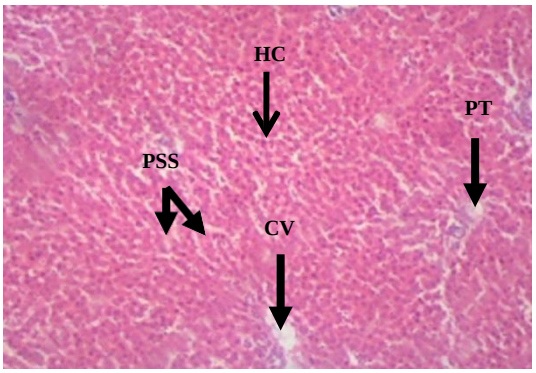

Plate 4.8.5: Photomicrograph of wistar liver (2A control group) treated with normal feed and distilled water showing Normal Hepatocytes (HC), Central Vein (CV), Peri-Sinusoid Spaces (PSS) and Portal Triads (PT). Stain used: Haematoxylin and Eosin.
Original magnification: X60

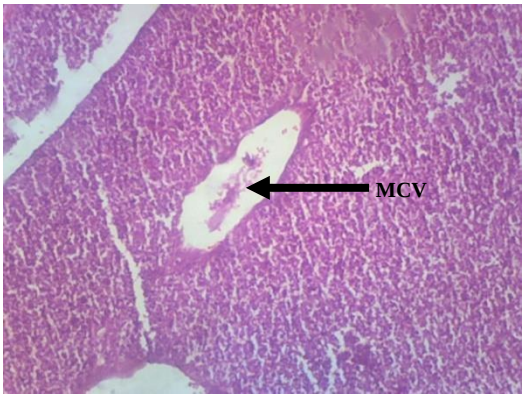

Plate 4.8.6: Photomicrograph of wistar liver (2B) treated with 1% of CaC_2 Ripened Banana showing Mild Congestion of the Vessels and Good Perfusion. Stain used: Haematoxylin and Eosin. Original magnification: X60

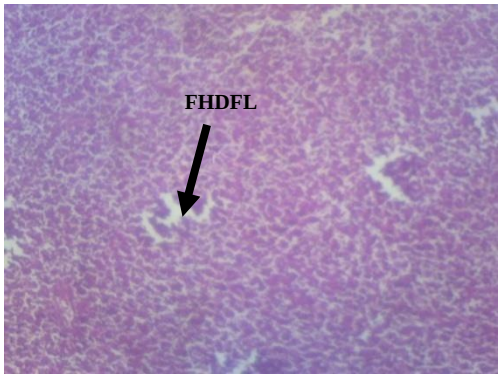

Plate 4.8.7: Photomicrograph of wistar liver (2C FOETUS) treated with 2% of CaC_2 showing Focal Hepatic Distortion of the fetal liver (FHDFL). Stain used: Haematoxylin and Eosin. Original magnification: X60

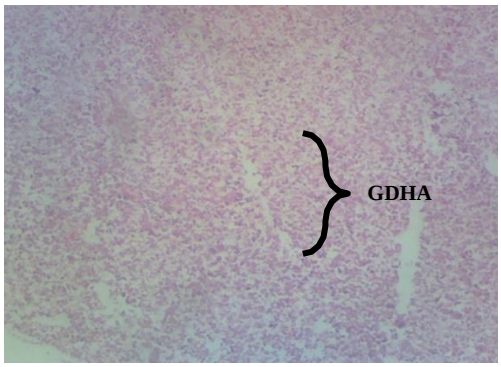

Plate 4.8.8: Photomicrograph of wistar liver (2D FOETUS) treated with Banana from market sample showing Generalized Distortion of Hepatic (SDHA), Abnormal Histological Appearance and Hepatic Parlour (No much blood seen). Stain used: **Haematoxylin and Eosin. Original**

DISCUSSION

The histological observation before and during pregnancy revealed infiltration of inflammatory cells which plays a major role in the progression of non-alcoholic steatohepatits to cancer and cirrhosis in the liver (Gao and Tsukamoto, 2016). It also showed a moderate congestion of hepatic vessels, distortion of hepatic tissues and architecture, extravasations of blood within the hepatocyte. This corroborates with the findings of Aladesanmi *et al*., (2020) who discovered that histopathology of liver in the acetylene exposed group of male rats revealed an altered histoarchitecture. Similarly, Bini *et al*., (2021) revealed that chronic exposure to industrial grade calcium carbide disrupted the architecture of internal organs like microvesicular fatty change in liver

The histological observation of the maternal and foetal liver during pregnancy showed increased perfusion with indistinctive borders, focal hepatic architectural distortion and disruption, congestion of hepatic vessels, increased perfusion, mild infiltration of inflammatory cells, hepatic parlour (no blood seen).

CONCLUSION

This study indicates that the consumption of Calcium carbide CaC2 causes harmful distortions of the maternal and foetal liver during histological examination and can lead to harmful related disorders.

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