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Development of Liver in Swiss Mice fed with *Solanum tuberosum, Cinnamomum zeylanicum and Cuminum cyminum* : From Juvenile to Adult (PND 21- 49)

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Abstract: The present research paper is in continuation of our previous research paper in which the importance of nutrients during pregnancy and lactation for both mother and their young ones were discussed at PND 1 and 21. It was concluded that whatever a pregnant female consumes, directly affects the liver of mother as well as the fetus, the same phenomena is also followed after lactation. The present research work was focused on the prolonged effects of high carb diet combined with cinnamon and cumin, given to mothers during period of gestation and lactation, and this also has its impact on later stages of life. Six groups were taken in the present investigation and they were group I Control (CN), group II high carbohydrate (HC), group III cinnamon group (Ci), group IV high carbohydrate + cinnamon group (HC+ Ci), group V cumin group (Cu) and last group VI high carbohydrate+ cumin group (HC+ Cu). Microstructure of liver of pups was studied at PND 49 and it was observed that nutrition obtained by mother during gestation and lactation also influenced the liver of the pups when these pups were fed on control diet after cessation of their lactation period. Results of the present study clearly demonstrate that diet taken during pre and postnatal stages of development also affects the health status during adulthood.

Keywords: pregnancy, nutrition, development, carbohydrate, antioxidant, liver.

1. INTRODUCTION

The field of investigating, the role of nutrition in various metabolic processes is very broad. As the research continues it is becoming clear that nutrition plays a major role in our survival. Nutrition is also called as nourishment or ailment (in the form of food) which is necessary for cells and organism to support life.

Maternal energy requirement increases during pregnancy[1]. During pregnancy there exist a direct correlation between diet, health and the outcomes of pregnancies[2]. Glucose is the main metabolic fuel for the developing embryo and fetus[3]. Body fuel which is needed for physical activity and also for proper organ function is provided by carbohydrates. Romson *et. al.,*[4] observed normal growth and development in dogs fed with carbohydrate-free triglyceride, but survival of fetuses was lower in this category than the dogs which fed with high carbohydrate diet.

Liver maintains or regulates homeostasis of the body. It contributes in many biochemical pathways *i.e.* growth, immunity, nutrient storage and supply. Liver is prone to damage because it has to detoxify lot of toxic substances that comes via food and other routes. Hepatotoxic chemicals, damage hepatic cells by generating free radicals. Tsukamoto and Lu[5] postulated that liver damage occur by lipid peroxidation, depletion of glutathione and formation of lipid radicals. This results in liver fibrosis and cirrhosis and can lead to the death of hepatocytes[6]. Several natural products with high antioxidant value are already reported to diminish the liver disorders caused by improper diet. In the present study stress induced by high carbohydrate diet on developing liver and the potential of herbal antioxidants as ameliorating agents were evaluated.

2. MATERIALS AND METHOD

The proposed experiments were conducted in the Environmental and Developmental Toxicology research Laboratory, Department of Zoology, University College of Science, Mohanlal Sukhadia University, Udaipur, Rajasthan, India; development of liver in Swiss mice fed with *Solanum tuberosum, Cinnamomum zeylanicum and Cuminum cyminum* from juvenile to adult (PND 21- 49).

2.1 Experimental Animal: This study was conducted on Swiss albino mice (*Mus musculus*). Animals were procured from the animal house of our department. The laboratory in which animals were kept was well-ventilated with relative humidity of 70-80%, with alternate 12 hours of light and dark periods. Animals were kept in a rectangular poly vinyl cages covered with steel grill and maintained on standard diet and water. The maintenance and handling of the animals were done as per guidelines purpose by Control and Supervision of Experimental Animals, Ministry of Environment and Forest, Government of India. The experimental protocol is approved by the Institutional Animal Ethical Committee of the University (No. CS/Res/07/759).In the present study healthy adult female Swiss albino mice (*Mus musculus)* 8-10 weeks of age and average body weights (BW) of 30 gm were selected. After reaching an average weight of 30 gm, four females were kept with one male in the ratio (4:1) for breeding. Then female mice were examined every morning for the vaginal plug and if it was present then, these females were isolated and followed by experimental diet because that day was counted as first day of gestation.

2.2 Diet Selection*:* the diet selection for experiment was based on its known nutritional value, medicinal value, cheap availability, and its common use domestically. *Solanum tuberosum* (potato) was taken as high carbohydrate diet, *Cinnamomum zeylanicum* (daalchini) taken as antioxidant and *Cuminum cyminum*

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(cumin) was taken as antioxidant and lactating agent. Diet was calculated according to the recommended dietary allowance (RDA) for humans.

***2*.3 Experimental Design:** selected females with vaginal plug were randomly separated into six experimental groups, each experimental group consist of 6 females.

- **Group I-** Control diet (CN): (Each mice is given 10gm (3.85gm Wheat + 3.85gm Maize + 1.55gm Gram + 0.75gm Groundnut).
- **Group II-** High Carbohydrate (HC): (*Solanum tuberosum*) (2.31gm Wheat + 2.31gm Maize + 0.93gm Gram + 0.45gm Groundnut + 4gm *Solanum tuberosum*)
- **Group III -** *Cinnamomum zeylanicum* (Ci) (bark) (3.74gm Wheat + 3.74gm Maize + 1.52gm Gram + 0.75gm Groundnut + 0.25gm *Cinnamomum zeylanicum*)
- **Group IV-** High Carbohydrate diet + *Cinnamomum zeylanicum* (HC+ Ci): (2.12gm Wheat + 2.12gm Maize + 0.85gm Gram + 0.41gm Groundnut+ 4gm *Solanum tuberosum* +0.50gm *Cinnamomum zeylanicum*)
- **Group V-** *Cuminum cyminum* (seeds) (Cu): (3.66gm Wheat + 3.66gm Maize + 1.47gm Gram + 0.71gm Groundnut + 0.50gm *Cuminum cyminum*)
- **Group VI-** High Carbohydrate diet + *Cuminum cyminum* (HC+ Cu): (2.12gm Wheat + 2.12gm Maize + 0.85gm Gram + 0.41gm Groundnut + 4gm *Solanum tuberosum* + 0.50gm *Cuminum cyminum*)

***2.4* Histopathological techniques:** The pups of control and experimental groups were sacrificed on PND 49 and liver was fixed in Bouins fixative for 24 hours and then transferred to 70% alcohol for prolonged washing to remove excess picric acid from the tissues**.**

Tissues were dehydrated, cleared in xylene and embedded in paraffin wax following routine procedure of block preparation. Routine thick sections were cut with a rotator microtome and fixed on clear and albumenized slides. These slides containing sections were stained with hematoxylin and eosin. Appropriate sections were observed under the microscope. Photomicrographs of the desired section were obtained using digital research photographic microscope.

3.0 RESULTS AND DISCUSSION

Both in animals and healthy human's hepatic enzymes are affected by the diet of an individual [7,8,9]. According to studies done by Wilborn *et. al*.,[10] genetic, physiological and behavioral factors contributes in the etiology of obesity. In the present study it was also observed that HC diet contributes to obesity in animals. Animals on HC diet showed significant increase in their body weight. Study done by Shiojiri[11], showed that all fetal hepatocytes were bipotent in terms of the differentiation of mature hepatocytes and intrahepatic bile-duct cells, and that the micro environment around portal veins plays an important role in bile-duct differentiation.

Decrease in hematopoietic cell is the key event in the development of liver. These cells become mature and move to blood stream as the development proceeds and at that moment all the liver components are clearly visible and identified. Reduction in hematopoietic cells in liver may be associated with increase in hepatic cells from birth to 30 days age[12].

In the present investigation hematopoietic cells were also not observed on PND 49 which was prominent in the cavity of portal vein at PND1. Portal tried i.e. portal vein, portal artery and bile duct were entirely visible at PND 49 which were in developing stage on PND 21 and were not identified as portal artery and bile duct.

In few studies, conducted previously it was postulated that ability to digest and metabolize carbohydrate in fishes is limited, hence high amount of carbs leads to nutritional problems[13,14]. High amount of carbs reduced growth rate and also contributed to poor food utilization in fishes[15].

Liver of HC group (II) in the present study had shrieked hepatocytes without proper cell boundaries and with small nucleus, where only some nucleuses have chromatin material. Even hepatic plates also shrieked due to which sinusoids became wide because of shrieked hepatocytes

Nutritive sweeteners made up of fructose were safe according to Food and Drug Administration, but even fructose intake above 25% of total energy results in hypertriglyceridemia and gastrointestinal symptoms[16]. In fructose-induced insulin resistance rats cinnamon extract treatment enhanced muscular insulin signaling, hence it improves metabolic syndrome related symptoms in rats[17]. In the present study it was noticed that, in cinnamon group (III) at PND 49, hepatocytes were increased in comparison to their regular size and they have two types of nucleuses in which only few nucleuses were normal and rest of them shrieked in size, with scanty chromatin material. Hepatic plates were organized with properly arranged hepatocytes and sinusoids were also normal. Mesh like structure still present like that of 21st day liver in the same experimental group due to intermingling of cytoplasm of hepatocytes.

Fifty six healthy male Wistar albino rats were given cumin for 60 days which were initially treated with carbon tetrachloride (CCl_4) for Sixty days[6]; and the authors concluded that cumin totally reversed the condition of increased lipid peroxidation, liver enzymes and decreased antioxidant enzyme levels induced by CCl_4, hence cumin is loaded with the potential to prevent liver from oxidative damage.

In cumin group on PND 1distribution of hepatocytes were not appropriate and they were also less in number, had distinct nucleus but not distinct membrane and were not arranged on hepatic plates. While on PND 49 organized hepatic plates with hepatocytes were noticed but there was little increase in size than normal, they have two types of nucleus where majority of nucleus were regular but some reduced in size. Nucleus in some of the cells was shifted to the corner (eccentric nucleus), but hepatic plates and sinusoids were well organized. Cells have granulated cytoplasm and at few places bile canaliculi were also observed.

When cinnamon was given in the combination with high carbohydrate diet, enhancement in the organization of liver components were noticed in comparison to HC group (II) and Ci group (III); here like that of PND 21, hepatic plates and sinusoids were well-arranged as compared to Ci group(III) on PND 49. Hepatocytes also have two type of nucleus normal sized nucleus with distinct chromatin material and reduced nucleus in which chromatin material was not visible. Cells have distinct margins; degeneration of cytoplasm was noticed in some cells. Sinusoidal space between two hepatic plates also increased.

Healthier synchrony in structure of liver was noticed with the help of cinnamon because it was reported that cinnamon treatment reversed the decrease in insulin sensitivity produced by HC in rats, deliberated in the study conducted by Richard *et.al*[18].This improvement in the liver components might be due to the antioxidant, anti-inflammatory & lipolytic activity of cinnamon[19].Cumin extract is an effective scavenger of superoxide anion radicals in a dose-dependent manner and thus prevents the formation of ROS[20].

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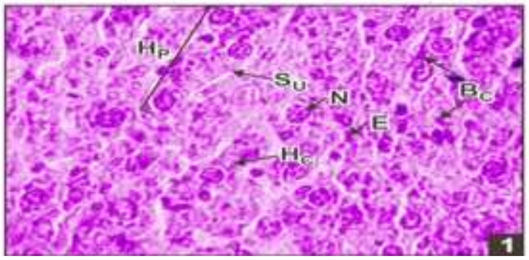


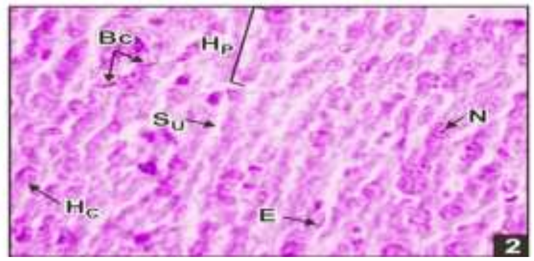
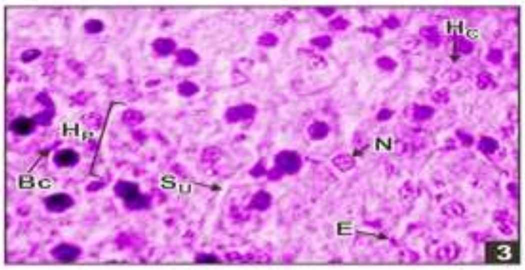


Fig. 1: CN: Hc with clear cell boundary and distinct N arranged on Hp; Bc and E cells visible**.**

Fig. 2: Hc with no clear margins; Hp shrinked due to which wide Su are visible



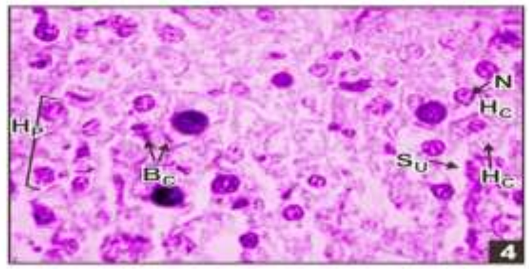
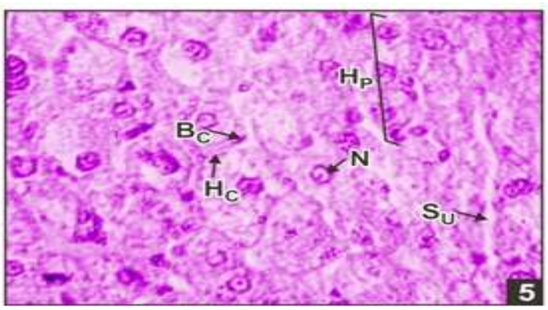


Fig. 3: Ci: Hc with distinct margins and N do not have chromatin material; Su between Hp shrinked due to increased size of Hc; Bc and E visible at few places.

Fig. 4: HC+ Ci: Hc with distinct cell boundary but with shirked N; sinusoid Su are few and are wide.



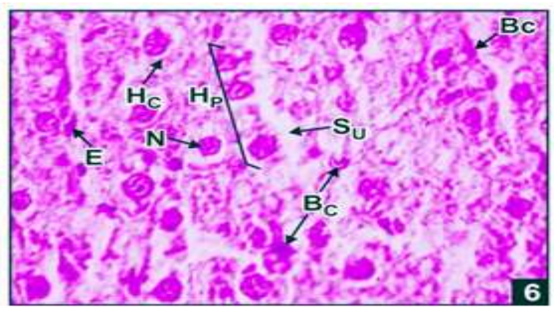


Fig. 5: Cu: Hc increased in size with distinct margins and N; some N shifted to corner of cells; Su not clearly visible; few Bc.

Fig. 6: HC+ Cu: Majority of Hc does not have clear boundaries but distinct N with chromatin material; Su few but wide; few E and Bc.

E= Endothelial cell, H_c= Hepatocyte, **H_p**= Hepatic plates, **N=** Nucleus, Su= sinusoid, **B_c**= Bile canaliculi, **CV**= central vein.

Plate 1: Photomicrograph of liver showing distribution of liver components on PND 49

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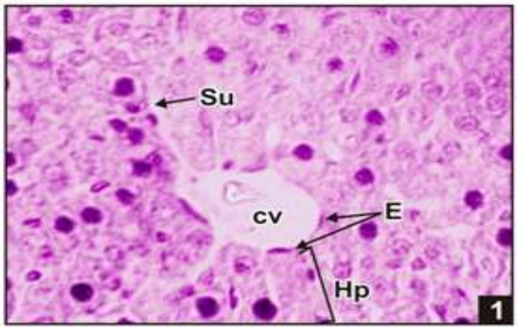


Fig. 1: CN: CV completely developed, lined by E and surrounded by properly arranged Su and Hp.

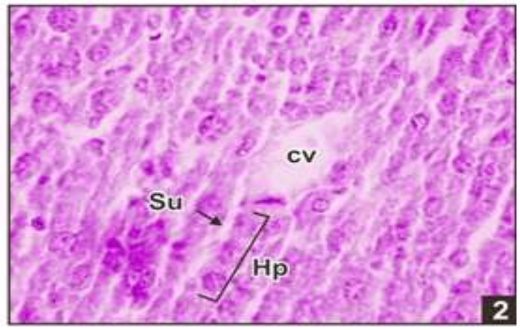


Fig. 2: HC: CV with distorted margins and surrounded by unsynchronized Hp and Su.

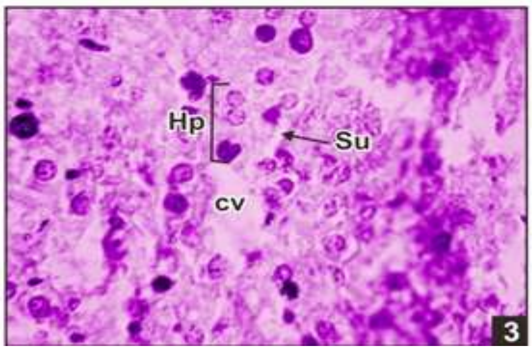


Fig. 3: Ci: CV clearly visible with irregular boundary; Su and Hp not properly arranged around CV.

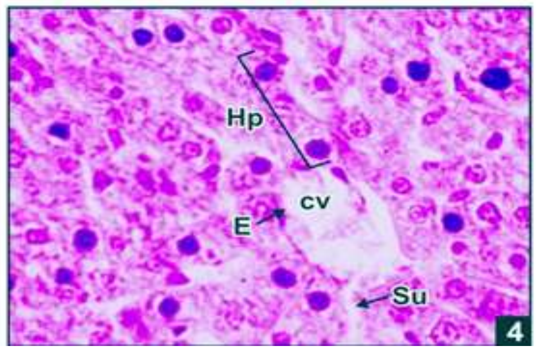


Fig. 4: HC+ Ci: CV has smooth margins, lined by few E; Su and Hp clearly organized around CV.

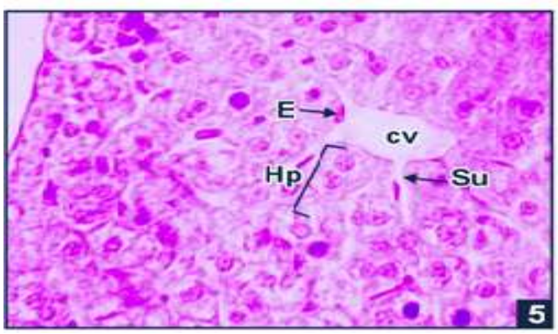


Fig. 5: Cu: CV completely developed, E-cells are clearly visible around CV and at margins of Su

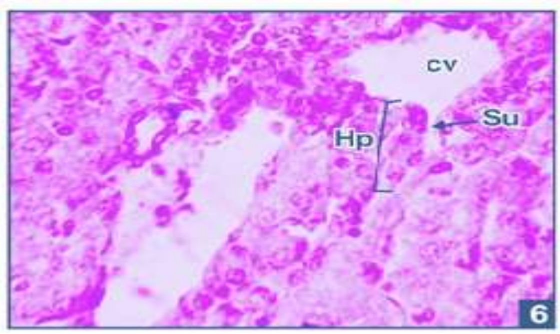


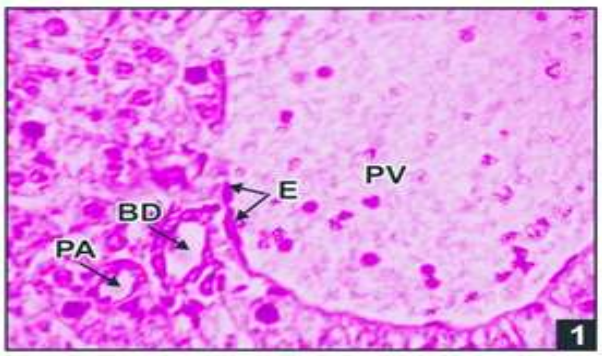
Fig. 6: HC+ Cu: CV have irregular margin; arrangement of Su and Hp is not in regular fashion..

E= Endothelial cell, **H$_c$**= Hepatocyte, **H$_p$**= Hepatic plates, **N**= Nucleus, **Su**= sinusoid, **B$_c$**= Bile canaliculi, **CV**= central vein.

Plate 2: Photomicrograph of liver showing central vein (CV) on PND 49

On the basis of above results of different studies it can be established that in combination group of high carbohydrate and cumin (VI) cells retained their normal shape when compared to Cu group (V), they had distinct cell boundaries, with normal sized nucleus having distinct chromatin material. Hepatic plates and sinusoids were well-arranged. Bile canaliculi and endothelial cells were visible and cytoplasm of cells was very less granulated. While in HC+ Ci and HC+ Cu group less granulated cytoplasm was observed which showed that Ci and Cu improves the glucose uptake which results in the decrease in glycogen deposition in liver and hence less granulated cytoplasm of hepatocyte was observed.

In the present study no major change in the structure of liver was observed like that of PND 1 and 21st due to selected diet plan. There were minor changes in the liver cells in every group which were mainly due to different diet given to animals and also due to the different developing stages of liver as this study was conducted during pregnancy and lactation, in which major change in the behavior of food consumption altered as the development proceeds.



F.g.1:CN: Completely developed portal area; PV has smooth margins and lined with E; fully developed BD and PA visible**.**

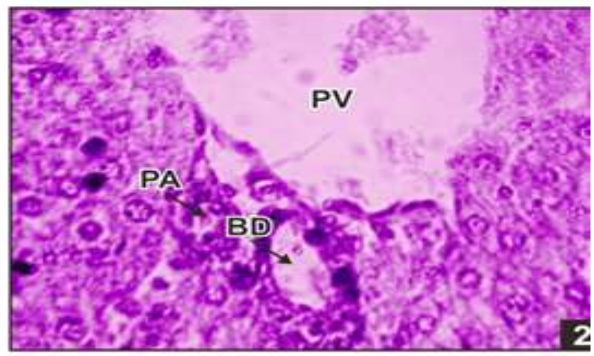


Fig. 2: HC: PV with irregular margin; BD and PA clearly visible but boundaries are not clear

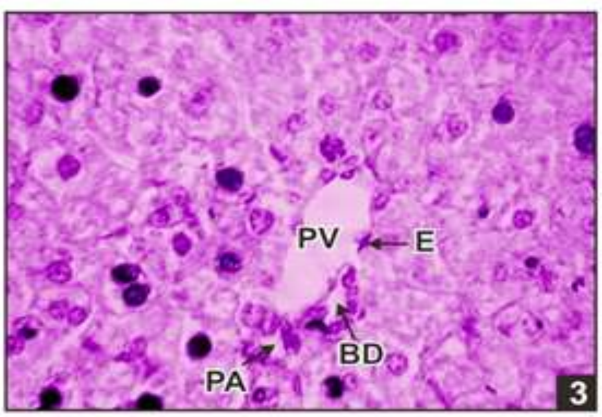


Fig. 3: Ci: PV is small in size than rest of the groups but has regular margins; BD and PA completely developed.

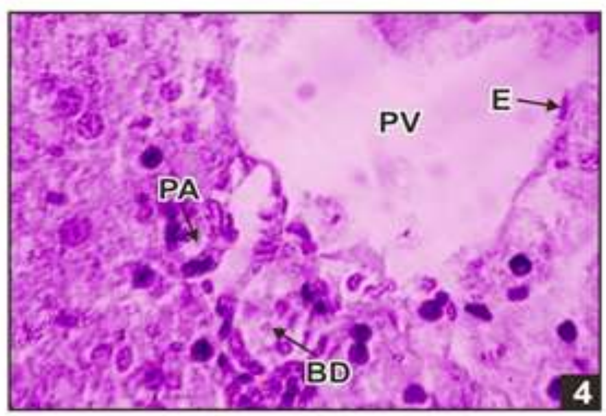


Fig.4: HC+ Ci: PV does not have smooth margins and not lined by E; BD and PA visible but with irregular boundaries.

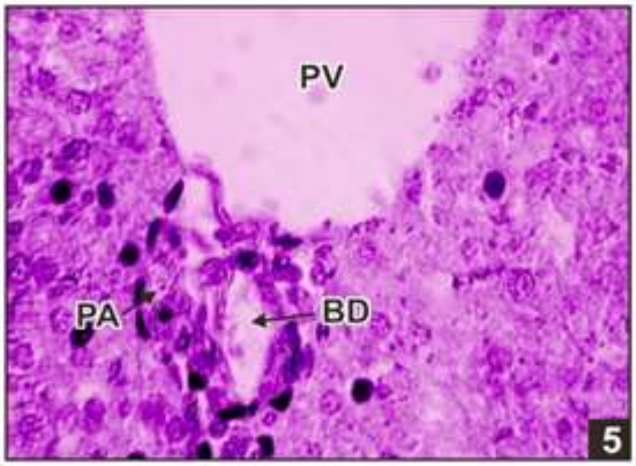


Fig. 5: Cu: PV with smooth margins but lack E lining at its margins; BD and PA visible but PA is not as clear.

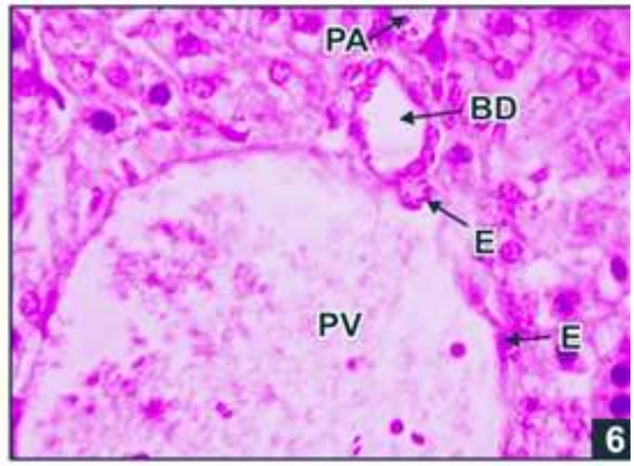


Fig. 6: HC+ Cu: PV with smooth margins and lining of E; BD and PA visible with clear boundaries

E= Endothelial cell, **PV**= Portal vein, **BD** = Bile duct, **PA** = Portal artery

Plate 3: Photomicrograph of liver showing portal area on PND 49

4. CONCLUSIONS

Variations in architecture of liver caused by HC diet due to increase in oxidative stress were nullified by the Ci and Cu, which are rich in antioxidants properties. These herbal products improved the architecture of liver on PND 49 in comparison to PND 1 and 21, when given in combination with HC. They reduced the oxidative stress caused by HC diet therefore better preserved hepatocytes with less granulated cytoplasm was observed on PND 49. The negative impact generated by HC diet on liver is gradually reduced with advancing age because the developing organs of neonates are more vulnerable to all types of environmental and nutritional insults. On the other hand once the organ systems are fully developed then they are comparatively more resistant to various types of challenges.

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