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Ultrastructural and Morphometrical Study of Gestational Induced Changes in Mouse Liver

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Abstract: ***This study was carried out to assess the ultrastructural findings of mice liver during pregnancy using transmission electron microscope. Thirty female adult mice, weighing 20–30 g, the mice were randomly into two groups A, B, (n=15, control & 15, pregnant), the liver tissues were collected and processed to paraffin block then sectioned and stained using transmission electron microscope to demonstrate fine structures in liver tissue. General morphological result showed changes of liver include increase in total body weight. Ultrastructural study revealed the changes in fine structure of hepatocytes with the presence of high number of mitochondria in pregnant group. Endothelial cell was large and more active, Ito cell showed increase in length and decrease in fat droplet.Widening of hepatic canaliculi in between hepatocytes and narrowing of space between hepatocytes and endothelial cell lining liver sinusoid. The study concluded that liver tissue shows changes during late stage of pregnancy mice.***

Keywords: Liver, Pregnancy, Hepatocytes, Transmission electron microscope, endothelial cell

1. Introduction

Pregnancy is the expression used to describe the period in which a woman carries a fetus inside of her [1]. Human pregnancy commonly lasts about 40 weeks or slightly more than 9 months, pregnancy is divided into three trimesters [2]. In mice the pregnancy lasts about (20-22) days, the period of pregnancy in mice is divided into first, second and third weeks. Body organs including liver corresponds to pregnancy in form of special adaptive changes [3].

The liver is the largest gland in the body of humans, vertebrates and other animals, its color is reddish to brown and contain four lobes of unequal size and shape in both human and mouse [4].

The mouse liver consists of four major lobes with a variable location pattern. The fibrous capsule of the liver projects connective tissue septa into the liver tissue, dividing the liver lobes into indistinct lobules with the central vein, in the middle and at the corners the portal triad (consisting of branches of the hepatic artery, the portal vein, and the bile duct) [5]. Liver parenchyma consists of hepatocytes, large polygonal cells with large central nuclei, arranged in cords, in between the hepatocyte cords are sinusoids lined by fenestrated endothelium. The Kuppfer cells, liver-resident macrophages. The apical surfaces of adjacent hepatocytes form the bile canaliculi, which join to form bile ducts lined by cuboidal epithelium [6]. Several studies that used mice as a model research on liver structures and functions assumed that there are wide similarities between the humans, mouse and rat liver [7],[8]. The liver has two main sources of blood, a hepatic portal vein and a hepatic artery [9], [10]. The pregnant woman experiences physiological changes to support fetal growth and development. In normal pregnancy, ultrasonographic examination reveals no dilatation of the biliary tract. The lithogenic or cholesterol saturation index of bile increases during pregnancy, which is considered as a cholelithogenic state [11].

The present study results of an empirical study of normal mouse liver, with emphasis on identifying the constituent changes in the population of cells in the liver during pregnancy and determining essential fine structural features of these cells.

2. Materials and Methods

Experimental animals and housing

The current study was conducted on thirty mature females albino mice *Mus musculus* collected from animal house in the AL-Nahrain university at 2016 AD. The mice (body weight average between 20- 30 gm and the age 8-10 weeks kept under conventional condition in acclimatized room with free access to water (fresh tap water) & food (standard pellet diet) and maintained under a 12h light :12h darkness cycle and constant temperature (22 ± 2 ˚C).The mice were divided into two groups:

1) **Group (A):** Control group has 15 mature, non- pregnant female mice.
2) **Group (B):** Pregnant 15 female mice between 15-19 gestations.

Each one female mouse had been placed in cage with one healthy male mouse and checked each morning until vaginal plug was found. The first day of gestation was the day after the formation of vaginal plug.

Sampling and tissue Preparation

The animals were anaesthetized with Ether; dissection was done to remove the liver under anesthesia and the animals were left to die by deep anesthesia. Under deep anesthetize the pregnant mice abdomen were dissected and the liver separated and put in containers , then the animals will be left to die by deep anesthesia .The sample became ready for histological preparation of fixation, dehydration, clearing, paraffin embedding and sectioning dewaxing and hydration. All according to (Bancroft *et al.*, 2013) [12].

3. Results

Analysis of the fine structures of the parenchyma of the liver showed different types of cells, the most numerous type of them was hepatocyte, other cells are considerd as non paranchymal cells including endothelial cells of liver sinusoid , Kupffer cells (liver macrophage) and Ito cells(liver satellite cell) (Figure: 1).

Examination of hepatocytes fine structures under the transmission electron microscope showed that they are large cells with large rounded nucleus and often binucleated or trinucleated (Figure: 2). The destinective feature of hepatocyte nucleus was the presence of heterochromatin usually situated adjacent to the external nuclear membrane very few apaptosis cells was seen (Figure: 1& 2).

Cytoplasm organelles of hepatocytes included a wide distribution of smooth endoplasmic reticulum, very few rough endoplasmic reticulum, large number of mitochondria, and numerous glycogen granules distributed all over the cytoplasm (Figure: 3A,B& 4).

Adjacent hepatocytes are separated by a very narrow space and these spaces wideness in certain location to form what's called the hepatic canaliculi (Figure: 5A). The space of disse is found to be located at the liver hepatocyte surface facing endothelial cells of liver sinusoid this space is usually folded with numerous microvilli.

In regard to the differences in the ultra structural elements of hepatocytes between pregnant and non-pregnant groups the following differences founded:
1. The number of nucleoli per cell nucleus was seen to be more in pregnant group (Figure: 5B).
2. The number of glycogen granule was seen less in pregnant group.

Other wise all other ultra structural of features was the same between to the two groups (Figure: 6 A&B). Regarding the non-parenchymal cells in this study there types of non-paranchymal cells, were identified.

Kupffer cells was the type seen usually near by or inside the liver sinusoid as a dark often irregular darkly stained cell which often was folded with very short pseudopodia. Kupffer showed large and often indented nucleus small dark rounded cytoplasmic numerous structures were seen representing large number of lysosomes (Figure: 6 A).

The other type of non -parenchymal cell which is most frequently seen is the endothelial cells; these cells have a very unique appearance of an elongated type of cell with a flat long nucleus. The endothelial cell is usually separated by very narrow space from the surrounding the hepatocytes this space seen to be narrower in pregnant than in non-pregnant group, the endothelial cell nucleus in pregnant group was more heterochromatic than those non-pregnant group and the amount of cytoplasm was more abundant (Figure: 7 A&B).

The third type of non-parenchymal cell is the Ito stellate cells usually located between hepatocyte and liver sinusoids they are small elongated cells and this cell is often contain fat droplets in their cytoplasm (Fig: 8).

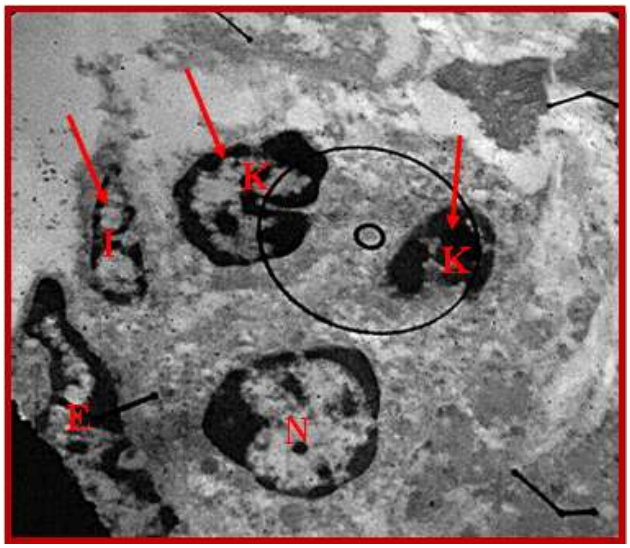

Figure 1: Section in the liver showing the nuclei of hepatocyte (N) Kupffer cell (K) (macrophage cell), endothelial (E) cell and Ito cell (I). TEM, pregnant group, Urinal Acetate. 10500).

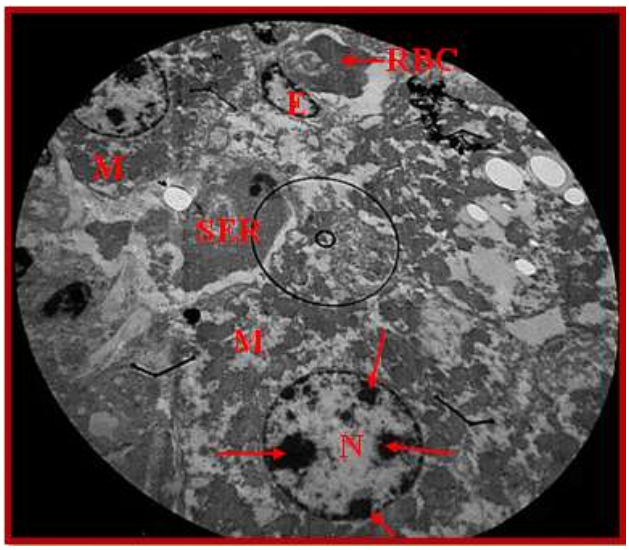

Figure 2: Section in the liver showing binucleated with chromatin arranges near the external nuclear membrane (H), RBC cell, endothelial cell of vessels (E),smooth endoplasmic reticulum (SER) and mitochondria (M). TEM, pregnant group, Urinal Acetate., 5800).

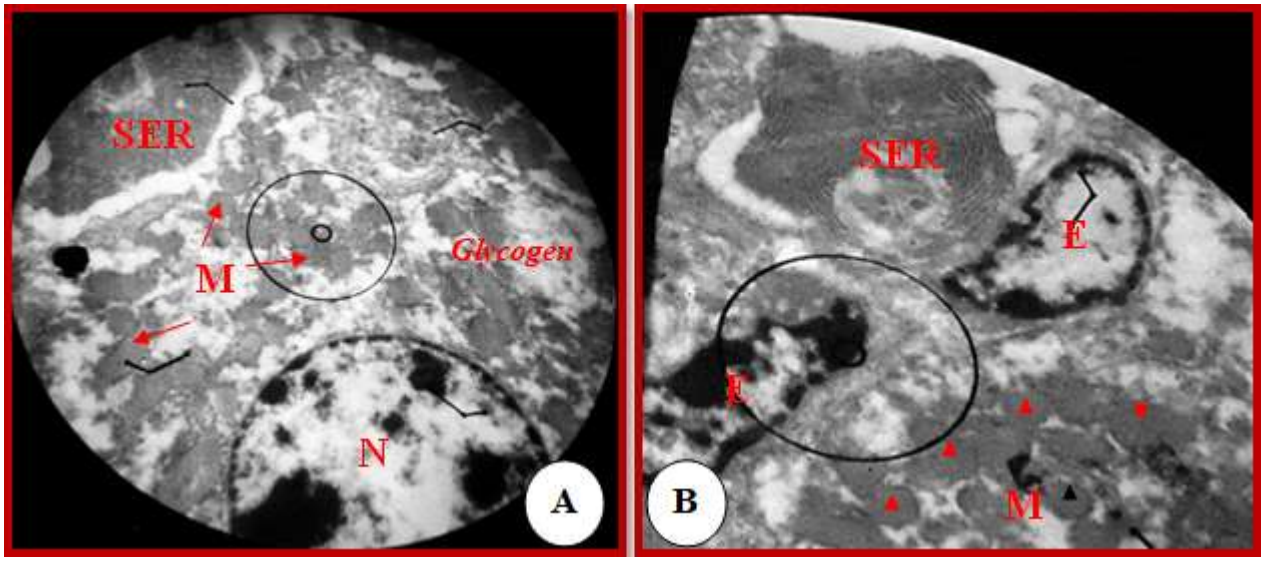

Figure 3: A Section in the liver showing the large number of mitochondria (red arrow) and nucleus of hepatocyte (N) .TEM, pregnant group, U.A, 14500. **B**: Showing the smooth endoplasmic reticulum (SER), endothelial cells (E) and number of mitochondria (M). TEM, pregnant group, Urinal. Acetate, 10500).

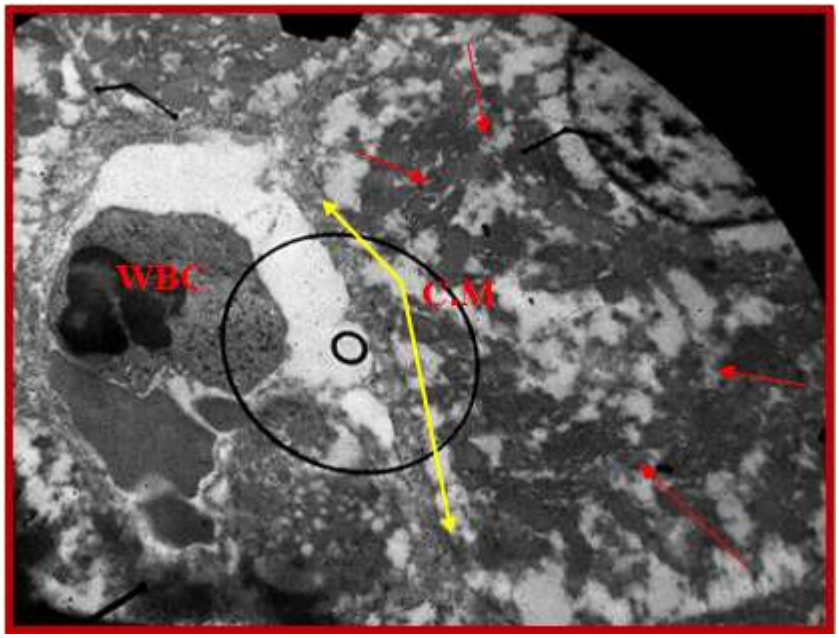

Figure 4: Section in the liver showing the large number of glycogen granules (red arrow), Cell membrane (C.M) and WBC. TEM pregnant group, Urinal. Acetate,10500).

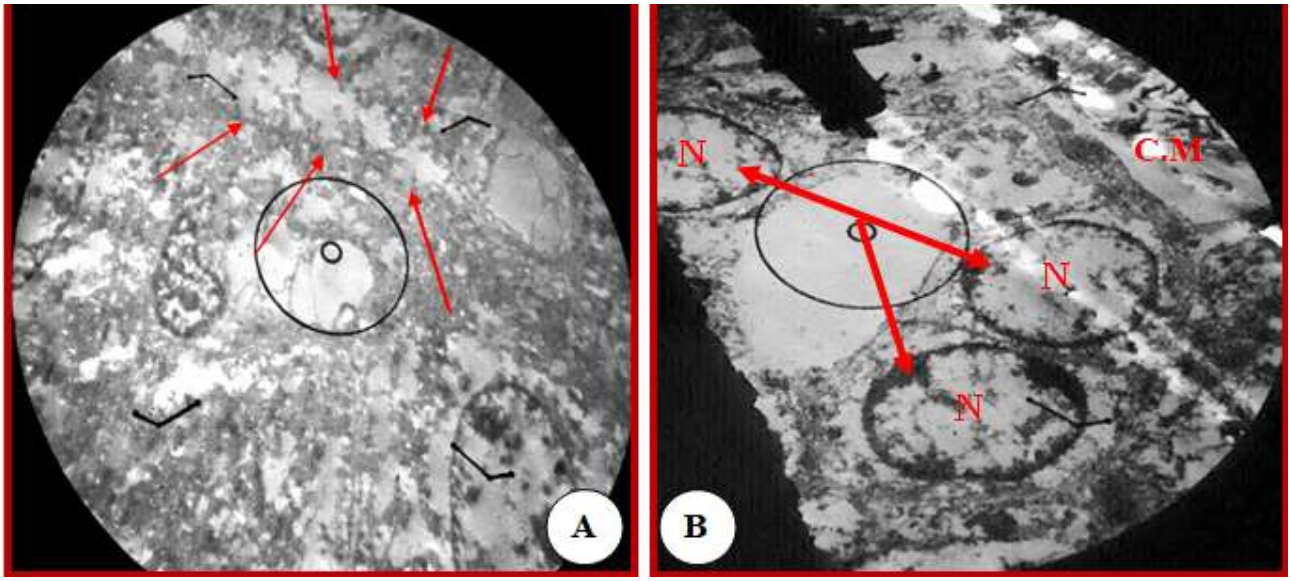

Figure 5: A: Section in the liver showing the bile canaliculi (red arrow), nucleus of hepatocyte (N) and Kupffer cell (K). TEM pregnant group, U.A, 8500). **B**: Showing the number of nucleoli to per nucleus (N) and the triple nucleus of the hepatocyte cell (red arrow) and cell mrmbrane (C.M). TEM, pregnant group, Urinal. Acetate, 10500).

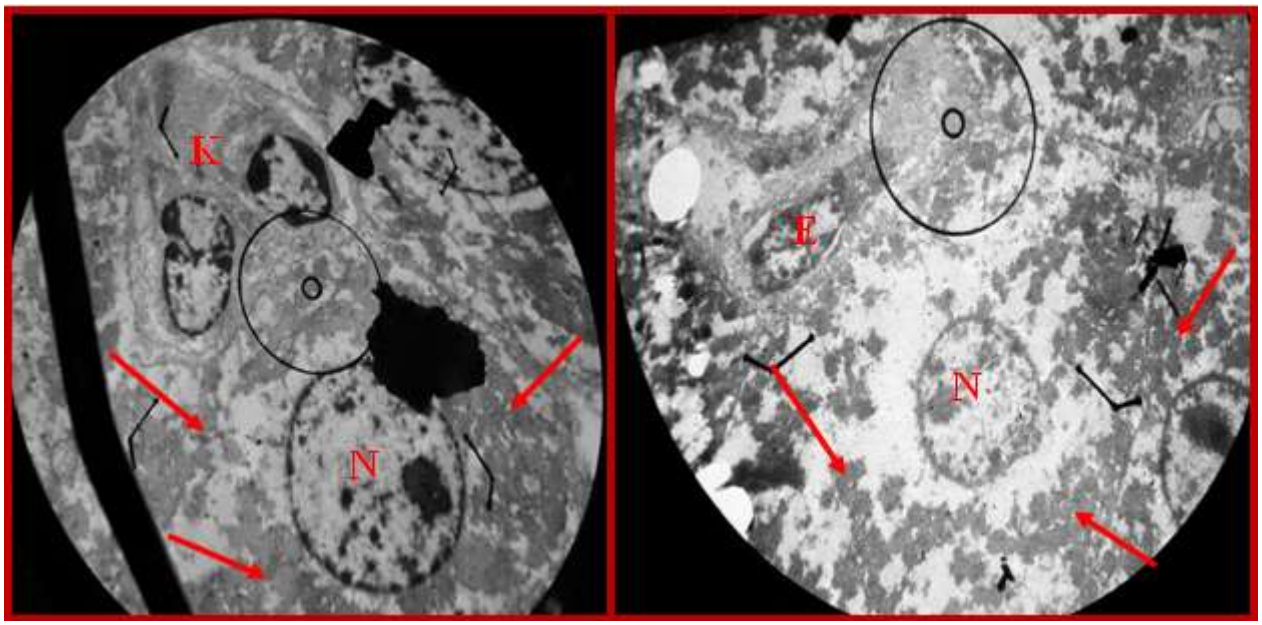

Figure 6: Section in the liver showing the ultastructure **A:** The Kupffer cell (macrophage cell) (K), and large number of mitochondria (red arrow). TEM, pregnant group, U.A, 5800), **B:** Showing the endothelial cell (E), nucleuos of hepatocyte (N) and large number of glycogen granules (red arrow). TEM, control group, Urinal. Acetate, 4800).

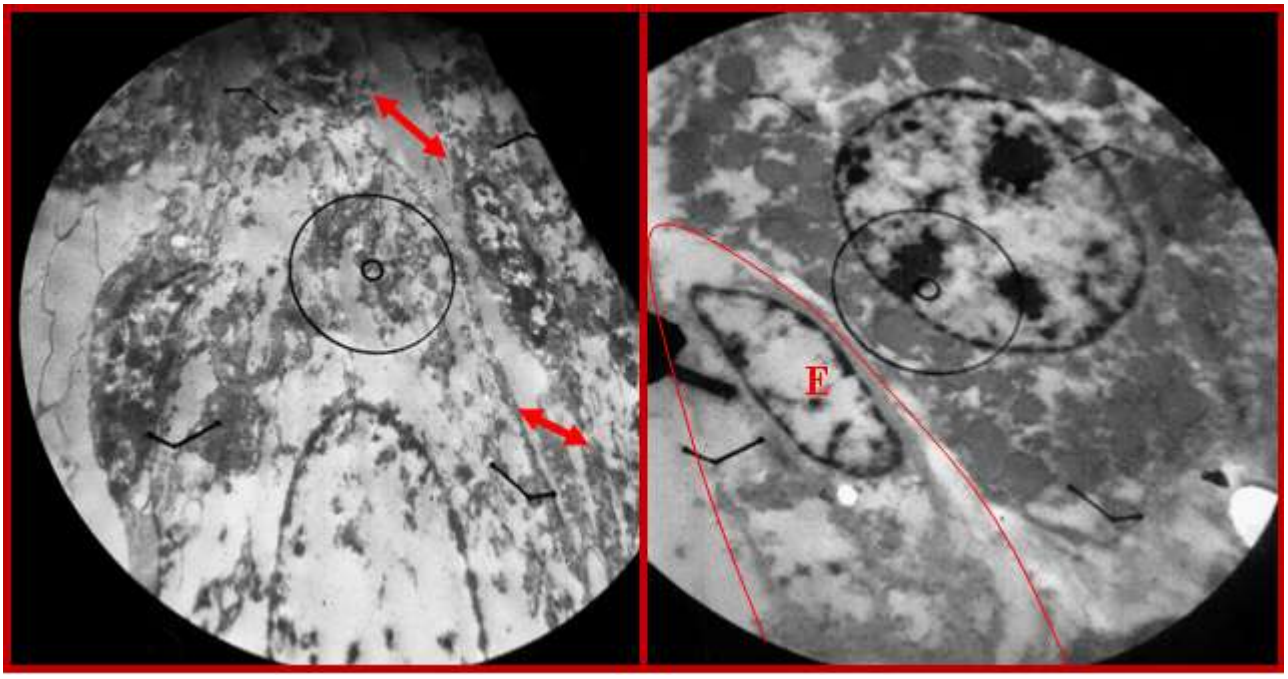

Figure 7: A: Section in the liver showing the wide space between two hepatocyte cell (red arrow). TEM, pregnant group, U.A, 2600), **B:** Showing the flat nuclei of endothelial cell (E). TEM, pregnant group, Urinal. Acetate,10500).

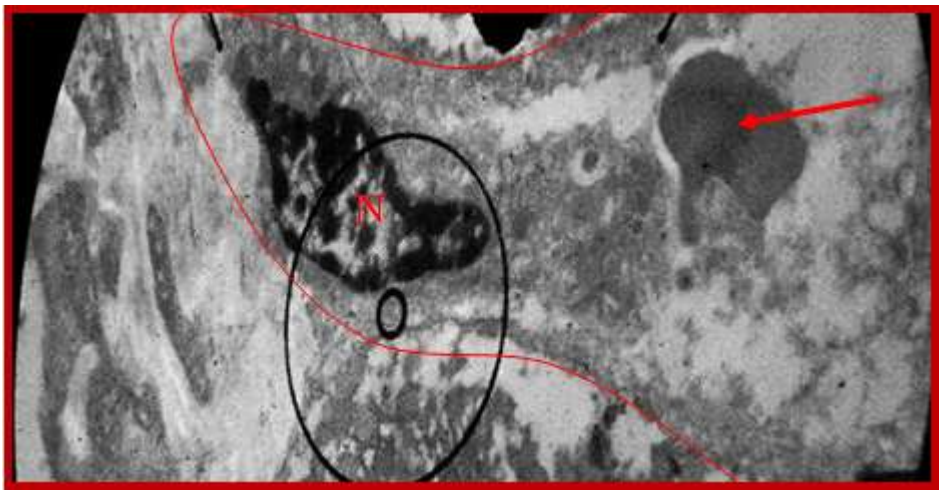

Figure 8: Section in the liver showing the Ito cell, nucleus of Ito cell (N) and fat droplet (red arrow). TEM, pregnant group, Urinal. Acetate, 10500

4. Discussion

The utilization of a technique such as TEM in the studying of liver morphological structures forms a great contribution to our knowledge regarding fine structures in the liver as it provides more detailed and accurate description for these structures.

TEM examinations in this study showed that many hepatocytes contained vesicles of differing size and shape this represented glycogen. Other changes such as proliferation of SER and accumulation of lipid droplets were also observed, other organelles including mitochondria and RER were easily detected. Ultra structural analysis of hepatocytes displayed fragmentation of RER and swollen mitochondria with disrupted cristae. The nuclear membrane was irregular with few dense heterochromatins' surrounding the nucleoli. Our observations demonstrated that EMR may adversely affect rat liver but the mechanisms involved need to be determined [13].

The fine structural features of mouse hepatocytes are like those announced for different species, large round nuclei, and occasionally two nuclei, are prominent, cytoplasm is occupied mainly by mitochondria, both smooth and rough endoplasmic reticulum, and scattered glycogen particles are present and numerous. As in other mammalian species, the apical portion of hepatocytes is associated with canaliculi; canaliculi are formed by a widening of the intercellular space, although the canalicular lumens are set off from the intercellular space by occluding junctions. Small profiles of Golgi apparatus were seen occasionally in the apical cytoplasm close to canaliculi [14], this study did not record the presence of supranucleur Golgi but wide intercellular space and wide space of diss was noted in pregnant group.

The present analysis indicated that a considerable portion of hepatocytes are double nucleated. Another approach taken in the present studies was to dissociate liver cells. This analysis yielded higher numbers, of approximately 35% double nucleated cells. These results are in agreement with other recent studies [15] that have reported that the population of hepatocytes includes many double nucleated cells. The functional significance of double nucleated cells, however, is not clear. No evidence was revealed to indicate that any other cell type within the liver included double nucleated cells.

Monocyte derived macrophages are found in virtually every organ and tissue of the body, and comprise the diffuse reticulo-endothelial system. In the liver these macrophages are termed Kupffer cells. Although originally, these phagocytic cells were likely confused with the stellate cells, later studies demonstrated that Kupffer cells can be identified by their ability to phagocytose tracer substances, including carbon, India ink, or latex microspheres, and also by their immunoreactivity to the F4-80 antibody and electron microscopic studies demonstrated this antibody labels a cell surface marker. Further, the results from studies of double labeling with fluorescently labeled latex microspheres and also from immunocytochemistry have demonstrated conclusively that the Kupffer cells are a population of cells distinct from the Ito stellate (fat storing) cells [16].

Kupffer cells are not distributed homogenously in the liver and appear to show some variation in regard to their phagocytic activity, it have been reported that Kupffer cells are more frequently encountered and are larger in regions around the portal areas than around the central venules. The present data corroborate this finding in the mouse, although the regional differences in the mouse liver appear not as great as the regional differences reported for rat liver [17].

Ito stellate cells are fat storing cells of the liver. Stellate cells are identifiable by their fine structural features of prominent intracellular lipid droplets and by cytoplasmic filamentous material. The intracellular filamentous material likely forms the basis of their immunoreativity to GFAP and to desmin. Quantitative estimates indicated that numbers of Ito stellate cells in rat liver were about 10–12% of hepatocytes. Further, reported that stellate cells were found more frequently in peri-portal areas than in pericentral areas. The present study noted that stellate cells were not distributed homogenously throughout the liver, but a consistent pattern between peri-portal and peri-central regions was not detected [18].

Endothelial cells are an important cell type in any organ, and certainly so in the liver. Liver endothelial cells are specialized, with the presence of fenestrations that appear aggregated into groups that form ‘sieve plates’. The very sparse nature of a basal lamina beneath the endothelial cells, along with the absence of diaphragmatic coverings of the fenestrations, allows for apparent relatively free movement of small molecules (less than 125 nm diameter) between the space of Disse and the capillary lumen [19].

5. Conclusion

Ultrastructural changes including high number of mitochondria, high number of nucleoli per cell nucleus, the nucleus of hepatocyte presence heterochromatin in pregnant group and the glycogen granules decreased in pregnant group compare with non-pregnant group.

References

[1] Arm, Fleischman; Oinuma, M.; Clak, S. I. Rethinking the definition of “Term Pregnancy”. Obstetric Anesthesia Digest, 2011, 31.3: 144-145.
[2] Lakshmi, B. N.; Indumathi, T. S.; Ravi, Nandini. An Hybrid Approach for Prediction Based Health Monitoring in Pregnant Women. Procedia Technology, 2016, 24: 1635-1642.
[3] Suckow, M. A.; Dannemann, P.; Brayton, C. The Laboratory Mouse (a volume in the laboratory animal pocket reference series). 2001.
[4] Junqueira, Luiz Carlos; Carneiro, José. Basic histology text and atlas. London: McGraw Hill, 2005, 2005.
[5] Tortora, Gerard J.; Derrickson, Bryan H. Principles of anatomy and physiology. John Wiley & Sons, 2008.
[6] Conti, Claudio J., et al. Atlas of laboratory mouse histology. National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland ACVP, American College of Veterinary Pathologists, 2004.
[7] Robertson, Richard T., et al. Liposomes incorporating a Plasmodium amino acid sequence target heparan sulfate binding sites in liver. Journal of pharmaceutical sciences, 2008, 97.8: 3257-3273.
[8] Longmuir, Kenneth J., et al. Effective targeting of liposomes to liver and hepatocytes in vivo by incorporation of a Plasmodium amino acid sequence. Pharmaceutical research, 2006, 23.4: 759-769.
[9] Agur, Anne MR; Dalley, Arthur F. Grant's atlas of anatomy. Lippincott Williams & Wilkins, 2009.
[10] Pannarale, Luigi, et al. Three-dimensional organization of the hepatic artery terminal branches: a scanning electron microscopic study of vascular corrosion casts of rat liver. Italian journal of anatomy and embryology= Archivio italiano di anatomia ed embriologia, 2006, 112.1: 1-12.
[11] Everson, Grecory T. Liver problems in pregnancy: distinguishing normal from abnormal hepatic changes. Medscape women's health, 1998, 3.2: 3-3.
[12] Bancroft, J. D., Floyd, A. D., & Suvarna, S. K. Bancroft's Theory and Practice of Histological Techniques, 2013, 53, 83, 93, 105-121, 433-517.
[13] Holovská, K., et al. Structural and Ultrastructural Study of Rat Liver Influenced by Electromagnetic Radiation. Journal of Toxicology and Environmental Health, Part A, 2015, 78.6: 353-356.
[14] Bedossa, Pierre; Paradis, Valérie. Liver extracellular matrix in health and disease. The Journal of pathology, 2003, 200.4: 504-515.
[15] Gupta, Sanjeev. Hepatic polyploidy and liver growth control. In: Seminars in cancer biology. Academic Press, 2000. P. 161-171.

[16] Bouwens, Luc, and et al. Liver cell heterogeneity: functions of non-parenchymal cells. Enzyme, 1992, 46: 155-168.
[17] Sun, Zhaoli, et al. Hepatic allograft-derived Kupffer cells regulate T cell response in rats. Liver transplantation, 2003, 9.5: 489-497.
[18] Sato, Mitsuru; Suzuki, Shinsuke; Senoo, Haruki. Hepatic stellate cells: unique characteristics in cell biology and phenotype. Cell structure and function, 2003, 28.2: 105-112.
[19] Pavelka, Margit; Roth, Jürgen. Functional ultrastructure: atlas of tissue biology and pathology. Springer, 2015.