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Study the Effect of different doses of Rapamycin on the liver development in the Swiss Albino Mice Embryos

Sada Ghalib Taher Al-musawi[1*], Ali Naeem Salman[1], Nahla A. Al-Bakri[2]

University of Thi-Qar College of Education for pure sciences Department. of Biology[1]
University of Baghdad, College of Education for Pure Science (Ibn Al-Haitham), Department of Biology[2]

Corresponding Author: 1*

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ABSTRACT

The current study, which extended from February 2020 to June 2021 at the University of Thi- Qar\ College of Education for Pure Sciences, aimed to follow the changes in external morphological features at different Embryonic Developmental stages in pregnant mice treated with different doses of Rapamycin (Rapa). Use In this study, 32 pregnant mice were divided randomly into four groups, each of which had eight pregnant mice. Each group received different dose of Rapa via intraperitoneally injection at different gestation days until the end of the specified periods, whereas the control group received a DMSO. Mice were administered under the same circumstances and dosages were determined based on body weight, as specified in pharmaceutical constitutions. The results demonstrate that different doses of Rapa caused various alterations such as a megakaryocytes around, hepatocytes with pyknotic nuclei, inflammation cells, isolated foci of hepatic cell necrosis, marginated chromatin in some nuclei, giant cells also observed, karyorrhexis and karyolysis were observed in necrotic nucli during histopathological examinations of mice embryos' liver. The findings of the current study revealed the fact that treatment of the early embryo by Rapamycin results in a histological changes demonstrating that rapamycin has teratogenic activity on embryo.

1. INTRODUCTION

Rapamycin (Rapa), also known as Sirolimus, was identified in an Easter Island soil sample in the early 1970s as a macrolytic location produced by the soil bacterium *Streptomyces hygroscopicus* (also known as Rapa Nui). [18] discovered that Rapamycin had immunosuppressive and antiproliferative effects in mammalian cells. Because of its toxic, anti-proliferative actions in yeast, it was found. About a decade after its development, it began to show anti-tumor effects in vivo. In yeast and mammalian cells, the target of Rapamycin (TOR) was discovered at the same time [14].

FK506-binding protein (FKBP12), a 12-kDa peptidyl-prolyl-isomerase that binds to the TOR1 and TOR2 mediators, forms a gain-of-function complex with Rapamycin [4]. The mammalian (or mechanistic) target of Rapamycin (mTOR) is a protein serine/threonine kinase that assembles into two structurally and functionally

different multi-protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [39].

The liver is the largest internal organ providing essential metabolic, exocrine and endocrine functions. These include production of bile, metabolism of dietary compounds, detoxification, regulation of glucose levels through glycogen storage and control of blood homeostasis by secretion of clotting factors and serum proteins such as Albumin [45].

The endoderm germ layer is established during gastrulation and forms a primitive gut tube that is subdivided into foregut, midgut and hindgut regions, fate mapping studies in the mouse embryo at day 8.0 of gestation (G8.0) indicate that the embryonic liver originates from the ventral foregut endoderm [41]. The first morphological sign of the embryonic liver is the formation of the hepatic diverticulum, an out-pocket of thickened ventral foregut epithelium adjacent to the developing heart at G9.0, the anterior portion of the hepatic diverticulum gives rise to the liver and intrahepatic biliary tree, while the posterior portion forms the gall bladder and extrahepatic bile ducts. At G9.5, the hepatic endoderm cells, known as hepatoblasts delaminate from the epithelium and invade the adjacent septum transversum mesenchyme (STM) to form the liver bud [15]. The STM contributes fibroblasts and stellate cells of the liver. Between G10–15 the liver bud undergoes a period of accelerated growth as it is vascularized and colonized by hematopoietic cells to become the major fetal hematopoietic organ [45].

2. Methods

2.1 Experimental animals preparation

Female Albino Mice, type Mus masculus, strain Balb /c, aged 11 to 12 weeks, were used in this study.30 2 gm obtained from the Animal House were returned to the Biology Department - College of Education for pure science / Thi- Qar University, Pregnant mice were placed in a room in plastic cages with metal lids and brushed with sawdust, in a well-organized and controlled environment with a constant Photoperiod (12 hour day/12 hour night) cycle, ventilation, and temperatures ranging from 20 to 24 C°. Mice were taken to ensure their health and free from diseases the vet and kept in clean cages with sawdust changes every two days, mice were kept under cleanliness conditions of the cages through a change sawdust once every two days, Animals were given a sufficient amount of water and food locally source (Wheat 34% , corn 25% ,barley 20% , powdered milk 10%,animal protein 10% , salt 1% all material grinding and mixing with some oil and water until they become a paste coherent) [40] and put in the designated place for the food in the cages, Animal breeding, then two adult females were confined together with one mature male overnight, and the females were checked for the presence of a vaginal plug on the next morning [38], On the cages, the date of mating was written which is Day zero (D0) of pregnancy, and the next day is the first day of pregnancy [3].

2.2 Rapamycin preparation

Pregnant mice were injected with therapeutic dose (1.5 mg to 1 kg) [42], 0.75 and 3 mg\kg Rapa via Intraperitoneal injection

2.3 Design of the study

The present study include 16 pregnant, divided into two groups according to the following arrangement:
1- The control group: consisting of 4 pregnant mice that were treated with DSMO by the Intraperitoneally injection.
2- Experimental groups: consisting of 12 pregnant mice for each group, they were treated with different doses of drug as follows:

- The first sub group: was treated with the dose 0.75 mg for each 1kg from the body weight.

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- The second sub group: was treated with the dose1.5 mg for each 1kg from the body weight.
- The third sub group: was treated with the dose 3mg for each 1kg from the body weight.

The pregnant mice injection with Rapa daily from the day eighth of pregnancy, the embryos were collect and dissected out at Embryonic day 16.

2.4 Isolate of the mice embryos

The pregnant mice, the pregnant mice were soaked in 70% ethanol to diminish the risk of contaminating the dissection with mouse hair. The skin was pinched and makes a small lateral incision at the midline with regular surgical scissors. The skin was Holed firmly above and below the incision and pulled apart toward the head and tail to expose the abdomen. The peritoneum was grasped with forceps and cut to expose the abdominal cavity. The reproductive organs located in the dorsal region of the body cavity, two uterine horns, the oviduct and the ovaries. The uterine horn was removed by grasping the uterus below the oviduct and cut it free along the mesometrium. Each embryo was separated by cutting between implantation sites along uterine horns. Sliding forceps were used to grab the muscular uterine lining between the surrounding muscle layer and the enveloped decidua tissues. The muscle layer was removed and a part of the decidua exposed, after which the embryo was shelled out with forceps tips and removed, then we examine the malformation and the changes after that we document results, then take a them a photograph by using a camera. The embryos were then preserved in a jar containing 10% formalin.

3. The Results

liver development at the day 16th of Gestation development (E16). Embryos' liver at gestation day 16 larger in size and more specific (Fi1A). Hepatoblasts had a larger, pale staining nucleus, whereas erythropoietic cells had increased in number, the majority of which were erythroid lineage and could be identified by intense, hyperchromatic nuclei and many of the hematopoietic foci at this developmental time took cord-shaped; in contrast, erythropoietic cells had increased in number, as the relative amount of hepatic cells increased, the sinusoids became smaller than in the preceding stage (E 13) (Figure1B).

Involuted hematopoietic foci were forced to migrate from inter-hepatocytic gaps to persinusoidal space as hepatocyte volume expanded rapidly. Hepatocytes grouped in single cell sheets known as hepatic plates surrounded the incomplete portal sections, separated by sinusoidal spaces connected to a network of blood vessels capillaries. There were still a lot many megakaryocytes around (Figue1B).

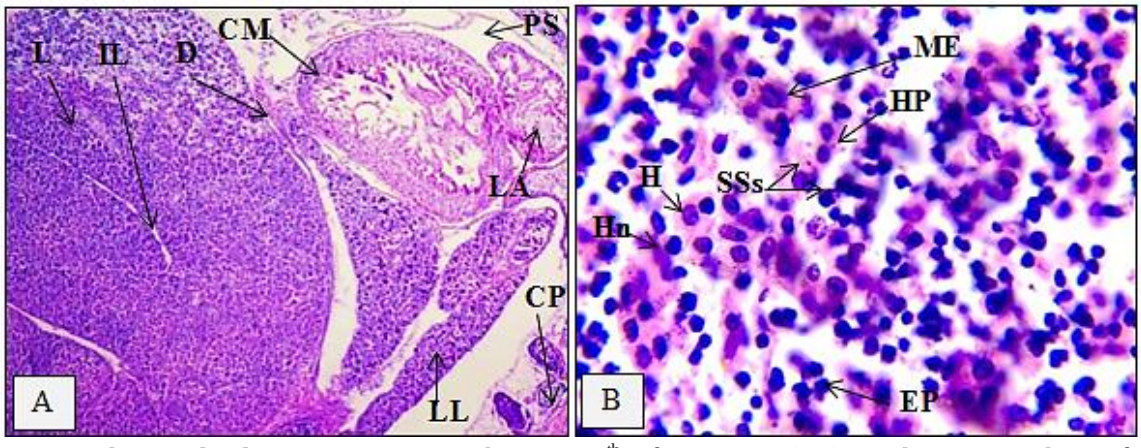

Figure 1: A:longitudinal section to mice embryo at 16th of gestation.(CP) cartilage primordium of ribs, (CM) Compact Myocardium,(D) diaphragm,(IL) Intraloubular space,(LA) lumen of atrium of heart,(L)

Liver (LL)lobes of the lung, (PS) pericardial space, (H&E)(4x).B:(EP) erythropoietic cell,(H) Hepatocyte,(Hn)Hyperchromatic nuclei,(HP) Hepatic plate,(SSs) Sinusoidal Spaces,(M) Megakaryocytes(H&E)(100x).

Examinations of the liver histopathology to mice embryo at 16th of gestation day treated with (0.75mg\kg Rapamycin) embryos of Treated group with 0.75 mg/kg of Rapamycin shows sinusoidal dilatation and cytoplasmic vacuolation, hepatocytes with pyknotic nuclei, Enucleated erythrocytes and megakaryocytes detected throughout the liver parenchyma Fig(2B).

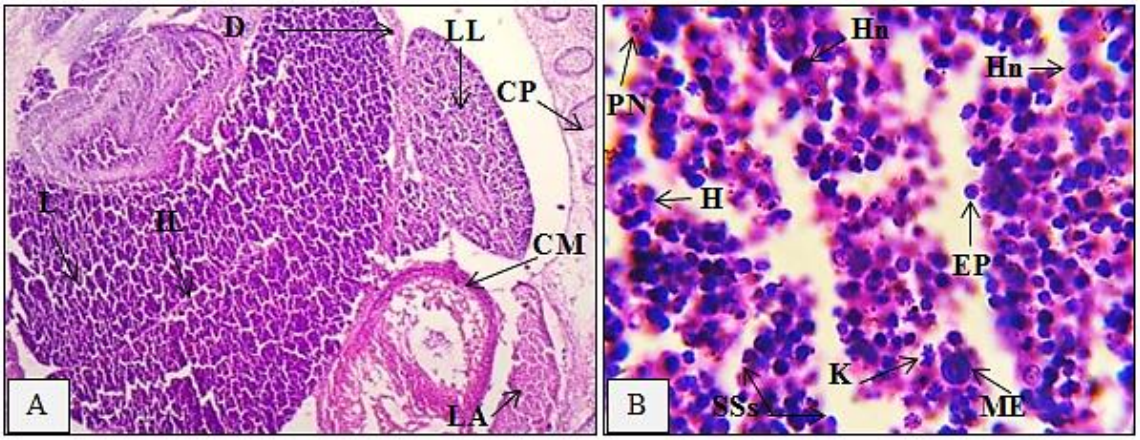

Figure 2: A:longitudinal section to mice embryo at 16th of gestation day treated with (0.75mg\kg Rapamycin).(CP) cartilage primordium of ribs, (CM) Compact Myocardium,(D) diaphragm,(IL) Intraloubular space,(LA) lumen of atrium of heart,(L) Liver (LL)lobes of the lung, (PS) pericardial space, (H&E) (4x).B:(EP) erythropoietic cell,(H) Hepatocyte,(Hn)Hyperchromatic nuclei,(PN) pyknotic nuclei,(SD) sinusoidal dilatation,(SSs)Sinusoidal Spaces,(M) Megakaryocytes (H&E)(100x).

There were developmental anomalies occurs to embryos at 16th of gestation day treated with (1.5mg\kg Rapamycin) include the compression of the chest and abdomen towards the back, causing the liver to drop from its normal position (Figure3A). The histological investigations, marginated chromatin was also identified in certain nuclei, giant cells, and isolated foci of hepatic cell necrosis, in addition to sinusoidal dilatation and cytoplasmic vacuolation (Figure 3B).

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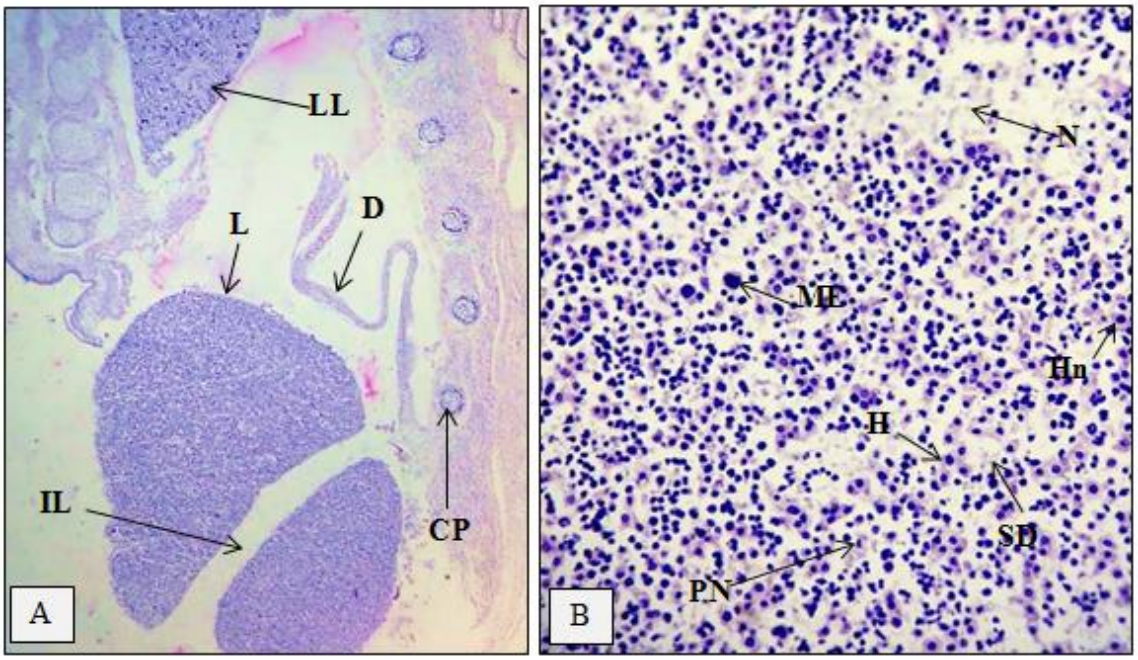

Figure 3: A:longitudinal section to mice embryo at 16th of gestation day treated with (1.5mg\kg Rapamycin).(CP) cartilage primordium of ribs, (D) diaphragm,(IL) Intraloubular space,(L)Liver (LL) lobes of the lung (H&E) (4x). B:(EP) erythropoietic cell,(H) Hepatocyte, (Hn) Hyperchromatic nuclei,(PN) Pyknotic Nuclei,(SD)sinusoidal dilatation,(M) Megakaryocytes, (N)Necrosis (H&E) (40x).

There were still a megakaryocytes around, hepatocytes with pyknotic nuclei, inflammation cells, isolated foci of hepatic cell necrosis, marginated chromatin in some nuclei, giant cells also observed, karyorrhexis and karyolysis were observed in necrotic nucli during histopathological examinations of mice embryos' liver at this group (Figure 4).

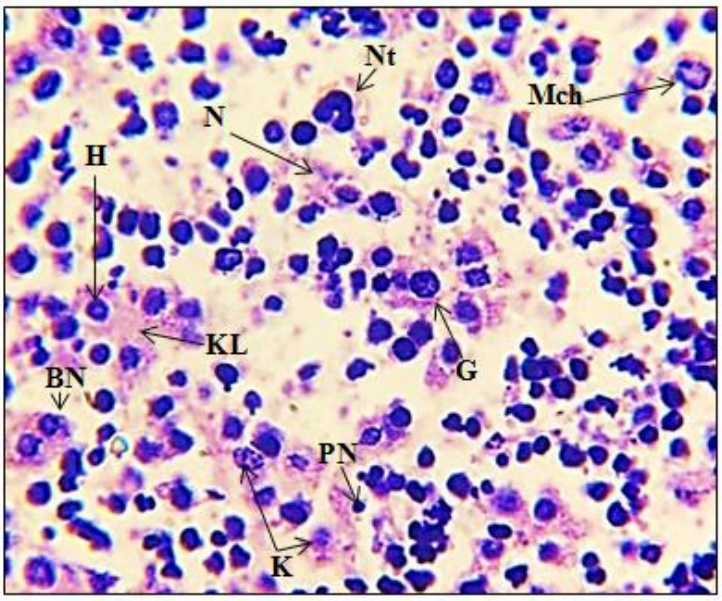

Figure 4: longitudinal section to mice embryo liver at 16th of gestation day treated with (1.5mg\kg\Rapamycin).(BN)Binucleated Hepatocytes,(G)Gaint cells,(K)Karyorrhexis,(KL) Karyolyis (N)Necrosis of hepatic Cell,(Nt)Neutrophilic cell ,(Mch) marginated chromatin nucleUS,(PN) pyknotic nuclei, (H&E) (100x).

There were no evedents about liver development at gestation day 16th because the embryos were die inside mothers uterus during the mothers treatment with 3mg\kg of Rapamycin (Figure5).

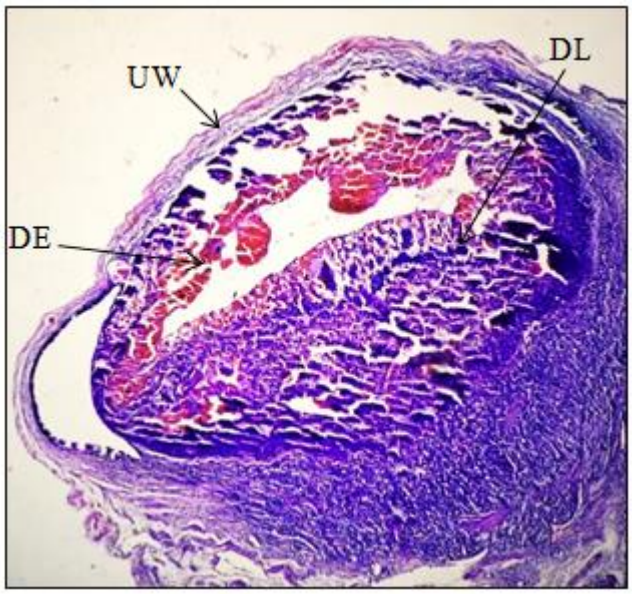

Figure 5: Lateral section to uterus at gestation day 16th from mother treated with (3 mg\kg Rapamycin) (DE) Dead Embryo, (DL) Distrupted Labyrinth, (UW) Uterus wall (H&E) (4x).

4. Discussion

The cells metamorphosis and follow a particular programming in their growth during the first three months of pregnancy in humans, which corresponds to the first week in pregnant women [16]. Any organ's most crucial period is when it is growing and forming its many structures [9]. Because this period will be extremely vulnerable to changing influences, it has been termed the critical period for organs and tissues [17]. The results of the current study have showed the occurrence of many changes and phenotypic malformations in the embryos which are treated with different doses of Rapamycin.

By conducting a literature review on the normal development of the liver of a mammalian embryo and fetus [11]. The liver in this study at E13 was mostly made up of hepatic cords (composed of undifferentiated hepatoblasts), hepatic cords were seen in layers, with big, basophilic nuclei and many nucleoli tightly packed together, making individual cell shape difficult to distinguish, at this stage, although Kupffer cells were thought to develop from bone marrow–derived monocytes in adults, their presence in the fetal liver preceded bone marrow development, and they may originate from the yolk sac, according to [8] who stated that although Kupffer cells were believed to develop from bone marrow–derived monocytes in adults, their presence in the fetal liver preceded bone marrow development, and they may originate from the yolk sac, there was also a lot of heterogeneity in the size and shape of hepatoblast nuclei as indicated before by [2].

Histopathological Examination for livers during current study shows various changes including hepatocellular damage involving necrosis in many nuclear changes Pyknosis, Karyorrhexis and karyolysis (E13 and E16 0.75mg\kg Rapa) that may be return to the inhibitory effects of Rapamycin on mTOR, maintaining hepatocellular homeostasis and suppressing hepatic injury, inflammation, and carcinogenesis require proper mTORC1 activity regulation, hepatocellular homeostasis is disrupted by either hyper or hypoactivation of mTORC1, resulting in liver injury, inflammation, and carcinogenesis [5].

Recent research suggests that specific internal and surface proteins are cleaved and/or released from

hepatocytes during these processes, piqueing interest in the creation of noninvasive markers to monitor liver cell death [6].

Rapamycin inhibits p70S6K activity, which reduces hepatic proliferation, It significantly improves hepatic that what was illustrated by [44]. In control hepatocytes, mTOR inhibitors administration showed a significant inhibitory effects on protein synthesis resulting in a 75% decrease that what was suggested by [12] when used metformin as an inhibitor of mTORC1 signaling their study demonstrates that metformin has a potent inhibitor of mTORC1 signaling and its control of protein synthesis in the liver.

Lipid metabolism, nucleotide metabolism, amino acid metabolism, vitamins, oxidative stress, carbohydrates metabolism, and corticosterone metabolism were the main pathways impacted by the mutation and/or therapy with low dosage Rapamycin, in the liver, these alterations were more pronounced [7].

Binucleation is caused by cell damage and the production of polyploidy, which is linked to cell regeneration, Dilated sinusoids and partial degradation of cytoplasm of the hepatocytes without clear borders between the cells were observed during administration of different doses of Rapamycin these results agree with what suggested. The endothelium's permeability may be disrupted, resulting in sinusoidal dilation [1].

The immunosuppresses drugs have a side effects causing apoptosis, which is a type of cell death that is ordered and genetically regulated. Chronic organ failure and/or morphological changes, such as chromatin condensation and marginalization, cell shrinkage, and plasma membrane blebbing, are all related with apoptosis. Because a significant number of intracellular proteases and endonucleases are activated, it can result in DNA fragmentation and the destruction of specific cellular proteins that what was concluded.

Rapamycin administration may cause oxidative stress through molecular pathways that result in hepatocyte cell death involved in initiating cell death, based on morphological criteria and ultimately lead to hepatic necrosis and liver injury.

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