Original Article

ISSN (0): 2635-0823; ISSN (P): 2635-0815

The Effect of Different Doses of Dexamethasone on The Hepatic Tissue of Pregnant Swiss Albino Mice Mus Masculus

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Abstract

The aim of the study is to see how Dexamethasone affects the liver of pregnant Swiss albino mice at various stages of development. The study included Fifty-four pregnant female mice that divided into three cohorts at random. There are 18 pregnant mice in each group. Each member was injected with different doses of Dexamethasone into a specific tail vein at different times. The control group, on the other hand, received standard saline injections. The mice were then killed at days 13, 15 and 17. And the results illustrated that when different dosages of Dexamethasone were used at different periods, the results demonstrated a negative effect on the liver of pregnant mice. These effects were stronger as the number and concentration of medication doses increased. Dexamethasone produces morphological abnormalities in the liver such as necrosis, vascular congestion, binucleated cells, and microabscesses involving hepatocytes and inflammatory cells. In conclusion, the use of Dexamethasone shows different changes in the liver tissue, including necrosis, Binucleated cells, vascular congestion and micro abscess when it was used for a long-term exposure or by repeating doses.

Keywords: Dexamethasone, Liver, pregnant Albino Mice Embryos.

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Received: 02 January 2024 **Revised: 25 February 2024** **Accepted: 09 March 2024** **Published: 31 March 2024**

Introduction

Glucocorticoids are a kind of steroid that is commonly used as an anti-inflammatory drug in individuals with allergies, autoimmune diseases, sepsis, asthma, and metabolic disorders. They can also be used in patients with malignancies.[1] One of the most popular steroid drugs used is a Dexamethasone (Dex.) which represents a long-acting synthetic glucocorticoid hormone that has 25 times stronger glucocorticoid effects than cortisol.[2] However, this class of drugs has a number of side effects, including hyperglycemia, which can result from increased insulin resistance and reduced glucose tolerance, leading to steatosis and fatty liver.[3]

The liver is the most effective organ for identifying toxicity and oncogenicity in Rodents.[4] According to previous studies, the hepatic lobule and the acinus represent the liver's structural and functional unit, respectively.[5,6] The hepatic lobule which represents a structural unit, is modelled on the blood flow inside the liver and itis frequently used for descriptive pathological and morphological diagnostics. The hepatic acinus, a functional unit, is based on blood flow and metabolism in the liver.[7] Many cell types, including endothelial, biliary, Kupffer, fat-storing, Ito (stellate), and pit cells, as well as hematopoietic cells in the sinusoids and blood arteries, make up the liver.[7] Polyhedral hepatocytes comprise around 60% of the liver biliary cells that create bile ducts in the portal sections and make up the portal triad, which also includes a hepatic artery and a portal vein.[8] The sinusoids are lined with fenestrated endothelial cells that produce prostaglandins. Kupffer cells are a type of self-renewing fixed macrophage that make up around 10% of all liver cells.[9] These cells produce inflammatory mediators and catabolize lipids and proteins.[8] Perisinusoidal cells called stellate cells have the ability to store vitamin A and represent a primary collagen source in the liver.[10] In addition, Pit cells, lymphocytes with natural killer activity, are found largely in the periportal area.[11] The goal of this study was to look at the histopathological effects of Dexamethasone on the livers of pregnant mice.

Subjects and Methods

Ethical statement: All experimental animal handling procedures were carried out, in accordance with guidelines authorized by the University of Southern China's Institutional Animal Care and Use Committee (No. 2014)12.

Experimental animals' preparation: Pregnant mice, Mus masculus, strain Balb/c, 11-12 weeks old and 30± 2 grams were used in this study. They were obtained from the animal house at the College of Education for Pure Science, Thi-Qar University, Iraq. The mice were housed in plastic breeding cages with metal covers and sawdust. They were kept in an organized and controlled environment with a ventilation, continuous photoperiod (12-hour through day/12hour through night) cycle, and temperatures ranging from 20- 24 C.[12]

A veterinarian examined the mice to ensure that they were healthy and free of diseases. Cleaning the cages and replacing the sawdust every two days helped the mice maintain optimal sanitary and environmental conditions. They were provided

with enough water and food, from a nearby source (34 %Wheat, 20 % barley, 25 % corn, 10 % animal protein, 10 % powdered milk, 1 % salt). These materials were crushed and mixed with oil and water until a cohesive paste was formed.[13] Two adult females were placed in each cage with one mature male overnight, and the females were inspected for the presence of a vaginal plug the next morning,[14] the mating day date was written on the cages, with the mating day being day zero (D0) of pregnancy and the next day being the first day (D1st) of pregnancy.[15]

Dexamethasone preparation: The experimental animals were given different dosages of dexamethasone sodium phosphate aqueous solution (8 mg / 2 mL). Mice were given intravenous injections via tail vein. The different concentrations of the drug was chosen based on the therapeutic dosage (8 ml to 70 kg),[13] which is 0.1 ml / 1 kg (0.002 ml / 25 g) of mouse weight. Each of the experimental groups consisted of twelve pregnant mice that were given different dosages of the medication as follows: The first group received a dosage of 0.1 mg per kilogram of body weight (equal to 0.002 mg per 25 g of mouse body weight), whereas the second group received a dose of 0.2 mg per kilogram of body weight (equivalent to 0.004 mg per 25 g of mouse body weight).

Histological studies: The preparation of solutions and dyes.[16]

To maintain the architecture of tissue samples, the liver samples were preserved in a 10% formalin solution for 2 days. After that the tissues were rinsed with tap water for 30 minutes to remove the overload fixer. After that, tissue sections were dehydrated for 2 hours in a sequence of rising concentrations of ethyl alcohol (70 %, 80 % 90 %, 95 %, and 100 %). The tissue samples were then soaked in two changes of xylene for an hour to make them clear. The tissue samples were placed in a 1:1 wax mixture melting at point of 56°C and xylene and baked at 60°C for 15 minutes. The samples were then withdrawn and placed in the melted wax for thirty minutes for each period.

These tissue samples were then immersed in molten wax paraffin, which was carefully poured into an ironic template in the letter (L) shape. Each tissue sample's information, including the dosage and pregnancy day, was recorded on a piece of paper and taped to the side of the template. The samples were allowed to be cooling and solidify before being used. A sharp knife was used to trim the wax templates. A microtome was used to cut tissue sections of 5 m thickness, which were then transferred to a water-bath at 37°C for tissue flatness, then tissue was lifted from warm water onto a slide, and marked with a diamonds pen (the embryonic day and the dosage of Drug), after that the slides being wiped with Mayer's albumin (as a sticky material), and they were placed on a warm hot plate at 40°C, then they left to be dry for approximately 24 - 48 hours.

To remove the paraffin from tissues, the tissue sections were heated or derided on a hot plate, then they were transferred straight to two changes xylene for 30 minutes. Tissue sections were then rehydrated using two 2-minute washes in each of a graded series of ethyl alcohol solutions (100%, 95%, 90%, 80% and 70% respectively) followed by a 2-minute rinse in ddH20.

The tissue slices were ready to stain using haematoxylin, which is used to stain the nucleus of cells, after the pre-treatment processes described above. Drops of haematoxylin stain were applied to tissue for 3 minutes before being washed under running water until the blue color developed. In ddH20, drops of 0.5 % eosin were then applied to slides for 2 minutes to stain the remaining the architectures of tissue, after which the sections were passed through a series of ascending concentrations of ethyl alcohol (70%, 80%, 90%, 95%, 100%) for two minutes at each concentration. The slides were then transferred to two changes xylene. Drops of D.P.X mounting media, were applied onto the tissue sections, then these slides were covered with coverslips and left to dry. At the next day, the slides (tissue sections) were ready to examine under the light microscope (Leica microscope, Germanic origin) equipped with a digital camera.

Results

This result showed that many changes has been shown in the liver tissue of the pregnant mice which were treated with different concentrations of Dexamethasone. These changes are shown as followings:

At the pregnancy day 13 (0.2mg \kg Dexamethasone)

There were many histological changes, including, binuclear hepatocytes, hyaline degenerative and inception of the nucleus division [Figure 1 A] degenerative changes with pyknotic nuclei, infiltration of inflammatory cells, there was a focal area of micro abscess consisting of inflammatory cells and necrotic debris binuclear cells and ballooning of hepatocytes [Figure 1 B].

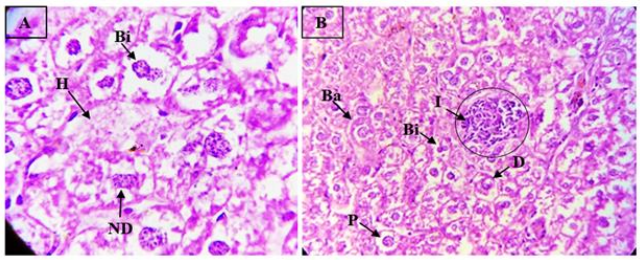

Figure 1: (A) mouse liver tissue sections show nucleus division (ND), hyaline degeneration (H), Binuclear (Bi)(H&E)100x. (B) Degenerative (D) Pyknotic nuclei(P), infiltration of inflammatory cells(I), Binuclear (Bi), a focal area of micro abscess (circle) consisting of inflammatory cells and necrotic debris (circle), Ballooning of hepatocytes (Ba) (H&E)20x.

2- At the pregnancy day 15 (0.2mg \kg Dexamethasone)

Different histopathological changes observed in pregnant mice, including infiltration of inflammatory cells, blood vessels congestion [Figure 2 A], atrophy in some hepatocytes, hypertrophy in some cells, binuclear cells and vacuolation in liver sinusoids at day 15 [Figure 2 B].

3- At the pregnancy day 17 (0.2mg \kg Dexametasone)

The histological changes were observed in the tissue sections such as blood vessels bleeding and infiltration of inflammatory cells [Figure 3 A & B], hepatocytes necrosis,

degenerative changes with pyknotic nuclei, binuclear cells and vacuolation in liver sinusoids at day 17 [Figure 2 B].

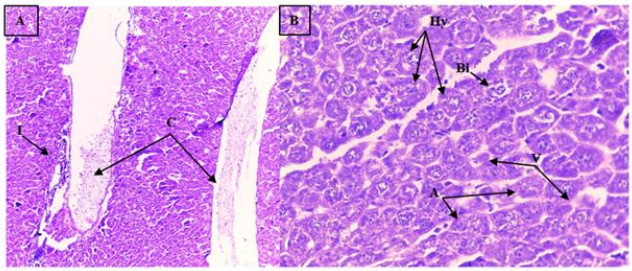

Figure 2: (A) mouse liver tissue sections show infiltration of inflammatory cells(I), congestion(C) (H&E)10x. (B) Hypertrophy (Hy), Binuclear (B), vacuolation (V), Atrophy (A) (H&E)20x.

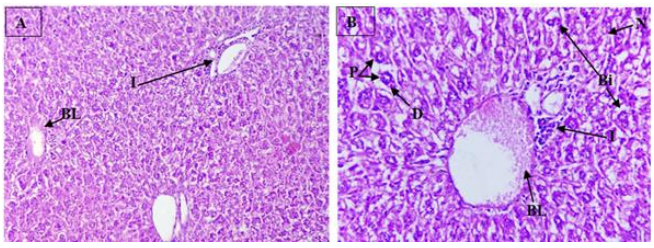

Figure 3: (A) Mouse liver tissue sections show infiltration of inflammatory cells(I), Bleeding (BL)(H&E)10x. (B) infiltration of inflammatory cells(I), Binuclear (Bi), degenerative(D), pyknotic (P), Bleeding (BL), Necrosis (N)(H&E)20x.

4- At the pregnancy day 13 (0.4mg \kg Dexametasone)

When Dexamethasone was raised to 0.4 mg/kg at day 13, histological alterations in the liver sections of pregnant women such as blood vessel constriction and inflammatory cell infiltration were shown [Figure 4A].

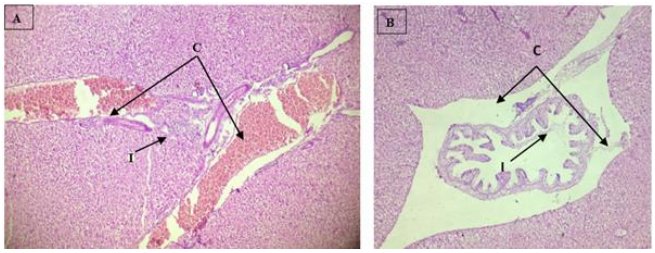

Figure 4: (A) Mouse liver tissue sections show infiltration of inflammatory cells(I), Congestive (C)(H&E) 10x. (B) infiltration of inflammatory cells(I), Binuclear (Bi), degenerative(D), pyknotic (P), Bleeding (BL), Necrosis (N)(H&E)10x.

5- At the pregnancy day 15 (0.4mg \kg Dexamethasone)

At this time and concentration, many microscopical alterations in the liver tissue were seen, including binuclear hepatocytes, hyaline degeneration, and nucleus division beginning [Figure 5 A]. There is a localized region of micro abscess composed of inflammatory cells and necrotic debris binuclear cells have degenerative alterations with pyknotic nuclei and infiltration of inflammatory cells [Figure 5 B].

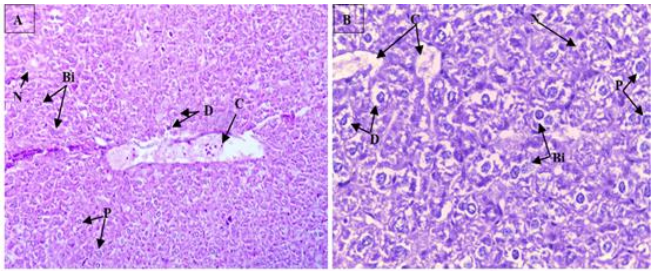

Figure 5: (A) (H&E)10x and (B) (H&E) 40x mouse liver tissue sections show infiltration of inflammatory cells(I), Bleeding (BL) infiltration of inflammatory cells(I), Binuclear (Bi), degenerative(D), pyknotic (P), Congestive (C), Necrosis (N) (H&E)10x.

6- At the pregnancy day 17 (0.4mg \kg Dexametasone)

On day 17 of pregnancy and 0.4mg \kg Dexametasone dose, the histopathological changes was observed in the liver tissue such as binuclear hepatocytes, congestive in blood vessels, infiltration of inflammatory cells, hyaline degenerative, necrosis and liver Sinusoids vacuolation in [Figure 6 A& B].

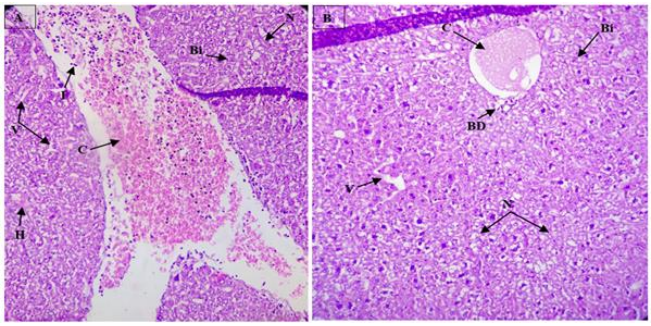

Figure 6: (A) and (B) (H&E)20x mouse liver tissue sections show infiltration of inflammatory cells(I), Binuclear (Bi), Congestive (C), Necrosis (N), Vacuolation(v), hyaline degenerative(H) bile duct (BD). (H&E)10x.

Discussion

The liver represents as a major organ for processing of nutrients absorbed via the gut and turning them into other compounds required by other organs.[17] It can be injured by a variety of factors, including medicines. Many medicines, including insulin,[18] glucagon,[19] and adrenal steroids,[20] have been shown in earlier investigations to induce liver injury.

Dexamethasone, a synthetic long-acting gluco-corticosteroid hormone consider as one of the most commonly recommended medications for an inflammatory conditions such as adrenal edema, hormone insufficiency, skin redness, arthritis, asthma, and kidney disease.[21] It is also utilized to improve the efficacy of anti-cancer drugs,[22] as well as to lower the risk of infant respiratory distress syndrome "RDS". It does, however, have a wide range of side effects in nearly the body systems.[23]

The current study treated mice in different periods of pregnancy with different doses of dexamethasone to study the morphological changes observed with each time and dose. Hepatocyte vacuolation and inflammatory cell infiltration were shown to be directly related to treatment

time and dosage in this investigation. Inflammatory and vascular abnormalities such as necrosis, congested sinusoids, and nuclear changes were also seen in this histological study, which were comparable to other studies.[24] The degeneration of hepatocytes may occur due to lipid accumulation. These changes can be appeared by using glucocorticoids which can cause increases in hepatic synthesis and secretion of very-low-density lipoprotein (VLDL).[25] The chemical drugs strongly indicating liver toxicity through increased generation of lipid peroxidation in liver tissues,[26] that destroys the cell nucleus and the cellular structure of their membranes and ultimately led to histopathological changes.

Dexamethasone suppresses the formation of arachidonic acid and prostaglandins, which acts as an antiaggregant agents in the body.[27] These, in combination with polycythemia and hypertension,[28] can result in sinusoidal dilation and congestion, as shown at the groups of treatments in this study, with significant variations between them showing dosage and duration dependency.

The anti-inflammatory effects of Dexamethasone are mainly due to activation of cytoplasmic glucocorticoid receptors that transcript of genes encoding a regulatory protein, which is why hepatocyte necrosis, degenerative sinus congestion, degenerative hepatocytes and inflammatory cells appear. These results were consistent with the,[29] who observed ballooning of hepatocytes in peripheral and mid- zones in the livers of dogs treated with dexamethasone and explained this due to the accumulation of fluid inside the cells that causes a watery swelling.

Conclusion

The use of Dexamethasone shows different changes in the liver tissue, including necrosis, Binucleated cells, vascular congestion and micro abscess when it was used for a long-term exposure or by repeating doses.

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How to cite this article: Taher SGH, Salman AN, Alghezi DA, Abdulamir HA. The Effect of Different Doses of Dexamethasone on The Hepatic Tissue of Pregnant Swiss Albino Mice Mus Masculus. Adv Clin Med Res. 2024;5(1): 4-8.

Source of Support: Nil, **Conflict of Interest:** None declared.