Effect of potassium Iodide on Liver of Albino Mice *Mus musculus* and their Pups

Suhad Ebraheem Mustafa , Aziz Khalid Hameed
Dept of Biology , College of Science , Tikrit University, Tikrit , iraq
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Abstract
The present study was designed to examine the efficus of potassium iodide for its efficacy to induce morphological malformation and histopathological lesions of liver in adult male mice , pregnant mice and pups *Mus musculus*.
The present study showed statistically a non-significant decrease of liver weight in therapeutic dose, and significant ($p \leq 0.01$) increase of liver weight in hypo and hyper dose group compared with control group in adult male mice and pregnant mice and their pups , and lung in hyper, hypo dose group compared with control group in adult male mice .
The histological examination showed many lesions in the liver of adult male mice , pregnant mice and pups The result also showed lymphocytic infiltration , degeneration, necrosis, atrophy and hemorrhage with kupffer cells fibrosis .

Introduction
Potassium iodide is a salt, similar to sodium chloride (NaCl), normal table salt. Its chemical symbol is KI, representing a compound of potassium (K) and iodine (I) [1] . It is ionic, K^+I^-. It crystallises in the sodium chloride structure, it is produced industrially by treating KOH with iodine. It is a compound made of 76% of the halogen iodine and 23% of the alkali metal potassium by weight [2]. KI is prepared by reacting iodine with a hot solution of potassium hydroxide, the product being subsequently reduced to iodide by heating the dry reaction mixture with carbon [3] .
Its readily absorbed in the intestinal tract and distributes rapidly through the extracellular space . Iodide concentrates in the thyroid gland, salivary glands, gastric mucosa, choroid plexus, mammary glands, and the placenta [4] .
When potassium iodide is introduced simultaneously at a short interval after each other, into the stomach Iodide is absorbed in the digestive tract [5] .

Materials and methods
1- The animals
One hundred albino mice *Mus.musculus* were used in this study. They were purchased from the center of Infertility and Embryology Research, Baghdad, Iraq.
Mice were kept in the animal house in wire-meshed stainless steel cages (i.e. two animals in one cage), but the cages were arranged in a manner that one animal could see at least several others. The animal house was controlled in which the temperature was maintained at (20-24C°), light schedule of 12:12 hours light: dark cycle with good ventilation.

2. The drug used
The drug used in the experiment was pure powder of potassium iodide . It was kept in a cool, dry place, away from direct heat and light. The experimental animals were administered potassium iodide in three oral dose, therapeutic, hypo and hyper doses using cavage as follows :
1. 0.04 mg/0.025 kg/day in a single daily dose (therapeutic dose) .
2. 0.08mg/0.025 kg/day in a single daily dose (hyper dose) .
3. 0.02mg/0.025kg/day in a single daily dose (hypo dose) [6] .

3. Animal grouping
The mice were used in this experiment aged 3 months, and their average body weight was 25±2gram at the beginning of the experiment.
They were divided into three main groups as follows :
- **Adult males**

Consisted of 20 animals divided into four subgroups each of 5 animals :
A- Control males received only normal diet and water .
B- Male mice were administered potassium iodide in an oral dose of 0.04mg/0.025 kg /day in a single daily dose for four weeks (therapeutic dose) .
C- Male mice were administered potassium iodide in an oral dose of 0.08mg/0.025 kg /day in a single daily dose for four weeks (hyper dose) .
d- Male mice were administered potassium iodide in an oral dose of 0.02mg/0.025 kg /day in a single daily dose for four weeks (hypo dose) .

- **Pregnant females**

Consisted of 20 animals divided into four subgroups each of 5 animals :
A- Control mothers received only normal diet and water .
B- Mothers were administered potassium iodide in an oral dose of 0.04mg/0.025 kg /day in a single daily dose, the treatment was continued from the 7th day of pregnancy to the 21st day of lactation .
C- Mothers were administered potassium iodide in an oral dose of 0.08mg/0.025 kg /day in a single daily dose, the treatment was continued from the 7th day of pregnancy to the 21st day of lactation .
D- Mothers were administered potassium iodide in an oral dose of 0.05mg/0.025 kg /day in a single daily dose, the treatment was continued from the 7th day of pregnancy to the 21st day of lactation .

4- Preparation for histological study

All animals were dissected under chloroform anesthesia. The thyroid gland weight was recorded after dissection for each animal using Sartorious sensitive balance (0.001 mg subdivision),and placed in fixative .

5- Body & Organs weight

The weights of organs were recorded at dissection of the animals. While the body weight for all pups was recorded before dissection with sensitive balance, the mean body weight and standard deviation were calculated for each group .

6- Histological sections

The tissues were processed according to [7], as follows :

1-Fixation

mmediately after removal and weighing of thyroid, both lobes were taken and fixed in 10% neutral buffered formalin solution (10ml of 40% formaldehyde + 90ml of tap water), for 18-22 hours at room temperature .

2-Dehydration

The tissues were removed from the formalin (10%) and then washed in running tap water for 30 minutes to remove traces of fixative. Half an hour for each change .

3-Clearing The purpose of this stage was to remove alcohol from tissue (dealcoholization) (30 minutes for each change) .

4-Infiltration and embedding

after clearing the tissues, they were passed through mixture of xylene and molten paraffin wax (melting 56-58C°) for 30 minutes, and then the tissues were kept in melted paraffin wax for one and a half hour for two changes to remove the xylene, the tissues were removed from the paraffin bathes to the molds and the molds were filled with wax after proper alignment of the tissue and labeling the blocks .

5-Tissue sectioning

rotary microtome (LKB-U.K.) with disposable blades was used. The section thickness used was 5µm. The ribbon was transferred into warm bath 44C°and then to the slides .

6- Tissue attachment

since formalin (10%) used as fixative, therefore section adhesive was needed using Mayer's glycerol-albumin mixture (prepared by mixing equal volumes of glycerol and albumin then the mixture was filtered using apiece of gauze ,finally few drops of thymol was added act as preservative substance) .

7- De-wax and hydration

de-wax was made using two changes of xylene (15 minutes for each change) and oven, hydration was made by immersing the slide in a descending concentration of alcohol bathes (100%,90%,80%,70%, 50%), 5 minutes for each bath, then slides were washed in distilled water .

8- Staining

haematoxylin and Eosin staining were used. Harris haematoxylin was used in regressive manner .

9- Mounting

this was made using DPX, cover slips were used to cover the sections .

Results

The results of the present study showed statistically a non-significant decrease of liver weight in therapeutic dose group as compared with the control group and there was a statistically significant ($p \leq 0.01$) increase in liver weight in hyper and hypo dose group as compared with the control group Results were analyzed statistically using Analysis of Variance (ANOVA) test [8] . Table (1) .

Table(4): Means± standard deviation of liver weight in different groups of adult mice

Group	Liver weight(g) Mean± SD
Control	1.50±0.29^{n}
Therapeutic dose (0.04mg)for 30 days	1.49±0.32^{n}
Hyper dose (0.08mg) for 30 days	1.80±0.20**
Hypo dose (0.02mg) for 30 days	1.91±0.42**

^{n}: non- significant

**: high- significant at P≤0.01.

Morphological changes of liver

The results of the present study showed that there is an effect of potassium iodide on morphology of the organs studied .

The morphological changes in liver of adult male mice showed after hyper dose when compared with liver of control group (Fig. 1), the morphological changes of adult male mice showed that the liver appearance become more pale with yellow area appeared extended peripherally (Fig. 2) .

In therapeutic dose the lesion represented by hemorrhage within liver, morphologically indicated by the appearance of dark coloured referred as hematoma nodule distributed externally on the lobes in addition the size became smaller in general and the color was pale as compared with the control (Fig. 3).

In hypo dose the morphological changes showed increasing number of odematous area in all liver in this case sever hemorrhage by the appearance of greenish area mixed red colour specially at the abdominal region, the shape also changed, the lobes differ from that of the control group in size and appearance, also there are aggregation of lobe on the liver tissue (Fig. 4) .

The morphological changes in pregnant mice showed in therapeutic dose when compared with control group, the liver changes in its size lobes and their colour odematous and greenish. The edges of the lobes are not clear with appearance of fat as a sheath on the surface of the lobes (Fig. 5).

In hyper dose when compared with control group showed that the size was large with clear appearance

of hematomus area and the fat sheath on the lobes of the liver, and the colour was pale in general (Fig. 6).
In hypo dose when compared with control group showed the lobes were not clear and hematomus area are obvious specially at the central portion and the fats are forming sheath at the peripheral region (Fig. 7).

Histological observations

Control group

The microscopic examination showed normal structure of liver and demonstrated normal central vein, normal arrangement of hepatocytes and sinusoids (Fig. 8).

Group A adult male mice administrated therapeutic dose 0.04 mg for 30 days

Under light microscopy the liver showed most of hepatocytes becomes atrophic which decrease in the size that lead to decreased the total size of the liver. Atrophy of hepatocytes associated with Pyknotic nuclei, also the Karyolysis are present. Atrophy resulting in swelling of sinusoids and dilation that lead to loss it's normal size. There was degeneration of some hepatocytes and hemorrhage are showed between the hepatocytes that caused by destruction and damage of central vein (Fig. 9).

Group B adult male mice administrated hyper dose 0.08mg for 30 days

Hyper dose of potassium iodide result in damaging of central vein wall and absence of endothelial layer that lining of central vein with swelling of sinusoids and most cells of hepatocytes also increasing size of kupffer cells . Hemorrhage indicated by the presence of red blood cells distributing in all liver, sinusoid and hepatocytes with different stages of cells necrosis some of them were cytoplasmic and other were nuclear (Fig. 10, 11).

Group C adult male mice administrated hypo dose 0.02mg for 30 days

The hepatocytes appear normal in many sections that prepared from this group and hepatocytes arrangement like radial plates which is a normal form the central vein appear normal and doesn't effected by this dose and it's endothelium were normal. The sinusoids that located between hepatocytes appeared normal in shape and size (Fig. 12).

Group D pregnant mice administrated therapeutic dose 0.04mg

The microscopic examination showed the hepatic cells are swelling with increasing the thickness of plasma membrane revealing the focal necrosis and nuclear degeneration, but in some lobules the hepatocytes are not differentiated or the cords are appeared with sinusoids (Fig. 13, 14).

Group E pregnant mice administrated hyper dose 0.08mg

The microscopic examination of cross section that prepared from this group showed extensive degeneration of hepatocytes and necrosis of some cells, also some of nuclei become small and concentrated that known Pykortic nuclei. The fatty droplets are present in the hepatocytes that lead to stenosis but the number of these fatty droplets are low (Fig. 15).

Group F pregnant mice administrated hypo dose 0.02mg

The microscopic examination showed the hepatocytes appeared normal in many section and arrangement like radial plates which is a normal form and the central vein appear normal and don't effected by this dose . The epithelium were normal and the sinusoids that located between hepatocyte are present normal in size and shaped (Fig. 16).

Group G control pups mice

The central vein are present normal and the hepatocytes form a radial plates which a normal form of arrangement and the sinusoids are present in normal size but it's filled with lymphocytes and red blood cells that normal because hemopoesis is occur in the liver during the fetal life (Fig. 17).

Group H pups mice administrated therapeutic dose

Hepatocytes not arranged like that of adult and large numbers of lymphocytes are infiltrated in all liver lobules with large kupffer cells fibrosis indicated by the presence of fibers and fibroblast nucleus that are elongated distributed in all of the liver lobules (Fig.18).

Group I pups mice administrated hyper dose

The microscopic examination showed necrosis at different stages with degeneration of nucleus disarrangement of the hepatocytes are not obvious and the sinusoid were in different size. Hemorrhage and hemolysis also occur (Fig. 19).

Group J pups mice administrated hypo dose

The microscopic examination showed Atrophy of some hepatocytes and increase in the size of some sinusoids, the number and amount of hepatocytes that formation in liver decrease even red blood cells were reduced and some sinusoid present with no hepatocytes or red blood cells (Fig. 20).

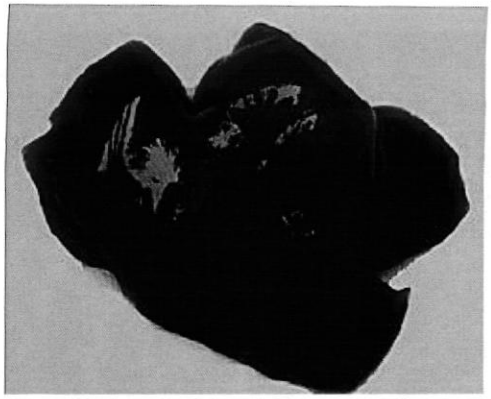

Figure (1): Liver of control male

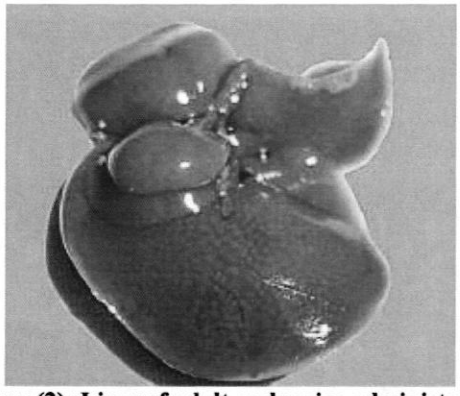

Figure (2): Liver of adult male mice administrated hyper dose for 30 days demonstrated scattered odema and yellow areas distributed through the liver

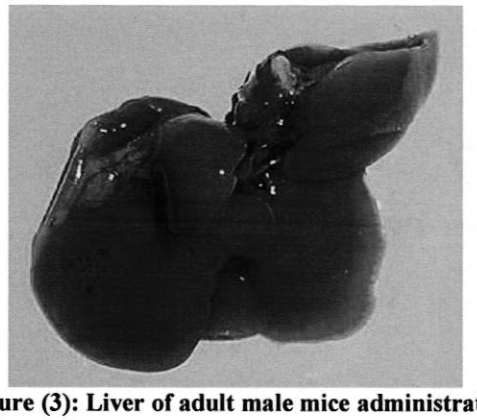

Figure (3): Liver of adult male mice administrated therapeutic dose for 30 days demonstrated hemorrhage within the liver and dark area referred as hematoma node

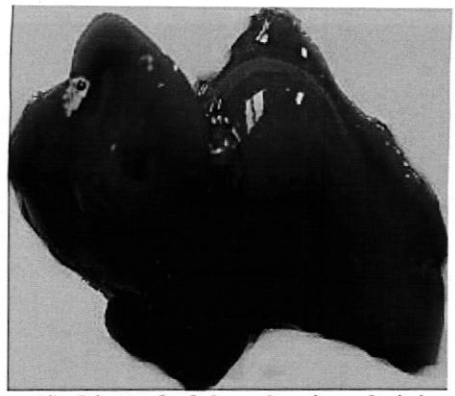

Figure (4): Liver of adult male mice administrated hypo dose for 30 days demonstrated edematous area in all liver and hemorrhage indicated by appearance of greenish area mixed red color

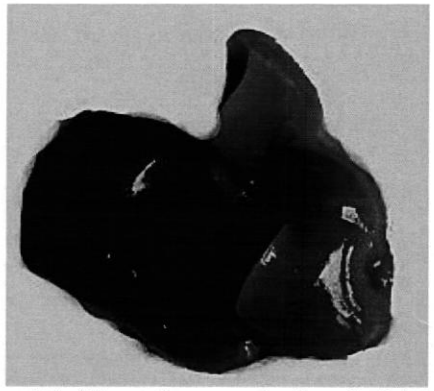

Figure (5): Liver of pregnant mice administrated therapeutic dose demonstrated odematus and greenish in lobe and the edges of lobe are not clear

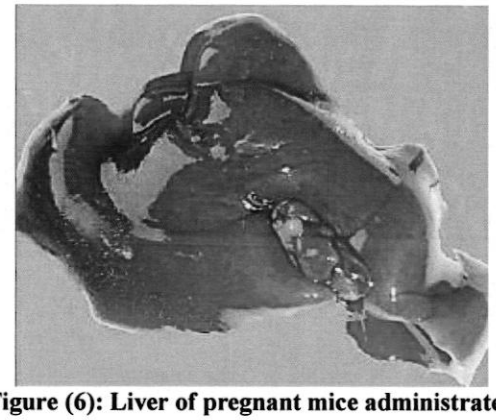

Figure (6): Liver of pregnant mice administrated hyper dose demonstrated hematoma area, the fat sheath on the lobes and the color was pale in general

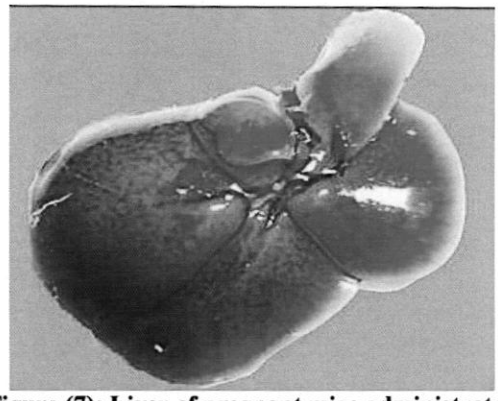

Figure (7): Liver of pregnant mice administrated hypo dose demonstrated haematomus areas and the fats forming sheath at the peripheral region

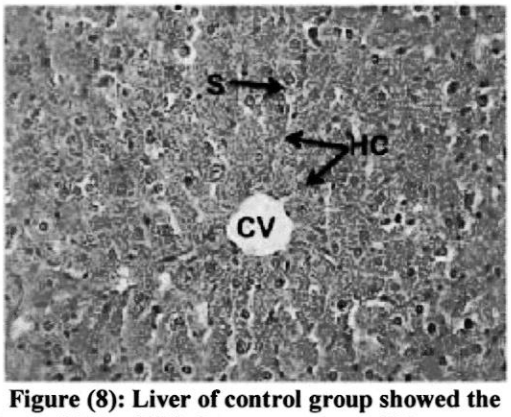

Figure (8): Liver of control group showed the Central Vein (CV) & arrangement of Hepatocytes (HC) and Sinusoids (S) (H&E 400X)

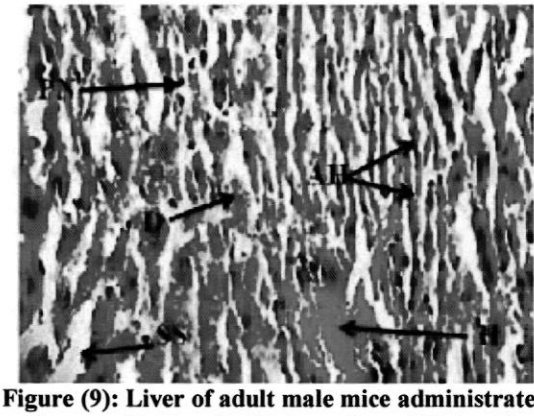

Figure (9): Liver of adult male mice administrated therapeutic dose for 30 days showed Pyknotic Nuclei (PN), Swelling Sinusoid (SS), Atrophy Hepatocytes (AH) and Hemorrhage (H) (H&E 400X)

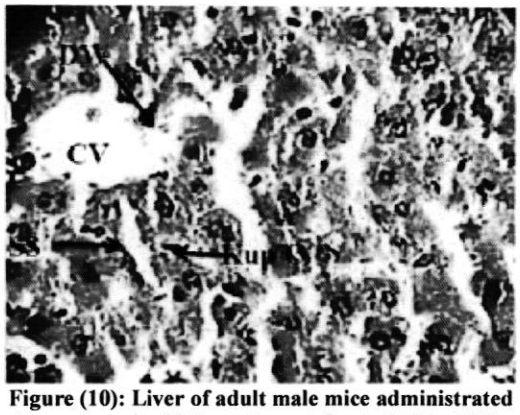

Figure (10): Liver of adult male mice administrated hyper dose for 30 days showed Central Vein (CV), Damage Wall (DW), Kuppfer Cell (Kup C) and Swelling sinusoid (SS) (H&E 400X)

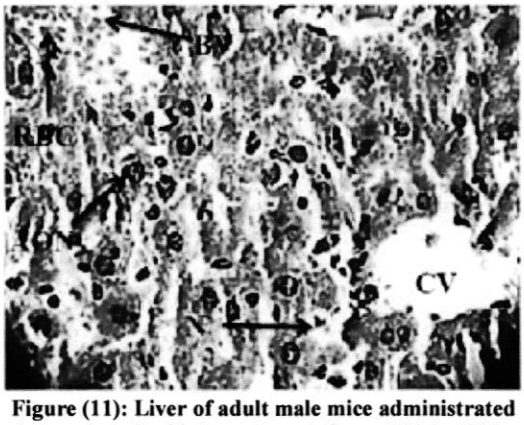

Figure (11): Liver of adult male mice administrated hyper dose for 30 days showed Central Vein (CV), Blood Vessels (BV), Red Blood Corpuscle (RBC), Necrosis (N) and Axues of nucleus (AON) (H&E 400X)

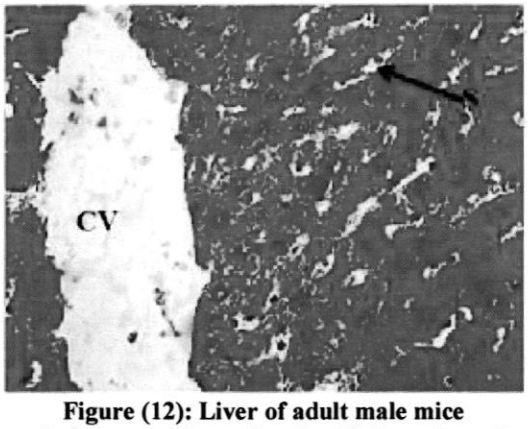

Figure (12): Liver of adult male mice administrated hypo dose for 30 days showed Hepatocytes (H), Sinusoid (S) and Central Vein (CV) (H&E 400X)

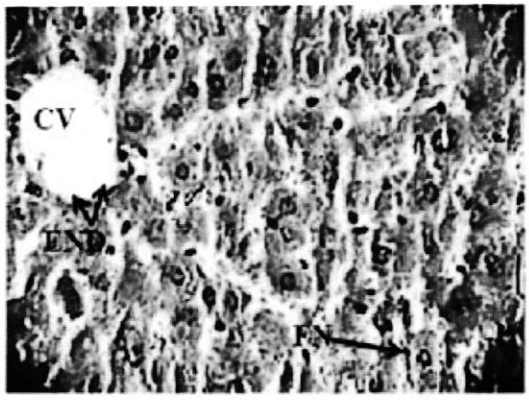

Figure (13): Liver of pregnant mice administrated therapeutic dose showed Central Vein (CV), Endothelial (END) and Focal Necrosis (FN) (H&E 400X)

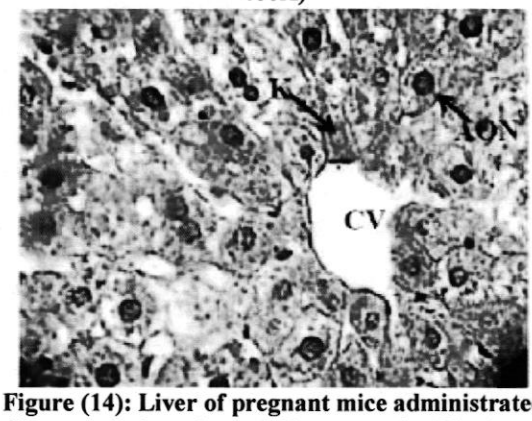

Figure (14): Liver of pregnant mice administrated therapeutic dose showed Karyocytosis (K), Axues of Nucleus (AON) and Central Vein (CV) (H&E 400X)

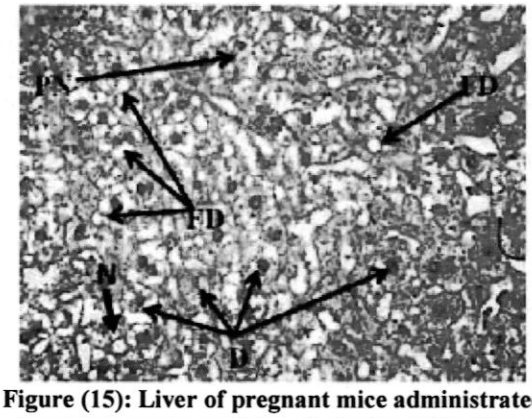

Figure (15): Liver of pregnant mice administrated hyper dose showed Pyknotic Nuclei (PN), Fatty Degeneration (FD), Necrosis (N) and Degeneration (D) (H&E 400X)

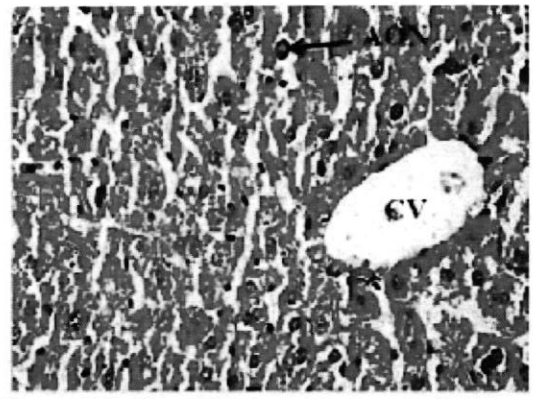

Figure (16): Liver of pregnant mice administrated hypo dose showed Axes of Nuclei (AON) and Central Vein (CV) (H&E 400X)

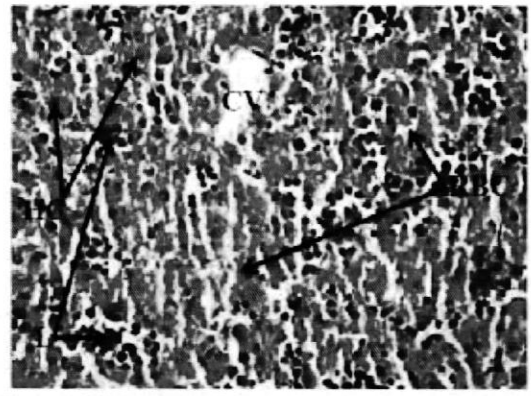

Figure (17): Liver of control pups showed Central Vein (CV), Red Blood Cells (RBC), Hepatocytes (HC) and Lymphocytes (L) (H&E 400X)

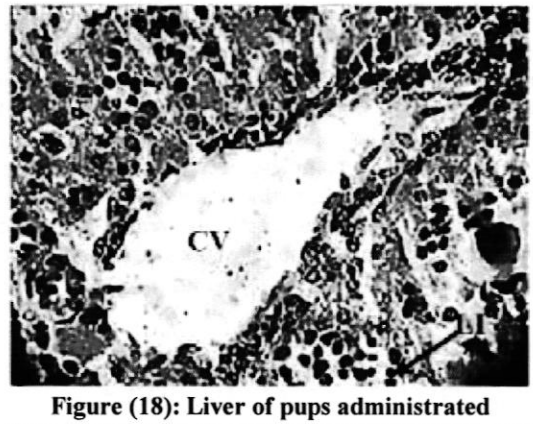

Figure (18): Liver of pups administrated therapeutic dose showed Central Vein (CV) and Lymphatic Infiltration (LI) (H&E 400X)

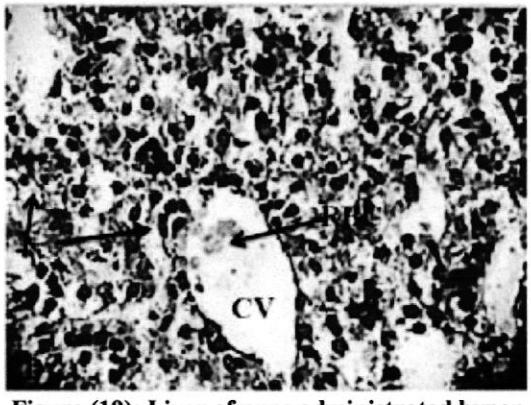

Figure (19): Liver of pups administrated hyper dose showed Red Blood Cells (RBC), Necrosis (N), Degeneration (D) and Central Vein (CV) (H&E 400X)

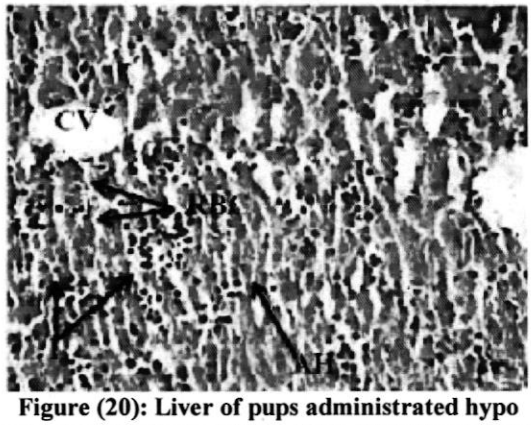

Figure (20): Liver of pups administrated hypo dose showed Central Vein (CV), Red Blood Cells (RBC), Lymphocytes (L) and Atrophy Hepatocytes (AH) (H&E 400X)

Discussion

The present study showed statistically a non-significant decrease of liver weight in therapeutic dose as compared with the control group, and significant ($p \leq 0.01$) increase of liver weight in hypo and hyper dose group as compared with the control group. Where as after study sections of the liver, it was found that there were degeneration of hepatocytes, necrosis and fatty droplets are present between hepatocytes were observed which may lead to increase in the weight of liver. In the present study, the results agree with [9] who stated that female albino rat treated by clomiphene citrate on liver cause fatty droplets, necrosis and infiltration of lymphocytes that cause increase of liver weight .

There are many morphological changes in the liver such as, redness in some areas and lightness in other areas. The causes of the light or pale area could be due to no arrival of sufficient amount of blood to these areas perfectly. The redness areas are caused by hemorrhage or congestion. There also appeared yellow nodules or yellow areas. The cause for this case appearance is complete necrosis of hepatocytes with condensation of the connective tissue. And there also appeared odematus results from increased movement of fluid from the intravascular to the interstitial space or decreased movement of water from the interstitium into the capillaries or lymphatic vessels [10] .

The histological study of the liver under the light microscope demonstrated that the liver showed different changes such as atrophy of hepatocytes associated with pyknotic nucles, also the karyolysis are present. Atrophy resulting in swelling of sinusoids and dilation that lead to loss its normal size . There is degeneration of some hepatocytes and hemorrhage are showed between the hepatocytes that caused by destruction and damage of central vein, also increasing size of kuppfer cells, sinusoid and hepatocytes with different stages of cells necrosis some of there are cytoplasmic and other are nuclear .

[11] elucidated that the one cause of necrosis is cytomembrane damage as caused by ATP deficiency, physical or chemical toxins , microbe toxins, complement factors, and lymphocyte preforming, which leads to a permeability disorder. As referred, the karyolysis is fading out of the nucleus due to chromatin dissolution. This is a late sign of induced cell death, while the Karyorhexis is a nuclear burst due to chromatin fragmentation. This is a late sign of programmed cell death, and the toxic effect of potassium iodide may lead to change in the membranes permeability of the cells and even the nuclei. Thus, that leads to destroy the cells and may lead to programmed cell death .

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تأثير آيوديد البوتاسيوم على الكبد في الفئران البيض واجنتها Mus musculus

عزيز خالد حميد ، سهاد إبراهيم مصطفى

قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

(تاريخ الاستلام: 25 / 9 /2012 ---- تاريخ القبول: 6 / 5 /2013)

الملخص

أجريت الدراسة الحالية للتعرف على تأثير فعالية آيوديد البوتاسيوم على أحداث التشوهات العيانية والآفات النسجية المرضية في الكبد لدى الفئران الذكور البالغين والفئران الحوامل نوع *Mus musculus* واجنتها.

أظهرت هذه الدراسة الإحصائية عدم وجود فروقات معنوية في انخفاض وزن الكبد لمجموعة الجرعة العلاجية، و زيادة معنوية عالية ($P \leq 0.01$) في وزن الكبد لمجموعة الجرع المفرطة والواطئة مقارنة مع مجموعة السيطرة في الفئران الذكور البالغين والفئران الحوامل، وفي الرئتين عند استخدام الجرع المفرطة والواطئة عند المقارنة مع ذكور الفئران البالغين .

أظهر الفحص النسيجي آفات عديدة في الكبد لمجموعة الفئران الذكور البالغين والفئران الحوامل واجنتها. تمثلت هذه الآفات با الارتشاح اللمفاوي ، تنكس، تنخر، ضمور مع نزف في بعض الخلايا مع تليف .