HISTOPATHOLOGICAL EFFECTS OF PURE CAFFEINE IN THE LIVER, HEART OF PREGNANT SWISS ALBINO MICE MUS MUSCULUS AND THEIR FETUSES

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ABSTRACT

The purpose of the present study was to evaluate the histopathological effects of caffeine on the liver and heart of pregnant white mice Mus musculus and their fetus. Thirty pregnant female mice were used, and they were divided into three experimental groups. The first one as a control group was given distilled water, while the second and third were given caffeine concentration 110, 130 mg/kg BW respectively once daily at 7th to 18th gestation day for 11 dosages in all time of experimental. The results showed several histopathological lesions in pregnant mice liver included Coagulative necrosis, dilated sinusoids, nucleus pyknosis, kupffer cells hypertrophy, focal necrosis, degenerated vaculate, and apoptosis, While the histopathological effects in fetal liver were similar to that of the pregnant mice. Shown Kupffer cells hypertrophy, inflammation cells infiltration, red blood cells Whereas for the heart, the results showed Hyaline necrosis and Rarefaction, hypoplasia, focal degeneration, giant cells and fibrin deposition and in fetal was Myocardial infarction and apoptosis.

Conclusion: Pure caffeine has histopathological effects on the liver and the heart during pregnancy, and it may cause significant effects on the organs of pregnant and her fetuses, as it crosses the placental barrier and reaches the fetus, especially "at high doses."

Keywords: Caffeine, Histopathological Lesion, Pregnant, Liver, Heart

I. INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is the most widely used activating chemical in the world. It works by blocking adenosine receptors, primarily A1 and A2A. Caffeine is absorbed almost entirely and extensively in the liver by phase I (cytochrome P450) enzymes, primarily CYP1A2, which appears to be polymorphically distributed in human populations. The principal caffeine metabolite in plasma is paraxanthine, while the main metabolites eliminated in urine are methylated xanthines and methyluric acids. (1). It's found in coffee, black tea, and chocolate since it's naturally created in the beans and leaves of the plants that make them. (2). In 1819, the German chemist Verdina Nderongo for the first time isolated a quantity of pure caffeine. In 1821 caffeine was isolated by French chemist Gabier and French pharmacists Pierre Joseph Balloté and Joseph Benim, world caffeine consumption is estimated at a du120,000 tons per year making it the most popular item in the world of stimuli (3).

Clarification was made the final composition of the caffeine near the end of the 19th century by Herman Fischer the chemical symbol of the caffeine is C8H10N4O2, its chemical name 3.7-dehydro-1,3,7,trimethyl-IH-purine-2.6-dione, (4) while its molecular structure is as follows:
Caffeine is an organic compound consisting of common elements such as carbonate, hydrogen, nitrogen, oxygen, caffeine commonly found in more than 63 plants worldwide. It is a direct stimulus for the nervous system, stimulates heart muscles, alters the respiratory system, and increases the speed of blood in the kidney (5).

The studies showed that excessive consumption of beverages, food, and drugs containing caffeine results in negative results, as it causes insomnia, headache, anxiety, and addiction, as well as consuming large quantities of caffeine at doses of more than 500 mg per day and for long periods leads to the pathological conditions known as caffeine poisoning (Caffeine tension) (6). The reported effect of prenatal caffeine consumption in rodents is most likely mediated by a decrease in acetylcholinesterase activity in the brain(7).

Studies have suggested that caffeinated substances during a period of pregnancy is associated with many effects and risk of congenital malformations due to caffeine crosses the placental barrier rapidly and slowly metabolites during pregnancy, and it's ability to accumulate in fetal and neonatal tissue (8). In addition to the contraction and reduction of the blood vessels in the placenta, which reduces the blood flow to the fetus and directly affects the development of the fetus (9), studies have indicated that the maternal caffeine is destroyed Adenosine action in embryos. In turn, the Adenosine acts as a protective " from the hypoxia of the fetus in the uterus, The half-life of caffeine in pregnant women is approximately 9-11 hours (10).

So the aim of study to identify the histopathological effects caused by different doses of caffeine in the liver and heart of pregnant mice and that fetus.

II. MATERIALS AND METHODS

Preparation of experimental animals and their mating

The Swiss albino mice Balb \ C, mice were obtained with an average weight of (23±2) g the mice were placed in plastic cages with metal covers meshed in a well-ventilated room and the temperature at (24 ± 2) °C and Photoperiod 12- hr light / dark (11). The special diet was given feeding mice and water (12), and for fertilization two females were placed with one male in the single cage overnight (13), and fertility was examined by observing sperms in the vaginal plug, The day of mating was the zero-day pregnancy.

The dasages of Caffeine

The study used pure caffeine its concentration (99%), and (110,130) mg/kg of the B.W which was prepared by dissolving it in 50 ml of distilled water. The doses were chosen based on the the lethal dose of pure caffeine is 127 mg/kg BW in the case of mice when utilizing oral dosing (14). pregnant mice were given a caffeine aqueous solution by mouth using a gavage Needle.

Experimental design

(30) pregnant females who owned the vaginal plug were isolated with separate plastic cages, and the date of mating placed on them, they given the aquatic solution from the 7th day of pregnancy (Organogenesis stages) until the 18th day of pregnancy (the day of the anatomy).

Experimental groups
A pregnant female mouse was divided into three groups, including a control group that was given distilled water, and the other two groups were given caffeine solution (110, 130) mg/kg BW, respectively.

<table>
<thead>
<tr>
<th>Dosing period</th>
<th>groups</th>
<th>Concentration mg / kg BW</th>
<th>Number of dosages</th>
<th>Number of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>An oral dose of the 7th day to the 18th day of pregnancy</td>
<td>control</td>
<td>Distilled water</td>
<td>11</td>
<td>10</td>
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<td></td>
<td>Experimental (1)</td>
<td>110</td>
<td>11</td>
<td>10</td>
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<tr>
<td></td>
<td>Experimental (2)</td>
<td>130</td>
<td>11</td>
<td>10</td>
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**Histological examination**

After anesthetizing mice with chloroform, the organs (liver, heart) were extracted and fixed in 10% formalin for (24-48) hours (15), tissue specimens were collected, dehydrate, cleared, embedded in paraffin sectioned at 5 µm thickness. The tissue sections stained and stains with hematoxylin and eosin (16). They examined under light microscope, final magnification was calculated according to a magnification of the ocular and objective lenses.

**III. RESULTS AND DISCUSSION**

In the liver, the pathogenic when given concentrations (110,130) mg/kg BW, coagulative necrosis were, dilated sinusoids (figure:1) and Vacuolated necrosis (figure:2) and these results are consistent with the study (17), of in there study of the chronic effect of energy drinks in liver of rat, the results revealed the appearance of hemorrhage, nucleus pyknosis, and hypertrophy of Kupffer cells (figure:3). The also were results are consistent with a study of (18) in its study of caffeine-containing drinks and their effect in white mice, This may be due to the role of the Kupffer cells in the body and their phagocytic function (19).

![Fig 1](image1.png)

**Fig 1:** Cross-section of the liver of a pregnant female mouse given an aqueous solution of caffeine at a concentration of 110 mg/kg of B.W showing coagulative necrosis (CN), Dilated sinusoids (DS) Inflammatory cell infiltration (IL), and blood vessel congestion (CON)( H&E 100X)

![Fig 2](image2.png)

**Fig 2:** Cross-sectional section of the liver of a pregnant female mouse given an aqueous solution of caffeine concentration of 110 mg/kg B.W showing vacuolated necrosis (VN), Inflammatory cell infiltration (IL)( H&E 100X)
Fig 3: Cross-section of the liver, concentration of 110 mg/kg of B.W showing Kupffer cells hypertrophy (KCH), Coagulative necrosis (CN), Degenerated Vacuolate (DV), and degenerated degeneration (VD). (D), hemorrhage (H), Nucleus pyknosis (ND) (H&E 400X)

The congestion in blood vessel, apoptosis (figure:4), apoptosis may have occure when caffiene caused inhibition of immune system or maybe a kind of "protection for liver tissue" by loss of some cells to preserve the rest of cells (20) and the appearance of hemolysis within the blood vessel (figure:5). The degeneration and phagocytes (figure:6) proliferation of Kupffer cells produce several inflammatory material such as reactive oxygen and nitric oxide, which serve to express the genotype of gluing molecules such as vascular cell adhesion Molecule (VCAM-1) in the liver, and these molecules activate inflammatory process, which stimulates inflammation of liver and thus damage and deformation of hepatic tissue (21).

Fig 4: Cross-section of the liver of a pregnant r female given an aqueous solution of caffeine, a concentration of 110 mg/kg BW, indicating necrosis (N), hemorrhage (H), vascular congestion (CON), and apoptosis (AP), Dilated sinusoids (DS) (H&E 400X)
Fig 5: a cross-section of the liver of a pregnant female mouse given an aqueous solution of caffeine at a concentration of 110 mg/kg B.W showing hepatocyte Diffuse necrosis (DN) and hemolysis (HE) (H&E 100X)

Fig 6: A cross-section of the liver of a pregnant female mouse given an aqueous solution of caffeine at a concentration of 130 mg/kg of B.W shows degeneration (D), Dilated sinusoids (DS), Kupffer cells hypertrophy (KCH), Degenerated Vacuolate (VD), Macrophages (M) (H&E 400X)

The results of given an aqueous solution of caffeine at a concentration of 130 mg/kg BW also showed the appearance of focal necrosis and swelling cells (figure:7), the blood vessel Rhesis (Figure: 8) caffeine concentrations may lead to disturbances in the metabolism of hepatocytes which make hepatic tissue more susceptible to harm (22) The lesions increased in severity with the appearance of Coagulative necrosis, rhesis, hemolysis inside a blood vessel, dilated sinusoids, vaculated degeneration, apoptosis (Figure,9), As a result of all the effects caused by caffeine, necrosis of all kinds occurs, which is the final result of injury to the liver tissue, and it is common in the case of acute and chronic liver disease and then fulfill the liver cirrhosis (23).

Fig 7: Cross-section of the liver of pregnant female mice, given an aqueous solution of caffeine, at a concentration of 130 mg/kg B.W, showing Focal necrosis (FN), and coagulative necrosis (CN), Vacuolated degeneration (VD) macrophages (M), swelling (S), and apoptosis (AP) (H&E 400X)
The histopathological changes that have occurred in the liver of the fetus of the mice were the Kupffer cells hypertrophy (Figure:10), Dilated sinusoids, infiltration of Inflammatory cells, Red blood cells (Figure:11), The reason may be due to the role of caffeine and its penetration through the placenta to the fetus and causing damage to the fetal hepatocytes (24).

Fig: 8 cross-sectional in the liver of a pregnant female mouse given an aqueous solution of caffeine, a concentration of 130 mg/kg B.W, showing the appearance of Kupffer cells hypetrophy (KCH), Rhexis (R), hemolysis (HE), Dilated sinusoids (DS) (H&E 100X)

Fig: 9 cross-sections in the liver of a pregnant female mouse given an aqueous solution of caffeine, the concentration of 130 mg/kg B.W showing Coagulative necrosis (CN), Rhexis (R), and hemolysis (HE), Dilated sinusoids (DS), Degenerated Vacuolate (VD) apoptosis (AP) (H&E 100X)

Fig: 10 cross-sections in the liver of pregnant mice embryos, 110 mg/kg of B.W showing Kupffer cells hypertrophy (KCH) Degenerated Vacuolate (VD), macrophages (M), and hemorrhage (H)( H&E 400X)
Fig: 11 cross-sectional sections in the liver of a pregnant female fetus given an aqueous solution of caffeine, a concentration of 130 mg/kg of body weight, indicating the Vacuolated (V), the incidence of hemorrhage (H), Dilated sinusoids (DS), Inflammation cells infiltration (ICF), Red blood cells (RBC) (H&E 400X)

In the heart, the study showed many histopathological lesions in the heart of female pregnant mice that given an aqueous solution of pure caffeine for both concentrations., Distinguished by the appearance of elongated nuclei with an irregular site (Fig:12) vacuolated necrosis (Fig:13). The appearance of vaculate degenerated and the hemolysis inside the blood vessels (Fig:14) and observed, contrast eosinophilic stain cell (Fig:15). The results of our study are consistent with what (25) they referred in your study of the effect of paracetamol on rats he attributed the cause of damage of the heart to high fluid in the kidneys, which leads to high blood pressure and this is due to its caused negative effects on the heart.

Fig: 12 cross-sections in the heart of a pregnant female mouse given an aqueous solution of caffeine concentration of 110 mg/kg B.W showing vacuolated necrosis (VN) (H&E 400X)
Fig: 13 cross-sectional in the heart of pregnant female mice given an aqueous solution of caffeine at a concentration of 110 mg/kg of B.W showing the vacuolated necrosis (VN) and the appearance of elongated nuclei with irregular position (arrows), and distended nuclei (arrow) (H&E 400X)

Fig: 14 cross-sectional sections in the heart of a pregnant female mouse given an aqueous solution of caffeine at a concentration of 110 mg/kg of B.W showing degenerated vacuolate (VD) and hemolysis (HE) inside the blood vessel (H&E 400X)

Fig: 15 cross-sectional cross-sections of a pregnant female mouse given an aqueous solution of caffeine at a concentration of 110 mg/kg of B.W showing Coagulative necrosis (CN), Eosinophilic stain cells (ES), and some nuclei are elongated (arrow), and Other some are irregular (arrow) (H&E 100X)

Also study were reported Haemorrhage (Fig:16), Hyaline necrosis and Rarefaction (Fig:17), Hypoplasia (Fig:18) Foamy appearance in the cytoplasm (Fig:19), The reason may be that caffeine inhibits adenosine receptors and releases catecholamine (26)
Fig: 16 cross-sectional in the heart of a pregnant female rat given an aqueous solution of caffeine, a concentration of 110 mg/kg B.W, showing hemorrhage (H), irregularity of the cardiac muscle fibers, and their enlargement of each other (arrow).

Fig: 17 cross-sectional cross-sections of a pregnant female mouse given an aqueous solution of caffeine at a concentration of 110 mg/kg of B.W showing degeneration (D), Eosinophilic stain in cell (ES), Hyaline necrosis (HN), and cardiac muscle fibrosis. Rarefaction (RR), and necrosis (N) (H&E 400X).

Fig: 18 cross-sectional in the heart of a pregnant female mouse given an aqueous solution of caffeine, the concentration of 130 mg/kg B.W, showing hemorrhage (H), Eosinophilic stain ES), Coagulative necrosis (CN), and Rarefaction (RR), Hypoplasia (HYP) (H&E 400X).

Fig: 19 cross-sectional in the heart of a pregnant female mouse given an aqueous solution of caffeine, a concentration of 130 mg/kg of body weight, showing the appearance of the cytoplasm with a foamy appearance (FA), and the appearance of (VD, HN) and (N), Nucleus pyknosis (ND)( H&E 400X).

Myocardial infarction, muscle hypertrophy(Fig:20), focal degeneration (Fig:21), giant cells and fibrin deposition(Fig:22), apoptosis (Fig:23). This study agrees with other study that have indicated that energy drinks containing caffeine lead to an increase in the incidence of heart infarction by 14% (27).
Fig: 20 cross-sections in the heart of a pregnant female mouse given an aqueous solution of caffeine at a concentration of 130 mg/kg of B.W showing the emergence of nucleated hypertrophy (arrows), myocardial infarction (IN), hypertrophy (HY) (H&E 400X)

Fig: 21 cross-sectional in the heart of a pregnant female mouse given an aqueous solution of caffeine at a concentration of 130 mg/kg B.W showing the appearance of (V), Hyalin necrosis (HN), and vacuolated degeneration (VD) (H&E 100X)

Fig: 22 cross-sectional sections in the heart of a pregnant female mouse given an aqueous solution of caffeine at a concentration of 130 mg/kg of B.W showing the appearance of Vacuolated (V), Hyaline necrosis (HN), Coagulative necrosis (CN), and the appearance of giant cells (GC), Fibrin deposition (FD) (H&E 400X)
Fig: 23 cross-sectional in the heart of a pregnant female mouse given an aqueous solution of caffeine at a concentration of 130 mg/kg of B.W showing the presence of Eosinophilic stain ES cells, (N), and apoptosis (AP) (H&E 400X)

While the pathological abnormalities of the heart tissue were in the fetuses of pregnant mice that were given the two concentrations, the appearance of Hyaline necrosis, Myocardial infarction, Nucleic condensation, and apoptosis (Fig: 24), Diffuse necrosis and edema (Fig:25) Studies indicate that caffeine use during pregnancy leads to an abnormal formation of blood vessels, where chronic caffeine treatment has been observed to have led to the abnormal activation of the renin-angiogenic system in fetal hearts (28).

Fig: 24 cross-sectional in the heart of a fetus in a pregnant mouse given an aqueous solution of caffeine at a concentration of 110 mg/kg of B.W showing the appearance of Hyaline necrosis (HN), Rarefaction (RR), nucleation condensation (ND), fibrin deposition (FD), apoptosis (AP) (H&E 100X)

Fig: 25 cross-sectional cross-sections of a fetus in a pregnant female rat given an aqueous solution of caffeine, the concentration of 130 mg/kg of body weight, showing the appearance of Diffuse necrosis (DSN), hemorrhage (H), vacuolated degeneration (VD), and the appearance of hypostasis or edema (OE) (H&E 100X)
IV. CONCLUSION

Pure caffeine had harmed both mothers’ fetuses’ liver and heart, especially if used during critical pregnancy stages such as the first trimesters.

RECOMMENDATIONS

Pregnant should not be unconscious of the risks of food, beverages and drugs contain caffeine and should not take high doses from it.

ACKNOWLEDGMENT

The author wishes to express her gratitude to the University of Mosul, College for Education for Pure Science, Department of Biology, for their assistance in improving the quality of this work.

CONFlict of interest

“The author declare that there are no conflicts of interest regarding the publication of this manuscript”.

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