Effect Of Methotrexate On Mice Embryo Liver

Huda Mahdy Al-khateeb[1],Abed Hassna Barraj[2] ,Zainab Abdulsalam Kadhim[2]*
[1]Department of Anatomy, College of Medicine, University of Baghdad,[2] Department of Biology, College of Science, University of Baghdad. Baghdad, Iraq

Abstract

Methotrexate (MTX)is a multiple therapeutic drug, it's used in treatment of ectopic pregnancy, neoplastic disease, autoimmune disorders, and inflammatory conditions. It's associated with a spectrum of side effect that includes damage to the (liver, kidney, lung and bone marrow). MTX has been associated with fetal malformations in(nervous, skeletal, gastrointestinal, and cardiac) system, and even fetal death. The study was conducted to test the effect of two doses of MTX (5and 10mg/kg) on the development of the mice liver during two gestational age (day11 and day 17) of pregnancy.18 healthy female mice were divided into three groups ,first group (control) was injected with normal saline ,second group injected with 5mg/kg and third group injected with 10mg/kg (all groups were injected on day 7 of gestation). Histology examination of control fetal liver on day11of gestation showed normal fetal development where cords of hepatoblast were formed and hepatic blood sinusoids were filled with erythroblasts ,fetal liver of animals dosed with 5mg/kg and 10mg/kg on day 11 of gestation showed ballooning degeneration with hepatoblast that have pyknotic nucleus .control fetal liver of day 17 of gestation showed normal hepatocyte arranged around a central vein, fetal liver of animals of day 17 of gestation dosed with 5mg/kg showed ballooning degeneration while fetal liver of 17 days of gestation dosed with 10mg/kg showed ballooning degeneration with inflammatory infiltration. Results of this study revealed that low dose MTX can affect internal organs causing damage during gestational age which might cause problems later in life after birth ,the effect have been proven in mice but does not necessarily apply on human but a possibility of such damage in human should be kept in mind.

تأثير عقار الميثوتركسيت على النمو الجنيني لكبد الفئران

هدى مهدي الخطيب[1]، عبد حسن براج[2]، زينب عبد السلام كاظم*[2]
[1]قسم التشريح، كلية الطب، جامعة بغداد،[2]قسم علوم الحياة، كلية العلوم، جامعة بغداد ، بغداد، العراق.

الخلاصة

الميثوتركسيت هو دواء متعدد الاغراض العلاجية حيث يستعمل لمعالجة حالات مثل الحمل الخارجي،الاورام السرطانية ، امراض المناعة الذاتية وحالات الالتهابات .الدواء له اثار جانبية مختلفة تشمل اضرارا للكبد والكلى والرئتين ونخاع العظم .الميثوتركسيت مسؤول عن التسبب بتشوهات جنينية في الجهاز العصبي والعظمي والهضمي والقلبي كذلك التسبب بموت الاجنة . تم اجراء الدراسة لاختبار تأثير جرعتين (5و10مغ/كغم) من عقار الميثوتركسيت على النمو الجنيني لكبد الفئران خلال مرحلتين جنينيتين (اليوم11 و17من الحمل)باستخدام جرعتين مختلفتين. 18انثى فأر بصحة جيدة تم وضعها ضمن ثلاث مجاميع الجموعة الاولى (حيوانات السيطرة) تم حقنها بمحلول ملحي ،المجموعة الثانية تم حقنها ب 5 مغ/كغم

*Email:Zainab.salam88@yahoo.com

والمجموعة الثالثة تم حقنها ب 10مغ/كغم من العقار (لكل المجاميع حقنت في اليوم السابع).الفحص النسيجي لعينات الكبد من الاجنة بعمر 11 يوم من الحمل لحيوانات السيطرة اظهر نمو جنيني طبيعي للكبد على هيئة ظهور الاورمات الكبدية حول الاوعية الدموية المليئة بالاورمات الدموية اما كبد الاجنة بعمر 11 يوم من الحمل للحيوانات المعالجة ب 5 مغ/كغم و 10مغ /كغم فقد اظهر انحطاط خلوي انتفاخي وخلايا ذات نواة متغلظة.كبد الاجنة بعمر 17 يوم من الحمل لحيوانات السيطرة اظهر نمو جنيني طبيعي بشكل خلايا كبدية طبيعية حول الوريد المركزي اما كبد الاجنة بعمر 17 يوم من الحمل للحيوانات المعالجة ب 5 مغ/كغم اظهر انحطاط خلوي انتفاخي و الكبد الجنينيي المجرع ب 10 مغ/كغم فقد اظهر انحطاط خلوي انتفاخي في النسيج ووجود التهاب واضح. نتائج التجربة اظهرت ان الجرع المنخفضة من الميثوتركسيت يمكن ان تسبب ضررا للاعضاء الداخلية اثناء النمو الجنيني لها وقد يسبب ذلك مشاكل فيما بعد الولادة ،التأثير تم اثباته في الفيران ولا يعني ذلك بالضرورة وجود نفس التأثير عند البشر لكن مع هذا فان ال تنبه الى امكانية حدوث الضرر واجبا.

Introduction

Methotrexate (MTX) was first synthesized for a specific chemotherapeutic purpose which was to inhibit folic acid synthesis in the cells of acute lymphoblastic leukemia in children ,Nowadays the drug is being used at low dose (5-25)mg to treat anti-inflammatory conditions and the treatment of various autoimmune diseases, including rheumatoid arthritis, lupus, psoriasis and juvenile idiopathic arthritis while high dosage is used to treat different types of malignancies [1,2].Moreover it's the first choice treatment for ectopic pregnancy[3].MTX is administered intravenously or intrathecally to treat malignant meningitis and orally or intramuscularly to treat some disease such as rheumatoid arthritis[4; 5]. Estimated Half life of low dose MTX is 10 hours but kidney and liver may store it for months[6].In the liver MTX is oxidized to its metabolite 7-hydroxymethotrexate,In human the majority of MTX and its metabolite are eliminated from the body by secretion in the urine (by the action of glomerular filtration and tubular secretion). Only a small part is excreted through the biliary tract[7; 8] ,Rats also utilize same methods but biliary excretion is favored[9] .MTX is transported via three routs :folate receptor, reduced folate carrier and proton coupled folate transporter [10].inside the cells MTX is converted to MTX polyglutamates which are the active compound that may be retained in the tissue for months[5].MTX is a structural analog of folic acid that can interfere with intracellular folate metabolism by binding to dihydrofolate reductase (DHFR) [10] resulting in decreasing bioavailability of tetrahydrofolate which is an important cofactor in thymidylate synthesis and de novo purine synthesis , thereby MTX Inhibits deoxyribonucleic acid (DNA) synthesis [11] and cause depletion of nucleotide which affects cells capability to carry out the excision repair of DNA damage [12]. In addition to the previous effect MTX by affecting folate levels can interfere with the enzyme methylentetrahydrofolate reductase resulting in reducing cellular 5-methyl tetrahydrofolate impairing methionine synthesis which is important for producion of S-adenosyl methionine which influence methylation reaction[13]. Since methylation of cytosine residue in the regulatory regions can affect gene activity ,any reduction in DNA methylation can have a negative effect on fetal development [14].In conclusion MTX affects DNA synthesis ,repair and methylation.Inhibition of DNA synthesis has been correlated with the concentration of unbound intracellular MTX , Factors that determine the quantity of total intracellular MTX. include extracellular MTX. concentration, cellular transport properties, degree of MTX. polyglutamate (MTXPGs.) formation and lysosomal degradation of MTXPGs to MTX [15; 16].MTX has been proved to be transferred through the placenata by the presence of the drug in the umbilical blood of a woman who received the drug hence it causes chromosomal aberrations in the neonate[17].Several studies addressed MTX. effect on pregnancy since .MTX selectively affects rapidly dividing cells, such as trophoblast cells, blood stem cells, also MTX.causes cells to arrest in metaphase[18]. studies suggested a species specific effect with embryotoxicity and teratogenecity in rats mice and rabbits ,the last two showed resistant [19]. The effect of several doses of MTX On developing zebrafish and found that MTX Caused an increase in apoptotic cells, S-phase delay, a decrease in the number of mitotic cells resulting in a decrease in the number of cells within the developing embryo [20]. Chemotherapeutic doses of MTX produces distinct pattern of central nervous system, craniofacial, and skeletal defects while The risk of

fetal abnormalities at rheumatological doses of MTX (5–25 mg weekly) is less clear, there have been several reports of increased rates of spontaneous abortion and major fetal anomalies, as well as successful pregnancies that result in a healthy infant [21].therefore the need to keep researching in the capability of this drug to cause embryotoxicity seems in place,our study relied on fetal hepatic development as indicative of MTX embryotoxicity since it differentiates in gradual steps in a process that covers all the periods before and after birth up to weaning and is linked directly to the physiological needs of organisms [22].

Material and method

A total of 18 healthy sexually mature female Swiss albino's mice *Mus musculus* of (8-12)weeks of age and (25) gram weight were bred and housed in the animal house of Medical College of Baghdad University under laboratory conditions(12 hours light,12 hours dark) with controlled temperature (29˚) ,good ventilation and fed normal rodent pallet (*ad Libitum*).The animals were grouped in a harem system (two or more females per male), and pregnancy was detected by checking for vaginal plug every morning, first day of gestation was considered the day after the plug was detected [23]. Pregnant mice were divided into three groups each group (n=6). group 1 received normal saline by intramuscular injection on day 7 of gestation(control group),group 2 received MTX 5mg/ kg body weight by intramuscular injection on day 7 of gestation ,group three females Received MTX 10 mg/kg body weight by intramuscular injection on day 7 of gestation. Open ether anesthesia was used to anesthetize pregnant mice , fully anaesthetized pregnant mice were dissected by longitudinal abdominal incision then graved uterus was dissected to extract embryos (from days 11,17). Embryos (above11 days)were dissected to get their liver. Collected tissues were fixed in 10% formalin dehydrated through graded alcohol series (80-100%) cleared by chloroform and embedded in paraffin wax and then was cut into sections of 6M mm thickness and stained with heamatoxylin and eosin [24].

Results and discussion

Control fetal liver of 11th day of gestation, figure-1, was occupied by hepatoblast which were arranged in cords around the large sinusoids that were filled with erythroblast .Erythroblast were an obvious sign of hematopoietic expansion in the liver after migration of hematopoietic cells from the yolk sac and aorta–gonad–mesonephros region to the developing liver which will become the main hematopoietic organ through the gestation time. the description of liver on this stage matched what was mentioned by[25-27] .The histopathology manifestation of liver of fetuses on 11 day of gestation dosed with 5mg/kg, figure-2, or 10 mg/kg, figure-3, showed ballooning degeneration with hepatoblast that have pyknotic nucleus, these findings agrees with [28] who mentioned that folic acid antagonists,which inhibit mitosis sometimes cause anomalies in the histological structure of the thickening region of the foregut floor ,cells during this period are continuously in need of folate substrate to support cellular replication [29] and since MTX inhibits folate metabolism by binding to dihydrofolate reductase causing tetrahydrofolate concentration to decrease [30] its expected for cells to die leaving areas of degeneration. Section of liver of 17^{th} day of gestation, figure-4, showed hepatocyte size increase as a result of glycogen accumulation which much what was concluded by [31, 32]. Hematopoietic activity through this period kept declining ,this result similar to the finding of [27,33],fetal liver of animals dosed with 5mg/kg on day 17 of gestation, figure-5, showed ballooning degeneration , fetal liver of animals dosed with 10mg/kg of day 17of gestation, figure-6, showed ballooning degeneration and inflammatory infiltration ,these findings can be probably explained by the indirect the effect of MTX on methylation process. It was mentioned by[13] that MTX reduces cellular 5-methyl tetrahydrofolate ,also MTX has been found to cause decrease in cholin metabolite in the liver[13] ,both 5-methyl tetrahydrofolate and cholin are derivatives fo S-adenosylmethionine which is the methyl donor in methylation process[34] , it's only logical to expect a negative effect of MTX on methylation which would interrupt liver development causing degeneration and ,additionally same manifestation were also found in livers of animals that had a hypomethylation as a result of methyl compounds starvation [35]. The inflammation occurred as a result of the cellular death as mentioned by [36]. Another possible mechanism is the effect of oxidative stress caused by MTX., some references confirmed the capability of MTX to cause oxidative stress and mentioned the DNA damage resulted by it in adults tissue such as [37, 38] yet there seem to be no clinical trial to test this mechanism in fetal tissue although of the fact that its well documented that the fetus is very sensitive to oxidative stress [39].

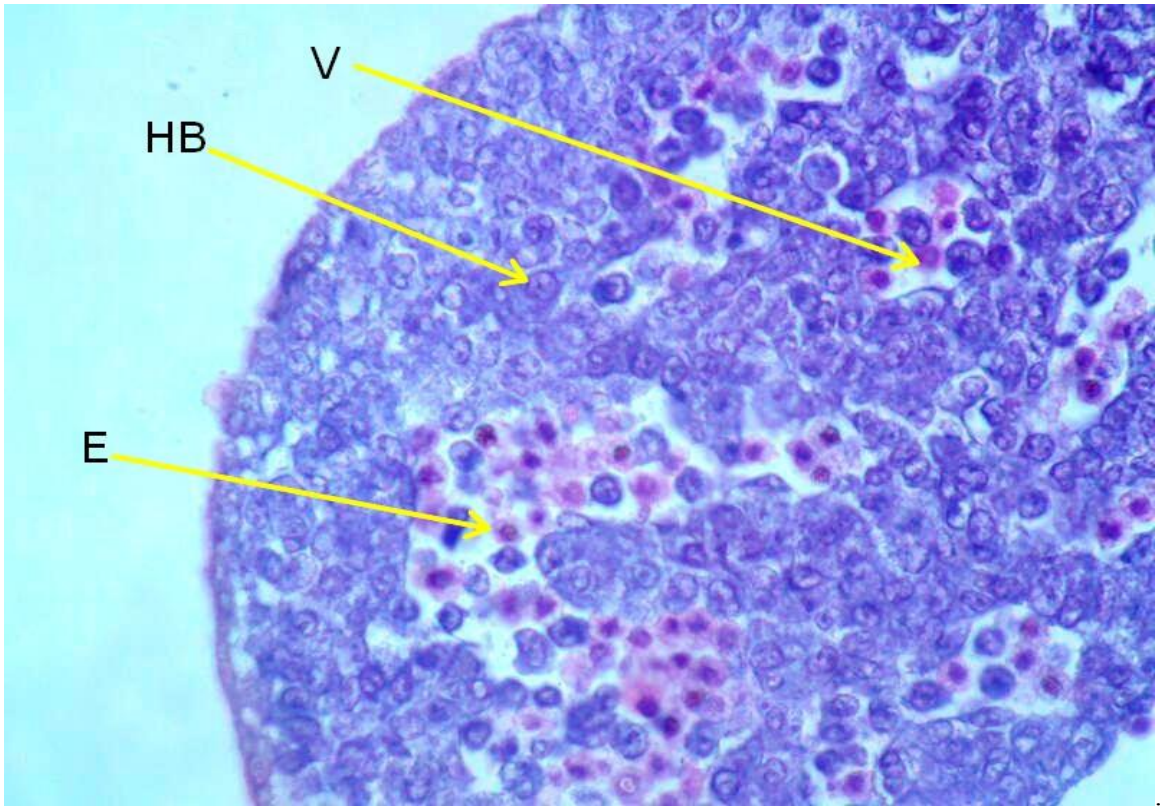

Figure 1- A Cross section in liver of control mouse embryo on day 11th of gestation showing erythroblast (E), hepatic blood vessel (V) and newly developed hepatoblast (HB) .(H & E) (40 x)

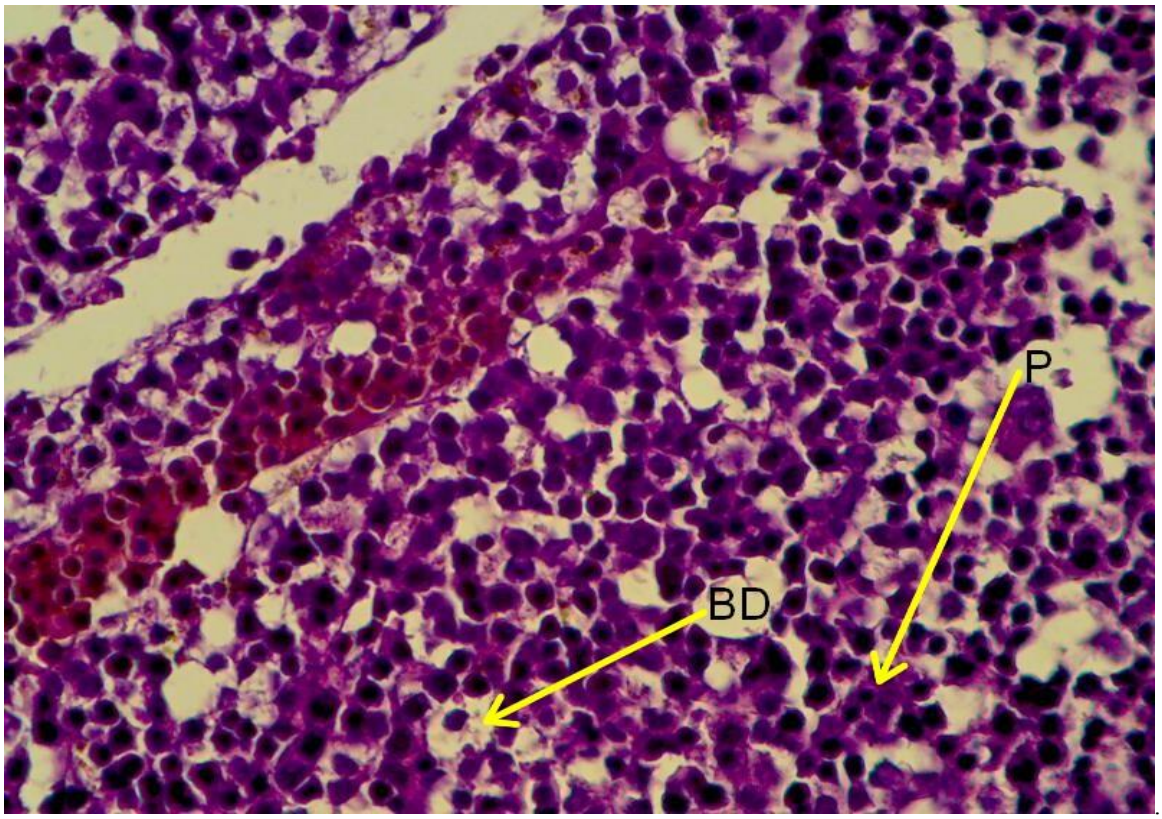

Figure 2- A cross section in liver of mouse embryo on day11 of gestation treated with5mg/kg of MTX. showing ballooning degeneration (BD). And hepatoblast with pyknotic nucleus (H&E) (40 x)

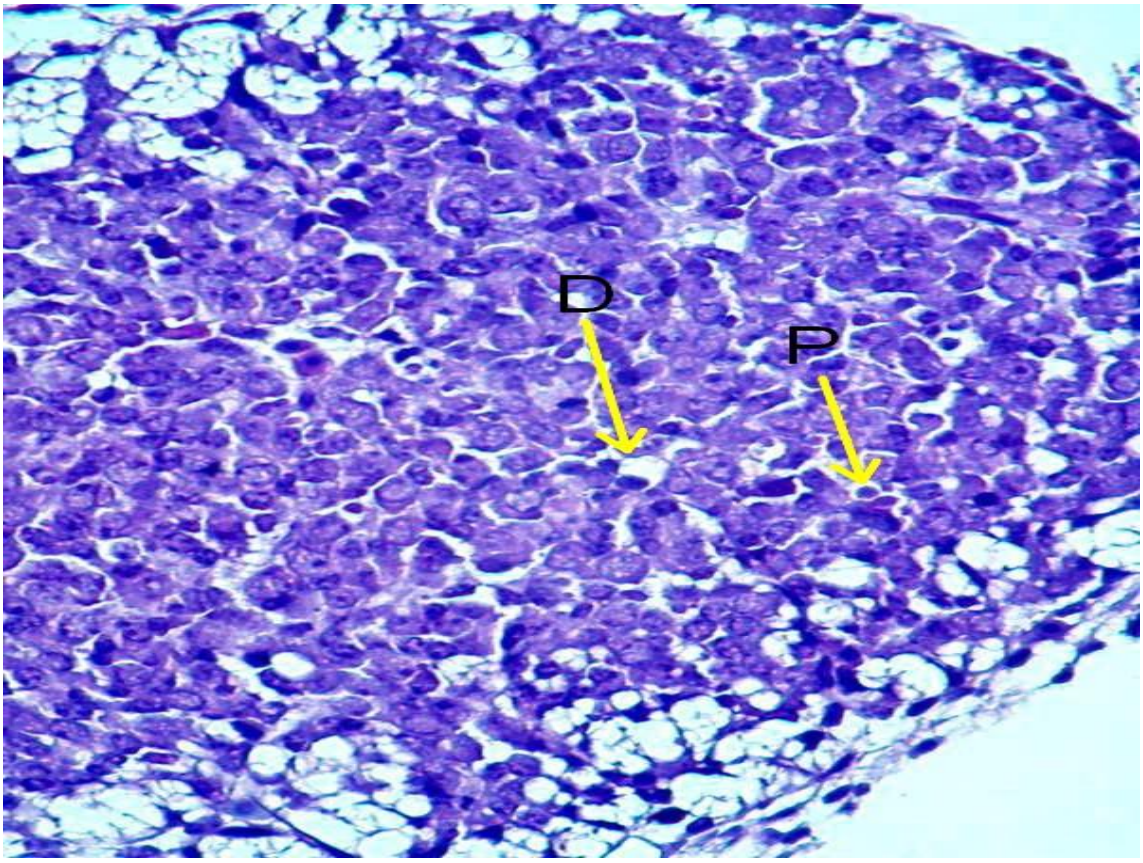

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Figure 3- Across section in liver of mouse embryo on day11 of gestation treated with 10mg/kg of MTX showing ballooning degeneration and hepatoblast with pyknotic hepatoblast (H & E)(40x**)**

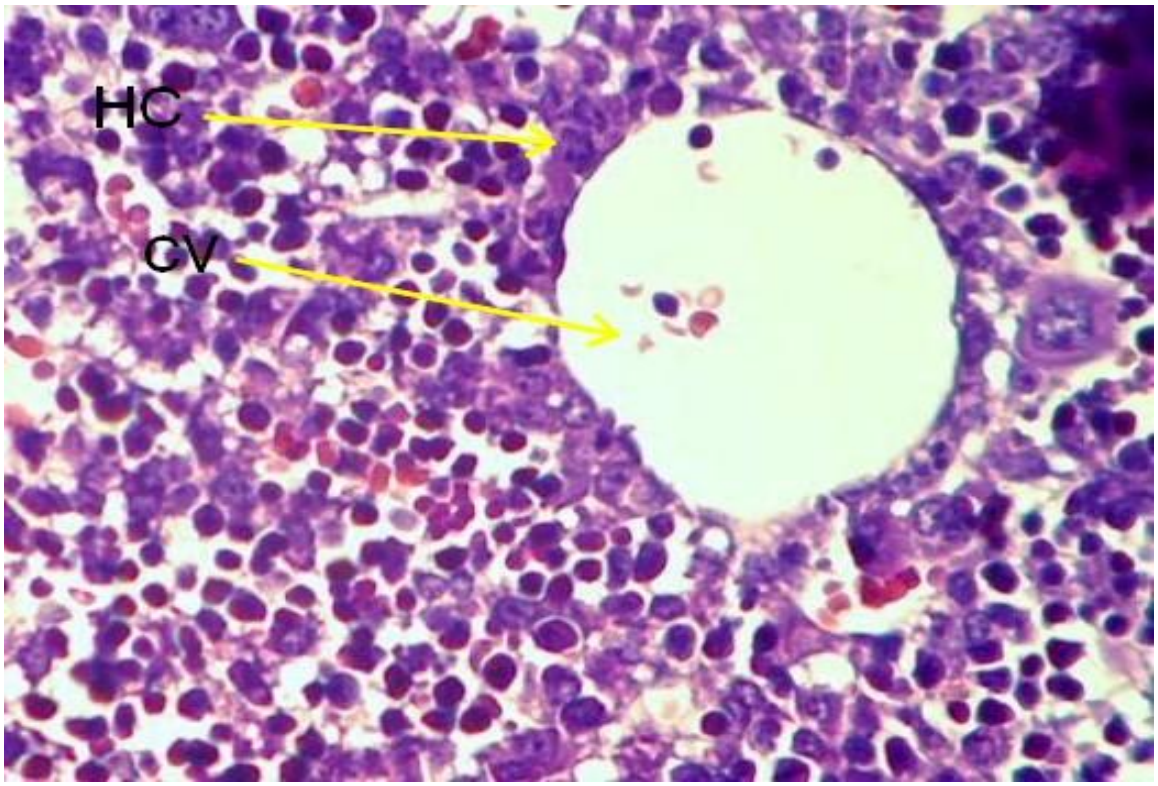

Figure 4- A cross section in liver of control mouse embryo on day 17 of gestation showing hepatocye(HC) around a central vein (CV) (H &E) (40X)

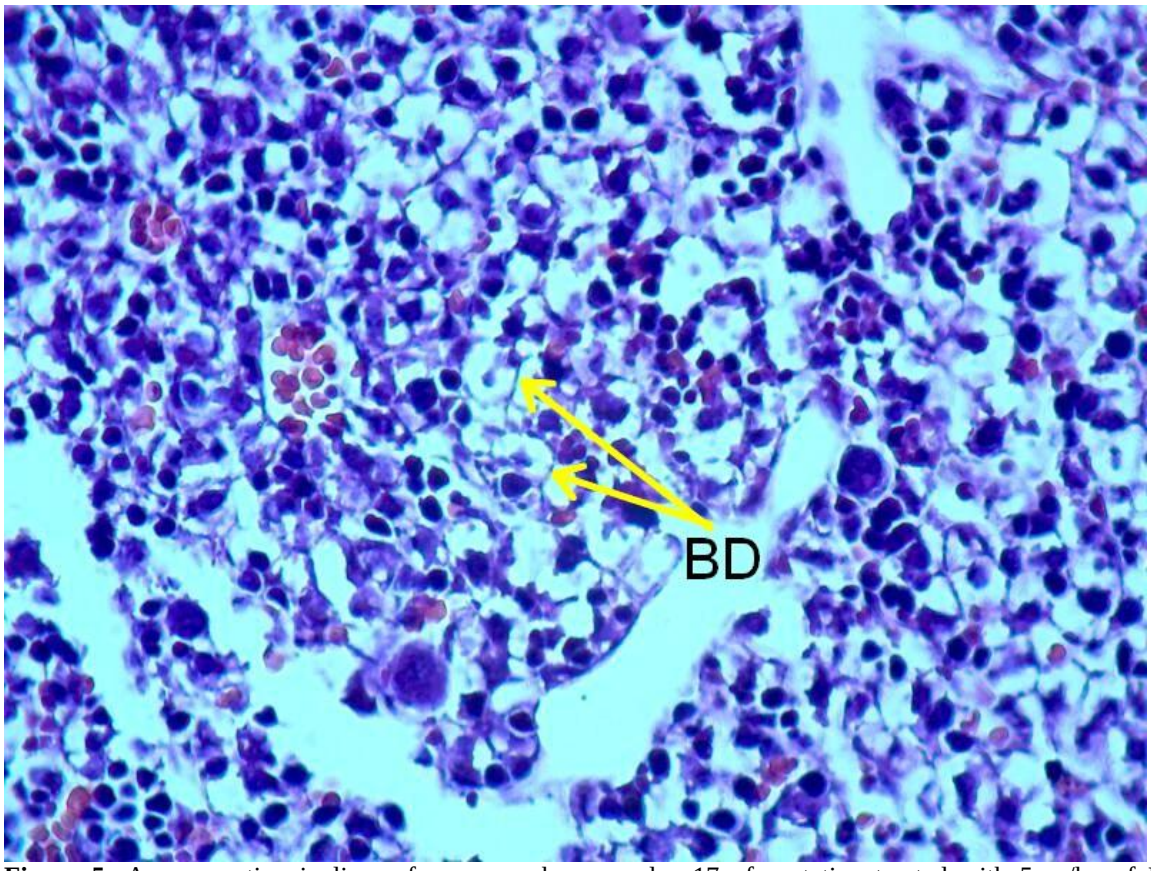

Figure 5- Across section in liver of mouse embryo on day 17 of gestation treated with 5mg/kg of MTX. Showing ballooning degeneration. (H & E) (40 x)

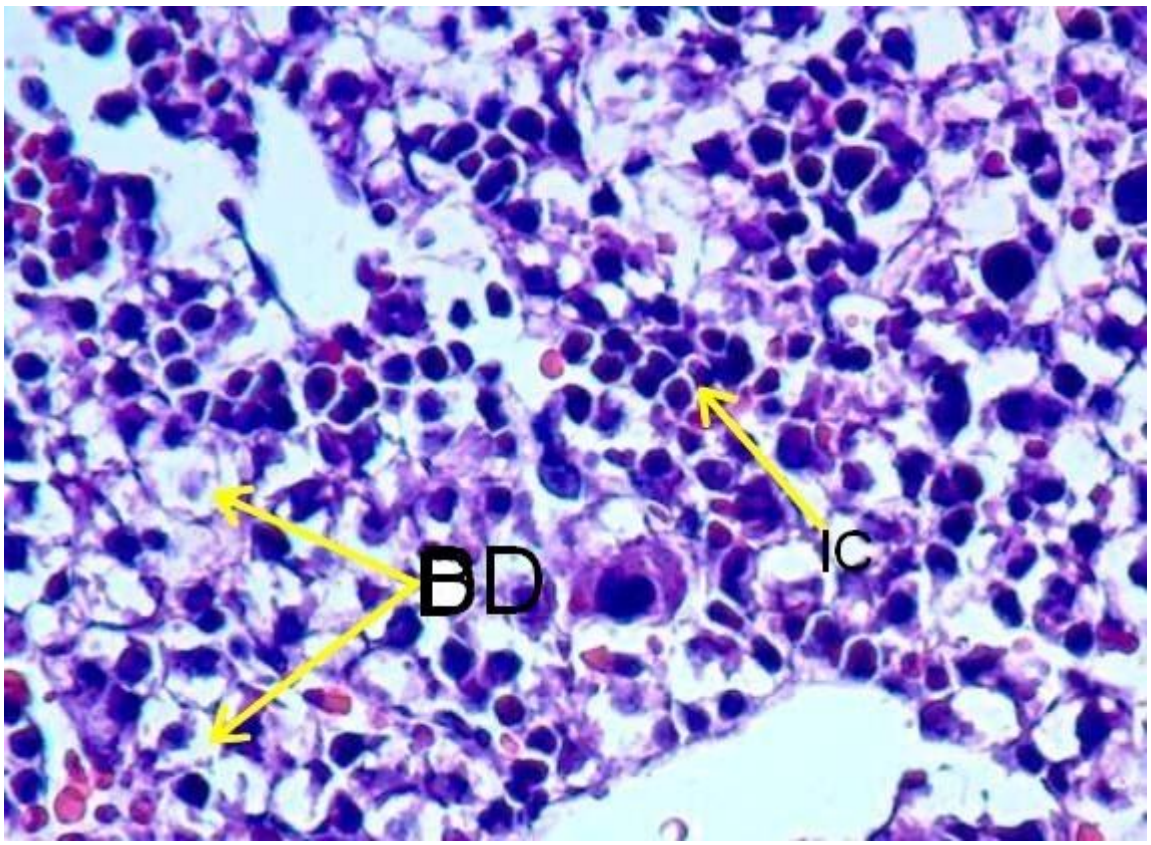

Figure 6- Across section in liver of mouse embryo on day 17 of gestation treated with 10mg/kg MTX showing ballooning degeneration with inflammatiory cells infiltration(IC) .(H & E) (40 x).

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