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Liver embryogenesis and physiological markers associated with induced cholestasis in fetus and pregnant rats

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***Abstract*---**Background**:** This study was designed to evaluate liver embryogenesis and some physiological biomarkers in cholestasis pregnant rats, and cholestasis rats treated with Arginine such as Total Bile acid (TBA), Estrogen hormone(ER) and Macrophage migration inhibitory factor (MIF). Methods**:** In this study, laboratory rats type *Rattus norvegicus* were used and after breeding them in the animal house of College of Science, University of Basrah, (90) virgin females were isolated and divided into three groups, with an average of (30) rats for each group, with average weight of (240 ± 25) g, and aged at (12 -14) week. The first group was considered as control group and was injected with physiological normal saline, second group was induced cholestasis group which injected into the peritoneal cavity with a bile acids dose of (5 mg/kg) of body weight dissolved in the neutral buffer solution for (8) days at rate (1) ml of the solution daily. As for the third group adopted as a treatment group, where cholestasis was induced as in the second group, then injected with a dose (5 mg/kg) of body weight of the amino acid arginine dissolved in the buffer solution for a period of (5) days daily after the induction process. Then rats were anesthetized using chloroform after (12.5, 14.5, 16.5, 18.5, 20.5) days after gestation for blood and tissue collecting. Results and discussion**:** The obtained results uncovered that there might have been statistically noteworthy expand over level for MIF, estrogen (ER) Furthermore bile acids (BA) over serum for prompted cholestasis assembly compared for approached group, which incorporates prompted cholestasis females treated with arginine, there might have been an noteworthy Contrast during (*P<0. 05*) compared with control one assembly.

***Keywords*---**Cholestasis, Cholic acid, Vitamin D3, Macrophage inhibitory factor (MIF), Estrogen hormone (ER).

Introduction

Liver might have been those practically paramount metabolic parenchyma organ about vertebrates with various functions, it receives oxygenated blood starting with the heart by means of the hepatic course Also nutrient-rich blood starting with those gastrointestinal tract by means of those portal vein, hepatocytes make up 70–85% of the liver's cell population, they would practical liver units with parts to metabolic results Furthermore bile arrangement ,synthesis about lipoproteins, serum proteins such as albumin, globulins, gloating Components ,exocrine and endocrine works as preserve glucose ,amino acids, ammodytidae Also bicarbonate homeostasis and change over poisonous substances under nontoxic materials by detoxification. (Hamel et al., 2006).

In rats, The root of the fetal liver is accepted should make an outgrowth of endoderma-derived phones from those incipient organism foregut under the mesenchymal septum transversum, hepatoblasts would depicted as those wellspring of liver progenitor units Throughout separate under Possibly hepatocytes alternately biliary epithelial phones (cholangiocytes) Throughout organogenesis (Shiojiri and Fausto, 1991), Different hepatic cells portions in the grown-up liver might make those wellspring of liver phones Throughout liver recovery. (Sell, 2001)

Cholestasis might be portrayed Concerning representation stagnation, alternately diminish carried out bile emanation Also flow, might be expected for hepatocytes utilitarian prevention alternately piece In at whatever level of the bile excretory pathway, beginning with the level of the hepatic parenchymal phones of the ampulla to Vater in the duodenum, cholestatic jaundice Might in this manner an opportunity to be requested under intrahepatic alternately extrahepatic cholestasis, depending upon the individuals level from claiming square will bile stream (Chen et al., 2018).

Cholestasis may be connected with expanded danger about fetal complications, prematurity, perinatal hypoxia, Also meconium-stained amniote fluid, and sudden passing intrauterine fetal passing , danger about adverse perinatal outcomes correlates for helter skelter bile corrosive focus (Geenes et al., 2014; Kawakita et al., 2015).

In conceived errors about (BAs) conjugation bring about those amassing for large amounts of unconjugated (BAs) ,since unconjugated (BAs) would passively Consumed readily, intraluminal BAs focuses What's more lipid dissolvability are reduced, bringing about extreme insufficiency for vitamins A, D, E, Furthermore k ,it camwood be predicted that unconjugated BAs might experience critical indifferent re-absorption in the bile conduit epithelium and inevitably bringing about a bile conduit driven kind for harm (Setchell et al., 2013; Nwabuobi et al., 2017).

Macrophage movement inhibitory variable (MIF) is a pleiotropic protein demonstrations Similarly as a cytosolic chaperone protein Also need enzymatic functions, including D-dopachrome, phenyl pyruvate tautomerase, and thiol-protein oxido reductase exercises (Petralia et al., 2020).

(MIF) gene polymorphisms have a impact on ailment Conclusion and light of glucocorticoid treatment, An close affiliation between incendiary phones Furthermore harmed bile ducts clinched alongside babies for biliary atresia indicated that incendiary phones assume a central part to ailment pathogenesis ,livers from claiming babies for biliary atresia need an expanded number What's more span for Kupffer phones close harmed ductal cells, also, (MIF) expressions correlates with unit separation (Berdeli et al., 2016).

A few hepatitis models for rodent likewise exhibited that (MIF) need An basic part Furthermore Push the (T helper) Th1 sort safe reaction in the improvemen from claiming intense hepatitis Also hepatic injury, which provided for those part of t lymphocytes and An Th1 cytokine IFN-g On biliary atresia pathogenesis (Wammers et al., 2018). Moreover Estrogen induced cholestasis syndromes is a very common form, this hormone can inhibit bile acids transportation by interfering with bile salts export pump (Chen et al., 2018).

Under cholestatic conditions, bile emission Also bile stream are exasperates and its focus expanded pretty nearly 66% more than solid conditions, cholestasis camwood be prompted by those obstacle from claiming bile ducts Toward tumors or gallstones, immune system reactions, pregnancy, drug-induced hepatotoxicity, autosomal-recessive issue and additionally liver infections (Wammers et al., 2018).

Cholestatic states need aid connected with hepatic aggravation it will be a critical characteristic for cholestatic liver infection over both people Furthermore test animals (Koeppel et al., 1997), Incessant aggravation of the liver, this incendiary procedure may be went with for Kupffer units activation, amassing for actuated safe phones Furthermore brings about hepatocellular apoptosis What's more necrosis, Additionally created burgeoning of bile conduit epithelial phones (Gujral et al., 2013).

Material and Methods

Experimental animals

Those study might have been done once solid grown-up virgin females Wistar pale rats (*Rattus norvegicus*) age (12-14) weeks with Normal weight (240 + 25) g, the animals were brought from the College of Education, Thi Qar University, the College of Veterinary Medicine, Basra University, and the College of Veterinary Medicine, Al-Kassim Al-Khadra University, then transferred to the animal house in the College of Science, University of Basra. The animals were housed in plastic with an adequate temperature (25-30) ℃ with nearly equal light cycle (12 - 12) hours, water and food were available throughout the experiments period (Almalak, 2004).

Mating and gestation period determination

Rats at the age range (12-14) weeks old were selected and the average weight was (240 ±25) g, after health & safety of rats were ensured, the adult females ready for fertilization were isolated and placed in a cages with males at ratio (1 male : 2

females) in every cage then let overnight, after being sure that mating took placed on morning by noticing the vaginal plug image (1), the females were isolated in individual cages, The day ahead which this plug might have been watched will be day zero of gestation (G0) (Balducci-Roslindo *et al.*, 2001).

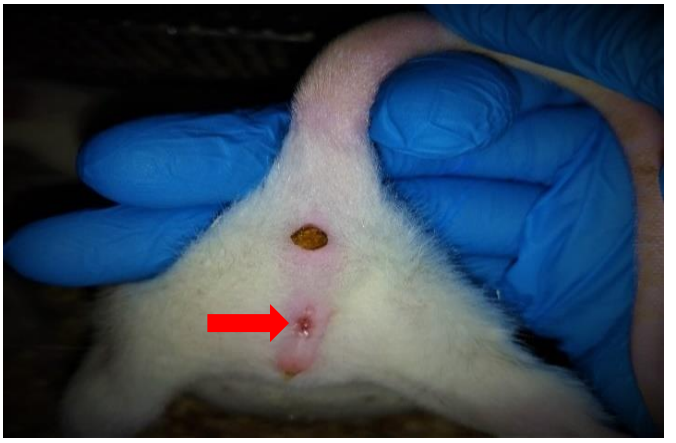

Image (1): Photo with respect to female rodent following mating, show those vaginal plug shaping. (➡) which revealed to the fertilization occurrence, (G0) gestation day.

Animals grouping and treatment:
Total number (90) of females Wistar albino rats (*Rattus norvegicus)* were randomly isolated and divided into three groups (fig. 2.1), with (30) rats on each group in a rate of (6) rats for each gestation period (12.5, 14.5, 16.5, 18.5, 20.5) days as follows:

Group I / control group
This group was regarded as a control group, it was injected with (1 ml) of normal saline intraperitoneally (IP) with one a day for 8 days, starting from the first day of gestation (G0) (Yu *et al.*, 2014).

Group II / Induced cholestasis group
Each female was injected (IP) with type of bile acids known the cholic acid (CA) to induce cholestasis, suitable dose 5 mg/ kg body weight (Bwt) was dissolved in (1ml) of buffer phosphate saline (BPS) to injected each rat with the equivalent of (1 ml) of solution once a day for 8 days, starting from the first day of pregnancy (G0), (CA) solution should be prepared freshly before injection (Yu *et al.*, 2014).

Group III / Treated group (Cholestasis + Arginine)
Pregnant females in this group were injected (IP) with the same dose of cholic acid (CA) once a day for (8) days, starting from (G0) day of gestation to induce cholestasis ,then at (9) day of gestation all rats were injected (IP) with arginine (Arg) at a dose of (5 mg) / 1 kg (Bwt) , dissolved in buffer phosphate saline (BPS) to injected the rats with the equivalent of (1 ml) of solution for each animal for (5) days (Yu *et al.,* 2014).

Sacrificed the experimental rats:
The animals were weighed on zero days (pretreatment) and at the end of the experiment (before scarified), all females from each group (control, induced and treated) were randomly sacrificed after being anesthetized with overdose of chloroform at each period of gestation (12.5, 14.5, 16.5, 18.5, 20.5) days, the fetuses and liver was examined, blood collected as follow.

Collection of blood:
Rats in all experimental groups were sacrificed at the end of each periods (12.5, 14.5, 16.5, 18.5, 20.5) days, after anesthesia with chloroform, suitable Cut in those rodent abdomen might have been done, those tests about blood were gathered starting with those heart by heart puncture for utilization of disposable syringes of (3-5)cc capacity, blood permitted on cluster through abandoning it In room temperature for(15-30) min., (5) ml of blood was poured into jelly test tubes free from anticoagulant and centrifuged at 3500 rpm about 10 min to isolated serum which then transmitted to Eppendorf tubes and stored at -20°C for biochemical tests (Cray *et al.,* 2009). Serum used to determine the biochemical parameters such as Estrogen hormone level(ER), Macrophage inhibitory factor(MIF)and Bile acid (BA) concentration.

Tissue samples:
Specimens from livers of pregnant rats in each experimental group was dissected post ( ) days of gestation and samples from fetuses were excised , washed with normal saline to removed blood and tissue debris then, cut into suitable size of specimens and transferred to fixed with (10%) formalin fixative to processing for histological examination .

Determination of Estrogen hormone(ER) concentration
Depending on the producer instructions, TOSOH, Japan, if estrogen, alternately progesterone those same manner). The test utilizes those aggressive restraint catalyst immunoassay technique, suitable microtiter plate wells for an immunizer particular to (estrogen) What's more horseradish peroxidase (HRP) Previously, estrogen unit.
Should figure the come about utilizing those professional delicate (curve expret 1. 3) to make a standard bend may be proposed.

Determination of Total Bile acid (TBA)
Bile acid test unit gives an advantageous fluorimetric methods with measure downright bile acids to living tests. Those coming about fluorescence force level (530 nm) is straight of the bile corrosive focus in the example. No wash Also reagent exchange steps need aid included. Could make promptly robotized for HTS fluid taking care of frameworks to preparing many tests for every day. At specimens Also norms ought to be run Previously, copy. Ultrapure water might have been utilized for those preparation from claiming Sample, internal Standard, and test spotless. Dark even lowest part plateswas utilized. Former with assay, bring at reagents will room temperature. Briefly axis catalyst tubes and continue ice Throughout test. Serum tests might a chance to be put away during –20 c to 3 weeks. Three wells necessary for every sample: Sample, inner Standard, Furthermore example spotless.

Calculation: bile corrosive focus of a example will be computed according to:.

$$[\text{Bile Acids } \mu M] = \frac{\text{F SAMPLE} - \text{F BLANK}}{\text{F STANDARD} - \text{F SAMPLE}} * 20 * n$$

Determination of Macrophage migration inhibitory factor (MIF)

A symptomatic unit for rodent macrophage relocation inhibitory variable (MIF) might have been used to measure the fixation about MIF Previously, serum in light of sandwich enzyme-linked immune-sorbent test engineering.

Results

Determination of Estrogen hormone (ER) concentration

The Mean values of Estrogen hormone in cholestasis rats at each period of gestation (12.5, 14.5, 16.5, 18.5, 20.5)days revealed to significant rise at ($p<0.05$), the mean rate got to (8.22, 36.91, 55.47, 67.92, 80.82) Pg/ml respectively associated to control set, table 1

Table 1 Mean value of estrogen hormone in all tested groups compared to control group at (12.5, 14.5, 16.5, 18.5, 20.5) days post gestation. All values expressed as mean ± SD

Period	Control group	Cholestasis group	Treated group
	Mean ± SD pg/ml	Mean ± SD pg/ml	Mean ± SD pg/ml
G12.5	5.78 ± 0.68	8.22 ± 0.93	6.85** ± 0.40
G14.5	6.75 ± 0.94	36.91* ± 1.45	7.74 ± 1.1
G16.5	6.98 ± 0.52	55.47* ± 2.12	17.51** ± 0.76
G18.5	7.73 ± 0.37	67.92* ± 2.12	51.54** ± 0.69
G20.5	8.49 ± 0.15	80.82* ± 5.66	55.41** ± 1.99

*/ revealed that there was a significant change in the average of estrogen concentration between the induced collection and the control collection

**/ revealed that there was a significant differences difference in the average of estrogen concentration between the treatment group and the control group

3.2 Determination of Total Bile acid (BA)

The results of the current study showed a significant rise at the probability level ($p<0.05$) in the average concentration of bile acids in the induced cholestasis group females compared with the control group females in all studied periods. A decrease in the concentration of bile acids was recorded as the pregnancy progressed in the control and treatment groups, while its concentration increased in the induced cholestasis group with the progression of pregnancy periods. Furthermore, no significant changes between the concentration of bile acids in the treatment group females compared with the control group females, Table 2.

Table 2 The mean value of bile acid in all tested groups compared to control group at (12.5, 14.5, 16.5, 18.5, 20.5) days post gestation. All values expressed as mean ± SD

Period	Control group	Cholestasis group	Treated group
	Mean ± SD (mm)	Mean ± SD (mm)	Mean ± SD (mm)
G12.5	12.48 ± 1.32	13.47 ± 1.59	13.39^{NS} ± 0.84
G14.5	13.93 ± 0.82	14.21^{*} ± 1.43	14.58^{NS} ± 1.14
G16.5	12.85 ± 1.55	15.06^{*} ± 1.6	15.86^{NS} ± 0.78
G18.5	12.06 ± 1.6	24.67^{*} ± 1.33	11.79^{NS} ± 0.61
G20.5	11.01 ± 0.89	25.5^{*} ± 0.91	10.88^{NS} ± 1.01

*/ showed that here was a significant change in the average of bile acid concentration among the induced group and the regulator group
NS/ showed that here was no significant changes difference in the average of bile acid concentration between the treatment group and the regulator group

Determination of Macrophage migration inhibitory factor (MIF)

Results on biochemical teat to determine (MIF) concentration revealed significant increase at ($p<0.05$) in serum of cholestasis rats at each period (12.5, 14.5, 16.5, 18.5, 20.5) day post gestation, the mean level reached to (0.18, 1.33, 1.85, 2.54, 4.26) Pg/ml respectively compare to the mean concentration of (MIF) of control rats at the same period of gestation, the mean value reached to (0.22, 067, 0.98, 1.35, 1.54) respectively (table 3).

Also the results showed significant difference in (MIF) factor in serum of cholestasis rats treated with arginine, the mean concentration reached to (0.39, 0.75, 1.08, 2.16, 3.6) Pg/ml at each period (12.5, 14.5, 16.5, 18.5, 20.5) day post gestation compare with cholestasis and control group (table 3).

Table 3 The mean value of macrophage migration inhibitory factor (MIF) concentration in all tested groups compared to control group at (12.5, 14.5, 16.5, 18.5, 20.5) days post gestation. All values expressed as mean ± SD

Period	Control group	Cholestasis group	Treated group
	Mean ± SD (Pg/ml)	Mean ± SD (Pg/ml)	Mean ± SD (Pg/ml)
G12.5	0.22± 0.02	0.81^{*} ± 0.04	0.39^{**} ± 0.26
G14.5	0.67 ± 0.07	1.33^{*} ± 0.42	0.75^{**} ± 0.15
G16.5	0.98 ± 0.48	1.85^{*} ± 0.13	1.08^{**} ± 0.02
G18.5	1.35 ± 0.18	2.54^{*}± 0.38	2.16 ± 0.3
G20.5	1.54 ± 0.08	4.26^{*} ± 0.03	3.6^{**} ± 0.14

*/ Demonstrated that there might have been a huge Contrast in the Normal from claiming MIF fixation the middle of the prompted aggregation and the control one assembly.
**/ Demonstrated that there might have been an noteworthy Contrast in the Normal of MIF centralization between the medicine one assembly and the control gathering.

Histological study

The study clarified the rats embryos liver of cholestasis group at the (12.5) days post gestation and figures showed reduced with liver parenchymal tissue, irregular of liver lobules, reduced in haemopoietic tissue which it is the most compartment in liver mass at this stage, degeneration of most epithelial cells that surrounded the hepatoblasts, congested of erythroblastic islands, degeneration of erythroblastic cells and unclear erythrocyte with nucleus, dilated of venous canals and portal branches (fig 1).

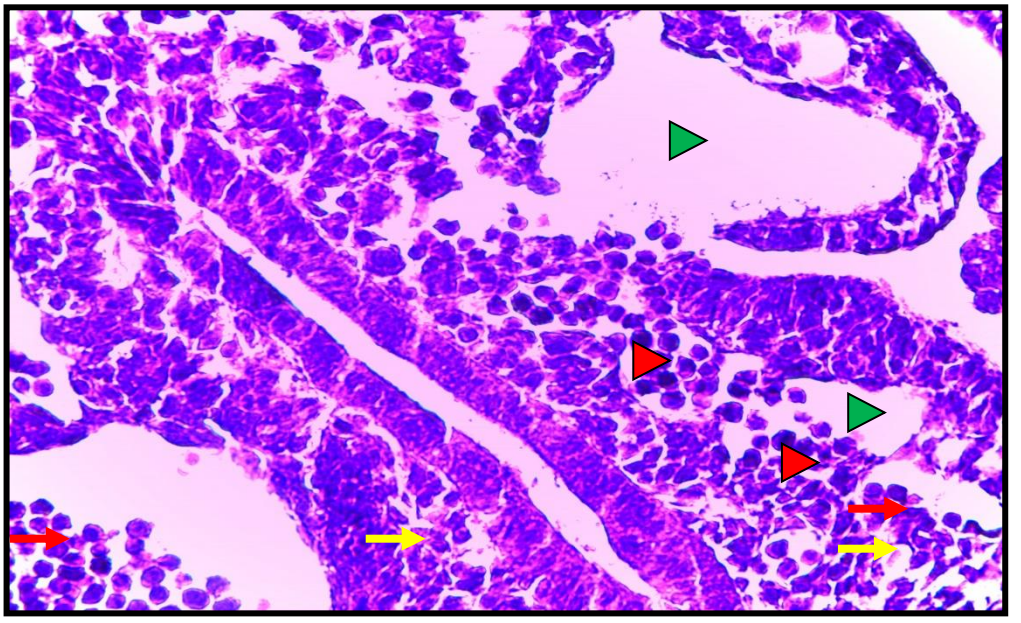

Figure 1: Transverse section in rat fetus from cholestasis group at (12.5) day post gestation showed structure of haemopoietic tissue (➡) composed of erythroblasts, most of erythroid with nucleus (▶) separated by cavities (▶) and reduced liver parenchyma (➡) (H&E) stain (100X).

Optical microscopy of tissue sections in the fetal liver of cholestasis group at (14.5) day showed degeneration of the endothelial cells lining the blood islands with the apoptosis of these islands, reduction in the size of the liver visceral tissue and the non-differentiation hepatobiliary cells, the absence of erythrocyte generating cells with nuclei inside the blood islands clearly and the expansion of the venous channel, sever congestion of central veins and dilated branches of portal ducts with infiltration of inflammatory cell (fig 2).

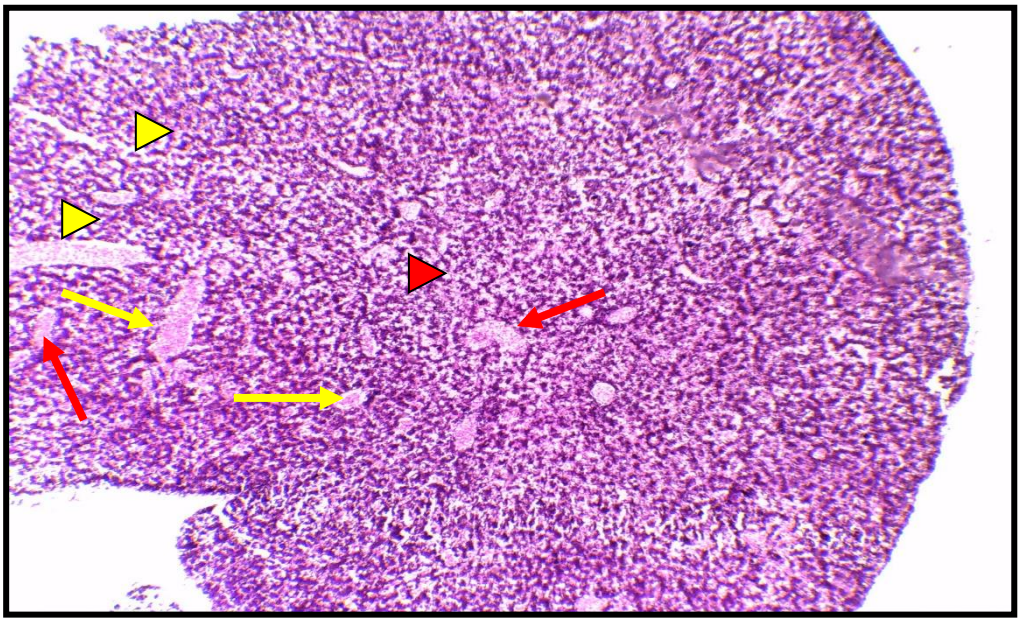

Figure 2: section in rat fetus from cholestasis group at (14.5) day post gestation ; 2showed reduced liver parenchyma ( ), sever congested in sinusoids and branches of portal duct ( ), the haemopoietic tissue appeared as foci ( ), congested venous sinuses ( ). (H&E) stain (100X).

Result on light microscopic exam of fetus liver sections from cholestasis group at (16.5) days post gestation showed irregular liver lobe, the hepatic parenchyma irregular with congested sinusoids and necrotic foci with sever inflammatory cells, most sinusoids with nucleated erythrocytes, increased thickness of transverse mesenchymal septum were observed, accumulation of blood cells and inflammatory cells within the portal tract, disorganized hepatocytes, increase with lining layer of venous and portal ducts and degeneration of endothelial lining layer of portal vein. (fig 3)

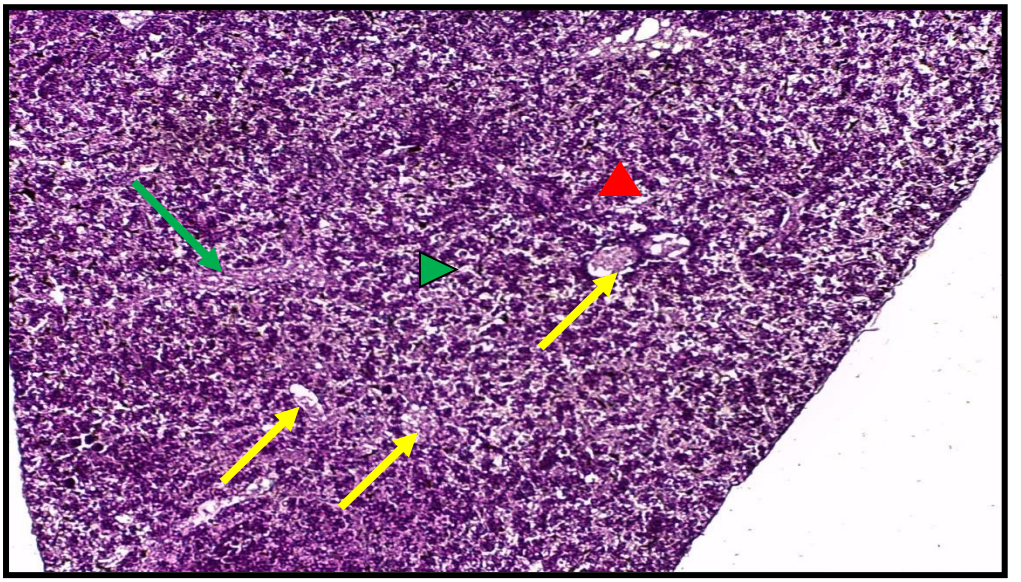

Figure 3: Section of fetus liver at (16.5) day post gestation from cholestasis rat showed hepatic reduced of liver parenchyma (▶), congestion of central vein (➡), hemorrhage within sinusoids (➡), irregular of hepatocytes (▲). (H&E) stain (40X).

Microscopic exam of fetus liver from cholestasis group showed incomplete maturation of the liver, irregular liver lobes with the reduction and disintegration of hepatic visceral tissue cells, leaving a space between them, degenerated hepatic cell non-differentiation of their boundaries sever congestion of portal vein and branches of portal ducts, disorganized of hepatocytes, reduced of liver parenchyma and degeneration of lining endothelial layer of portal veins, still inflammatory cells and erythrocytes within sinusoids and haemopoietic tissue appeared as blood islands foci, moreover, degeneration of capsule surrounding the liver lobe, also thick mesenchyme tissue that must give latter the diaphragm, in addition sections in lung, stomach and vertebrae was noticed (fig 4).

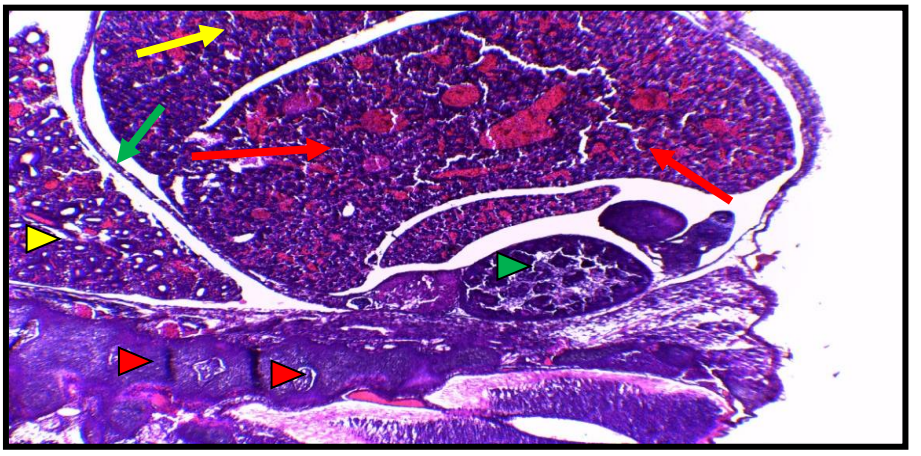

Figure 4: Section in rat fetus liver at (18.5) day post gestation related to cholestasis group showed irregular and incomplete liver lobes ( ), loose liver stroma ( ), surrounded by mesenchyme tissue develop to diaphragm ( ), section in stomach ( ), lung ( ), and vertebrae ( ) observed. (H&E) stain (40X).

Figures on fetal liver at (20.5) day of gestation from induced cholestasis group revealed to reduction in the size of the liver, undifferentiating of hepatic lobes and their irregular division, reduction of the hepatic visceral parenchyma, degeneration of hepatocytes, severe blood congestion within venous canals and lysis of the endothelial cells lining these canals, and resorption of inflammatory cells. Also, mild steatosis observed, hemorrhage within sinusoids, bile ducts hyperplasia (fig 5)

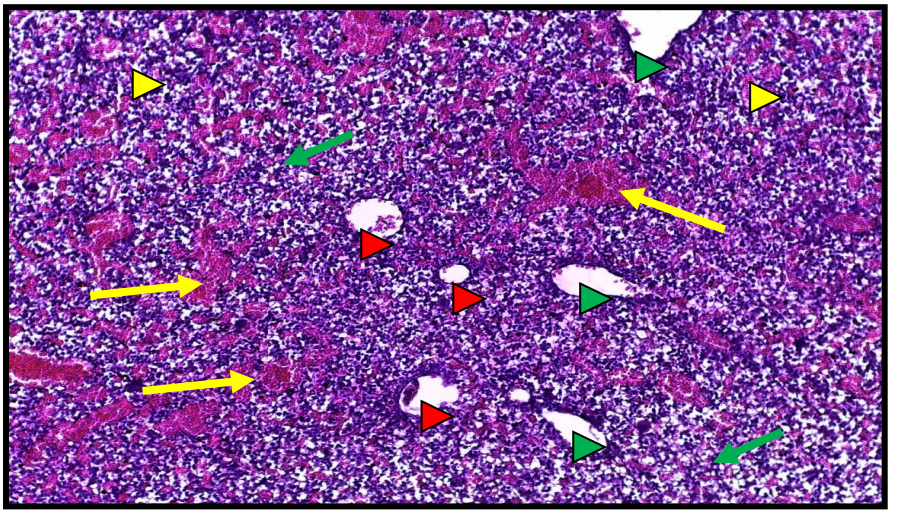

Figure 5: Section in fetus at (20.5) day post gestation from cholestasis group showed reduced in liver parenchyma size ( ), dilated portal tract ( ), with lining layer hyperplasia, hemorrhage ( ) within liver stroma, mild steatosis ( ) and congestion of central vein ( ). (H&E) stain (40X).

Discussion

Cholestatic liver infection means any circumstances connected with impeded bile stream accompanying with a poisonous bile corrosive aggregation in the liver or systemic circulation, could be subdivided under different sorts as stated by its clinical phenotype, for example, such that biliary atresia, drug induced cholestasis, gallstone liver disease, intrahepatic cholestasis of pregnancy, essential biliary cholangitis What's more grade sclerosing cholangitis (Gijbels *et al.*, 2021).

Determination the concentration of Estrogen hormone

The results of the current study showed a rise in the level of estrogen in the blood serum with a statistically significant difference ($P<0.05$) in the induced cholestasis group compared with the control group and the treatment group, and also showed significant differences between estrogen concentration in female rats in induced cholestasis group compared with treatment group. The reason is due to the increased concentration of bile acids affects the functional activity of hepatocytes, thus obstructing estrogen metabolites to be converted into steroids.

Estrogen camwood restrain bile acids transportation from hepatocytes under bile canaliculi Toward meddling with the bile salt send out pump Also multidrug resistance-associated transporter, and also blacks it likewise inhibits Na+-taurocholate cotransporting polypeptide Also Na+-independent natural anion-transporting polypeptides in the basolateral film of hepatocytes with meddle with the uptake for bile acids (Jiezhong *et al.*,2013).

Estrogen-induced cholestasis camwood likewise happen Throughout pregnancy; intrahepatic cholestasis for pregnancy is those the vast majority regular liver infection for pregnant women, cholestasis brings about hepatic What's more systemic amassing of cytotoxic bile acids, this induces liver harm went with Toward pruritus and indigestion, extreme frisbee prompting biliary fibrosis and cirque which prompt liver brokenness, affect hormones metabolism, liver enzymes synthesis and lost homeostasis (Pan & Jeong, 2015).

Determination of Total Bile acid (TBA)

Bile acids would amphipathic sterols synthesized starting with cholesterol in the liver Furthermore discharged under the digestive system the place they assume a fundamental part Previously, emulsifying dietary lipids (Li and Chiang, 2014).

The results of the current study showed a significant increase in the level of bile acids at G12.5, G14.5, G16.5, G18.5 and G20.5 pregnancy periods in induced cholestasis group compared with control group the increase in bile acids concentration in the blood serum was due to accumulation of bile acid concentrations, during the first 8 days of pregnancy led to necrosis of hepatocytes and liver normal structure breakdown, which caused the release of bile acids into bloodstream and obstructed liver physiological function, these results agree with the study (Wammers *et al.*, 2018), which showed increased levels of bile acid in female rat with induced cholestasis.

Moreover our results also discussed in other studies pointed that Bile acids are profoundly cytotoxic, and the over the top amassing might actuate a few abnormalities for example, cholestatic liver injury, it will be referred to that the bile corrosive digestion system alters Throughout pregnancy bile acids would significant courier atoms that not just partake in the digestion system of glucose, lipid, energy, pills as well as includes clinched alongside safe regulation, however, bile acids would profoundly cytotoxic because of their cleanser properties , those over the top amassing for hydrophobic bile acids, for example, cholic corrosive (CA), chenodeoxycholic corrosive (CDCA) Furthermore murine bile corrosive (MCA) camwood actuate aggravation What's more cell demise for liver, prompting cholestatic liver harm Furthermore actually cirque (Lee *et al.*, 2016; Taoka *et al.*, 2016)

Determination of Macrophage migration inhibitory factor (MIF)

Current study results showed that the induced cholestasis group showed a statistically significant increase in macrophage migration inhibitory factor (MIF) at different pregnancy periods compared with control group, the reason for increase in level of this growth factor MIF may be due to its increased secretion from organs close to liver and the inability of the liver to filter blood from it, as well as its excretion by some cells in the liver, such as hematopoietic cells and Kupffer cells, this study agrees with the results of several studies (Arikan *et al.*, 2006) confirmed that the concentration of growth factor MIF in the blood serum of animals exposed to induced cholestasis bile stasis increases compared to the control group animals.

Macrophage relocation inhibitory figure (MIF) will be a incendiary cytokine What's more a paramount controller from claiming intrinsic resistant responses, serum focuses from claiming MIF would connected with sickness seriousness What's more result for patients for decompensated cirque and acute-on-chronic liver disappointment (ACLF), moreover, circle (MIF)concentrations associate for sickness seriousness for immune system and cholestatic liver infection (Wirtz *et al.*, 2021).

Histological study

The results of histological examination of livers of females in control group showed the presence of hepatocytes with a normal, nucleated, healthy cytoplasm and a distinct central vein around which the hepatocytes are arranged in ropes of radial arrangement between which the bloody areas are confined and surrounded by a thin capsule from which barriers extend between the lobes, and this is ;consistent with the study of (Ober & Lemaigre, 2018) which showed that the normal histological structure of the adult liver in rats is branded by the existence of hepatic lobes consisting of ropes of hepatocytes originating from the central vein and heading to the periphery in a radial arrangement and are present between these cells, as well as the presence of portal veins and also extend the bile ducts that penetrate the hepatic lobes.

The results of the current study of tissue sections in the livers of induced cholestasis group females indicated necrosis and hepatocellular degeneration and

nucleation disintegration with central vein congestion, hematopoiesis, and resorption of inflammatory cells, as well as enlarged hepatocellular nuclei and increased vacuoles in some cells. The reason for the tissue changes in livers of the induction group is to increase the concentration of acids Bile, which has a negative effect on the membranes of hepatic cells and blood vessels, and what may lead to damage and blockage of the bile ducts and consequently damage to the liver, and these results are similar with what (Yu *et al.*, 2014) mentioned about the effect of injection and increase of bile acids and the damages it causes such as hepatocytes necrosis with steatosis and cellulitis.

In addition, Wammers *et al.*,(2018) indicated that the hepatic damage resulting from the injection of bile acids causes several tissue changes, including necrosis of the liver lobes, with the appearance of edema, lipid changes, congestion of blood vessels, and resorption of inflammatory cells.

It was observed in the present study that the histological sections of the livers of the female livers of the treatment group which treated with bile acids and then arginine showed more regular liver cells with almost normal visceral tissue, with a decrease in infiltration of inflammatory cells, the presence of a central vein and almost regular blood vacuoles, and this shows the importance of the amino acid arginine in the healing of the liver from damage, and this was supported by (Ozsoy *et al.*, 2011) who indicated that the damaged liver tissue is characterized by the presence of a number of histological changes, including the presence of clear gaps in the cytoplasm of hepatocytes with proliferation of nucleolus cells and the expansion of blood sinuses and portal veins, while these changes were less apparent when using acid Amino-arginine, as the histological structure of the liver was closer to normal, and this is due to the importance of arginine in maintaining the stability of the cell membranes of hepatocytes and reducing the toxic effect of increasing the concentration of bile acids.

Arginine protects the liver by activating the heapto-detoxicity process via its anti-oxidant property thus protecting the liver from damage and lipolysis (Hofmann, 2004). Arginine also plays a role in reducing hepatic necrosis and inhibiting programmed cell death by producing NO, regulating liver enzyme levels and inhibiting the oxidative process (Wu et al., 2009).

Cholestatic liver fibrosis may be connected with bile acid-induced oxidative stress and lipid peroxidation, furthermore, oxidative stress disturbs liver fibrosis by means of stellate cell activation, moreover, the bile salts would mostly answerable for the plasma film harm which prompts further oxidative anxiety (Sokolovic *et al.*, 2013).

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