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Histopathological effect of amoxicillin on the liver and kidney of pregnant mice *Mus musculus*

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Abstract

The current study aimed to investigate the effect of amoxicillin trihydrate on inducing histopathological lesions in the maternal livers and kidneys, weights of maternal bodies and selected organs, as well as behavioural changes of pregnant mice *Mus musculus*. The doses were given orally from 7th day till 17th day of pregnancy. A total of 21 pregnant mice were used in this study. Animals were divided into three groups, 7pregnant mice/ group. Group I, was received orally distilled water during experimen period, while groups II and III received 1500 and 3000 mg/kg b.w.orally of amoxicillin trihydrate during the same period above, respectivelly. Histopathological examination of liver sections of group II revealed congestion, dilatation of the central vein, moderate infiltration of inflammatory cells and sinusoids dilatation, while degeneration and necrosis of hepatocytes were highlighted in the group III. Kidney sections in the group II showed swelling and degeneration of some renal tubules, as well as degeneration of glomerular cells, expansion of Bowman's space and acute swelling of the proximal tubules were observed in group III. Forthermore, groups II and III revealed decrease in the weight of maternal bodies and selected organs, both groups II ,III showed behavioral changes including; isolation, irritability agrression and excessive movement comparing to the control group. In conclusion, using high doses of amoxicillin trihydrate during pregnancy impact on maternal body in many harmful ways as confirmed in the current study. Thus, it seems to be important to use this drug after the physician consultation and knowing the prescribed doses.

Keywords: Amoxicillin, histopathology, pregnancy, liver, kidney, mice

الخلاصة

هدفت الدراسة الحالية الى التحري عن تأثير الاموكسيسيلين ترايهيدريت عند الجرع 1500 و3000 ملغم/كغم من وزن الجسم، في حث التغيرات النسجية المرضية في

اكباد وكلى الفئران الحوامل، التغيرات الوزنية لاجسام الامهات والاعضاء المختارة، فضلا عن التغيرات السلوكية للفئران الحوامل *Mus musculus*. اعطيت الجرع فمويا من اليوم السابع الى اليوم السابع عشر من الحمل. استخدم في الدراسة الحالية 21 فأر حامل، اذ قسمت الى ثلاث مجاميع، 7 فأر حامل لكل مجموعة. المجموعة الاولى جرعت فمويا بالماء المقطر خلال فترة التجربة. في حين جرعت المجموعتين الثانية و الثالثة بالجرع 1500 و3000 ملغم/كغم من وزن الجسم من عقار الاموكسيسيلين ترايهيدريت خلال نفس الفترة المذكورة في اعلاه . اظهر الفحص النسجي لمقاطع الكبد للمجموعة الثانية احتقان وتمدد الوريد المركزي، ارتشاح معتدل للخلايا الالتهابية وتوسع الجيبانيات، في حين لوحظ التنكس والنخر في الخلايا الكبدية في المجموعة الثانية. اظهر الفحص لمقاطع الكلية للمجموعة الثانية تورم وتنكس بعض النبيبات الكلوية ،في حين لوحظ التورم الحاد لبعض النبيبات الكلوية، تنكس الخلايا الكبيبية وتوسع فسحة بومان. علاوة على ذلك لوحظ انخفاض في وزن الامهات والاعضاء المختارة في المجموعتين الاولى والثانية فضلاعن، تسجيل تغيرات سلوكية تضمنت: الانعزال، التهيج، العدوانية، فرط الحركة مقارنة مع مجموعة السيطرة. لهذا نستنتج، ان استعمال الجرع العالية من الاموكسيسيلين ترايهيدريت خلال الحمل من الممكن ان يؤثر على جسم الام وبعدة طرائق ضارة كما توصلت اليه الدراسة الحالية. وبهذا، يبدو من المهم ان يتم استخدام هذا العقار تحت استشارة طبية مع التعرف على الجرع الموصوفة.

الكلمات المفتاحية :اموكسيسيلين ، امراض النسج ، الحمل ، كبد ، كلية ، فئران

1. Introduction

Amoxicillin, is a prescription antibiotic, belong to a class of medication called penicillins (Beta-Lactam family antibiotic). Due to its high potency, amoxicillin can be used in the treatment of infectious diseases including; Otitis media, skin infections and Lyme illness which are caused by specific bacteria [1 , 2]. In the early 1970's, amoxicillin is first introduced in the UK as an antibacterial agent for oral administration [3]. Amoxicillin is known by several names that differ from country to another, where it is known as BAN in the UK and in the USA known as USAN, whilst in Australia known as AAN [4]. Amoxicillin is synthetically manufactured in two forms, the first one is amoxicillin trihydrate, which is available as tablets, regular capsules and syrups

for oral usage, and the second form is amoxicillin sodium salts which is used for intravenous injection [5]. As like as most of drugs, administered amoxicillin is metabolized by liver, filtered by kidney and excreted into the urine [6]. Despite the benefits of amoxicillin treatment, many medical reports mentioned to its harmful effects during long term usage which can increase during pregnancy [7]. Recently, it is clearly confirmed that the physiological changes which occur during pregnancy can modify the pharmacokinetics of drugs by which may reduce the safety of drugs and became more toxic, so this may have direct effect on maternal liver and kidney as they known as the main contributors in metabolism and excretion of drugs, respectively [8].

There were many studies referred to the toxic effects of amoxicillin especially during pregnancy and the main maternal organs that affected including; liver, kidney and brain. The drug affects the fetus severely at high doses, but this extends to the mothers bodies as well [9, 10, 8]. It has been shown that short and long term treatment of amoxicillin during pregnancy can impact on the weights of the mother and fetus [11]. Furthermore, High doses of most B-lactam antibiotics can interfere with brain function and impact on rodent behavior. For example, high doses of neomycin may cause an increase in the mice nervous excitement, but not locomotor activity [12], while administration the mice with ampicillin for 6 weeks caused increase of immobility and enhance anxiety and administration of mice with cephoperazone lead to decrease in movements and impairs recognition memory [13].

Although several decades of wide usage of amoxicillin, its safety in individuals, especially in pregnancy cases is poorly understood, thus this study was established to find out the impact of amoxicillin at 1500 and 300 mg/kg b.w. doses from 7^{th} till 17^{th} day of pregnancy on the histology of the maternal liver and kidney, weight changes in both maternal body and selected organs as well as behavioral profile of pregnant mice *Mus musculus*.

2. Materials and methods

2. 1. Animals husbandry

Twenty one (21) pregnant mice *Mus musculus* were enrolled in the current study (a middle aged mouse from 8-14 weeks, weight between 27-30 gm). The aminals that used in this study bought from the college of Veterinary medicine/ University of Mosul/ Mosul, Iraq, and the mice were kept at the animal house of Department of Biology/ College of Education for Pure Science/ University of Mosul in asutible plastic cages which provided with free access to food (pellets) and tap water at 23±3 °C and exposed to a natural light-dark cycle. The mice females were mated with males (3 females:1 male/cage). Vaginal plug was considered as a marker for mating and this day confirmed as a zero day [14]. All animals were sppourted with a special humanity care during the study period.

2. 2. Antibiotic preparation

Amoxicillin trihydrate (a-amino-p-hydroxybenzyl penecillin) was used in the current study with a chemical formula C16H19N3OS. Physically, the drug appears in the form of white to a white off cloured powder [6]. The drug used at two doses including; 1500 and 3000 mg/kg b.w. These doses were prepared by dissolving each concentration in 5 ml of distilled water. The volume of the given doses were 0.13-0.15 ml depending on the animal weight. The studied doses had been chosen depending on the LD50 of amoxicillin in mice which is 5000 mg/kg b.w. The drug was administered to the mice orally . Amoxicillin that tested in the current study was in the form of 500 mg/kg capsules manufactured by Bristol laboratories Ltd., Bristol House, Unite 3. Canal Side, Northbridage Road, Berkhamsted Hertfordshire, United Kingdom (info@ bristol. labs. co.uk).

2. 3. Experiment design

At the 7th day of pregnancy, the mice were divided into three groups (7 mice/group) as the following; group I (control group); the pregnant mice were orally administered with distilled water from the 7th till 17th day of pregnancy. In group II and group III, the pregnant mice were orally administered with 1500 and 3000 mg/kg b.w. from 7th till 17th day of pregnancy, respectively.

2. 4. Specimens preparation

After last dose on 17th day of pregnancy, pregnant mice were prepared for dissection and histological sampling. Following the dissection process of mice, liver and kidney were removed, weighted, washed with distilled water and fixed with 10% formalin for 48 hr. The specimens of liver and kidney were routinly processed and stained with Delafield's Hematoxyline-Eusin and mounted with D.P.X. [15]. All sections were examined using light microscope and photographed with a digital camera (Sony, Japan) connected to an optical lens.

2. 5. Data analysis

The data of body and organ weights were expressed as mean±standard deviation. The data were statistically analyzed using one-way analysis of variance (One-way ANOVA) with Dunnett's test (multiple comparison procedure) were used to compare the means of both experimental groups II and III with the mean of group I. The recent data was statistically evaluated using GraphPad Prism5 v5.0 software (GraphPad Software, Inc., San Diego, California, USA). The data variation was considered as statistically significant at probability value (p<0.05).

3. Results

3. 1. Light microscopic observations of the liver of the pregnant mice

Microscopic examination of liver sections of control group revealed normal histological architecture of maternal live, including; nomal central vein and normal features of hepatic parenchymal cells (Fig. 1). The histopathological examation of the liver of the pregnant mice that gevin amoxicillin orally at the dose of 1500 mg/kg b.w.

from 7th till 17th day of pregnancy showed severe histological injuries characterized by congestion and dilatation of the central vein and moderate infiltiration of inflammatory cells which were seen near the wall of expanded central vein (Fig. 2). Additionaly, sinusoids dilatation with degenerative alterations of hepatocytes had been highlited (Fig.3). The lesions were increased as the dose of amoxicillin increased. The central vein damage represented by severe congestion and dilatation at the dose of 3000 mg/kg b.w. of amoxicillin with increase of hepatocytes degeneration was observed (Fig. 4). These alterations were accompanied by moderate perivascular infiltration of inflammatory cells in the portal area (Fig. 5). Furthermore, ballooning degeneration and necrosis of hepatocytes were also observed (Fig. 6).

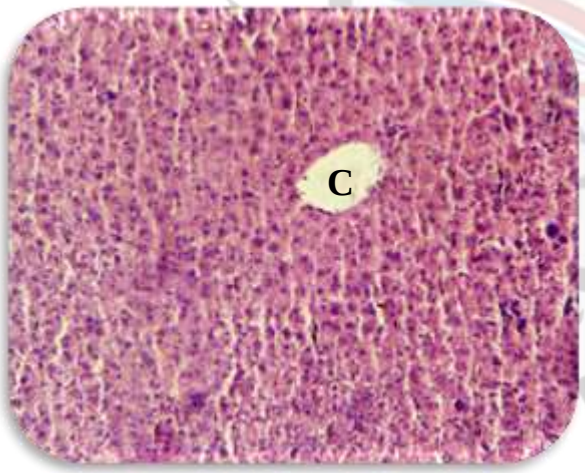


Fig.1: Cross-section of liver from control pregnant mouse *Mus musculus* showing intact central vein (C) with other normal histological features (H&E, 100X).

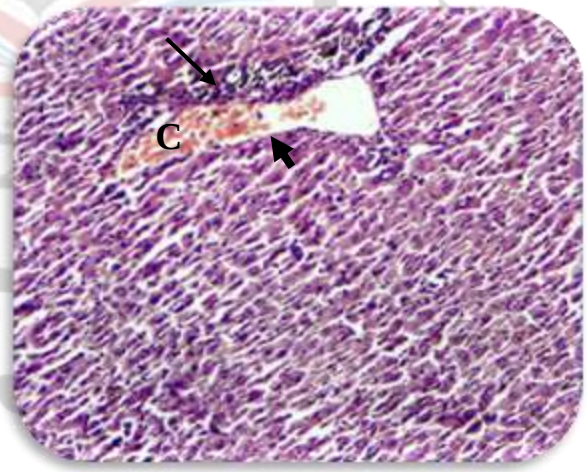


Fig.2 Cross-section of liver from pregnant mouse *Mus musculus* that orally administered with 1500 mg/kg b.w. of amoxicillin trihydrate from 7th till 17th day of pregnancy, showing central vein dilatation (thick arrow) and congestion (C) with moderate infiltration of inflammatory cells (thin arrow) located next to the central vein wall (H&E, 100X).

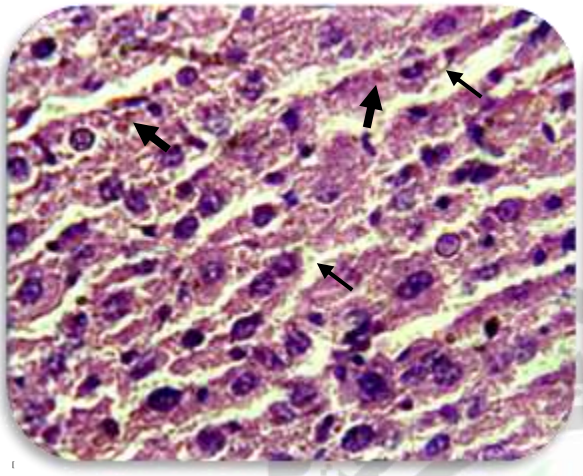


Fig.3 Cross-section of liver from pregnant mouse *Mus musculus* was orally administered with 1500 mg/kg b.w. of amoxicillin trihydrate from 7th till 17th day of pregnancy, showing sinusoids dilatation (thin arrows) and degenerative changes (thick arrows) in hepatocytes (H&E, 400X).

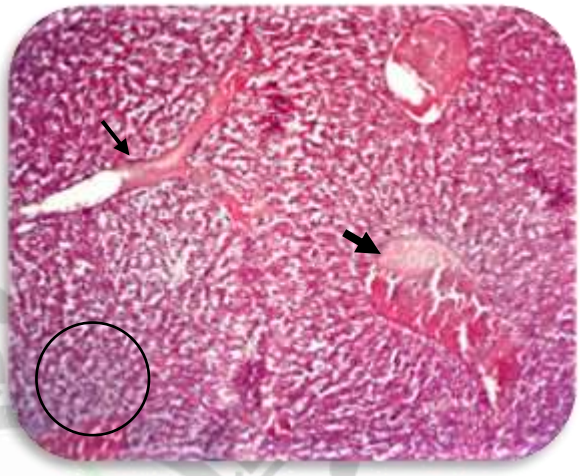


Fig.4 Cross-section of liver from pregnant *Mus musculus* was orally administered with 3000 mg/kg b.w. of amoxicillin trihydrate from 7th till 17th day of pregnancy, showing severe congestion (thick arrow) and dilatation of central vein (thin arrow) with an increase of hepatocytes degeneration (circle) (H&E, 100X).

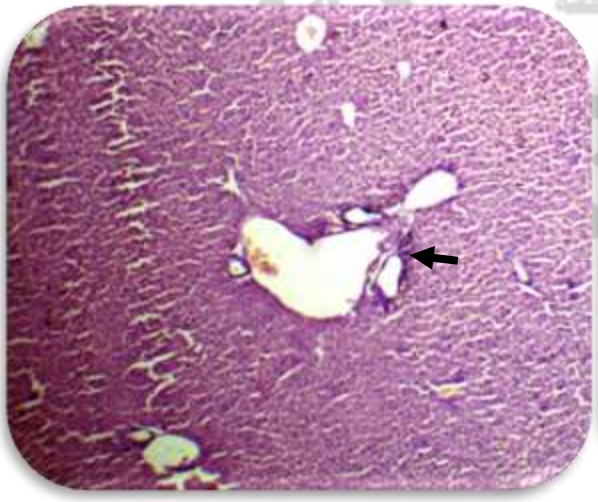


Fig.5 Cross-section of liver from pregnant mouse *Mus musculus* was orally administered with 3000 mg/kg b.w. of amoxicillin trihydrate from 7th till 17th day of pregnancy, showing moderate

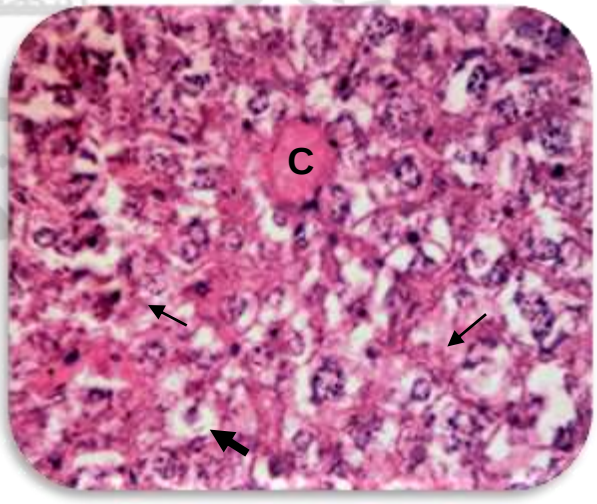


Fig.6 Cross-section of liver from pregnant mouse *Mus musculus* were orally administered with 3000 mg/kg b.w. of amoxicillin trihydrate from 7th till 17th day of pregnancy, showing congested central vein (C), ballooning changes

infiltration of perivascular inflammatory cells (thick arrow) in the portal area (H&E, 100X).

of some hepatocytes (thick arrow) and with necrosis of most hepatocytes (thin arrows) (H&E, 400X).

3. 2. Light microscopic observations of the kidney of the pregnant mice

Microscopic examination of the kidney sections of control group indicated normal histological features of the kidney (Fig. 7). Light microscopic examination of kidney from pregnant mice that orally administered with 1500 mg/kg b.w. of amoxicillin showed swelling of some proximal tubules and necrotic feature of the lining epithelium of the renal proximal tubules (Fig. 8). Moderate infiltration of inflammatory cells and degeneration of some epithelial cells that line renal tubules were demonstrated (Fig. 9). Light microscopic examination of kidney from pregnant mice that orally administered with 3000 mg/kg b.w. of amoxicillin at the same period above showed detectible histopathological lesions represented by degeneration of glomerular cells and epithelial cells that line the proximal tubules. Furthermore, karyolysis of some renal tubule cells nuclei and expansion of Bowman's space were reported (Fig. 10). Additionally, microscopic examination of kidney sections from mice administered with 3000 mg/kg b.w. also showed severe alterations in the kidney tissue including; acute swelling of renal tubules, necrosis of renal tubule cells, pycnosis and karyolysis of many renal epithelium cells nuclei (Fig. 11)

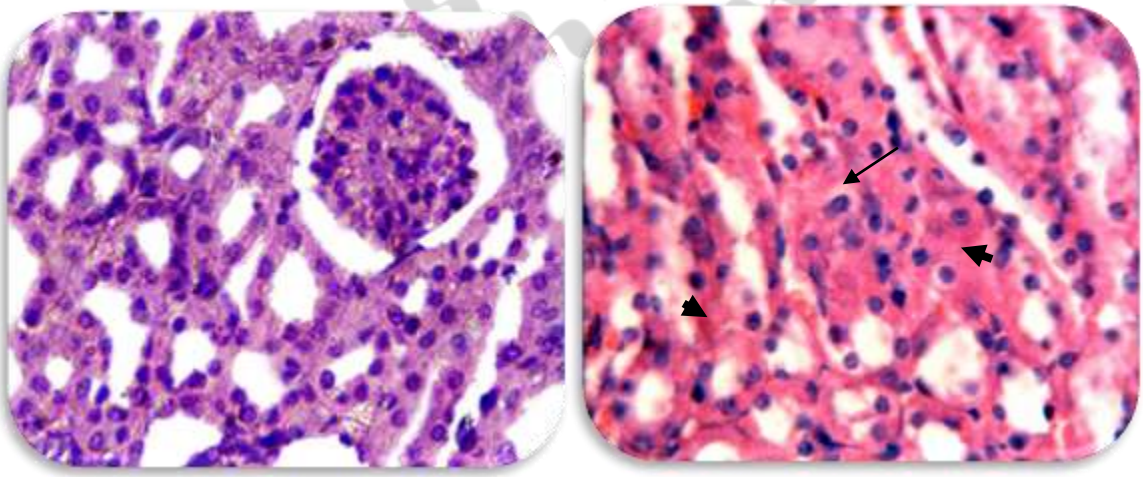
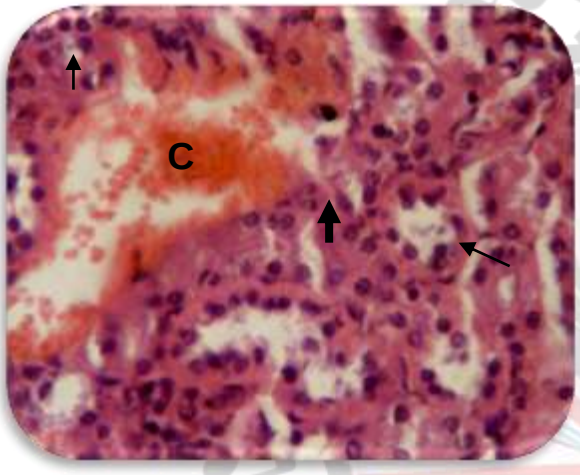


Fig.7: Cross-section of kidney from control pregnant mouse *Mus musculus* showing normal histological features (H&E, 100X).

Fig.8: Cross-section of kidney from pregnant mouse *Mus musculus* was orally administered with 1500 mg/kg of b.w./day of amoxicillin trihydrate from 7th till 17th day of pregnancy, showing degeneration of some proximal tubules (thin black arrow) and necrosis (arrow head) of most renal tubules epithelium (H&E, 400X).



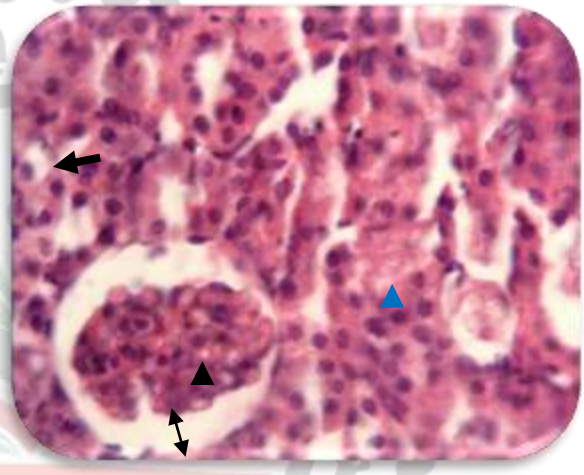


Fig.9: Cross-section of kidney from pregnant mouse *Mus musculus* was orally administered with 1500 mg/kg of b.w. of amoxicillin trihydrate from 7th till 17th day of pregnancy, showing congestion (C) and moderate infiltration of inflammatory cells (thick arrow) in the renal tissue and degeneration of some epithelial cells that line renal tubules (thin arrows) (H&E, 400X).

Fig.10: Cross-section of kidney from pregnant mouse was orally administered with 3000 mg/kg of b.w. of amoxicillin trihydrate from 7th-17th day of pregnancy, showing degeneration of glomerular cells (black arrow head), many cells of proximal tubules epithelium (thick black arrow),karyolysis (blue arrow head) and expansion of Bowman's space(arrow with two heads) (H&E, 400X).

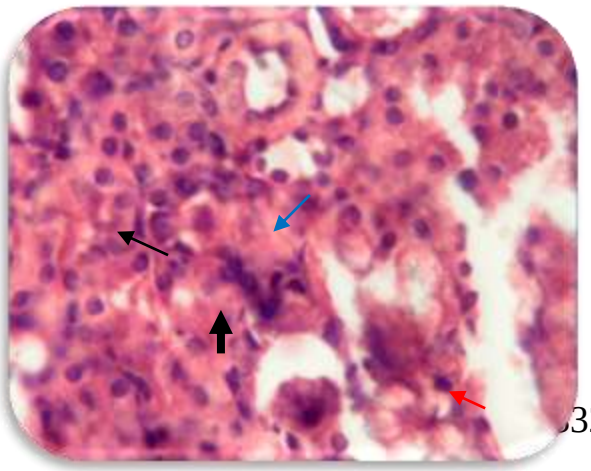


Fig.11: Cross-section of kidney from pregnant mouse *Mus musculus* was orally administered with 3000 mg/kg of b.w. of amoxicillin trihydrate from 7th till 17th day of pregnancy, showing acute swelling of the renal tubules (thin black arrow), necrosis of renal epithelial cells (thick black arrow), pyknosis (red arrow) and karyolysis (blue arrow) (H&E, 400X).

3. 3. Effect of amoxicillin trihydrate on the pregnant mice behavior

For investigating behavioral disturbances that associated with antibiotic abuse, amoxicillin-treated pregnant mice were underwent to daily observations during amoxicillin exposure period (from 7th day till 17th day of pregnancy) and continued for one week after last dose of antibiotic administration. The behavioral changes were as the following; excessive movement and nervousness were reported particularly in group II (1500 mg/kg b.w.) after 10 min of amoxicillin administration. Additionally, convulsions, irregular movement of limb and truculence were markedly observed in group III (3000 mg/kg b.w.).

3. 4. The effect of amoxicillin on the weights of body and selected organs of pregnant mice

To investigate the influence of amoxicillin on the weight of maternal body and studied organs (liver and Kidney), the pregnant mice from three groups I, II, III were weighted in the 7th day, before amoxicillin had been given, then the body weights remeasured on the 17th day of pregnancy. The findings of the current study indicated noticeable decrease in body weight in both groups II and III comparing to group I (control group). The loss of body weight in amoxicillin-treated pregnant mice was dose-dependent manner (Table 1). Furthermore, investigation of amoxicillin effectiveness on the maternal liver and kidney weights was as follows; the organ weights of pregnant mice on the 17th day were measured. The findings of current study indicated dose-based loss in the liver and kidney weights in both group II and III compared to control group. The loss of weight was only significant in body and kidney ($p < 0.05$) on the 17th day of pregnancy at 3000 mg/kg b.w. (Table 1).

Table 1: Shows the effect of different doses of amoxicillin trihydrate on weight of whole body, liver and kidney during pregnancy.

	Mean±SD

Parameters	Control (n=7)	1500 mg (n=7)	3000 mg (n=7)
body weight on 7th day	25.97±0.50	25.47±1.04	25.81±0.55
body weight on 17th day	30.38±0.63	29.01±0.95	28.02±0.96 *
liver weight on 17th day	2.04±0.22	1.68±0.58	1.35±0.59
kidney weight on17th day	1.56±0.07	1.28±0.19	1.08±0.13 *

-The loss of weight was only significant in the body and kidney on the 17th day of pregnancy at 3000 mg/kg b.w. * $p < 0.05$ (compering to control group).

4. Discussion

In the present study, light microscopic examination of the liver sections from pregnant mice that treated orally with different doses including; 1500 and 3000 mg/kg b.w. of amoxicillin trihydrate during from 7th till 17th day of pregnancy showed remarkable histopathological lesions at both doses. At 1500 mg/kg b.w., congestion of central vein, dilatation of sinusoids, infiltration of inflammatory cells with hepatocytes degeneration were noticed, whilst at 3000 mg/kg b.w., lesions such as perivascular infiltration of inflammatory cells in the portal area, ballooning degeneration and necrosis of hepatocytes were observed. The findings of the current study were similar to that previously reported [16, 17, 18, 19]. Furthermore, the current findings of histopathological lesions in the liver and kidney of amoxicillin-treated mice were comparable to that obtained by [20]. The histopathological effects of amoxicillin in individuals and rodents especially during pregnancy may associate with induction of oxidative stress by reactive oxygen species formation including; superoxide anion (O^-), hydrogen peroxide (H2O2) and hydroxyl radical (OH^-), where they are known as main contributors in destruction of cellular proteins, lipids and genetic materials [21, 22]. The liver histopathological changes in current study were some what similar to that obtained by Al-Khafaji and Al-Sultany [23].

The examination of kidney sections showed swelling of the proximal tubules, moderate infiltration of inflammatory cells, necrosis of renal tubules as well as expansion of Bowman's space, pyknosis and karyolysis of renal epithelial nuclei and degeneration of glomerular cells, were reported at 1500 and 3000 mg/kg b.w. The recent findings are consistent with the data obtained previously [20, 21, 24, 25], which they all confirmed that using high doses of amoxicillin during pregnancy induce various lesions in the kidneys of human and rodents. As well as the recent findings were contrary to what indicated by some previous studies [26, 27]. Since the most of drugs pass through the kidney to be excreted, drug dosing adjustment is essential to avoid the therapeutic failure of kidney, which can occur in patients receiving high doses of numerous antibiotics and drugs [28]. Additionally, renal damage that occurred at high doses of amoxicillin in the recent study may be related to disturbance in the regulation

of enzymatic and non-enzymatic anti-oxidant systems which represents as protective barriers in the body leading to induce oxidative stress in the kidney tissues [29].

Moreover, the current data indicated abnormal behavioural changes in the pregnant mice from both groups II and III, where they showed excessive movement, nervousness, irregular movement of the hind limb, convolution and truculence. Those behavioural changes in the amoxicillin given-mice at doses 1500 and 3000 mg/kg b.w. in the current study may be due to imbalance in the neurotransmitter levels by which cause enhancement of oxidative stress in the brain cells leading to neurotoxicity that significantly contributed to behavioral disorders [30], or may due to the ability of some antibiotics (penicillin family) to trigger the changes in the biochemistry of brain and blood-brain barriers causing behavioral alterations [31]. Furthermore, the convulsions in groups II and III mice may have occurred due to increase in the concentration of glutamate in the brain cells by amoxicillin, as well as the aggression or truculence may associate with increase in the levels of cytokines in a particular area of brain that located in the frontal cortex, where this area that control on numerous behavioral features [30].

The current findings revealed that the loss in the maternal body weight was only significant (p<0.05) at 3000 mg/kg b.w., which is comparable to the data obtained by some studies [9, 32, 33]. The current data was differed from some previous results [34, 35, 36]. The current findings may be due to reduce of intestinal microbiota population by high dose of antibiotic which may cause decrease in microbe-induced obesity [37], while low doses of antibiotic can lead to weight gain [38]. Additionally, reduction of maternal liver weight in both groups II and III were partially agreed with the data obtained by Thiim and Friedman [39], where they found an increase in the liver weight of female rats with decrease in liver weight of male rats following administration with 200, 500 and 2000 mg/kg b.w. for 26 weeks. The reduction of liver weight of rat, cat and dog following administration with 500 mg/kg b.w amoxicillin for 21 days were confirmed previously [40]. This reduction of liver weight in amoxicillin-treated animals may be associated with disturbance of gastrointestinal flora which involve in altering some related metabolic pathways [31]. Moreover, a significant low kidney weight which was reported in the current study in pregnant mice treated with 3000 mg/kg b.w. of amoxicillin is comparable to that indicated by Thiim and Friedman [39] in male rats. Such results may be due to that amoxicillin induces oxidative stress in kidney of rats (or may in all rodents) and perturbed Ca^{+2} homeostasis due to generation of free radicals which may impact on the histological composition of the kidney and that may be explain the reduction in their weight in the recent study [41].

5. Conclusion

Collectively, although some antibiotic like amoxicillin was generally considered as a safe drug during pregnancy, our data indicated that excessive use and high doses of amoxicillin in pregnancy cause loss of weights; in the maternal bodies, livers and kidneys, disturbance in the structure of some maternal organs which may impact on

functions of mentioned organs. Thus, usage of amoxicillin particularly during pregnancy should be under specific medical supervision.

Biography of presenting auther

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Summary: A lecturer at the college of Education for pure Science/ Mosul University/ Mosul IRAQ. I have taught many subjects like histology, embryology, and invertebrates to the students of undergraduate education. I supervised a number of graduation projects. I gave lectures in physiological psychology for three years at the college of Humanities. Bachelor Degree of biology, college of Education for pure science/ Department of biology/ University of Mosul, Iraq from 2000–2004. Master degree (MA) in Zoology/ histology and histiochemical changes in crustaceans (fresh water craps), college of Education for pure science / department of biology / University of Mosul, Iraq from 2004-2007. PhD in Zoology /Embryology. College of Education for pure science/ department of Biology/ University of Mosul, Iraq from 2008-2013.

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References

[1] Geddes AM, Klugman KP, Rolinson GN. Introduction: historical perspective and development of amoxicillin/clavulanate. International journal of antimicrobial agents; 30: 109-112, 2007.

[2] Venekamp RP, Sanders SL, Glasziou PP, Del Mar CB, Rovers MM. Antibiotics for acute otitis media in children. The Cochrane Database of Systematic Reviews (6): CD000219, 2015.

[3] Kaur SP, Rao R, Nanda S. Amoxicillin: a broad spectrum antibiotic. International Journal of Pharmacy and Pharmaceutical Sciences; 3(3): 30-7, 2011.

[4] Gillies M, Ranakusuma A, Hoffmann T, Thorning S, McGuire T, Glasziou P, Del Mar C. Common harms from amoxicillin: a systematic review and meta-analysis of randomized placebo-controlled trials for any indication. Cmaj; 187(1): E21-E31, 2015.

[5] Farazuddin M, Chauhan A, Khan RM, Owais M. Amoxicillin-bearing microparticles: potential in the treatment of Listeria monocytogenes infection in Swiss albino mice. Bioscience Reports. 31 (4): 265–72, 2011.

[6] Roy J. An introduction to pharmaceutical sciences: production, chemistry, techniques and technology. Elsevier; 2011.

[7] Sabry SA. Histological and ultrastructural studies on the effect amoxicillin on the stomach of mouse fetuses. The Egyptian Journal of Hospital Medicine; 67(1): 366-376, 2017.

[8] Nahum GG, Uhl K, Kennedy DL. Antibiotic use in pregnancy and lactation: what is and is not known about teratogenic and toxic risks. Obstetrics & Gynecology; 107(5): 1120-1138, 2006.

[9] Abou-Tarboush FM. Teratogenic and toxic effects of Hiconcil (amoxicillin) on mouse fetuses. *Arab Gulf Journal of Scientific Research*; 12:133–140, 1994.

[10] Czeizel AE, Rockenbauer M, Sorensen HT, Olsen J. Augmentin treatment during pregnancy and the prevalence of congenital abnormalities: a population-based case–control teratologic study. European Journal of Obstetrics & Gynecology and Reproductive Biology; 97(2):188–192, 2001.

[11] Bookstaver PB, Bland CM, Griffin B, Stover KR, Eiland LS, McLaughlin M. A review of antibiotic use in pregnancy. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy; 35(11): 1052-1062, 2015.

[12] Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, Deng Y, Blennerhassett P, Macri J, McCoy KD, Verdu EF. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology; 141(2): 599-609, 2011.

[13] Ceylani T, Jakubowska-Doğru E, Gurbanov R, Teker HT, Gozen AG. The effects of repeated antibiotic administration to juvenile BALB/c mice on the microbiota status and animal behavior at the adult age. Heliyon; 4(6): e00644, 2018.

[14] Thaete LG, Levin SI, Dudley AT. Impact of anaesthetics and analgesics on fetal growth in the mouse. Laboratory animals; 47(3): 175-183, 2013.

[15] Suvarna KS. Bancroft's theory and practice of histological techniques. 7th Edition. Elsevier, Churchill Livingstone, Beijing, China, 1–654, 2012.

[16] Amin A, Hamza AA. Oxidative stress mediates drug-induced hepatotoxicity in rats: a possible role of DNA fragmentation. Toxicology; 208(3): 367-375, 2005.

[17] Olayinka ET, Olukowade IL. Effect of amoxycillin/clavulanic acid (Augmentin 625®) on antioxidant indices and markers of renal and hepatic damage in rats. Toxicol. Environ. Health Sci, 2, 85-92, 2010.

[18] Shahin MA, Rashad HI. Histological and Ultrastructural studies on the effect of amoxicillin on the liver of mice foetuses. Egyptian Journal of Histology, 2019.

[19] El-Sherbiny GA, Taye A, Abdel-Raheem IT. Role of ursodeoxycholic acid in prevention of hepatotoxicity caused by amoxicillin-clavulanic acid in rats. Annals of hepatology; 8(2): 134-140, 2009.

[20] Elsayed MGA, Elkomy AAA, Gaballah MS, Elbadawy M. Nephrotoxicity of cefepime: A new cephalosporin antibiotic in rats. Journal of pharmacology & pharmacotherapeutics; 5(1): 33-38, 2014.

[21] Fahmy MA, Farghaly AA, Omara EA, Hassan ZM, Aly FA, Donya SM, Ibrahim AA, Bayoumy EM. Amoxicillin–clavulanic acid induced sperm abnormalities and histopathological changes in mice. Asian Pacific Journal of Tropical Biomedicine; 7(9): 809-816, 2017.

[22] Nita M, Grzybowski A. The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxidative Medicine and Cellular Longevity, 2016.

[23] Al-Khafaji MA, Al-Sultany HH. Influence of chitosan on hematological and histopathological changes in mice infected with Brucella melitensis immunized with Rev-1 vaccine. Iraqi Journal of Veterinary Sciences;34(1):23-29 2020.

[24] Finco DR. Kidney function. In Clinical Biochemistry of Domestic Animals 4th Ed. (ed Jiro J. Kaneko). Academic Press Inc. California. Holt. 1997.

[25] Nathanson S, Moreau E, Merlet-Benichou C, Gilbert T. In utero and in vitro exposure to β-lactams impair kidney development in the rat. Journal of the American Society of Nephrology; 11(5): 874-884, 2000.

[26] Galla S, Chakraborty S, Cheng X, Yeo J, Mell B, Zhang H, Mathew AV, Vijay-Kumar M, Joe B. Disparate effects of antibiotics on hypertension. Physiol Genomics; 50(10): 837–845, 2018.

[27] Sjovall J, Westerlund D, Alvan G. Renal excretion of intravenously infused amoxycillin and ampicillin. British journal of clinical pharmacology; 19(2): 191-201, 1985.

[28] Verbeeck RK, Musuamba FT. Pharmacokinetics and dosage adjustment in patients with renal dysfunction. European journal of clinical pharmacology; 65(8): 757-773, 2009.

[29] Olayinka ET, Olukowade IL, Oyediran O. Amoxycillin/clavulanic acid combinations (Augmentin (R) 375 and 625 tablets) induce-oxidative stress, and renal and hepatic damage in rats. African Journal of Pharmacy and Pharmacology; 6(33): 2441-2449, 2012.

[30] Atli O, Demir-Ozkay U, Ilgin S, Aydin TH, Akbulut EN, Sener E. Evidence for neurotoxicity associated with amoxicillin in juvenile rats. Human & experimental toxicology; 35(8): 866-876, 2016.

[31] Akst J. Behavioral changes in mice given antibiotics in early life. The scientist; 3(2): 101-102, 2017.

[32] Gérard P. Gut microbiota and obesity. Cellular and molecular life sciences; 73(1): 147-162, 2016.

[33] Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J, Kau AL, Rich SS, Concannon P, Mychaleckyj JC, Liu J. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science; 339(6119): 548-554, 2013.

[34] Allen HK, Stanton TB. Altered egos: antibiotic effects on food animal microbiomes. Annual review of microbiology; 68: 297-315, 2014.

[35] Subbiah M, Mitchell SM, Call DR. Not all antibiotic use practices in food-animal agriculture afford the same risk. Journal of environmental quality; 45(2): 618-629, 2016.

[36] Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teillant, A, Laxminarayan R. Global trends in antimicrobial use in food animals. Proceedings of the National Academy of Sciences; 112(18): 5649-5654, 2015.

[37] Murphy EF, Cotter PD, Hogan A, O'Sullivan O, Joyce A, Fouhy F, Clarke SF, Marques TM, O'Toole PW, Stanton C, Quigley EM. Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity. Gut; 62(2): 220-226, 2013.

[38] Cox LM. Antibiotics shape microbiota and weight gain across the animal kingdom. Animal Frontiers; 6(3): 8-14, 2016.

[39] Thiim M, Friedman LS. Hepatotoxicity of antibiotics and antifungals. Clinics in Liver Disease; 7(2): 381–399, 2003.

[40] Schwarze C, Schmitz V, Fischer HP, Sauerbruch T, Spengler U. Vanishing bile duct syndrome associated with elevated pancreatic enzymes after short-term administration of amoxicillin. European journal of gastroenterology & hepatology; 14(11): 1275-1277, 2002.

[41] Adesanoye OA, Ifezue AOC, Farombi EO. Influence of chloramphenicol and amoxicillin on rat liver microsomal enzymes and lipid peroxidation. African Journal of Biomedical Research; 17(3): 135-142, 2014.