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Fetal Teratogenic and Histopathological Influences of Tramadol on White Rat Fetuses

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ABSTRACT

Tramadolisan effective opioid analgesic that is widely used in the world to relieve moderate to severe pains, because there are not insufficient information's of this drug on fetal parameters and histological effects on the fetuses, this study wasdesigned, 12 pregnant white rats(*Rattus rattus*) were used in current study to indicate the fetal teratogenic and histopathological changes of someorgans(brians and livers)of fetuses for a pregnancy period of 20 days,the means ofbody weight and age of pregnant rats were (210.5gand 11.5weeks)respectively, these pregnantanimals were individed into 3 groups:the first group was treated with normal saline (control group)(n=4), second group was treated with tramadol drug with concentration of 125mg/kg (n=4) and third group was treated with tramadol drug with concentration of 200mg/kg (n=4),pregnant rats in all groups were administrated from the 1st pregnancy day and dissected on the 20thday of it.

Current study results revealed a significant increase($P < 0.05$) in the number ofdead fetuses andnumber ofundeveloped fetuses in treated groups with tramadol drug of two doses(125 mg/kg and 200 mg/kg)respectively,whereas number offetuses in two uterine hornsand number oflivefetusessignificantly decreased($P < 0.05$) in treated groups with tramadol drug of two doses(125 mg/kgand 200mg/kg) respectively compared with control animals group, as histological study showed that of different pathological changes occurrence in the organs of fetuses whose their mothers were treated with two doses of tramadolduring pregnancy when compared with control rats group,and these effects were more severe at the higher drug dose.

The conclusion/based on the results of our current study which in it tramadol drug was administered from the first day of pregnancy, this drug had a toxic influences on the various fetusgenesis processes and stimulatedvarious pathological changes inbrain and liver tissues of fetuseswhich increased with increasing drug dose.

KEYWORDS

Fetal,Teratogenic, Histopathological, Tramadol.

Introduction

Tramadol is a powerful medicinal pain analgesic that belongs to the group of opioid analgesics because it affects the morphine receptors themselves and even competes onthem,it isknown by several brand names such as madol, tradol, tamul, tidol, tradol, tramal,zamadol and others (Subedi*et al*.,2019),this drug was discovered in 1977 and used for medical purposes in relieving and sedating the acute and moderate pains associated with some chronic diseases such as diseases of the bones, muscles, joints and cancerous tumorsin addition to pains of the accidents and severe burns (Kean *et al*.,2009; Wojciech,2009),tramadol is classified as a type 2 pain reliever, but it has a greater analgesic effect than type 1 analgesic drugs so this drug should not be used without a prescription because it is a very dangerous narcotic drug that stimulates many neurological diseases (Bravo *et al*., 2017), this drug is completely absorbed by the intestineand has a half-life of about (5 - 7) hours when the drug calms the pain after (1) hour after taking it orally, while its effect reaches its peak between (2-4) hours, and the drug remains effective for (6) hours depending on the dose concentration and condition of the patient's body, tramadolcan be found in a form of syrup, injections, pills, eye dropsin addition to rectal suppositories(Beakley*et al*.,2015).

Tramadol mainly metabolized into the metabolite O-desmethyl-tramadol which is the primary recipient of the opioid receptor, while tramadol inhibits the reabsorption of norepinephrine and also absorption of serotoninand these pathways improve the effectiveness of the drug in response to the pain (Subedi*et al*.,2019),the principle action oftramadol is based on the chemical similarity in composition with the hormone naturally created in the body, which is called the endorphin responsible for relieving pain when it binds to specific receptors in the brain causing the reduction of pain signals sent from the person's body to his brain, so that tramadol competes with endorphin on his receptors performing his work in the same way as endorphin in pain relief (Solimane*t al.*,2017),the use of tramadol in high doses without a prescription or continuing to take it for a long time despite cure of the medical reason that led to taking it leads to the addiction that causes various effects such as impotence, reduced fertility of both sexes, menstrual disorders(El-Ghawet,2015;Babalonis*et al*.,2013), hallucination, dry mouth. increased sweating, tingling and paresthesia in the limbs, loss of appetite, a sense of paranoia, loss of ability of intensification and sleep, severe

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anxiety, panic seizures in addition to buzzing in the ears, and the incidence of these negative effects of the drug increases in a variety of cases such as obesity, malnutrition and aging, while the symptoms of withdrawal syndrome appear when the person stops taking the drug (Barsotti*et al*.,2003; Alshammary*et al*.,2020). This drug is not taken with alcohol because it increases its effectiveness and prevents taking this drug in people with severe liver disorders, or those who take psychiatric and mental drugs in addition to other analgesics and sleeping pills (Abbas *et al*.,2020). Some studies have indicated that tamadol should not be taken during pregnancy for a long time because it may increase the chances of miscarriage and stimulate reversible withdrawal symptoms in newborns, because the drug has the ability to pass to the fetus through the placenta stimulatingthe body offetus to adopt on it during his fetal life in the mother's womb and stopping taking the drug after his birth leads to infection of the fetuses with neonatal abstinence syndrome which is accompanied with severe and dangerous side effects that may cause death of the fetuses(Kallen and Reis,2015),and this drug also should be avoided during the lactation as a result of it passing into infants with milk causing multiple dangerous effects of them such as difficulty feeding and excessive sleepiness in addition to some severe respiratory disordersthat occurs when tramadol is taken specially during the first three days of breastfeeding or when taken in high concentrations and are fatal of infants(Bloor *et al*., 2012,).

Materials and Methods

24 albino rats of *Rattus rattus* species (12 female with body weight mean 240.8 gandage 11.5 weeks and 12 male with body weight mean 237.6 gandage 10.5 weeks) were used in this study,the female and male were placed in plastic cages under laboratorial symmetric circumstances and providedfreelywith the water and chow,then females were married with the males by placing one female rat with one male rat overnight in the mating cagesand in the morning the mated females were examined by observing the presence of mating plug in the female's vagina or in the cage, the day when the mating plug was seen,and this day was 0 day of pregnancy(Fox *et al*., 2006).

Drug and Administration

Tramadol pills were brought from the pharmacies, two concentrationsoftramadol (125 mg /kg and 200 mg /kg) were used in this study,12 pregnant female of rats were dividedinto two major majorgroups, the first was a control group,contained 4 pregnant rats, administrated with normal saline and dissected at 20^{th} day of pregnancy,the secondwas a treated group, contained 8 pregnant rats which were dividedinto two subgroups each of themincluded (4) pregnant rats, administrated with two concentrations of tramadol drug (125mg/kg and 200 mg/kg) respectively and were dissected at 20^{th} day of pregnancy, the pregnant experiment ratsin all groups were administrated from first pregnancyday and daily single dose by stomach tube.

The Anatomy and Tissue Study

On the 20^{th}day of pregnancy, pregnant females in all studygroupswere numb with diethyl ether, the pregnant females were fixed on the dissection board and abdominal cavity was opened, the wombs (uterine horns)were removed from the body with the fetuses,then the uterine horns were opened for extracting the fetuses from them,the numbers of fetuses(in two uterine horns,dead, undeveloped and the live) were counted, then fetuseswere anesthetized with diethyl etherand vivisectedfor removingthe livers andbrains which were placed in formalin solution (10%) for (48) hrs for histological sections which were preparedaccording to method ofHumason (1972), the tissue sections ofthe studied fetal organswere examined by compound microscope and then photographed by the same type of microscope after providing it with a camera that contains a film.

Study data were statistically analyzed by (ANOVA) analysis, values were asmean and slandered error (M±SE) and L.S.D at probability level ($p<0.05$) was relied to find significant differences.

Results

The present study results indicated that a significant decrease($P <0.05$) in number offetuses in two uterine hornsand number oflivefetuses,while the number of undeveloped fetuses significantly increased($P <0.05$) in treated groups with tramadol drug of two doses(125 mg/kg and 200mg/kg) respectively when comparedwith controlrats, and there

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were significant differences between the treated groups with two doses of the drug, alsothe results recordedaincrease significant (P <0.05) in number ofdead fetuses in treated group with tramadol drug of dose (200mg/kg) comparedwith treated group with tramadol drug of dose (125mg/kg) and with control animals,but there was no any significant differences between the treated group withtramadol drug of dose (125mg/kg) and control group(Table 1),the histological study results showed that the fetus organs whose mothers were treated with different doses oftramadol during pregnancy had suffered from pathological changes in the tissue structure of brians and liversof fetuses whose their mothers were treated with tramadol during pregnancy with concentration of 125 mg / kg such as thickened astrocytes, necroptotic neurons,appearance of the clustered microglial cells, appearance of the oligodendrocytes thicken,necrosis of nervous tissue andnecrotic nerve cells in the brianas shown in the figures(2,3,4,5,6)when compared with control group figure (1),while there were thickened liver cells, a shattered hepatic vein with hemorrhage, necrotic hepatic cells, expansion of blood vessles andnecrosis hepatic tissue in the liveras shown in the figures(14,15,116,17,18)compared with control group(13),whereas these histopathological effects in thebrains and livers of fetuses become more severe in concentration of 200 mg / kg as shown in the figures of brian (8,9,10,11,12)andfigures ofliver (20,21,22,23,24,25,26,27,28)when compared with control groupfigures (7,19)respectively.

Table 1. Influences of tramadol on some fetal parameters

The treatments	Fetuses number in two uterine horns	Deadfetuses number	undevelopedfetuses number	Livefetuses number
Normal saline(control group)	**11.21± 0.02**	**0.00± 0.00**	**0.00± 0.00**	**11.21± 0.02**
Treated group withtramadolof 125mg/kg	**10.11± 0.04c**	**0.00± 0.00**	**1.85± 0.03c**	**9.00± 0.05c**
Treated group withtramadolof 200mg/kg	**8.02± 0.03ce**	**1.88± 0.02ce**	**2.06± 0.03ce**	**5.00± 0.01ce**
LeastSignificant Difference(L.S.D)	**1.70**	**0.75**	**0.65**	**0.44**

Value: Mean ± Standard Error.

c: Significant differences with control group ($p<0.05$).

e: Significant differences between two treated groups with drug($p<0.05$).

Figure 1. Cross section of the brain of a pregnant rat fetus of the control group showing: (1) normal astrocytes, (2) normal nerve cells, (3) normal nervous tissue. H and E Stain -100X.

Figure 2. Cross section of the brain of a pregnant rat fetus of the treated group with tramadol at a conceration of 125mg/ kg showing:- (1) thickened astrocytes, (2) necrotic nerve cells, (3) congregated microglial cells, (4) oligodendrocytes thicken,(5) necrosis of nervous tissue. H and E Stain -100X.

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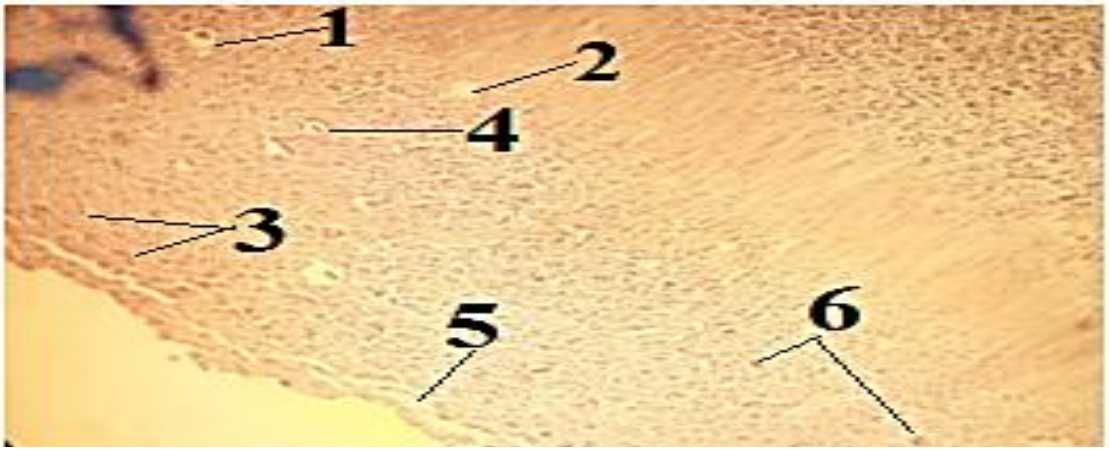

Figure 3. Cross section of the brain of a pregnant rat fetus of the treated group with tramadol at a conceration of 125mg/ kg showing:(1) thickened astrocytes,(2) necrotic astrocytes, (3) congregated microglial cells, (4) oligodendrocytes thicken (5) necrosis of nervous tissue, (6) necrotic nerve cells. H and E Stain -100X.

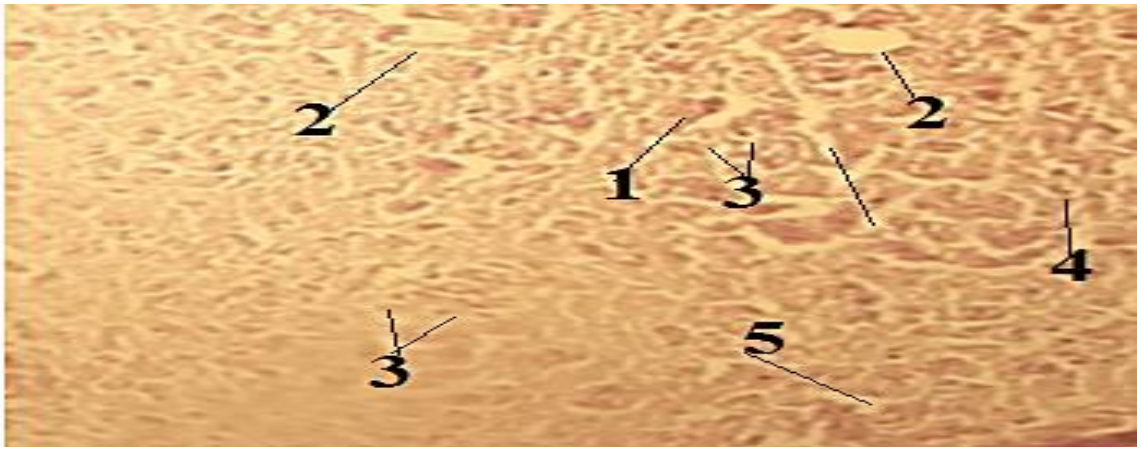

Figure 4. Cross section of the brain of a pregnant rat fetus of the treated group with tramadol at a conceration of 125mg/ kg showing:- (1) thickened astrocytes, (2) necrotic astrocytes, (3) congregated microglial cells, (4), oligodendrocytes thicken,(5) necrotic nerve cells. H and E Stain -100X.

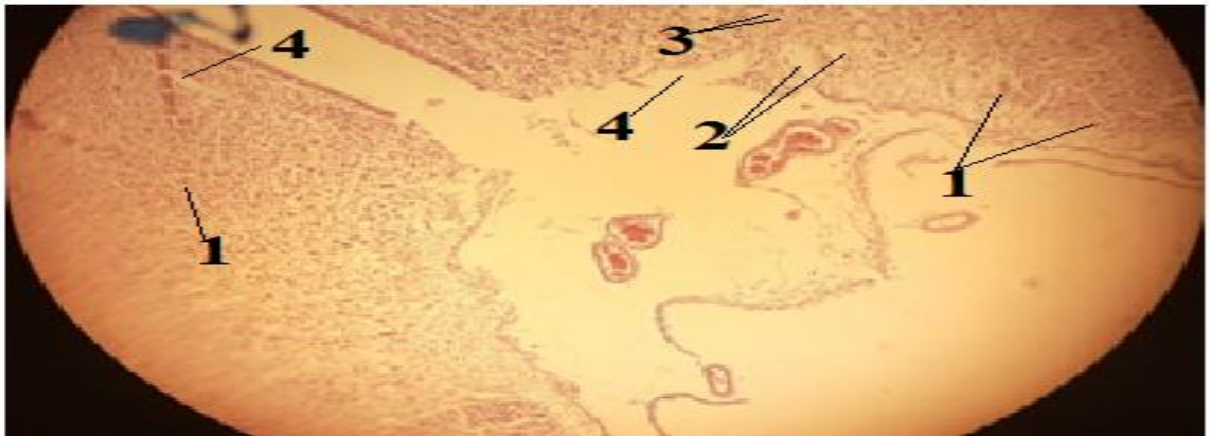

Figure 5. Cross section of the brain of a pregnant rat fetus of the treated group with tramadol at a conceration of 125mg/ kg showing:(1) thickened astrocytes, (2) necrotic astrocytes, (3) congregatedmicroglial cells, (4) necrosis of nervous tissue. H and E Stain -100X.

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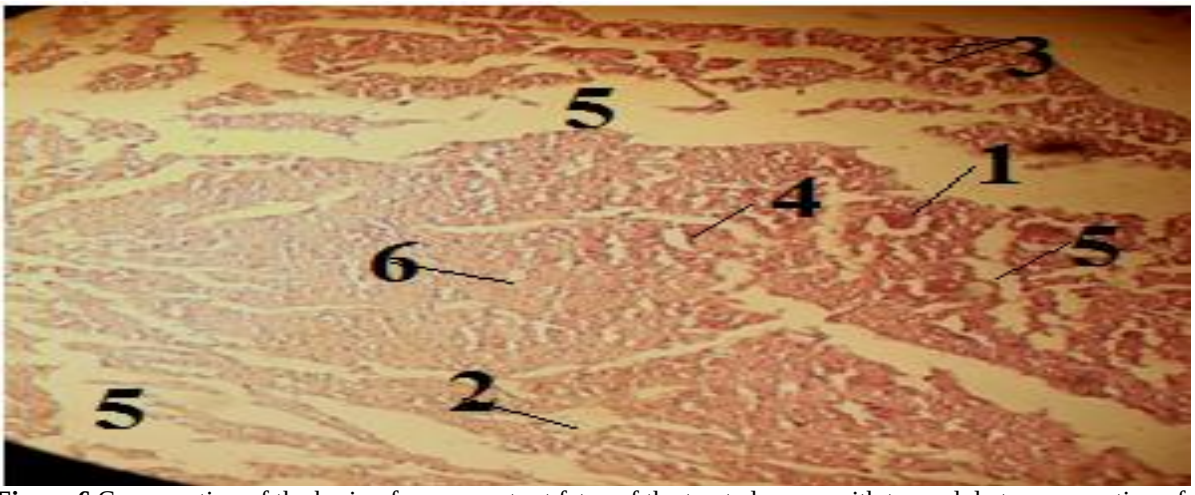

Figure 6. Cross section of the brain of a pregnant rat fetus of the treated group with tramadol at a conceration of 125mg/ kg showing: (1) thickened astrocytes, (2) necrotic astrocytes, (3) congregated microglial cells, (4) oligodendrocyte thicken, (5) necrosis of nervous tissue, (6) necrotic nerve cells. H and E Stain -100X.

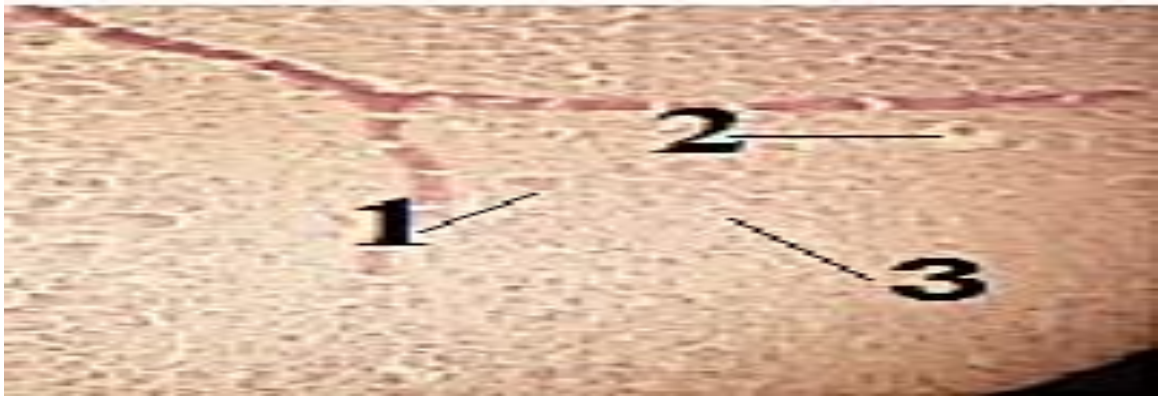

Figure 7. Cross section of the brain of a pregnant rat fetus of the control group showing :(1) normal astrocytes, (2) normal nerve cells,(3) normal nervous tissue. H and E Stain -100X.

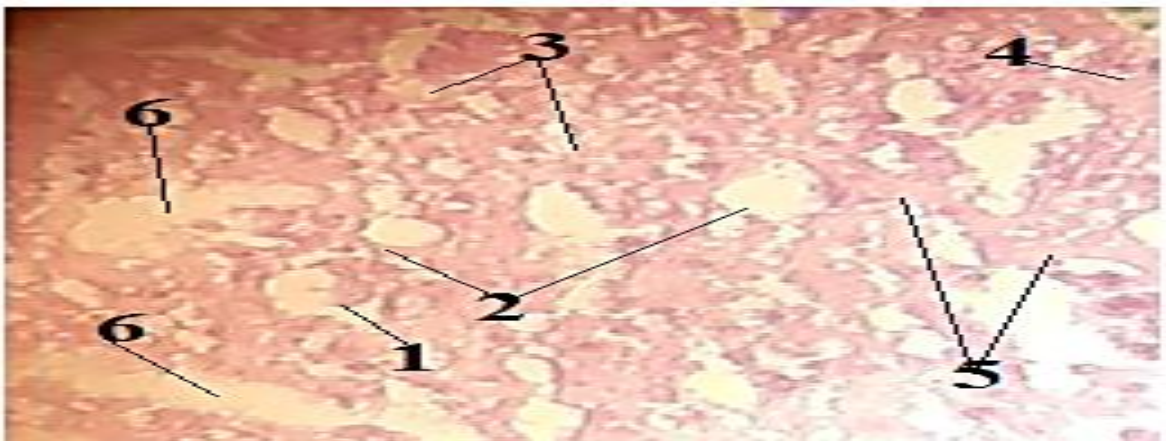

Figure 8. Cross section of the brain of a pregnant rat fetus of the treated group with tramadol at a conceration of 200 mg/ kg showing:(1) thickened astrocytes, (2) necrotic astrocytes, (3) necrotic neurons(4) congregated microglial cells,(5) hemorrhage in nervous tissue,(6) necrosis of nervous tissue. H and E Stain -100X.

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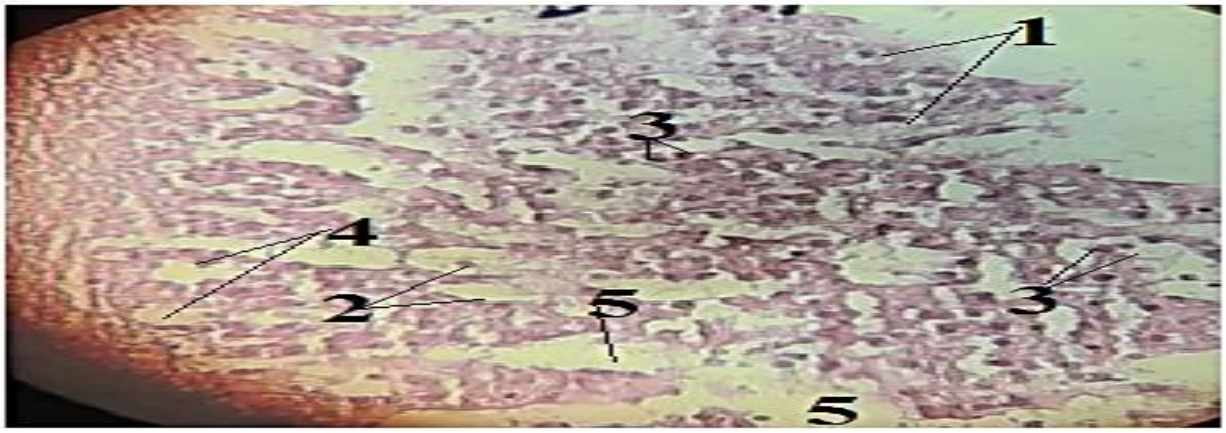

Figure 9. Cross section of the brain of a pregnant rat fetus of the treated group with tramadol at a conceration of 200 mg/ kg showing:- (1) thickened astrocytes,(2) necrotic astrocytes, (3)congregated microglial cells, (4) severe necrotic nerve cells, (5) severe necrosis of nervous tissue. H and E stain -100X

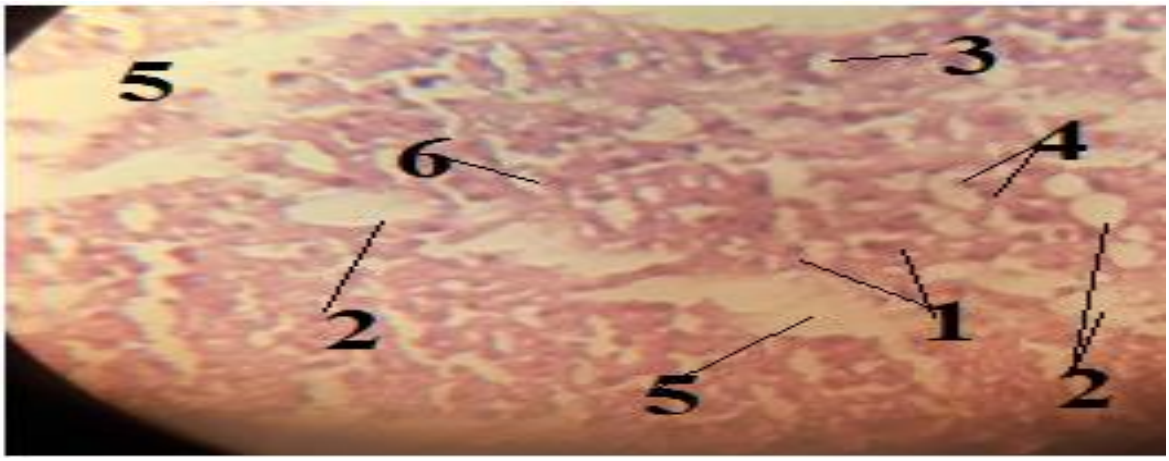

Figure 10. Cross section of the brain of a pregnant rat fetus of the treated group with tramadol at a concertation of 200 mg/ kg showing:(1) thickened astrocytes, (2) necrotic astrocytes, (3) oligodendrocyte thicken,(4)congregated microglial cells, (5) severe necrosis of nervous tissue, (6) severe necrotic nerve cells.
H and E Stain -100X

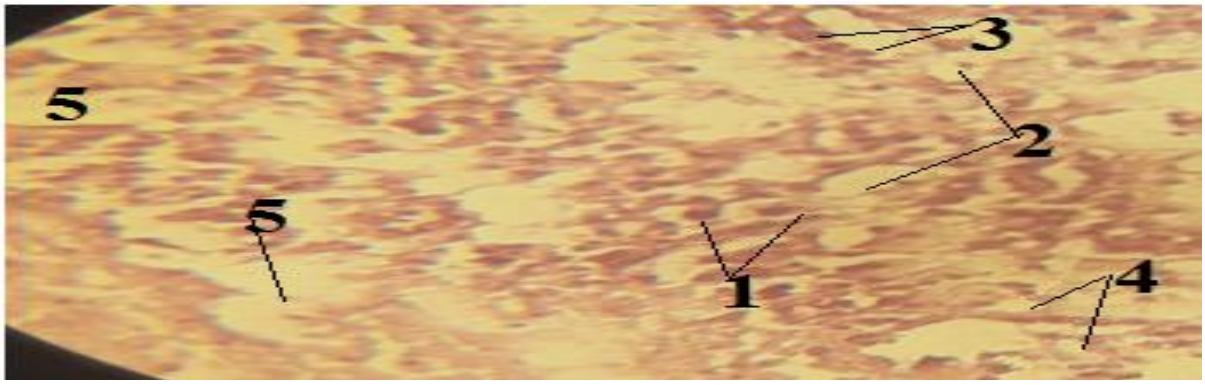

Figure 11. Cross section of the brain of a pregnant rat fetus of the treated group with tramadol at a conceration of 200 mg/ kg showing:(1) thickened astrocytes, (2) necrotic astrocytes, (3) congregated microglial cells, (4) severe necrotic nerve cells, (5) severe necrosis of nervous tissue. H and E Stain -100X

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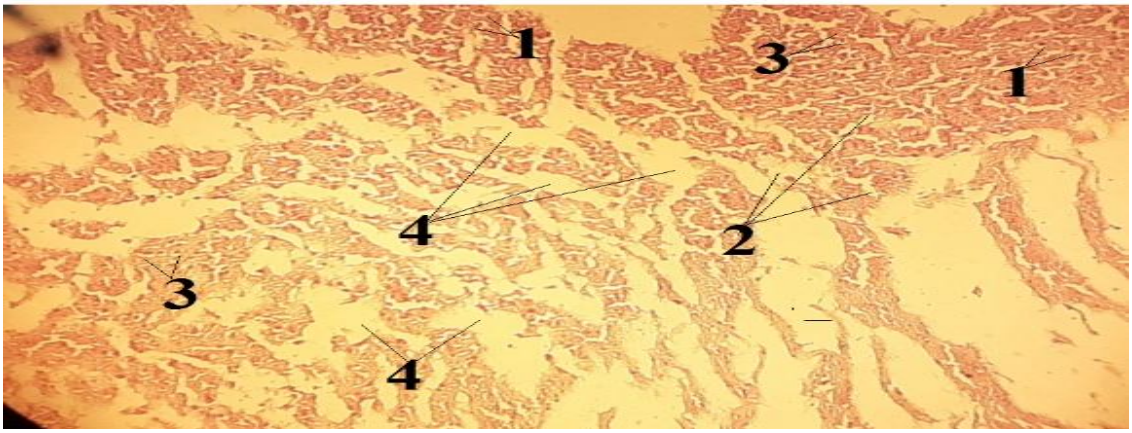

Figure 12. Cross section of the brain of a pregnant rat fetus of the treated group with tramadol at a conceration of 200 mg/ kg showing:(1) thickened astrocytes, (2) necrotic astrocytes, (3) congregated microglial cells, (4) severe necrosis of nervous tissue. H and E Stain -100X.

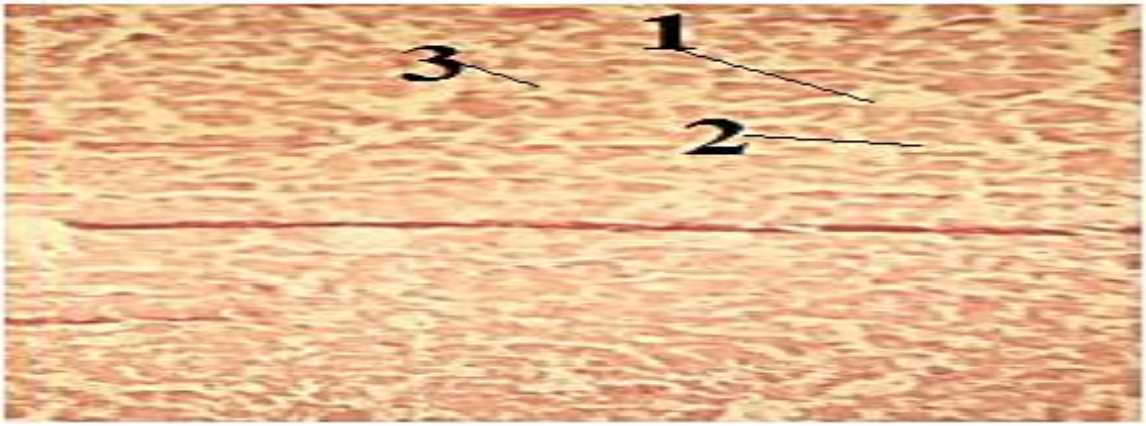

Figure 13. Cross section of liver of a pregnant rat fetus of a control group showing:(1) normal central vein, (2) normal hepatocytes, (3) normal sinusoids. H and E Stain -100 X

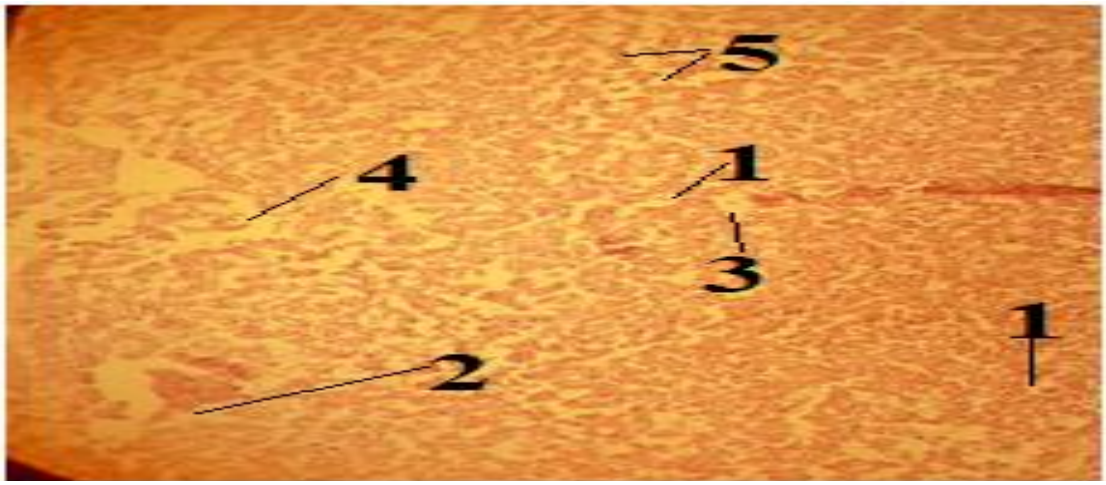

Figure 14. Cross section of the liver of a pregnant rat fetus of the treated group with tramadol at a conceration of 125mg/ kg showing:(1) thickened hepatocytes, (2) shattered central vein with hemorrhage, (3) necrotic hepatic cells, (4) expansion of blood vessle, (5) necrosis of hepatic tissue. H and E Stain -100 X

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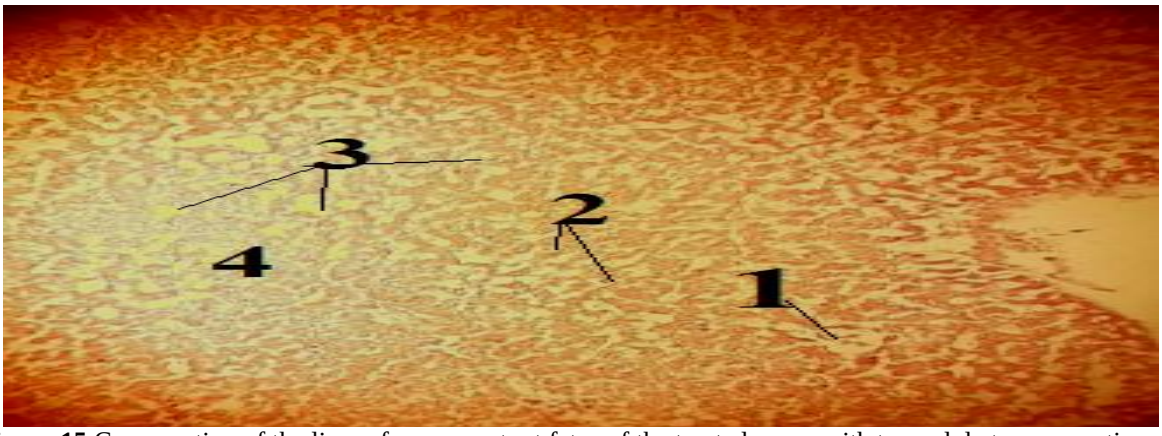

Figure 15. Cross section of the liver of a pregnant rat fetus of the treated group with tramadol at a conceration of 125mg/ kg showing:-(1) shattered central vein (2) thickened hepatocytes, (3) necrosis of hepatocytes, (4) clearing of hepatocytes. H and E Stain -100 X

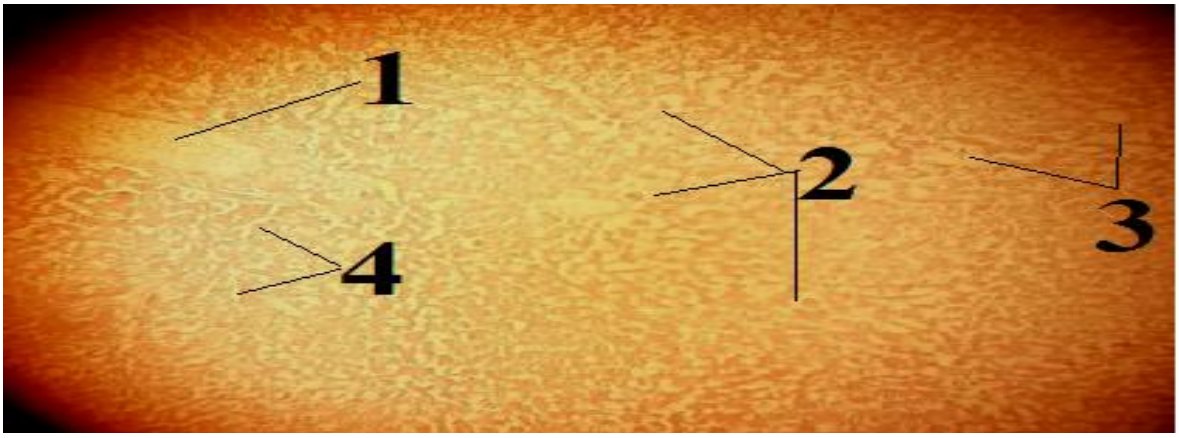

Figure 16. Cross section of the liver of a pregnant rat fetus of the treated group with tramadol at a conceration of 125mg/ kg showing:-(1) expansion of blood vessle (2) thickened hepatocytes, (3) necrosis of hepatocytes, (4) expansion of sinusoids. H and E Stain -100 X

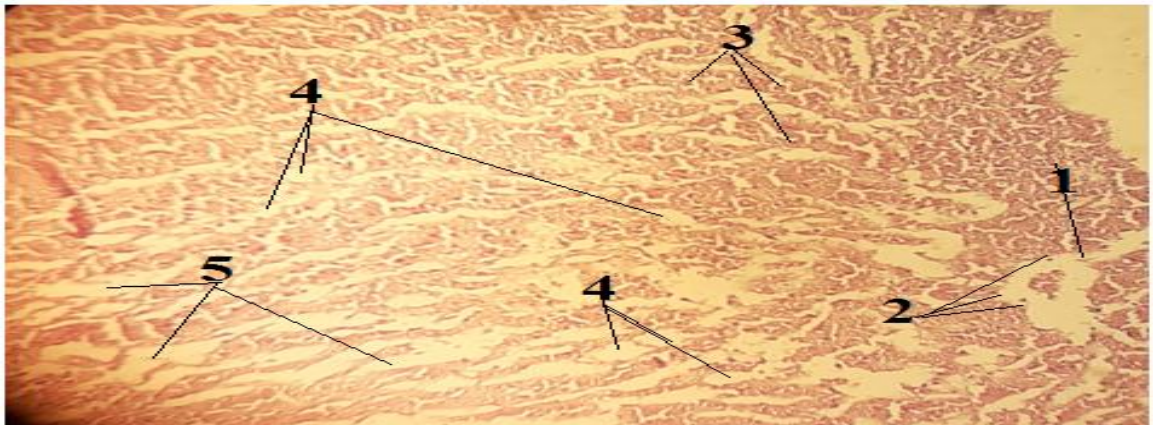

Figure 17. Cross section of the liver of a pregnant rat fetus of the treated group with tramadol at a conceration of 125mg/ kg showing:-(1) shattered central vein, (2) infiltration of inflammatory cells, (3) thickened hepatocytes, (4) necrosis of hepatocytes, (5) necrosis of the hepatic tissue. H and E Stain -100

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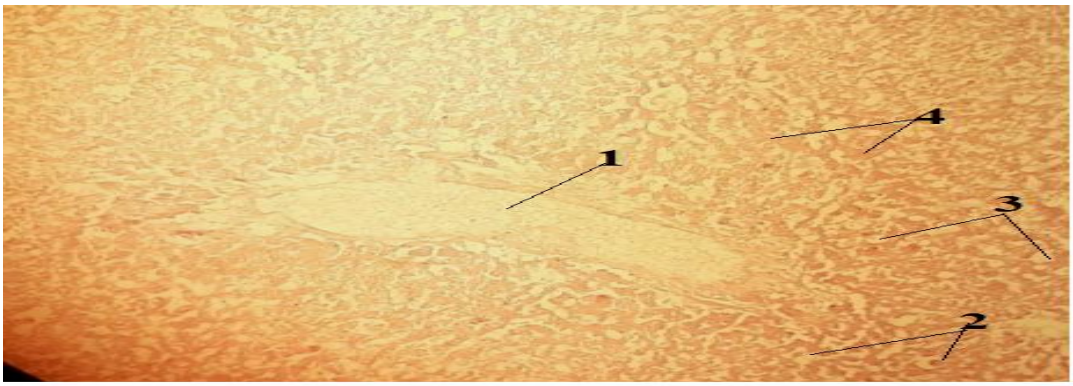

Figure 18. Cross section of the liver of a pregnant rat fetus of the treated group with tramadol at a conceration of 125mg/ kg showing:(1) expansion of blood vessle, (2) thickened hepatocytes, (3) necrosis of hepatocytes, (4) hemorrhage in hepatic tissue. H and E Stain -100 X

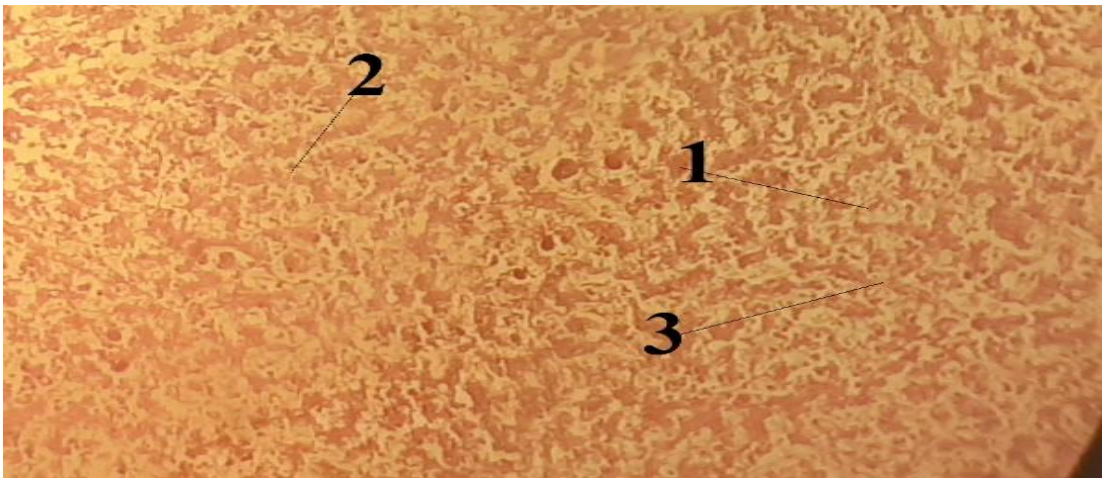

Figure 19. Cross section of the liver of a pregnant rat fetus of the control group showing:(1) naormal central vein, (2) normal hepatocytes, (3) normal sinusoids. H and E Stain -100 X.

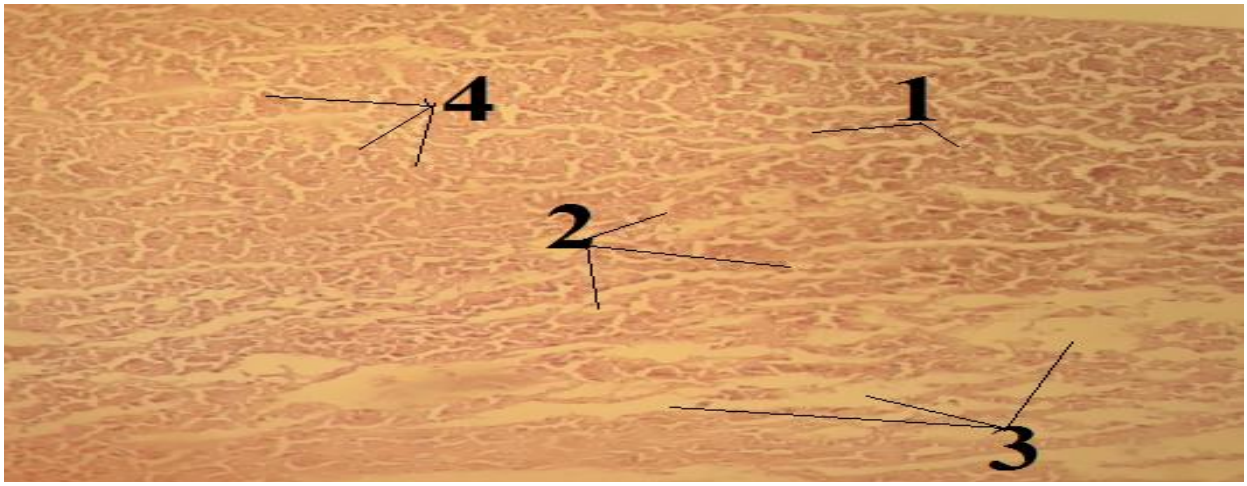

Figure 20. Cross section of the liver of a pregnant rat fetus of the treated group with tramadol at a conceration of 200 mg/ kg showing: (1) thickened hepatocytes, (2) necrotic hepatocytes, (3) necrosis of hepatic tissue,(4) sexpansion of sinusoid spaces. H and E Stain -100 X.

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Figure 21. Cross section of the liver of a pregnant rat fetus of the treated group with tramadol at a conceration of 200 mg/ kg showing:(1) central vein dilation, (2) necrosis of hepatic tissue, (3) infiltration of inflammatory cells, (4) hemorrhage into hepatic tissue,(5) severe dissolution of hepatic tissue. H and E Stain -100 X

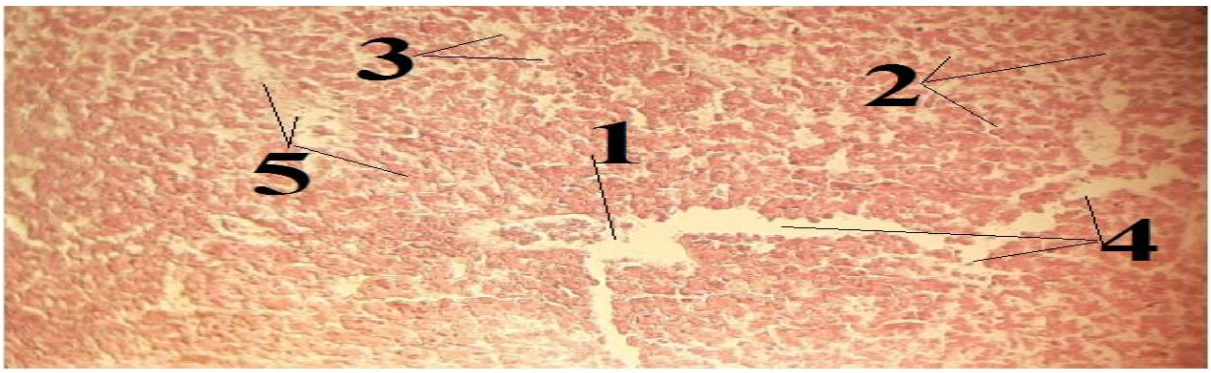

Figure 22. Cross section of the liver of a pregnant rat fetus of the treated group with tramadol at a conceration of 200 mg/ kg showing:(1) central vein dilation and destroy the wall of it, (2) thickened hepatocytes, (3) necroic hepatocytes, (4) necrosis of hepatic tissue, (5) infiltration of inflammatory cells. H and E Stain -100 X

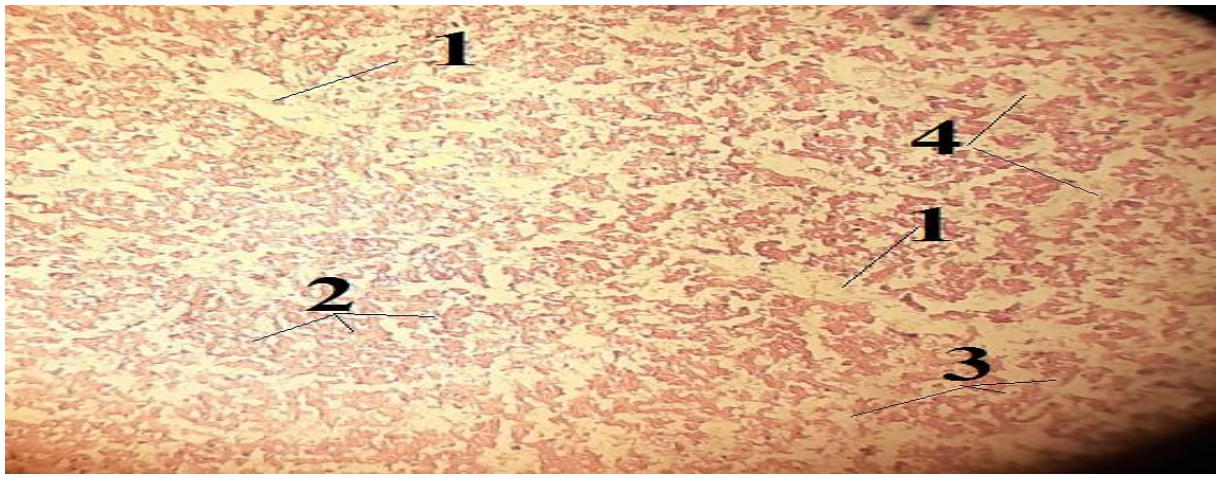

Figure 23. Cross section of the liver of a pregnant rat fetus of the treated group with tramadol at a conceration of 200 mg/ kg showing:-(1) expansion of blood vessle, (2) thickened hepatocytes, (3) necrotic hepatocytes, (4) severe dissolution and necrosis of hepatic tissue. H and E Stain -100 X.

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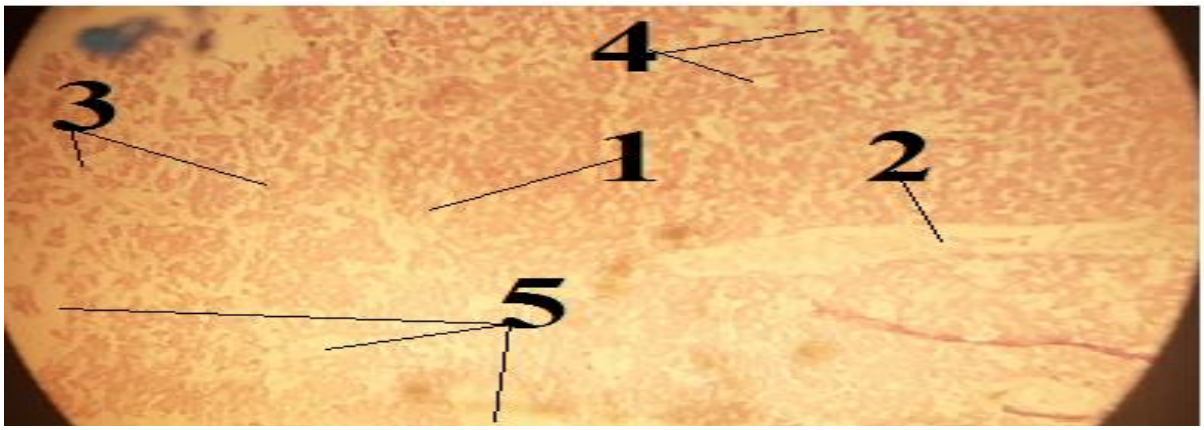

Figure 24. Cross section of the liver of a pregnant rat fetus of the treated group with tramadol at a conceration of 200 mg/ kg showing :(1) central vein dilation and destroy the wall of it,(2) expansion of blood vessle, (3) thickened hepatocytes, (4) necrotic hepatocytes, (5) necrosis of hepatic tissue. H and E Stain -100 X.

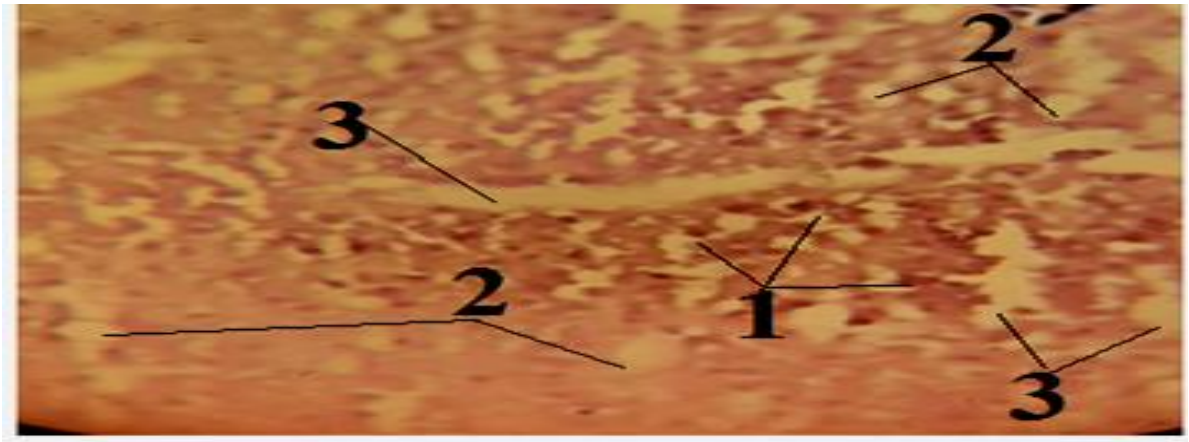

Figure 25. Cross section of the liver of a pregnant rat fetus of the treated group with tramadol at a conceration of 200 mg/ kg showing:(1) sevsre thickened hepatocytes, (2) severe necrotic hepatocytes, (3) necrosis of hepatic tissue. H and E Stain -100 X

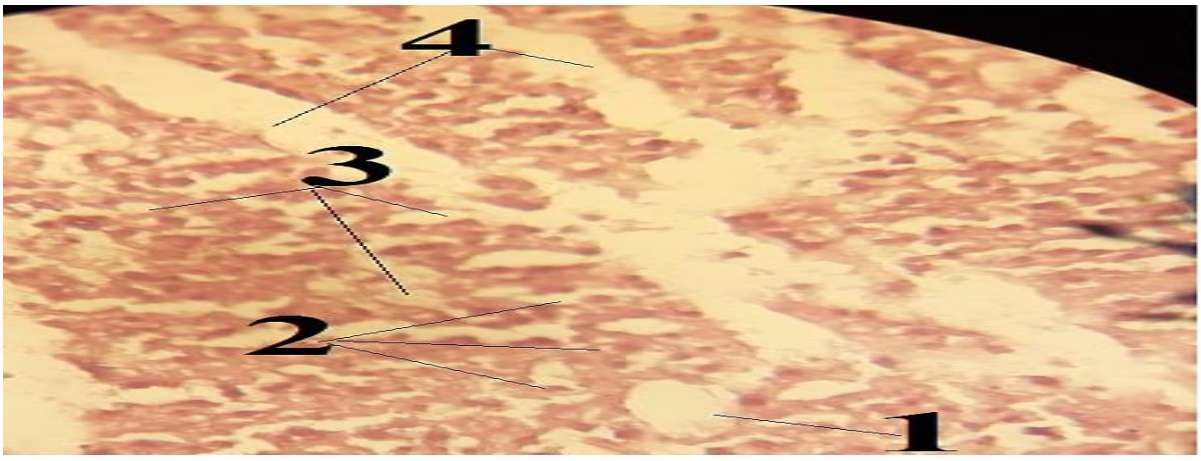

Figure 26. Cross section of the liver of a pregnant rat fetus of the treated group with tramadol at a conceration of 200 mg/ kg showing:(1) shattered central vein, (2)thickened hepatocytes, (3)severe necrotic hepatocytes, (4) sevsre necrosis of hepatic tissue. H and E Stain -100 X

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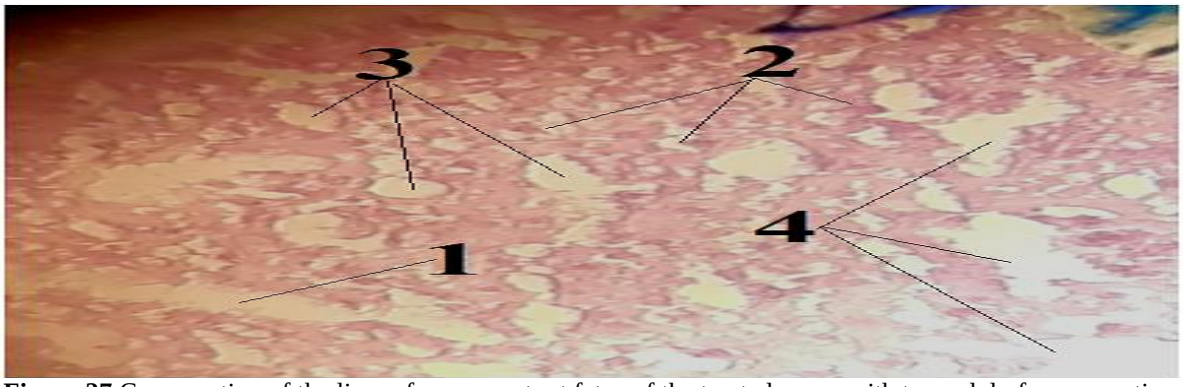

Figure 27. Cross section of the liver of a pregnant rat fetus of the treated group with tramadol of a conceration 200mg/ kg showing:(1) expansion of blood vessle, (2) severe thickened hepatocytes, (3) severe hepatocytes necrosis, (4) severe necrosis of hepatic tissue. H and E Stain -100 X

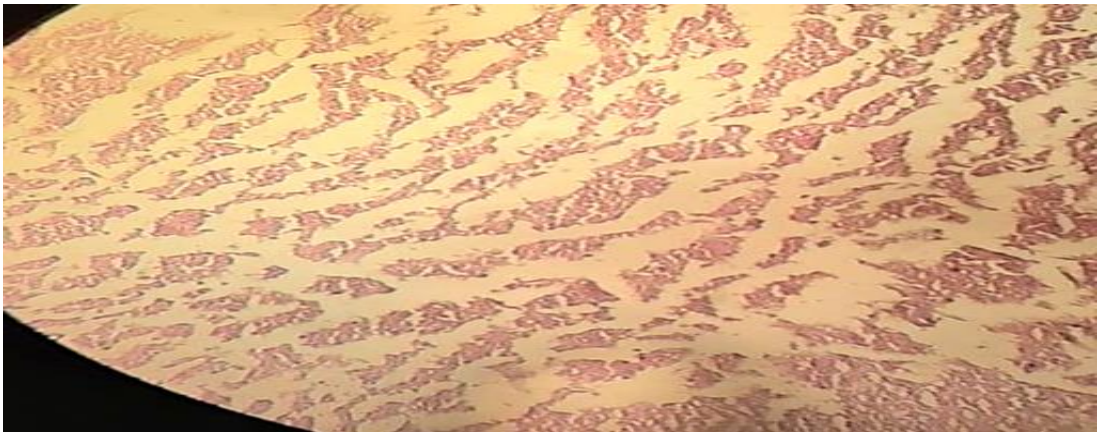

Figure 28. Cross section of the liver of a pregnant rat fetus of the treated group with tramadol at a conceration of 200 mg/ kg showing:severe necrosis and damage of hepatic tissue. H and E Stain-100 X

Discussion

The studies have shown that deforming factors stimulate various defects of the fetuses and newborns, the severity of these distorting effects depends on the way of obtaining, doses, time and duration of exposure and others individual factors such as age,health status and others(Shojaeifard*et al*.,2017), the drugs are dangerous teratogenic agents that cause various defects and congenital malformations of fetuses when administered during pregnancy,tramadol is one of the effective opioid analgesicsthat may stimulate various structural changes leading to deformities of the fetuses and prevent their growth and development during pregnancy stages which reflects on the newborns causing congenital deformities of them (Shipton2000), the results of this study were shown by some studies that pointed to the treatment ofpregnant rats during the early pregnancy stage with tramadol led to a toxic effects on the fetuses causing their absorption and remain them undeveloped in the endometrium of the uterus (Buthina*et al*., 2018), also the administration of pregnant mice with tramadol caused a delay in fetal development in addition to pathological effects in the structure of the tissues of the some organs such as livers and brainsof the fetuses leading to their non-development (Elonazsirous*et al*.,2020),the results of our study can be interpreted onthe oxidative stress that tramadol generates it causing the oxidative breakdown in different organs of mothers and fetuses in their uterus stimulating the absorption and non-development and consequently the death of them andas well as causes pathological changes in the tissues of fetuses because of the ability of tramadol to pass through the placentato the fetus during pregnancy leading to harmful effects of fetuses as a result of increased lipid oxidation in maternal and fetal cell membranes and stimulates various reproductiveand histological effects in all stages of pregnancy, and this was shown by a study ofFaria*et al*(2017) which signified that tramadol stimulated toxic oxidative effects in different organs of rats and

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caused an increase in oxidation process of proteins in them leading to the destruction of the heart, lung and the damage of nerve cells in the brain, another study indicated that the tramadol drug stimulated oxidative stress which,led to the oxidative damage in the fetuses cerebellum whose their mothers were treated during pregnancy with this drug (Chomchai*et al.*,2019), Alsostudyof Abdullah (2018)showed that tramadol dosingofthe rats during pregnancy stimulated severe shrinkage in many areas of the brain of the fetusesbecause the drug passes easily through the placenta as well as the blood-brain barrier and subsequently accumulated in the tissues of the fetuses due to the immaturity of the kidneys and the enzymatic functions of them, other studies attributed the reason for these results especially the pathological changes in the liver tissue to the fact that this organ is responsible formetabolism process of the tramadol drug, since the liver metabolizes this drug to O-desmethyltramadol which is the active compound of the drug and ithas a greater affinity to opioid receptors than this drug in its original form causing abnormal anatomical and histologicaleffects in the liver and stimulating hepatotoxicity (Youssef and Zidan,2016;Abbas *et al*.,2020).

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