

HISTOLOGICAL AND MOLECULAR STUDIES OF THE EFFECTS OF TRAMADOL ON BRAIN, LIVER AND KIDNEY OF ADULT RABBITS

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ABSTRACT

This study was to examine the histological changes in liver and kidney in tramadol -induced rabbits . This study was conducted on 10 adult male rabbits. Rabbits. were divided evenly into two groups: control group, received 1 ml normal saline 0.9% Treated group received 0.3 ml of tramadol subcutaneously for 30 days. Results: The histological analysis of the liver showed that the thickening wall with minimal fibrosis in the periportal area infiltration of inflammatory cells in necrotic change of the hepatocytes massive vacuolation of the hepatocyte, kupffer cell were present intensity in the sinusoid. The histopathological examination of kidney revealed lymphocyte infiltration , congestion ,glomerulus and tubular damage. The tubular was containing hypertrophied epithelial cell which block the lumen .Brain tissues in treated groups showed degeneration of pyramidal cells in the cortex , congestion of the blood vessels in the cortex and medulla. The cortex certain pyramidal cell with slight enlarged. The random amplified polymorphic DNA (RAPD) based on Polymerase Chain Reaction (PCR) with 5 primers were applied, used to estimate fingerprinting and Genetic Distance for 3organs . the results showed clear difference in the number of packets as well as in the genetic dimension compared to the control group. Conclusion: The histopathological and molecular alterations of liver, kidney and brain tissues threw lights on the possible risks of increased hepatic, renal and neurological damages evoked by repeated administration of tramadol for long periods.

Keywords: Tramadol, histopathological changes and Rabbits.

عبد الله

مجلة العلوم الزراعية العراقية -2018: 49(6):1083-1089

دراسة التأثير النسيجي والجيني لعقار الترامادول على الاعضاء(الدماغ والكبد و الكلية) في الارانب البالغة

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المستخلص

هدف الدراسة هو فحص التغيرات النسيجية والخلوية للكبد والكلية والدماغ للارانب التي جرعت بعقار الترامادول . نفذت الدراسة باستخدام 10 ارانب ذكور . قسمت الى مجموعتين .المجموعة الاولى جرعت 1مل من الملح الطبيعي .9% والمجموعة الثانية عولجت .3.0مل من عقار الترامادول.النتائج:-شاهد من خلال الفحص النسيجي تثخن في جدار الكبد وتليف قليل وانتشار خلايا التهابية وتنكسات في خلايا الكبدية . اما في الكلية وجود خلايا التهابية واحتقانومتوت النبيبات كذلك في الماغ هناك تحطم في خلايا الهرميه في القشرة واحتقان في اللب والقشرة.كذلك استخدمت تقانة التضاعف العشوائي المتعدد (ريد)المتعدد على تقانة (بي سي ار) باستخدام 5 برايمرات بينت النتائج للتحليل الوراثي هناك اختلافا واضحا في عدد الحزم وكذلك في البعد الوراثي مقارنة مع السيطرة.

كلمات مفتاحية:-ترامادول، تغيرات نسيجية، ارانب

INTRODUCTION

Tramadol is (1RS;2RS) -2- (dimethylaminoethyl) -1- (3-methoxyphenyl)-cyclohexanol -hydrochloride. used as analgesics and is listed in many medical guidelines to treat both acute and chronic pain. Absorption of tramadol is more of than morphine , and rapidly absorbed in the small intestine. Its metabolized in the liver by two main pathways: O-demethylation to O-desmethyltramadol (M1) and N-desmethylation to N-desmethyltramadol (M2) by cytochrome P450 enzyme system, then excreted unchanged by the kidneys^(13,7,12). In the present study was carried out to investigate the histological changes in the liver, kidney, and brain of rabbits exposed to high doses of tramadol.

MATERIAL AND METHODS

The experimental study was carried out on 15 mice (about 1.5-2.1 kg), during the period from June to May 2016, it has been achieved in the animal house of the college of veterinary medicine / Tikrit University. The animals were maintained under controlled environmental conditions. They were provided a free access to standard pellet diet and tap water. The animals were divided into 2 groups each group consists of 5 animals: The drug tramadol HCl (Trabilin Ampoule) 100mg/2ml (manufactured by Mepha company purchased from Iraqi pharmacy).

Group 1 (G1): healthy control mice.

Group 2 (G2): mice received 0.3 ml of tramadol subcutaneously.

Treatment was done once daily for continuous 30 days.

Histological study

After sacrifice, livers and kidney were obtained from the mice and immediately fixed in 10% formalin. The tissues were dehydrated in graded ethanol solutions (50, 70, 80, 90, two changes each of 100%), cleared in xylene and embedded in paraffin wax . Sections of 5 microns were cut and stained with eosin and hematoxylin according to⁽²⁾. Photomicrographs of the stained slides were taken using digital camera attached to light microscope.

RAPD analysis

RAPD analysis was performed according to the protocol of Williams *et al.* 1990⁽¹⁷⁾. This

study was undertaken for genetic analysis within the 3 organs (liver, kidney, brain) from rabbit by using RAPD markers the method involves an extraction of DNA, were analyzed using Random Amplified Polymorphic DNA (RAPD). Total 5 primers were used as, G-PRMER, M-PRMER, P-PRMER, U-PRMER, W-RMER

RESULTS AND DISCUSSION

The control group, showed the structure of liver structure the central vein and hepatocytes were arranged in radial form and normal size and shape also sinusoid appeared normal in the size as well as Kupffer cell, as in figures (1): The present study we showed after administration the parenchyma of the liver was containing hypertrophied cell appeared balloon . and the cytoplasm of these cell was not containing and stain , certain number of these cells was not contain any parenchyma of the liver and in the portal area .the bile ducts were containing hyper epithelialization in portal area .the sinusoid were containing numerous Kupffer cells , however there was another area containing normal hepatocyte with polygon shapes eosinophil cytoplasmic, as in figures (2-5).

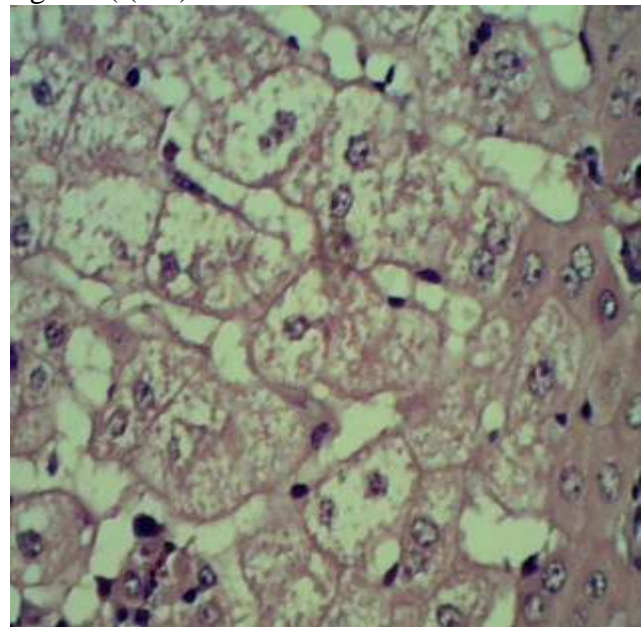


Figure 1. Liver of Rabbit (control)(H&E 40 X).

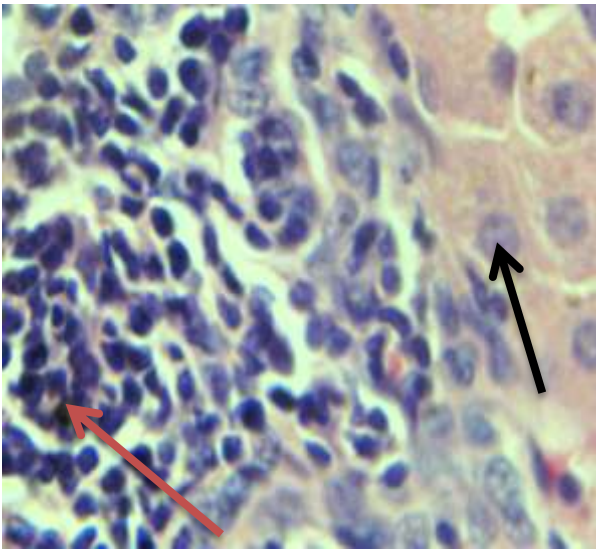


Figure 2. Liver of Rabbit administration with(tramadol 0.3 ml) for 30 days ,showed (red arrow)focal aggregation of lymphocyte ,(black arrow) the parenchyma of the liver with hypertrophy of liver cell (H&E 40 X).

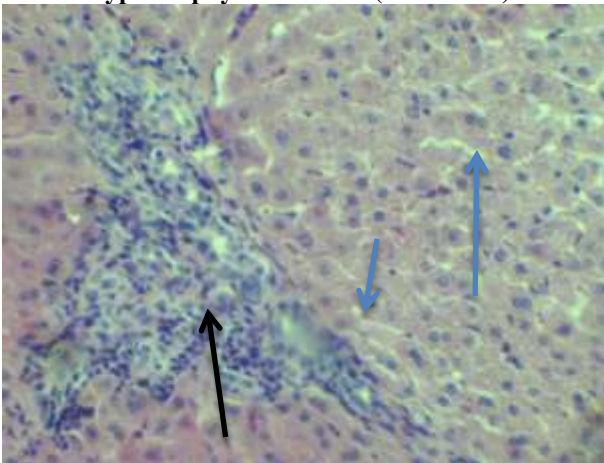


Figure 3. Liver of Rabbit administration with(tramadol 0.3 CC) for 30 days, showed (black arrow)lymphocyte aggregation and increase fibrosis , (blue arrow)the liver was containing hypertrophied cell (H&E 40 X).

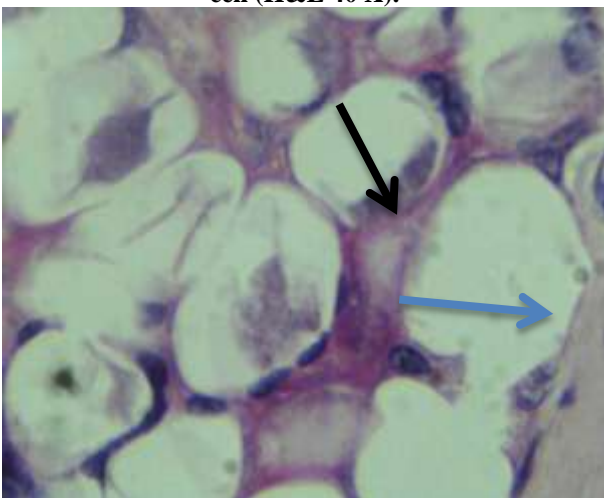


Figure 4. Liver of Rabbit administration with(tramadol 0.3 CC) for 30 days, showed, (blue arrow)he liver was necrosis (black arrow)degeneration (H&E 40 X)

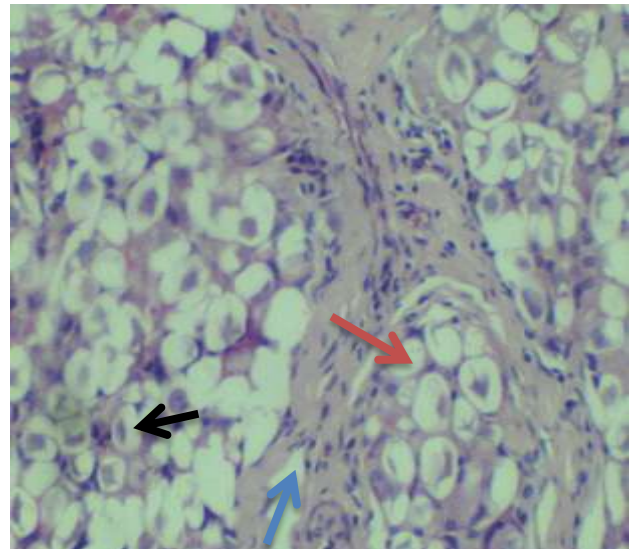


Figure 5. Liver of Rabbit administration with(tramadol 0.3 CC) for 30 days, showed, (blue arrow)he liver was necrosis (black arrow)degeneration (red arrow) fibrosis (H&E 40 X)

In the control group, showed the structure of kidney ,the glomerular appeared size and shape and tubules brush border and basement membrane, as in figures (6).The histological study of the kidney showed, the cortex was containing hypertrophy of glomeruli with increase the epithelial cells on the glomeruli. The proximal tubular was containing hypertrophied epithelial cell which block the lumen of the these tubular the B.V in between were highly congestion with blood with thickening of the walls of these blood vessels .the medulla was containing increase the interstitial C,T. between the collecting tubules as in figures (7-9).

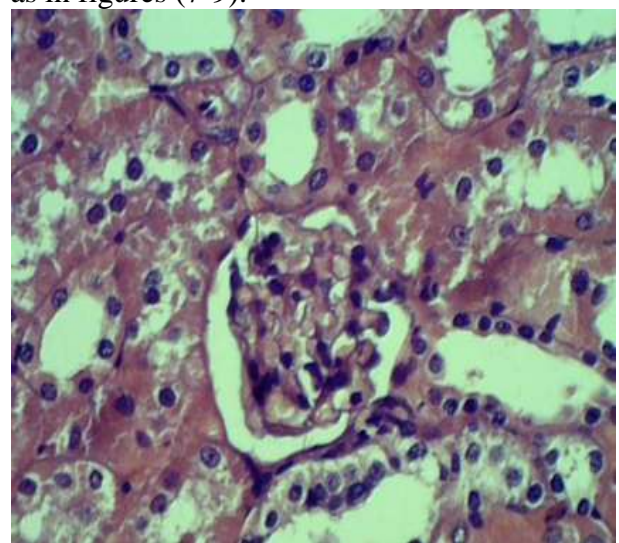


Figure 6. Kidney of Rabbit (control)(H&E 40 X)

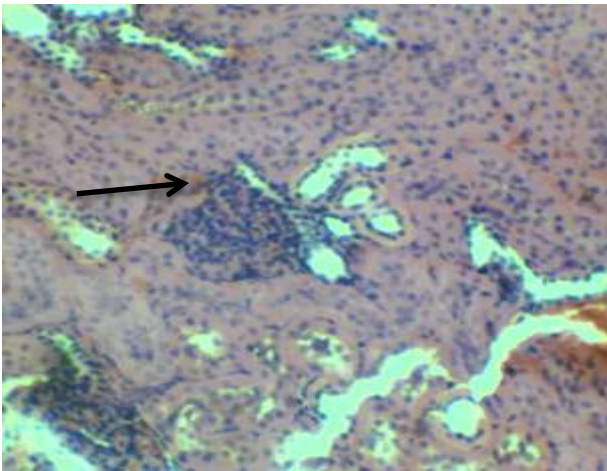


Figure 7. kidney of Rabbit administration with(tramadol 0.3 CC) for 30 days, (black arrow)showed, The tubular was containing hypertrophied epithelial cell which block the lumen of the these (H&E 40 X)

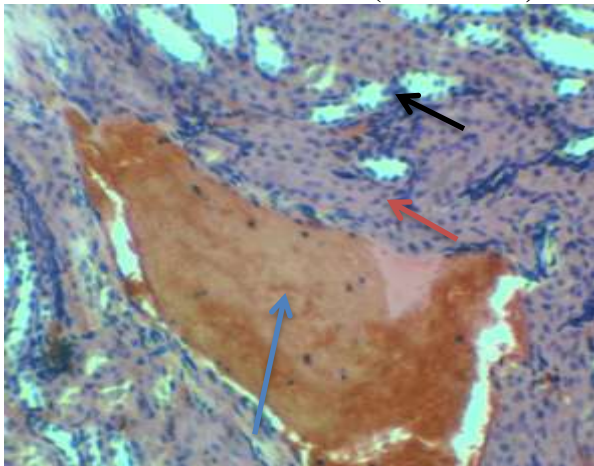


Figure 8. Kidney of Rabbit administration with(tramadol 0.3 CC) for 30 days, showed (black arrow)lymphocyte infiltration ,(blue arrow) congestion ,glomerulus and (red arrow) tubular damage (H&E 40 X).

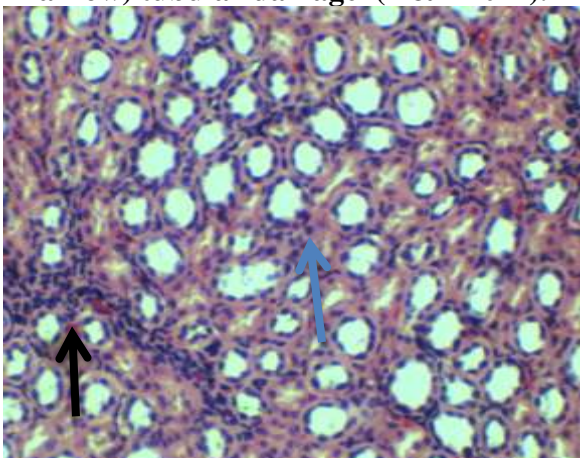


Figure 9. Kidney of Rabbit administration with(tramadol 0.3 CC) for 30 days, showed (black arrow)lymphocyte infiltration ,(blue arrow) tubular damage (H&E 40 X),

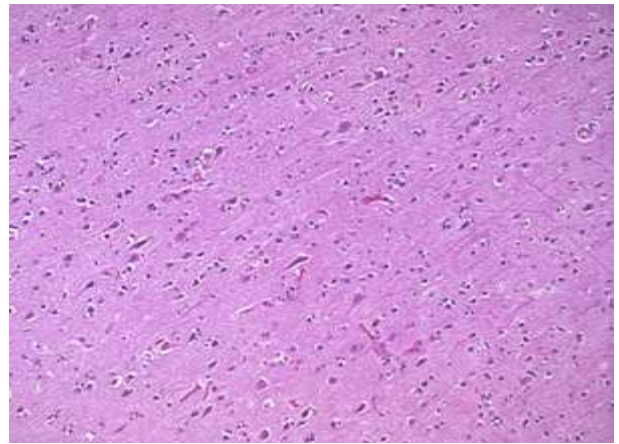


Figure 10. showed brain control, small pyramidal cells, granule (stellate) cells

In the present study the brain showed ,tissues was containing congestion of the blood vessels in the cortex and medulla (white matter). The cortex certain pyramidal cell with slight enlarged , as in figures ((11,12).

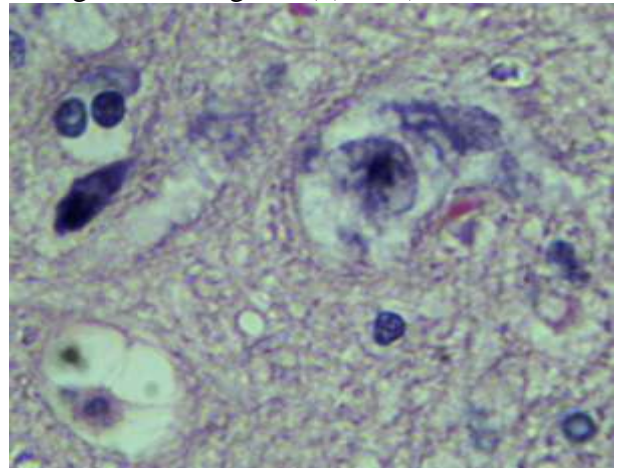


Figure 11. Brain of Rabbit administration with(tramadol 0.3 CC) for 30 days, showed degeneration of pyramidal cells in the cortex ,surrounded by large vascular(H&E40X).

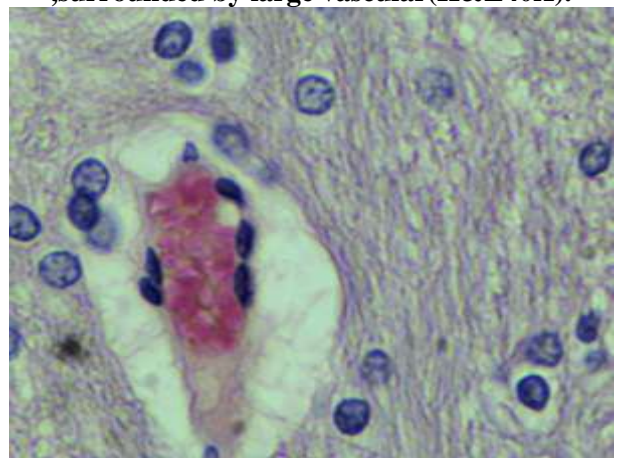


Figure 12. Brain of Rabbit administration with(tramadol 0.3 CC) for 30 days, showed, tissues was containing congestion of the blood vessels in the cortex and medulla (white matter). (H&E 40 X).

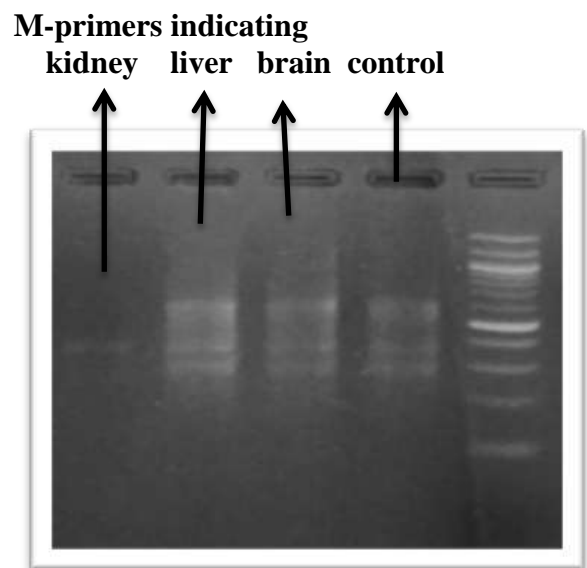
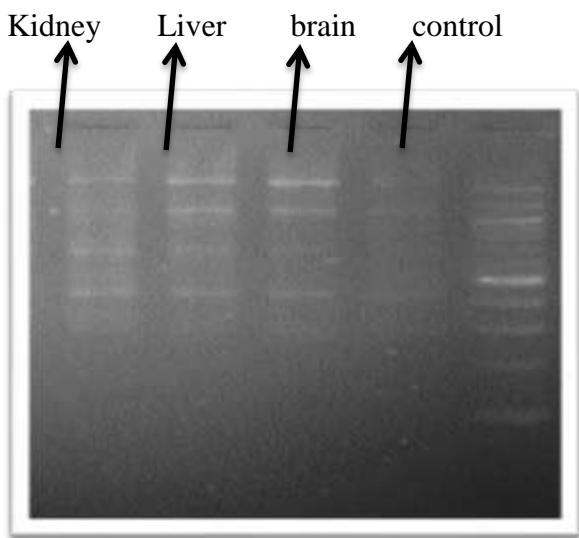
RAPD analysis Screening of specific RAPD amplicon

Table 1,fig-13. In a RAPD analysis a total of bands was scored from 5 primers out of which bands were polymorphic was found from 5

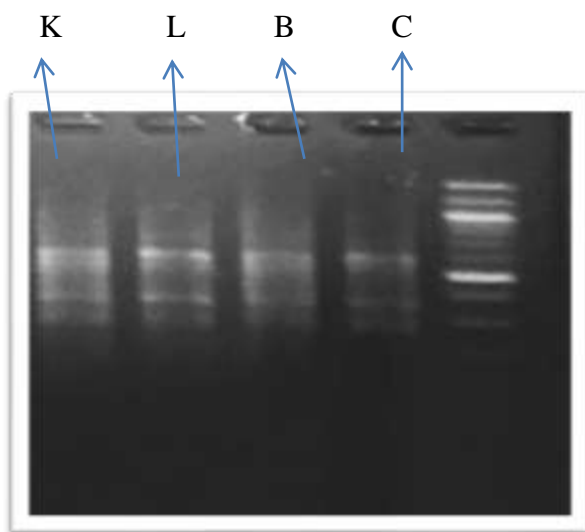
primers. (Table 2) showed Genetic distance of tramadol on 3 organs (brain ,liver ,kidney) with **Similarity = $2nx / nx + ny$**
Genetic distance = $1 - (2nx / nx + ny) \times 10$

Table 1. Table shows RAPD markers of the control with 3 organs treatment by tramadol with 5 RAPD primers

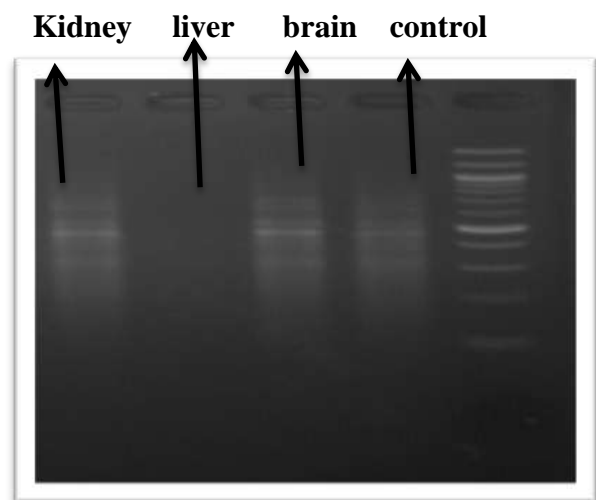
primers / organs	control	Brain	liver	Kidney	Total
Primer G	3	3	3	2	8
Primer -M	2	1	1	1	3
Primer-P	2	2	2	0	6
Primer-U	2	2	0	1	5
Primer-W	2	2	2	2	8

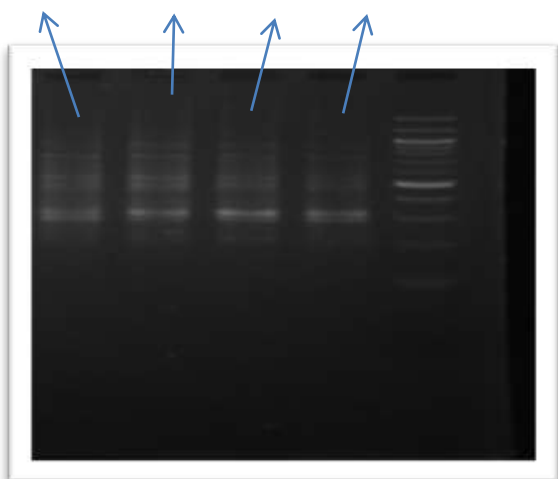


G-primers indicating



P-primers indicating



U-primers indicating**Kidney liver brain control****W-primers indicating****Fig 13. RAPD reactions on an agarose gel(5 RAPD Primers).****Table 2. Genetic distance of tramadol on 3 organs (brain ,liver ,kidney) with control**

Kidney	liver	brain	organs
58.8	57.8	1.083	Control

DISCUSSION

Studying of the histopathological effects of toxicity of tramadol on liver tissue shown necrosis , degeneration , lymphocyte aggregation and increase fibrosis , and the liver was containing hypertrophied cell .This could be elucidated by the fact that the liver is responsible for the metabolism and excretion of tramadol ⁽¹⁴⁾. Which in accordance with results of Atici and his associates⁽¹⁾. who stated focal and necrosis congestion in the rat liver due to long term use of tramadol. Activated Hepatic stellate cells proliferate dynamically and secrete a large amount of extra-cellular matrix which contributes to hepatic fibrosis in response to injury⁽⁸⁾. The brain is chiefly susceptible to oxidative damage due to its high levels of oxygen consumption, and low levels of antioxidants⁽⁹⁾. Results of the present study showed that treatment of rabbits with tramadol lead to congestion of the blood vessels in the cortex and medulla ,degeneration of pyramidal cells in the cortex .this is in agreement with Sakurai-Yamashite Y⁽¹⁵⁾, El-Naggar et al. ⁽⁴⁾. demonstrate that tramadol administration for long period in rats lead to a significant rise in the level of serotonin in the brain cortical tissue . Also Rabei HM. ⁽¹¹⁾ concluded that the

oxidative stress prompted by the tramadol in brain. The kidneys are involved in the secretion of several, toxins and therefore they are liable to liberate high quantities of free radicals which contribute to high oxidative stress that is involved in Causing kidney damage ⁽⁵⁾. Histopathological examination of sections of kidney tissue of rats treated with tramadol showed lymphocyte infiltration , congestion, glomerulus and tubular damage. The above observations may be confirmed by the suggestions of Jassen-ortho inc. ⁽⁶⁾. and Wu et al. ⁽¹⁵⁾. who indicated that Tramadol and its metabolites are excreted via kidneys, so it may cause nephrotoxicity.The multiple effects of opioids on neuronal structure (cytoskeleton) due to long term use of morphine and other opioids lead to brain damage. in the lymphocytes and mouse spleen, lung and heart via activating opioid receptor ,It may induce the mRNA expression of pro-apoptotic receptors. They observed a large amount of apoptotic cells of these rats, and the expressions of the apoptosis related proteins (Fas, Bcl-2 and caspase-3) presented with alteration., the expressions of Fas and caspase-3 increased markedly and Bcl-2 expression reduced significantly in morphine addiction group and morphine abstinence group. When compared with the control group ,These suggest that long term use of morphine as an example of opioid drug may increase the apoptotic neurons via apoptosis related signaling pathway. This also confirmed by a result presented previously by (18)the essential role of the liver in drug metabolism ,has been associated with hepatotoxicity due to (16).Metabolites may have a higher activity and/or a greater toxicity than the original drug. These metabolites excreted via kidneys a lead to kidney dysfunction, may also cause a cellular damage(10)(16). our results are coincided with results of Elmanama et al.2015(3).who suggested that treatment with tramadol is of more harmful to the liver and causes a serious cellular toxicity and a liver failure. in the present study, investigate that tramadol-induced damage in tissues . (brain, liver and kidney). Our findings pointed out the risk of hepatic and renal damage due to long term use of tramadol. Although this drugs are reported to be effective in pain management,

their toxic effects must be kept in mind during chronic usage.

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