Effect of Tramadol administration on the liver of juvenile and adult male albino mice: Comparative histological and histochemical study

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Abstract:

Background: Tramadol is a broadly used opioid drug; it is an effective analgesic agent for the treatment of moderate to severe pain. The liver is one of the organs responsible for the metabolism and excretion of opioids, which may cause hepatotoxicity.

Objectives: The present study was designed to evaluate the effects of tramadol on the liver of both juvenile and adult experimental mice.

Materials and methods: Twenty juvenile mice aged 3 weeks and twenty adult mice aged 2 months, were used in the present study. Each group was divided into two subgroups; control and tramadol-treated group (40 mg/kg b.wt., orally for 30 consecutive days). Liver specimens were taken for histopathological examination.

Results: This study showed that tramadol treatment induced histopathological changes in both groups (juvenile and adult), but these changes were more prominent and obvious in juvenile than adult group. These changes were manifested by disorganization of liver architecture, cellular degeneration, with congested central and portal veins, associated with inflammatory cells infiltration.

Conclusions: Tramadol has harmful effects on the liver of both juvenile and adult albino mice and these effects were more obvious in juvenile than in adult mice. Toxic effects of tramadol should keep in mind, even though, the important role it plays as a powerful pain killer.

Keywords: Tramadol, Histopathology, Liver.

Introduction:

Tramadol is a drug which is considered as a synthetic and centrally acting analgesic, available in Europe countries since 1977 and available in the United States since 1995. Tramadol is used for treatment of pain syndrome previously amenable only for opiate analogues **(Hafez etal.,2015).**Tramadol is known to have a dual mode of action. Its analgesic effect is due to its partial affinity for the μ-opiate receptor and also to its inhibition of norepinephrine and serotonin re-uptake **(Shadnia et al., 2008)**. Tramadol is rapidly absorbed after oral administration. The mean peak plasma concentration occurs after 2 hours and its bioavailability is approximately 70% as a result of the first-pass metabolism in the liver **(Bloor et al.,2012,Eassa and El-Shazly,2013, and Lavasani etal.,2013).**Its elimination is totally by the kidneys; approximately 30% of the dose is excreted in urine as an unchanged form, however 60% of the dose is excreted as metabolites **(Eassa and El-Shazly,2013, Lavasani et al.,2013)**. Tramadol is considered as a safe drug as it is devoid of many side effects of traditional opioids. However, recently, abuse and dependence of tramadol beside toxicity and even tramadol-related deaths have been reported **(Tjäderborn et al., 2007).**Liver is the main organ responsible for the metabolism of tramadol this process

is occurred via cytochrome isoenzymesP450 2D6, and P450 2B6 and P450 3A4(**Lavasaniet al.,2013).**Despite that tramadol abuse is not a newly introduced issue to the society of Egypt, especially among the young adults, the availability and illegal transactions are associated with its abuse, making tramadol being the most easily accessible drug and also at a cheap cost **(Fawzi,2010).** This study aimed to compare the histological effects of tramadol on liver of juvenile and adult male albino mice.

Materials and methods:

Animals: A total number of 40 apparently healthy albino mice(20 juvenile , weighting 10-15 gm, aged 3 weeks and 20 adult, weighting 20-30 gm, aged 2 months) were purchased from animal house of Assiut University. Animals were kept under controlled laboratory conditions of humidity, temperature and light: dark cycle. Mice were fed standard food, and allow free access to water.

Drugs and chemicals:

Tramadol-hydrochloride (Tramundin) 200 mg tablets from Mundi Pharma Egypt by official request to the dean of Faculty of Medicine through pharmacy of Qena University Hospital.

Experimental design:

The mice were allocated into two groups as following:

Group I (Juvenile group):

The juvenile mice were randomly allocated into 2 equal subgroups (10 each) as follow:

Group Ia(control group): The mice in this group received isotonic saline orally once daily for one month.

Group Ib (tramadol treated group): The mice in this group administered 40mg/kg b. wt./day tramadol, orally once daily for one month. The tablets were smashed and dissolved in normal physiological saline 0.9%.

Group II (Adult group):

The adult mice were randomly allocated into 2 equal subgroups (10 each) as follow:

Group IIa (control group): The mice in this group received isotonic saline orally once daily for one month.

Group IIb(tramadol treated group):The mice in this group administered 40mg/kg b. wt./day tramadol, orally once daily for one month. The tablets were smashed and dissolved in normal physiological saline 0.9%.

Histological examination:

For light microscope: Liver specimens from all groups were taken, and then fixed in 10%formaline for 72 hours, washed in 70% alcohol, dehydrated in ascending grades of alcohols, embedded in paraffin, and sectioned by a microtome at (5µm) thickness**(Bancroft and Gamble,2008)**and stained with the following stains:

General H&E stain: for routine histological examination.

Siruis red: for demonstration of collagen fibers.

PAS stain: for demonstration of glycogen inside the cells. All the staining was done according to **(Bancroft et al, 2013)**

For semi thin section examination:

Liver specimens were taken and immersed in 4% glutaraldehyde in cacodylate buffer (pH 7.4) for 24hours and post fixed in 1% osmium tetroxide in phosphate buffer for two hours. Tissue sections were rinsed in the same buffer, dehydrated with alcohol, cleared with propylene oxide then embedded in Epon-812 substitute. Semi-thin sections (0.5-1µm) were cut with glass knives on the ultratome and finally stained with 1% toluidine blue (pH 7.3) for light

microscopic examination **(Ayacheet al,2010)**

Results and discussion:

In juvenile control group (Ia)and adult control group(IIa) the liver appeared normal with normal hepatic cells, normal portal area and normal central vein (Fig.1,3).The collagen fibers appeared with normal distribution around the portal and central veins (Fig.5,7), PAS-reaction showed an existence of a considerable amount of glycogen in the hepatocytes (Fig. 9,11).

In tramadol treated groups (juvenile Ib) and (adult IIb) there were alterations in the tissues but the alterations and changes were more prominent in juvenile group than in adult group, those alterations were in the form of hepatic cellular necrosis and degeneration, inflammatory cell infiltration was apparent around the portal structure, also portal veins appeared dilated, cytoplasmic vacuolation of some hepatocytes also noticed (Fig.2,4). Examination of Sirius red stained tissue sections; collagen fibers showed an increase in its distribution in tramadol group when compared with the control group (Fig.6,8). Examination ofPAS stained tissue sections;a weak PAS-reactivity of most of hepatic cells in tramadol treated group when compared with the control group (Fig.10,12).

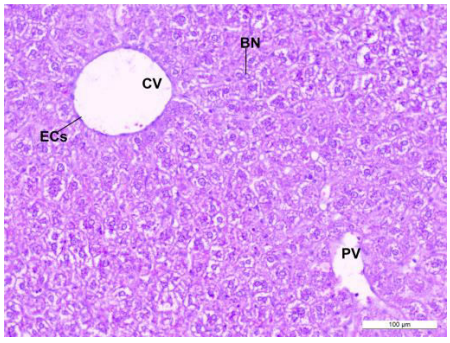

Fig.1:A photomicrograph of the liver from juvenile control group (group Ia) showing normal central vein(CV) with normal hepatocytes and normal endothelial cells (ECs), normal portal vein (PV), some hepatocytes are binucleated (BN)**(H&E×200).**

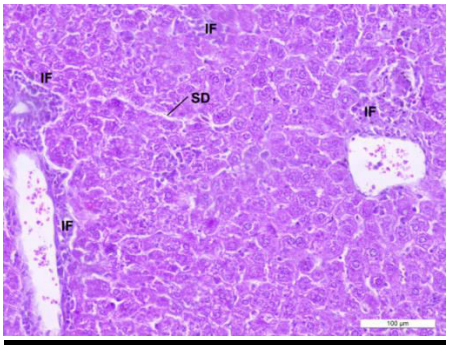

Fig.2:A photomicrograph of the liver from juvenile tramadol treated group (group Ib) showing massive inflammatory cell infiltration (IF) appears in the portal area, around the sinusoids and around the CV, mild sinusoidal dilatation is observed (SD)**(H&E×200).**

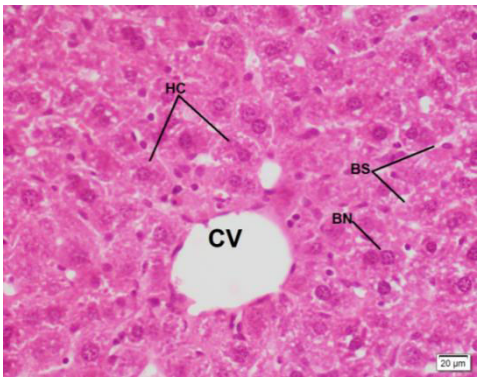

Fig.3: A photomicrograph of the liver from adult control group (IIa) showing normal central vein(CV) with normal polygonal hepatocytes(HC),normal vesicular nuclei, some hepatocytes are binucleated (BN), normal blood sinusoids (BS) **(H&E×400).**

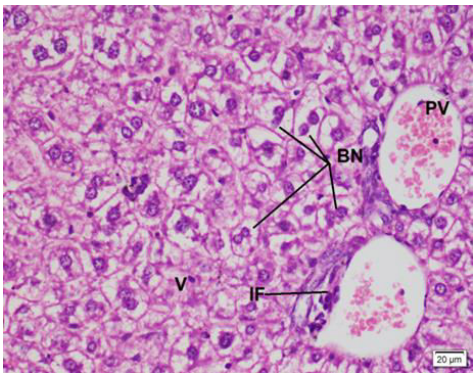

Fig.4: Aphotomicrograph of the liver from adult tramadol treated group (IIb), showing dilatation and congestion in the portal vein (PV),inflammatory cell infiltration (IF) around the PV, cytoplasmic vacuolation(V) and binucleation (BN) in the hepatocyte**(H&E×400).**

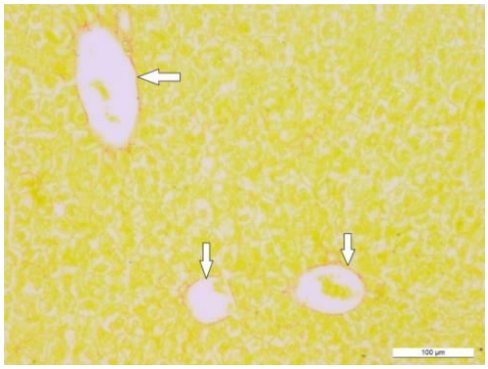

Fig.5:A photomicrograph of the liver from juvenile control group (group Ia) showing normal mild distribution of collagen fibers around the central vein and the portal vein (white arrows) **(sirius red x200).**

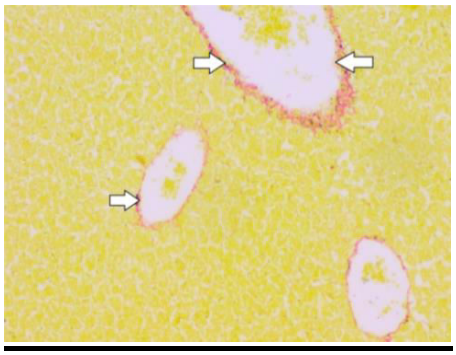

Fig.6:A photomicrograph of the liver from juvenile treated group (group Ib) showing increase in the distribution of collagen fibers around the central vein and the portal vein (white arrows)**(sirius red x200).**

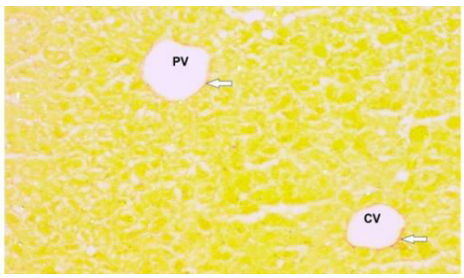

Fig.7:A photomicrograph of the liver from adult control group (group IIa),showing normal distribution of collagen fibers around central and portal veins (white arrows)**(siruis red x200**).

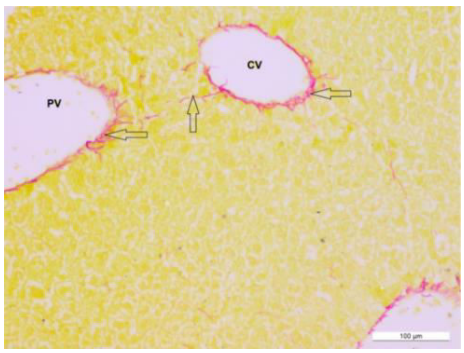

Fig.8: A photomicrograph of the liver from adult treated group (group IIb) showing increase in the distribution of collagen fibers around the central vein and the portal vein (white arrows) **(sirius red x200).**

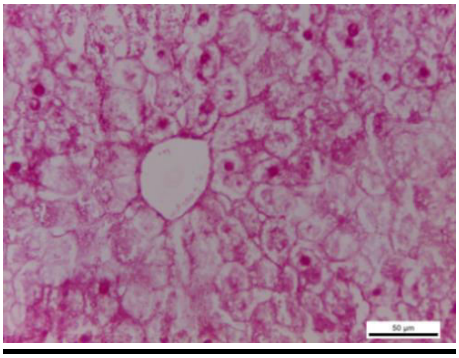

Fig.9:A photomicrograph of the liver from juvenile control group (group Ia) showing normal distribution of glycogen inside the hepatic cells **(PAS x400).**

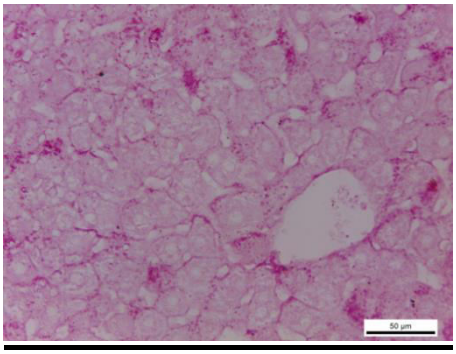

Fig.10: A photomicrograph of the liver from juvenile tramadol treated group (group Ib) showing marked depletion of glycogen inside the hepatic cells **(PAS x400).**

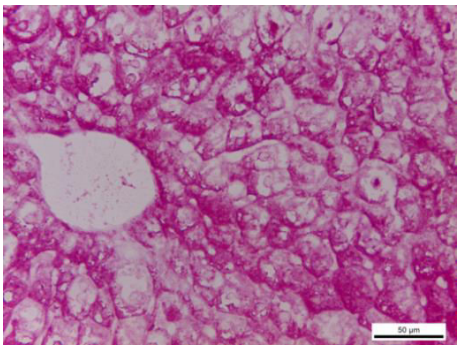

Fig.11: A photomicrograph of the liver from adult control group (group IIa) showing normal distribution of glycogen inside the hepatic cells **(PAS x400).**

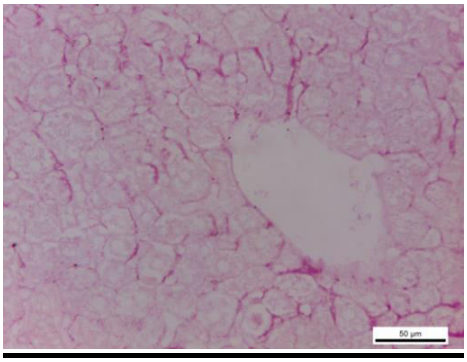

Fig.12: A photomicrograph of the liver from adult tramadol treated group (group IIb) showing marked depletion of glycogen inside the hepatic cells **(PAS x400).**

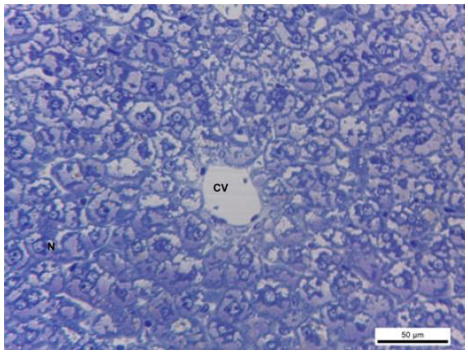

Fig.13: A photomicrograph of a semi-thin section of the liver from juvenile control group (Ia) showing normal central vein(CV) and normal hepatic cells with vesicular nuclei(N) **(Toludine blue X400).**

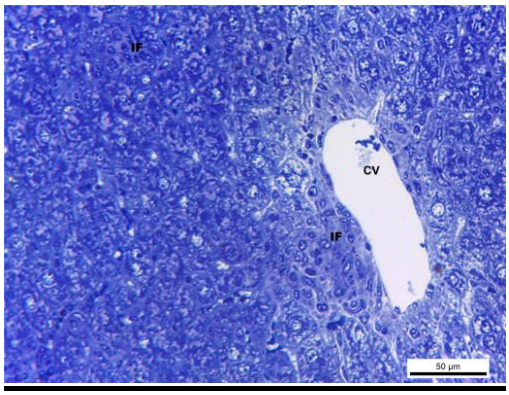

Fig.14: A photomicrograph of a semi-thin section of the liver from juvenile tramadol treated group (Ib) showing inflammatory cell infiltration (IF) around the central vein and in between the hepatic cells **(Toludine blue X400).**

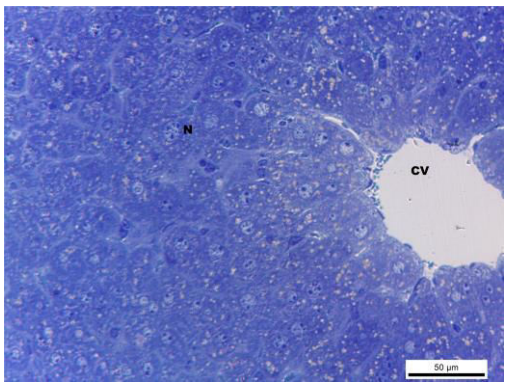

Fig.15: A photomicrograph of a semi-thin section of the liver from adult control group (IIa) showing normal central vein(CV) and normal hepatic cells with normal vesicular nuclei(N) **(Toludine blue X400).**

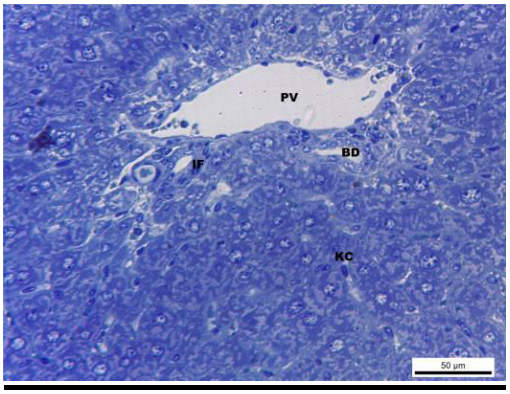

Fig.16:A photomicrograph of a semi-thin section of the liver from adult tramadol treated group (IIb) showing, portal vein (PV) with inflammatory cell infiltration (IF) around the portal area **(Toludine blue X400).**

Discussion:

Although opioids are widely used since very long time, their long-term effects especially at cellular level, are not clearly understood **(Aticiet al.,2005**).

The findings in this study are consistent with other studies that found degenerative changes of the hepatocytes after tramadol administration in mice **(Sheweita et al., 2018).** The congestion in the central vein and sinusoids was present in both juvenile and adult groups. This result was corresponded with that observed by **(Aticiet al.,2005 and Buhariet al.,2012).** Congestion in the central vein may bedue to the harmful effect of tramadol on heart. It is known that the mammalian heart is usually affected by opioids administration **(Tanushet al.,2015).** The appearance of inflammatory cells in the liver tissue after tramadol administration may suggest that tramadol could interact with proteins and enzymes of the hepatic tissue and interfere with the antioxidant defence mechanism and leading to reactive oxygen species (ROS) that may cause an inflammatory reactions**(Joharet al.,2004).**The expression oxidative stress is used to describe various deleterious processes result from the imbalance between the excessive formation of ROS and limited antioxidant defences**(Pizzinoet al.,2017**)but

in our study we found that the appearance of the inflammatory cells were most prominent in juvenile group more than in the adult group.In this study, vacuolation of the liver cells is supported by a study done by(**Awadalla and Salah-Eldin,2015).**The vacuolation appeared in the cytoplasm of liver cells is mainly a result of disturbance in lipid and fat metabolism occurring after pathological changes(**Dkhil et al.,2010).** Vacuolation of liver cells were noted by other studies following treatment with tramadol (**Youssef and Zidan,2016).** The present data agreed with **(Samaka et al.,2012)**, who found liver parenchymal alteration in the form of focal necrosis, Portal changes, marked inflammatory cellular infiltrate and mild fibrosis in group treated with tramadol.Regarding the present data concerned with collagen fibers in the hepatic tissues, the present study found that there were increased in the collagen fibers in the hepatic tissue of the tramadol-treated groups (group Ib) and (group IIb) comparing with control group with more affection of juvenile than adult group. This result is in agreement with **(Altindag et al.,2007)**, who explained that the increased collagen fibbers occur due to decreased collagen metabolism that may be related with oxidative stress.

The data collected from the present study suggest that depletion of liver glycogen after tramadol administration might be attributed to the effect of tramadol on glucose absorption or on the enzymes involved in the process of glycogenesis and glycolysis **(Jarrar and Taib,2012).**Serum glucose levels is generally increased in mice in response to tramadol administration(**Elyazjiet al.,2013).**

Conclusion: From this study, it can be concluded that tramadol has harmful effects on the liver of juvenile and adult albino mice and these effects were more obvious in juvenile than in adult mice.

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