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| Original Article | The Hazards of Tramadol on the Liver Structure of Adult Male Albino Rats and the Possible Protective Role of N-acetylcysteine: Histological and Immunohistochemical Study <i>Marwa M. Ahmad¹, Amira I. Alsemeh¹, Hayam E. Rashed² and Dalia-El Shafei³</i> <i>¹Department of Human Anatomy, ²Department of Pathology, ³Department of Community, Faculty of Medicine, Zagazig University, Egypt</i> |
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ABSTRACT

Background: Tramadol administration is associated with hepatic congestion, apoptosis and induces oxidative damage in tissues. The thiol group in N-acetyl-cysteine (NAC) interacts directly with reactive oxygen species leading to cellular protection against such damage.

Aim of Work: The present study was designed to highlight the hazards of tramadol (TR) on liver structure of adult male albino rat and the efficacy of NAC on the histological structure, liver functions for protection against such possible induced hazards.

Materials and Methods: Forty male albino rats were used. Rats were divided into four groups with 10 rats per each group. Control group received normal saline 2ml/day orally by gavage for 30 days. NAC-treated group received 150 mg/kg/day of NAC dissolved in normal saline by oral gavage for 30 days. TR- treated group received TR 40mg/kg/day dissolved in normal saline by oral gavage for 30 days. TR + NAC group were administered TR and NAC in similar doses as in TR and NAC groups' respectively for 30 days. The livers were removed and processed for light microscopic examination and different biochemical markers in addition to immunohistochemical examination of both Bax and 8- hydroxyl-2-deoxy guanosine (8-OHDG).

Results: The TR- treated group revealed disrupted arrangement of the hepatic cords together with congestion of central vein and mononuclear perivascular infiltration. Most of the hepatocytes showed necrosis and apoptotic hepatocytes with bile duct duplication. There were diffuse fibrous tissue formation and a significant increase in the number of Bax and 8-OHDG positive cells. As regard to liver functions, there were highly significant elevations of serum levels of aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Serum alkaline phosphatase (ALP) and bilirubin and reduction of serum albumin. As regard to the antioxidant enzymes, there was an increase of malondialdehyde (MDA) and decrease in superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) in the liver tissues. In contrast with TR+NAC group where liver parenchyma restored its uniform architecture with minimal collagen fibers, significant reductions in the number of Bax and 8-OHDG positive cells. Liver functions and antioxidant enzymes level nearly restored its normal values.

Conclusion: The TR is suggested to have hepatotoxic effect and NAC might have protective effects against such induced hepatotoxicity.

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Key Words: 8-OHDG positive cells, bax, liver, N-acetyl cysteine, tramadol.

Corresponding Author: Marwa M. Ahmad, Department of Human Anatomy, Faculty of Medicine, Zagazig University, Egypt, **Tel.:** +20 12257 01437, **E-mail:** Marwaahmed515@gmail.com

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INTRODUCTION

Drug addiction is a social and health problem all over the world. Analgesics especially the opioids are among the commonly abused drugs (Do Quang-Cantagrel, *et al.*, 2000).

TR is a synthetic opioid analgesic agent that acting centrally. It is used orally and parentally for treatment of moderate to severe pain (Johnson, 2005). Usual doses are up to 200 mg/day and the maximum allowed daily dose is 400 mg (McKeon *et al.*, 2011).

Repeated TR administration might lead to accumulation of toxic metabolites in their bodies and decrease the clearance of TR thus increasing its potential for toxicity (Tjaderborn, 2007). The toxic effects of TR are expected during long-term therapy especially in large doses (Watson, *et al.*, 2004).

TR abuse, dependence as well as acute overdose -related deaths have been increasingly reported especially in young male adults (Lee, *et al.*, 2013). Moreover, an increasingly alarming rate of TR abuse has been reported in the Egyptian community in the last few years (Fawzi, 2011).

The liver plays an important role in detoxification and metabolism of the drugs, so that it's expected that every drug is associated with a degree of hepatotoxicity (Atici, *et al.*, 2005). TR is converted in the liver to at least one active metabolite (O-dimethyl tramadol; M1). Such metabolites may have higher activity and/or greater toxicity than the drug itself (Tolman, 1998).

The toxic effects of such abused drugs are commonly associated with oxidative stress, mitochondrial dysfunction, apoptosis, in addition to other mechanisms (Cunha-Oliveira *et al.*, 2010).

Oxidative stress is a pathological cellular insult which is associated with increase in the production of free radicals and reactive oxygen species (ROS) and decreases antioxidant capacity both exogenous & endogenous antioxidants enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione capacity (GSH). Measurement of the levels of ROS production & metabolites of lipid peroxidation such as malondyaldehyde (MDA) and the antioxidant capacity may give information about antioxidant status (Pereska *et al.*, 2006).

Apoptosis plays a prominent part in the pathogenesis of toxic liver injury due to a variety of agents. The Bcl-2 family is a major protein family controlling cell survival or cell death in the molecular pathway of apoptosis (Youle and Strasser, 2008).

The NAC is a sulfur-containing amino acid that possesses many biological properties considered one of the most extensively studied agents. It has multiple therapeutic applications (Braakhuis, 1995). NAC could significantly interfere with the pathophysiology of free radicals of drug inducing oxidative stress (Raza, 2003). As a precursor of GSH, NAC can play an essential role in preventing the oxidative damage (Prakash and Kumar, 2009).

The detoxification capability of GSH is directly related to its reduced thiol group (Singh, 2002). It is well-reported that the thiol group in NAC interacts directly with reactive oxygen species leading to cellular protection against oxidative damage (Zafarullah *et al.*, 2003).

This study was aimed to clarify the possible hazards of TR on the rat liver structure and the role of NAC in protection against such changes.

MATERIALS AND METHODS

Chemicals

Tramadol tablets, each contain 150 mg tramadol hydrochloride, were obtained from Medical Union Pharmaceuticals (MUP), (Giza, Egypt) dissolved in 37.5 cm³ normal saline to obtain a solution for each 1 cm³ contains 4 mg and given 2cm³ /100 gm./day as a single dose by oral gavage. NAC powder was obtained from SEDICO pharmaceutical company in Egypt each sachet contains 600 mg of NAC and dissolved in 40 cm³ normal saline to obtain a solution for each 1 cm³ contains 15 mg and given 2cm³ /100 gm. / day as a single dose by oral gavage. Kits for lipid peroxidation (MDA) and all other anti- oxidant enzymes were purchased from Bio-diagnostic Co. All other chemicals were of the highest quality available.

Animals

Forty adult male albino rats (180-220mg) were used in this study. They were obtained from the Zagzaig Scientific and Medical Research center (ZSMR) of Faculty of medicine Zagazig University. The animals were kept in a controlled light room with a photoperiod of 12 hours dark and 12 hours light at a temperature of 28±2 °C. The

experimental design was approved by the animal care and use committee, Zagazig University. Rats were divided into four groups with 10 rats per each as follow:

Group 1: (Control group): consisted of 10 rats received 2ml of normal saline (solvent of both NAC and TR)

Group 2: (NAC- treated group): consisted of 10 rats received 150 mg/kg/day of NAC (*Varma, et al., 2004*).

Group 3: (TR- treated group): consists of 10 rats which were treated daily with TR 40 mg/kg/day (*El Sawy and Abdel Malak, 2015*).

Group 4: (TR + NAC treated group): consisted of 10 rats which were treated daily with similar doses of both TR and NAC as in groups 3&4 respectively.

At the time of sacrifice, rats were anesthetized using intra-peritoneal injection of thiopental 30mg/kg (IACUC, 2007) and then the blood samples were collected and centrifuged for 10 minutes at 5000 rpm to harvest the clear serum. Then, all animals were dissected and their livers were isolated. Liver from both control and treated rats were minced and homogenized (10% W/V) separately in ice-cold saline, sucrose buffer (0.25 M sucrose, 1 mM EDTA and 0.05 M Tris-HCl, pH 7.4) in a Thomas Sci. Co. glass-type homogenizer (Teflon pestle). The homogenization of tissues was carried out in Teflon-glass homogenizer with a buffer containing 1.15% KCl to obtain 1:10 (W/V) whole homogenate. Homogenates were centrifuged at 5000 rpm for 50 min they were used for measurement of MDA content and antioxidant enzymes (CAT, SOD, and GSH) activities. The other part was rinsed in phosphate-buffered (pH 7.5), fixed in phosphate-buffered formalin for histopathological examination.

Biochemical Study

- a. Detection of liver functions indices: (ALT) and (AST) activities were measured in IU/l (Thomas, 2007). (ALP) enzyme in IU/l, albumin mg/dl and bilirubin level in mg/dl were measured (*Perry, et al., 2000*).
- b. Determination of lipid peroxidation and antioxidant enzyme activities:

Lipid peroxidation level (MDA content) was measured in the liver as described by (*Placer et al., 1966*). CAT activity was assayed by the method reported by (*Aebi, 1974*). The activity of

SOD was estimated (Paolotti and Mocali, 1990) while GSH was determined by the method of (*Maral et al., 1977*).

Histopathological Study

After scarification, specimens from the liver were taken from the four studied groups. They were fixed in 10% neutral buffer formalin, embedded in paraffin, sectioned at 5 microns (*Hegazy and Hegazy, 2015*).

The sections were stained with hematoxylin & eosin (H & E) and Mallory trichrome stains (*Bancroft and Gamble, 2008*). All sections should be examined using light microscope.

Immunohistochemical study for Bax and 8-OHdG

Immunostaining was performed on serial sections of paraffin blocks, 4 µm thicknesses. The tissue sections were deparaffinized and rehydrated in graded ethanol. Deparaffinized tissue sections were treated with hydrogen peroxide for 10 min to block nonspecific peroxidase reaction. Microwave antigen retrieval was performed for 20 min in citrate buffer 0.01 M (pH 6.0). After washing with phosphate buffer saline (PBS), the slides were incubated for 60 min at room temperature with rabbit monoclonals; Anti-Bax antibody (E63, 1:250, abcam, UK) and 8-OHdG antibody (mouse anti-8-OHdG antibody, monoclonal 15A3; Santa Cruz Biotechnology, USA (sc-66036)). Binding site of primary antibodies was visualized by using the Dako EnVision™ kit (Dako, Copenhagen, Denmark). The peroxidase reaction was visualized by incubating the sections with diaminobenzidine (DAB) for 15 min.

The sections were counterstained with Mayer's hematoxylin. Specimens were considered positive for Bax when more than 20% of hepatocytes cells were positively stain (*Matsumoto et al., 2004*). Immunohistochemical examination for detection (8-OHdG) was performed according to the method described by Toyokuni (*Toyokuni et al., 1997*).

8-OHdG immunostaining was evaluated as four groups: group 1: negative (<5% of cells showing nuclear positivity); group 2: weak (5%–20% of cells showing nuclear positivity); group 3: moderate (21%–80% of cells showing nuclear positivity); and group 4: strong (>80% of cells showing nuclear positivity).

Statistical analysis

The data were statistically analyzed using SPSS (Kirkwood & Sterne, 2003). They were expressed as the mean (\pm SD) and analyzed by means of one-way analysis of variance (ANOVA). Statistical evaluation of data was done following Student's t-test in addition to Chi-square and post hoc Tukey's test for comparison between different values. A difference was considered significant at $P < 0.05$.

RESULTS

1- Histopathological results

Haematoxylin and Eosin

Group 1 (Control group)

Examination of liver sections revealed normal architecture of the liver. It consisted of cords of hepatocyte alternating with blood sinusoids radiating from the central vein. The blood sinusoids incompletely lined with flattened endothelial cells von Kupffer cells (Figure 1). Hepatocytes appeared polygonal with acidophilic granular cytoplasm and vesicular central, rounded nuclei. The portal tract contained branches of portal vein, and hepatic artery and bile ducts in a sleeve of connective tissue (Figure 2).

Group 2 (NAC-treated group)

No histological differences were noticed in examination of the liver sections of this group when compared with the control group.

Group 3 (TR-treated group)

Examination of liver sections of rats of TR -treated group revealed that the radial arrangement of the hepatic cords was disrupted and there was congestion of central vein with mononuclear peri-vascular infiltration. Most of the hepatocytes showed extensive cytoplasmic vacuolation indicated hydropic degeneration of hepatocytes (Figure 3). Also there were congested expanded portal veins with bile duct duplications (Figure 4). There was massive mono nuclear cellular infiltration around the portal vein and some hepatocytes exhibited pyknotic nuclei (Figure 5).

Group 4 (TR + NAC-treated group)

Histological examination of H&E-stained liver sections of rats of this group showed improvement of the normal architecture of hepatocytes around the dilated central vein. The blood sinusoids were

still dilated. Many hepatocytes showed acidophilic granular cytoplasm with vesicular nuclei (Figure 6). The portal vein was less expanded with mild mononuclear peri-vascular infiltration (Figure 7).

Mallory trichrome stain

The liver sections of both control group and NAC-treated group revealed thin layer of collagen fibers around the portal tract and the central vein. Few collagen fibers are seen radiating along the blood sinusoids (Figures 8,9).

Dense bundles of collagen fibers were detected in the portal area and extending between the adjoining hepatocytes in TR- treated group as expressed by intense blue staining of Mallory trichrome (Figure 10). The central vein of TR-treated animals showed congestion with slight increase in collagen fibers deposition (arrow) (Figure 11). There was dilatation of central vein with the collagen fibers extending in between the adjoining hepatic sinusoids (Figure 12).

The liver sections of TR + NAC-treated group revealed decreased amount of collagen fibers around the portal vein compared to tramadol-treated group (Figure 13)

Immunohistochemical results

BAX

Immunohistochemical staining of BAX in liver section from a control rat and NAC treated rat showed negative immunostaining (Figure 14), while immunohistochemical staining of BAX in TR treated rats showed strong positive brown staining indicating apoptosis in liver tissue in response to tramadol (Figure 15). The TR + NAC treated group showed attenuated expression of BAX with weak immunostaining (Figure 16). Significant differences < 0.001 were observed in the degrees of BAX immunostaining among the tested groups (Table 1).

In stereo logic estimation, Bax positive cell density of control group was the little than other groups ($p < 0.05$). Bax positive cells density was higher in TR-treated group than in other groups ($p < 0.05$).

8-OHdG

Immunohistochemical staining of 8-OHdG in liver section from the control rat and NAC -treated rats showed negative immunostaining (Figure 17), while immunohistochemical staining

of the 8-OHdG in TR-treated rats showed moderate to strong positive brown staining indicating increased oxidative DNA damage in response to tramadol (Figure 18) while TR + NAC- treated group showed attenuated expression of 8-OHdG with weak immunostaining (Figure 19). Significant differences <0.001 were observed in the degrees of 8-OHdG immunostaining among the tested groups (Table 2).

2-Biochemical results

Statistical comparison between control group and NAC- treated group as regard liver function tests and oxidative stress markers revealed no significant difference ($p > 0.05$) in the mean values of the liver function tests and oxidative stress markers.

The measured data of ALT, AST, ALP, Albumin, total bilirubin & direct bilirubin as regard liver function tests are summarized in (Table 3).

There was a significant increase in the mean values of serum ALT, AST, ALP, and total bilirubin, in TR-treated group as compared to the control group ($p < 0.05$). The TR and NAC-treated group revealed a significant decrease in the mean values of serum ALT, AST, ALP, total bilirubin, when compared with TR-treated group ($p < 0.05$). There was a significant decrease in the mean values of serum Albumin in TR-treated group as compared to the control group ($p < 0.05$), however in TR and NAC-treated group a significant increase in the mean values of serum albumin was observed when compared with TR-treated group.

Results indicated that MDA concentration showed non-significant differences between control group and NAC-treated group. On the other hand, a significant increase in the MDA level was observed in TR-treated group when compared to the control group. TR and NAC-treated group showed a significant decrease in the MDA level in comparison to the TR-treated group. On contrary, TR treatment significantly

($P < 0.05$) decreased the level of SOD in liver tissue. Also, TR treatment significantly decreased the level of GSH in the liver tissue ($P < 0.05$). In addition, TR-treated group showed significant decrease in the level of CAT activity ($P < 0.05$). In the liver tissue of TR and NAC-treated group there was significant increase in SOD, GSH and CAT level in liver tissue in comparison to the TR-treated group (Table 4).

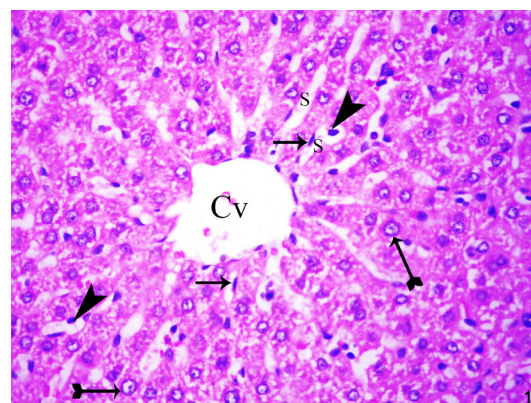


Fig. 1: A photomicrograph of section in rat liver of control group showing sheets of normal hepatocytes with acidophilic granular cytoplasm and vesicular nuclei (bifid arrows) radiating from central vein (Cv) and separated by blood-sinusoids (S) with von Kupffer cells (arrow head) and endothelial cells in their wall (arrow) (H & E X400)

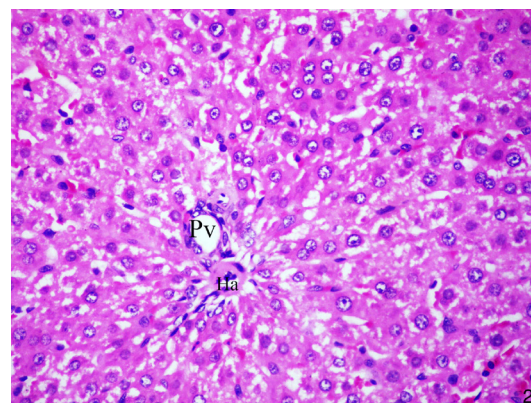


Fig. 2: A photomicrograph of section in rat liver of control group showing branch of portal vein (Pv) and branch of hepatic artery (Ha). Normal hepatocytes with acidophilic granular cytoplasm and vesicular nuclei (bifid arrows) are noticed. (H & E X400)

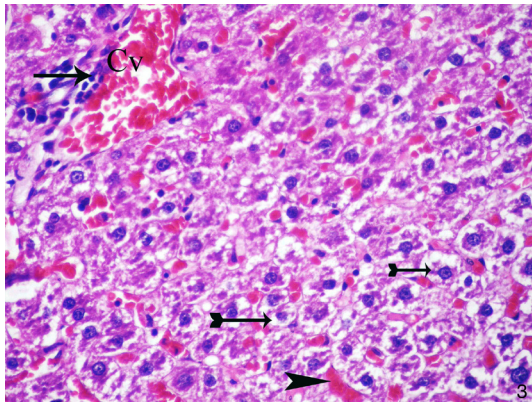


Fig. 3: A photomicrograph of section in rat liver of TR-treated group showing disturbed liver architecture around the congested central vein (Cv) with mononuclear peri-vascular infiltration (arrow) Most of hepatocytes have vacuolated cytoplasm (bifid arrows), with congested dilated blood sinusoid (arrow head) (H & E X 400)

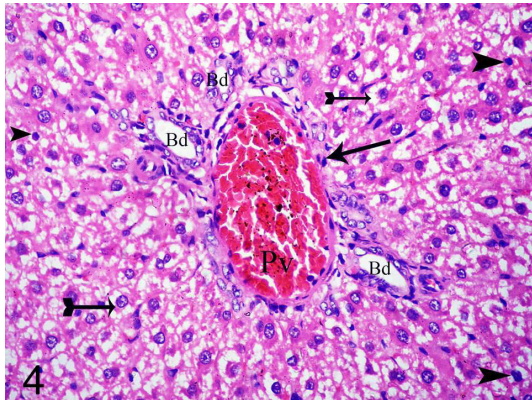


Fig. 4: A photomicrograph of sections in rat liver of TR-treated group showing congested expanded portal vein with mild mononuclear peri-vascular infiltration (arrow) around portal vein (Pv) & bile ducts (Bd) duplication. Vacuolation of cytoplasm is also seen (arrowed line). (H & E X 400)

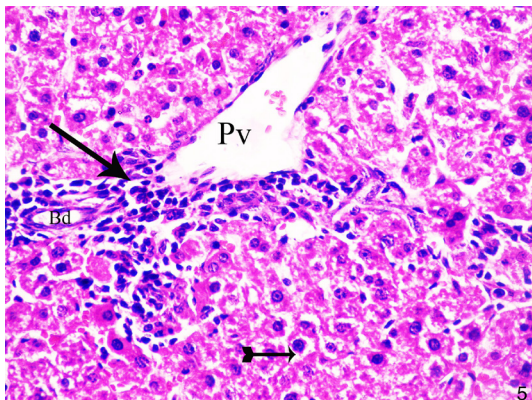


Fig. 5: A photomicrograph of sections in rat liver of TR-treated group showing expanded portal vein (Pv). Severe mononuclear peri-vascular infiltration (arrow). Some hepatocytes exhibited pyknotic nuclei. (H & E X400)

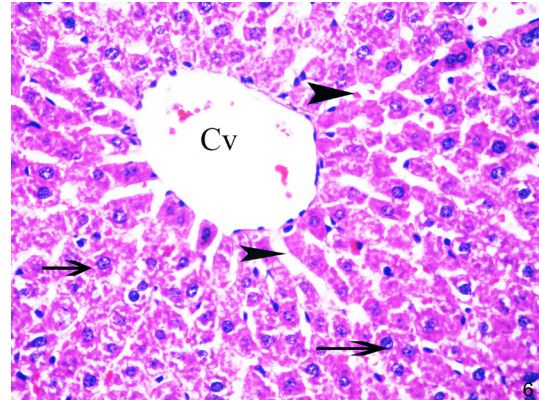


Fig. 6: A photomicrograph of sections in rat liver of TR + NAC-treated group showing hepatocytes with eosinophilic cytoplasm and vesicular nuclei (arrows) radiating from dilated central vein (CV). The blood sinusoids are still dilated to some extent with minimal congestion (arrows head) (H & E X400)

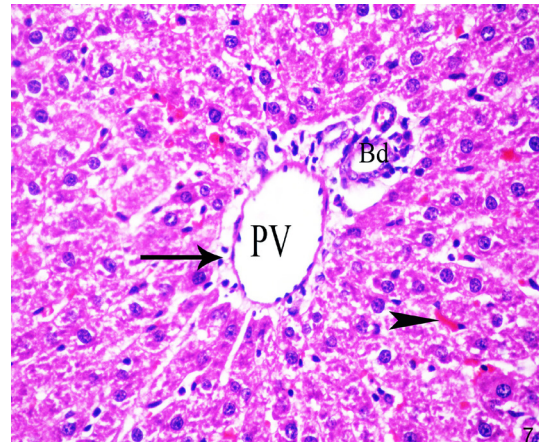


Fig. 7: A photomicrograph of sections in rat liver of TR + NAC-treated group showing less expanded portal vein (Pv) with minimal mononuclear peri-vascular infiltration (arrow) and Blood sinusoids are less dilated with minimal congestion (arrow head). Bile ducts are also seen (Bd) (H & E X 400)

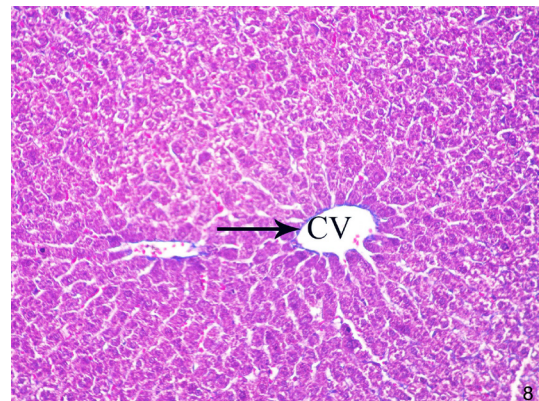


Fig. 8: A Photomicrograph of section in rat liver of control group showing normal pattern of collagen deposition (arrows) around the central vein (Cv) & the adjoining blood sinusoids (S). (Mallery trichrome X200)

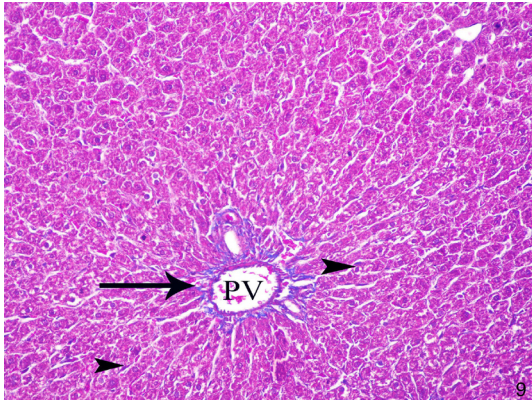


Fig. 9: A Photomicrograph of section in rat liver of NAC-treated group showing a normal pattern of collagen deposition (arrow) around the portal area (Pv) (Mallory trichrome X 200)

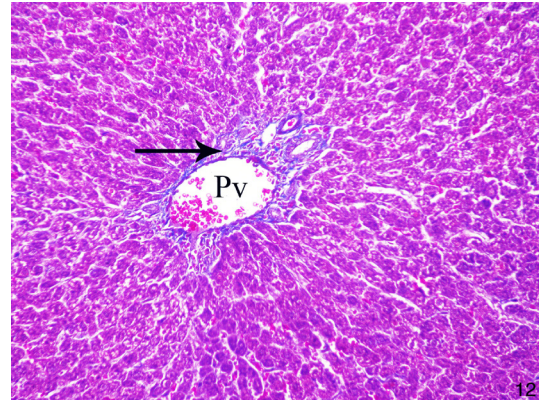


Fig. 12: A photomicrograph of section in rat liver of TR-treated group showing dilated central vein (Cv) with slight increase in collagen fibers deposition (arrow) (Mallory trichrome X200)

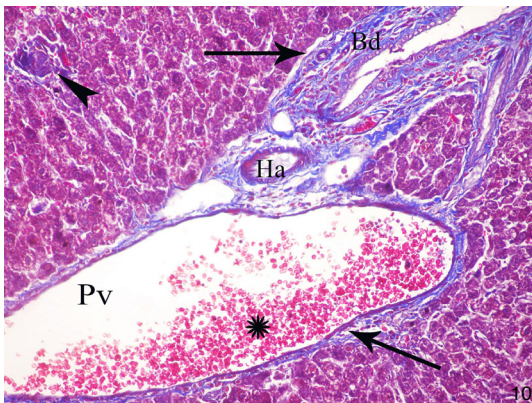


Fig. 10: A photomicrograph of section in rat liver of TR-treated group showing abundant collagen fibers (arrows) around the dilated congested portal vein (Pv), bile duct (Bd) and branch of hepatic artery (Ha) and extending between the hepatic sinusoids (arrows head) (Mallory trichrome X200)

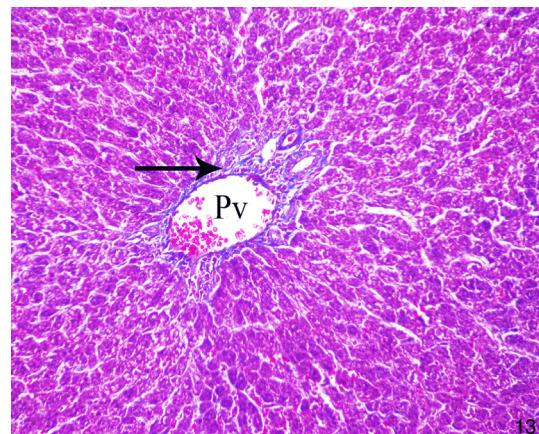


Fig. 13: A photomicrograph of section in rat liver of TR + NAC-treated group showing few collagen fibers (arrow) around the portal area, portal vein (Pv) (Mallory trichrome X 200)

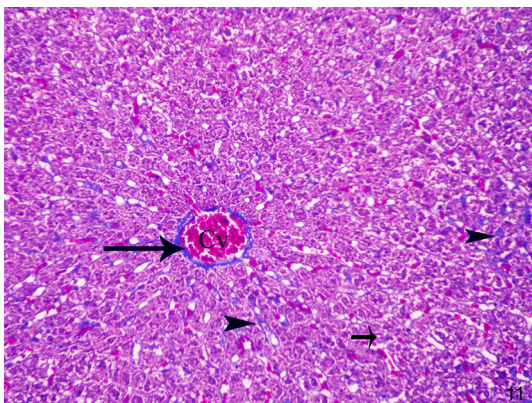


Fig. 11: A photomicrograph of section in rat liver of TR-treated group showing slight increase in collagen fibers deposition (arrow) around the congested central vein (Cv) in addition to collagen fibers extending in between the adjoining hepatic sinusoids (arrow head) (Mallory trichrome X200)

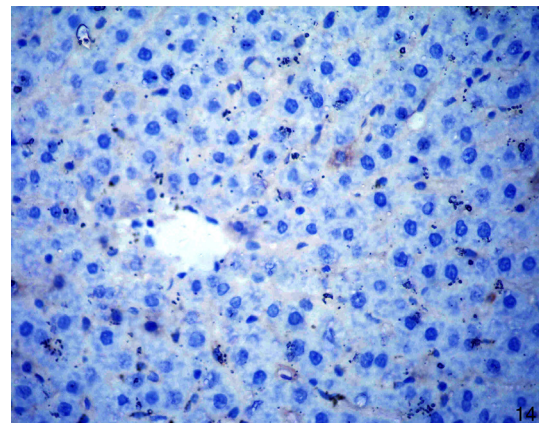


Fig. 14: A photomicrograph of a section in rat liver of control and NAC-treated groups showing negative Bax-reaction (Bax immunostaining \times 400)

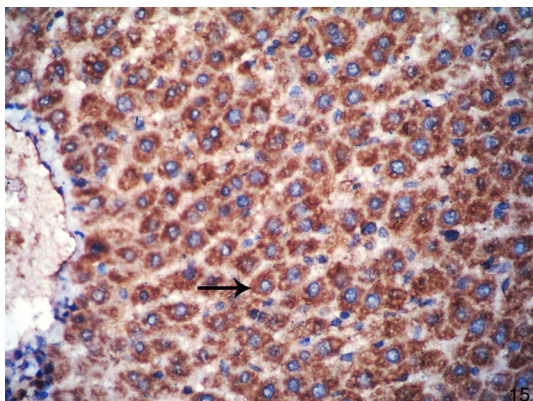


Fig. 15: A photomicrograph of a section in rat liver of TR-treated group showing intense Bax-positive reaction in the cytoplasm (arrow) (Bax immunostaining $\times 400$)

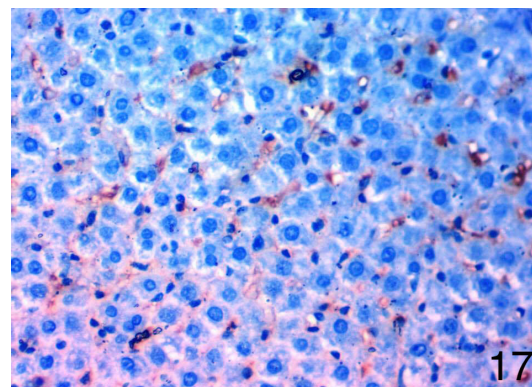


Fig. 17: A photomicrograph of a section in rat liver of control and NAC-treated groups showing negative 8-OHdG reaction (8-OHdG immunostaining $\times 400$)

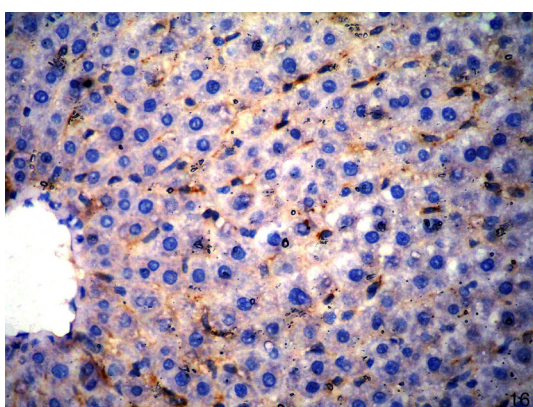


Fig. 16: A photomicrograph of a section in rat liver of TR + NAC-treated group showing mild Bax-positive reaction in the cytoplasm (Bax immunostaining $\times 400$)

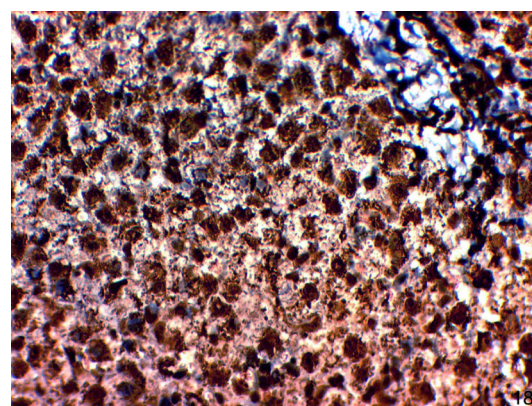


Fig. 18: A photomicrograph of a section in rat liver of TR-treated group showing intense 8-OHdG-positive reaction in the nuclei and cytoplasm (8-OHdG immunostaining $\times 400$)

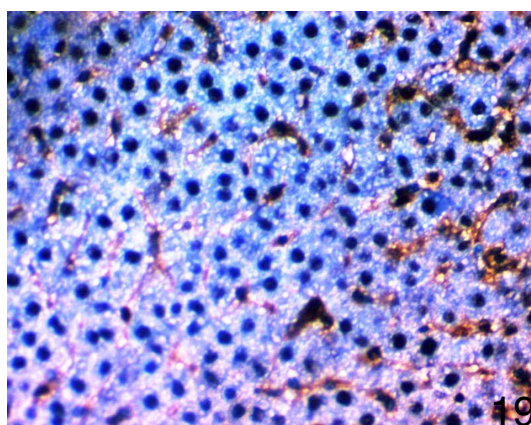


Fig. 19: A photomicrograph of a section in rat liver of TR + NAC-treated group showing mild 8-OHdG-positive reaction in nuclei. (8-OHdG immunostaining $\times 400$)

Table 1: Comparison between degrees of Immunohistochemical staining of Bax in the different studied groups by

| Variable | Control group (I) | | NAC group (II) | | TR group (III) | | TR + NAC group (IV) | | χ^2 | P |
|----------|-------------------|-------|----------------|-------|----------------|------|---------------------|------|----------|--------|
| | N | % | N | % | N. | % | N. | % | | |
| -ve | 10 | 100.0 | 10 | 100.0 | 1 | 10.0 | 7 | 70.0 | 33.553 | <0.001 |
| +ve | 0 | 0.0 | 0 | 0.0 | 9 | 90.0 | 3 | 30.0 | | |

In stereo logic estimation, Bax positive cell density of control group was the little than other groups ($p<0.05$). Bax positive cells density was higher in TR-treated group than in other groups ($p<0.05$).

Table 2: Comparisons between degrees of Immunohistochemical staining of 8- OHdG in the different studied groups

| Variable | Control group (I) | | NAC group (II) | | TR group (III) | | TR + NAC group (IV) | | χ^2 | P |
|----------------|-------------------|-------|----------------|-------|----------------|------|---------------------|------|----------|--------|
| | N | % | N | % | N. | % | N. | % | | |
| Negative | 10 | 100.0 | 10 | 100.0 | 1 | 10.0 | 4 | 40.0 | 42.452 | <0.001 |
| Weak stain | 0 | 0.0 | 0 | 0.0 | 1 | 10.0 | 3 | 30.0 | | |
| Moderate stain | 0 | 0.0 | 0 | 0.0 | 3 | 30.0 | 2 | 20.0 | | |
| Strong stain | 0 | 0.0 | 0 | 0.0 | 5 | 50.0 | 1 | 10.0 | | |

Table 3: Comparison among control, NAC- treated group, TR-treated group ,and TR + NAC -treated group as regard the mean values of serum ALT (IU/L), AST (IU/L), albumin (mg/dl), total bilirubin (mg/dl,) and direct Bilirubin (mg/dl) using ANOVA test.

| Biochemicals | Control group (I) | NAC group (II) | TR group (III) | TR + NAC group (IV) | P | LSD |
|--------------------------|-------------------|----------------|----------------|---------------------|-------|--|
| Catalase (U/L) | 984.66±49.98 | 977.39±39.76 | 870.12±99.95 | 934.67±61.99 | <0.05 | 0.764 ^a <0.05 ^{b,c,d,e,f} |
| ALT (U/L) | 38.69±4.2 | 35.99±3.45 | 147.55.4±9.45 | 84.46±9.08 | <0.05 | 0.678 ^a <0.05 ^{b,c,d,e,f} |
| AST (U/L) | 118.34±5.34 | 121.87±6.32 | 244.34±6.39 | 163.86±9.34 | <0.05 | 0.835 ^a <0.05 ^{b,c,d,e,f} |
| ALP (U/L) | 88.64±25.87 | 90.33±23.45 | 488.65±39.45 | 302.98±41.62 | <0.05 | 0.807 ^a <0.05 ^{b,c,d,e,f} |
| Albumin (mg/dl) | 502.34±51.34 | 497.75±48.42 | 357.99±44.21 | 404.77±51.43 | <0.05 | 0.498 ^a <0.05 ^{b,c,d,e,f} |
| Total Bilirubin (mg/dl) | 0.25±0.02 | 0.24±0.01 | 0.76±0.05 | 0.49±0.04 | <0.05 | 0.747 ^a <0.05 ^{b,c,d,e,f} |
| Direct Bilirubin (mg/dl) | 0.15±0.01 | 0.15±0.02 | 0.20±0.002 | 0.18±0.01 | <0.05 | 0.738 ^a <0.05 ^{b,c,d,e,f} |

Values are expressed as mean ± standard deviation (SD) of n = 10 animals/group a Group I versus Group II, b Group I versus Group III, c Group I versus Group IV, d Group II versus III, e Group II versus IV, f Group III versus Group IV.

Table 4: Comparison of the mean values of oxidative stress markers: MDA (nmol/g tissue), SOD (U/g), CAT (U/g) and GSH (mmol/g) of the control, NAC, TR and TR &NAC groups

| Biochemicals | Control group (I) | NAC group (II) | TR group (III) | TR + NAC group (IV) | P | LSD |
|----------------------|-------------------|----------------|----------------|---------------------|-------|--|
| MDA (nmol/g. tissue) | 49.03±11.21 | 43.13±8.09 | 74.13±18.54 | 51.75±14.46 | <0.05 | 0.745 ^a <0.05 ^{b,c,d,e,f} |
| CAT (U/g. tissue) | 32.56±9.79 | 30.04±8.52 | 17.82±6.76 | 23.43±7.09 | <0.05 | 0.488 ^a <0.05 ^{b,c,d,e,f} |
| SOD (U/g. tissue) | 10.47±4.33 | 9.33±3.87 | 6.34±2.13 | 7.64±3.01 | <0.05 | 0.769 ^a <0.05 ^{b,c,d,e,f} |
| GSH (mmol/g. tissue) | 4.42±1.32 | 4.40±1.42 | 3.25±1.03 | 3.91±1.07 | <0.05 | 0.575 ^a <0.05 ^{b,c,d,e,f} |

Values are expressed as mean ± standard deviation (SD) of n = 10 animals/group a Group I versus Group II, b Group I versus Group III, c Group I versus Group IV, d Group II versus III, e Group II versus IV, f Group III versus Group IV.

DISCUSSION

The histopathological results of this study demonstrated that, TR administration in rats causes liver damage in the form of disturbed liver architecture, congested dilated central veins, hydropic degeneration of hepatocytes, congested dilated sinusoids, necrotic signs in some hepatocytes (apoptotic nuclei) compared with control group.

In addition to portal changes which included marked peri-vascular infiltration, bile duct proliferation together with hyperplasia and fibrosis of the blood vessel wall. These findings are in parallel with Awadalla and Salah-Eldin (2015) who reported that the toxic effect of TR on the liver in the form of hydropic degeneration, with congested central veins and necrotic signs in some hepatocytes. Similar results were demonstrated by the studies of Atici, *et al.*, (2004) who found that TR treatment induced central vein dilatation, necrosis and vacuolization in the hepatocytes. Their findings observed only in peri-venular areas (centro-loboular zone).

This was in contrast with Tolman, (1998) who stated that the metabolites produced as a result of tramadol metabolism had little pharmacological activity and can be easily removed from the body.

In the present work, Mallory trichrome stained sections revealed increasing in collagen fibers content in the portal area and extending between the hepatocytes compared with the control group. This result is in agreement with Elkhateeb *et al.*, (2015) that revealed increased collagen fibers in Masson's trichrome-stained sections of the liver and kidneys of TR-treated rats. Altindage *et al.*, (2007) also suggested that the increase in collagen fibers is due to decrease in collagen metabolism that may be attributed to oxidative stress. Surazynski *et al.*, (2008), stated that collagen is not only a structural component of extracellular matrix, but it has also been recognized as a ligand for integrin receptors, which play an important role in signaling that regulate ion transport, lipid metabolism, kinase activation and gene expression

In contrast co-administration of NAC along with TR in the present study revealed that treatment with NAC reduces the damage caused by TR administration. This is clearly appearing in the form of mild dilatation of blood sinusoids and cellular infiltration apparently decreased in the portal area. Some hepatocytes regained their acidophilic cytoplasm with minimal fibrosis in portal area and around the central veins.

Similar results were demonstrated by the studies of Kamalakkannan *et al.*, (2005) who supported the improvement in the liver by the effect of the NAC which used as antioxidant. In the current study, there was highly significant increase in the serum ALT, AST, ALP and total bilirubin in the TR-treated group when compared with control group. These findings of the current study are in agreement with those of El-gaafarawi *et al.*, (2013) who recorded a significant increase in the ALT and AST activities in rats after administration of 40 mg/kg and 80 mg/kg TR than control group.

Yang, *et al.* (2009) also reported that there was an increase in the level of ALT indicating the malfunctioning and damage of liver tissues due to repeated TR use. Increase in the AST level can occur in connection with damages of the heart or skeletal muscle as well as of liver parenchyma; however, liver-specific enzyme ALT has been shown to be only significantly elevated in hepatobiliary disease (Vozarova *et al.*, 2002). Different results were observed by (Mona, 2016) who stated that tramadol can decrease liver enzymes such as AST and ALT in the case of hepatic ischemic/reperfusion injuries in rats suggesting that TR can reduce both inflammation and oxidative stress in such studied cases. In the current study co-administration of NAC along with TR reduced the level of the serum ALT, AST, ALP and total bilirubin and increased the level of serum albumin, this agrees with (Kamalakkannan *et al.*, 2005) who reported that administration of NAC reduced the level of these enzymes in CCl₄ treated rats. This could be explained that NAC can prevent liver damage by suppressing the leakage of enzymes through cellular membranes. This preserves the integrity of the plasma membranes and thus restores these enzymes levels.

Lipid peroxidation has been used as an indirect marker of oxidant-induced cell injury. MDA appears to be the most mutagenic product of lipid peroxidation and it is considered a useful measure of oxidative stress status (Pan, *et al.*, 2008).

Oxidative stress was not only by augmenting lipid peroxidation but also by inhibiting the antioxidant enzymes activities (Bekheet *et al.*, 2011). The current study recorded that TR treatment induced oxidative stress, expressed by highly significance increase in MDA and highly significant decrease in GSH, SOD and CAT enzymes levels in liver tissue compared with the control group. Similar findings were obtained by

Awadalla and Salah-Eldin (2015) who reported that TR treatment lead to an increase in MDA level, while there is a decrease in the activities of GSH, SOD and CAT enzymes in both liver and kidney tissues. Recent studies have demonstrated that ROS and the resulting oxidative stress play a pivotal role in apoptosis Kannan (2000). However, there are conflicting results in the literature concerning the effects of opioids on apoptosis. *In vitro* studies using specific cell lines showed that opioids might induce or enhance apoptosis Singhal (1998).

Moradi *et al.*, (2014) reported that, under the oxidative stress situation, CAT, GSH and SOD activity significantly decreased in opium-addicted hamsters compared to controls. The co-administration of NAC along with TR restored the changes brought by TR. These findings are in agreement with Wong *et al.*, (2003) who reported that the ability of NAC in regulating GSH concentration and thus protect liver damage from reactive metabolites formed fromCC14. Also Yalcin *et al.* (2008) reported that NAC is known to increase the intracellular stores of glutathione thereby enhancing the endogenous antioxidant level.

Alturfan, *et al.*, (2012) Suggested that the protective effect of NAC on liver tissue is due to its ability to increase glutathione production by providing more substrate for reactive intermediates that promote detoxification mechanisms. This also may be the reason for the restoration of other antioxidant enzymes such as SOD and CAT.

The imbalance between ROS and antioxidant defenses results in oxidative stress (Turrens, 2003). Many studies demonstrated that TR and its active metabolites may produce excessive release of ROS leading to single or double strand DNA breaks. The 8-OHdG acts as an established biomarker of oxidative stress as it is produced by the oxidative damage of DNA by reactive oxygen and nitrogen species. In this work, there was a significant increase in the expression of 8-OHdG in the TR- treated group in comparison to decreased expression in TR and NAC- treated group. This indicates increased oxidative DNA damage in response to TR and also highlights the protective role of NAC. In the current study, there was a significant increase in the expression of BAX in the TR- treated group in comparison to decreased expression of BAX in TR and NAC-treated group. This accelerated the process of apoptosis. Our data coincide with the results of

khourdy *et al*; (2010) who reported increase the Bax expression besides decrease Bcl-2 expression in rats treated with high dose of TR rather than those treated with low dose of TR indicating dose dependent effect of tramadol.

Awadalla and Salah-Eldin (2015) reported that upregulation of Bax besides down-regulation of Bcl-2 in rat's liver and kidney treated with TR suggested that TR-induced damage in a manner of activation of apoptotic cell death pathway in these tissues.

CONCLUSION

It was concluded that TR is suggested to have hepatotoxic effect in adult male albino rats moreover it supports possible protective role of NAC in TR- induced hepatotoxicity. This might be due to its ability to stabilize cell membranes, scavenge free radicals and antioxidant properties.

ABBREVIATIONS

TR: Tramadol; **NAC:** N-acetylcysteine; **CAT:** Catalase; **GSH:** Glutathione; **SOD:** Superoxide dismutase; **MDA:** Malondialdehyde; **ALT:** Alanine aminotransferase; **AST:** aspartate aminotransferase; **(ALP):** Serum alkaline phosphatase; **Hx&E:** Hematoxyline and Eosin, **8-OHDG:** 8- hydroxyl-2-deoxy guanosine.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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مخاطر عقار الترامادول على تركيب الكبد في ذكور الجرذان البيضاء البالغين والدور الوقائي ل N أستيتيل سيستن : دراسة نسيجية ومناعية

مروة محمود أحمد^١، أميرة ابراهيم السمح^١، هيام راشد^٢ وداليا الشافعي^٣
^١ قسم التشريح والأجنة، ^٢ قسم الباثولوجيا، ^٣ قسم طب المجتمع، كلية الطب – جامعة الزقازيق

ملخص البحث

المقدمة: ان تناول الترامادول يرتبط مع الاحتقان الكبدي، موت الخلايا المبرمج ويحفز الأوكسدة المضرة في الأنسجة. وتتفاعل مجموعة ثيول في N أسيتيل سيستن مباشرة مع أنواع الأوكسجين التفاعلية مما يؤدي إلى حماية الخلايا من الضرر الناجم من مثل هذه الأوكسدة.

الهدف من الدراسة: هذه الدراسة تهدف إلى تسليط الضوء على مخاطر الترامادول على بنية الكبد في ذكور الجرذان البيضاء البالغة وكذلك وظائف الكبد والتي تتمثل في إنزيمات الكبد ومضادات الأوكسدة وفعالية N أسيتيل سيستن في حماية الكبد.

المواد والطرق المستخدمة: أجريت هذه الدراسة على أربعين من الجرذان البيضاء البالغة مقسمة بالتساوي على أربع مجموعات .

-المجموعة الضابطة: تلقت ٢ مل محلول ملحي (المذيب لكلا العقارين الترامادول و N أسيتيل سيستن) وتم اعتبارها كمجموعة ضابطة

-مجموعة N أسيتيل سيستن: تلقت ١٥٠ مج / كغم /يوميًا من N أسيتيل سيستن المذاب في محلول ملحي عن طريق الفم لمدة ٣٠ يوم

-مجموعة الترامادول: تم معالجتها بالترامادول عن طريق الفم ٤٠ مج /كغم يوميًا

-مجموعة الترامادول مع N أسيتيل سيستن: والتي تم معالجتها بجرعة مماثلة من الترامادول بالتزامن مع N أسيتيل سيستن بنفس الجرعة وهي ١٥٠ مج/كغم/يوميًا لمدة ٣٠ يومًا. تم بعدها إزالة الكبد ومعالجته لفحصها بالمجهر الضوئي وفحص وظائف الكبد.

النتائج: أظهر الفحص لمجموعة الترامادول عن تعطل خلايا الكبد جنبًا إلى جنب مع احتقان الوريد المركزي إلى جانب تسلل خلوي حول الأوعية. وأظهرت معظم خلايا الكبد نخر في أنسجة الكبد و تلف مبرمج للخلايا مع ازدواج القناة الصفراوية. كما لوحظ وجود زيادة في الاليف الغروية منتشرة حول الوريد البابي إلى جانب زيادة كبيرة في عدد من باكس و ٨-أوهديغوسيتيف في الخلايا. أما فيما يتعلق بوظائف الكبد، فقد كان هناك ارتفاع كبير في مستويات انزيمات الكبد المختلفة والبيليبروبين وانخفاض مستوى الألبومين. أما بالنسبة للإنزيمات المضادة للأوكسدة فقد كانت هناك زيادة في مالونديالدهيد وانخفاض في ديسموتاز، الجلوتاثيون والكاتالاز في أنسجة الكبد. على النقيض من مجموعة الترامادول و N أسيتيل سيستن حيث استعاد الكبد النسيج الخلوي الطبيعي تقريبا مقارنة بالمجموعة الضابطة و كذلك الحد الأدنى من ألياف الكولاجين، وانخفاض كبير في عدد من باكس والخلايا إيجابية ٨-أوهديغ. ووظائف الكبد ومستوى الإنزيمات المضادة للأوكسدة استعادت تقريبا قيمها العادية.

الخلاصة: أتضح من نتائج الدراسة ان الترامادول له تأثير ضار على تركيب كبد الجرذان البيضاء البالغة وأن N أسيتيل سيستن له دور وقائي ضد هذا الضرر الناجم عن تناول الترامادول.