

THE POSSIBLE PROTECTIVE ROLE OF N-ACETYLCYSTEINE AGAINST TRAMADOL-INDUCED NEPHROTOXICITY IN THE ADULT ALBINO RATS (LIGHT AND ELECTRON MICROSCOPIC STUDY)

By

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ABSTRACT

Background: Tramadol is nephrotoxic in over doses N-acetylcysteine is effective against inflammatory processes and oxidative stress.

Objective: Study of tramadol- induced nephrotoxicity on the rats and assess the possible protection of N-acetylcysteine.

Material and Methods: Forty adult albino rats were obtained from the breeding animal house of Faculty of Pharmacy, Al-Azhar University. The rats were housed in the animal house at Faculty of Pharmacy, Al-Azhar University under the suitable and standard conditions of temperature and feeding from January, 2021 till February, 2021. The rats were divided into four groups **Group I (control group):** the rats of this group was given 30 ml /kg saline daily for thirty days orally; **group II (treated with tramadol):** the rats of this group were given 15.5 mg /kg tramadol orally daily for thirty days; **Group III (Toxic dose tramadol):** The rats of this group were given 30 mg /kg tramadol orally daily for thirty days; **Group IV (treated with toxic dose of tramadol and N-acetyl cysteine):** The rats of this group were given 30 mg /kg tramadol and N-acetylcysteine 100 mg/kg orally daily for thirty days. The rats at the last day of the experiment were killed and their kidneys were collected for histological evaluation using hematoxylin and eosin and Masson trichrome, and examined under light and transmission electron microscope. Also, Blood samples were taken for measurement of urea and creatinine. The data obtained from biochemical results for all groups were expressed as means and subjected to statistical analysis.

Results: Treatment of rats with toxic dose of tramadol (group III) showed marked damage in the renal tissues, The damage in the renal tissues was improved in rats that were given N- acetylcysteine together with tramadol (group IV). However, tramadol that was given in a therapeutic dose (Group II) did not cause any damage in the kidney.

Conclusion: N-acetylcysteine partially improves the tramadol –induced nephrotoxicity.

Keywords: Tramadol; N-acetyl cysteine; Oxidative stress and nephrotoxicity.

INTRODUCTION

Tramadol is considered a centrally acting analgesic. It is wide spreadly used throughout the world. It has opioid, noradrenergic and serotonergic properties. Various data suggest that, in addition to its analgesic effect, tramadol may have also antidepressant and anxiolytic-like effects (*Berrocoso et al., 2011*).

Tramadol produces its analgesic property via the inhibition of reuptakes of both serotonin and nor-epinephrine (*Reeves and Burke, 2011*).

The liver is the main site of the metabolism of tramadol. The products of the metabolism are excreted via the kidney, so the kidney is the main target for the toxicity of tramadol. Biotransformation of tramadol occurs in the liver, firstly by demethylation of tramadol and then conjugation of the demethylated compounds occur resulting in about ten metabolites (*Dickman, 2012*).

Repeated tramadol administration might lead to accumulation of toxic metabolites, increase the risk for pharmacokinetics interactions and/or decrease the clearance of tramadol, thus increasing its potential for toxicity (*Senay et al., 2011*).

Oxidative stress is considered the major damaging process resulting from the imbalance between the reactive oxygen species (which have been produced in an excess manner) and decreased antioxidant defences (*Turrens, 2013*).

These reactive oxygen species (such as H_2O_2 , O_2^- and CO_3^-), which produced in an excess manner, cause much damage

in DNA and also produce harmful effects in cell membranes via lipid peroxidation (*Droge, 2012*).

N-acetyl cysteine (NAC) is an n-acetylated derivative of the naturally occurring amino acid cysteine (*Gass and Olive, 2013*). It is a synthetic precursor of glutathione (GSH), which can stimulate the intracellular synthesis of GSH and enhances glutathione S-transferase (GST) activity (*Tylicki et al., 2015*). It is highly effective in the intracellular neutralization of free radicles (*Aruoma et al., 2015; Harrison et al., 2010*).

AIM OF THE WORK

The aim of this work was to demonstrate the various toxic effects by tramadol abuse on the renal cortex and also to study if N- acetyl cysteine has a role in improvement of these dangerous toxic effects and protect the kidney from nephrotoxicity.

MATERIAL AND METHODS

I. Material:

Animals:

The present study was performed on forty adult albino rats of 4-6 months old. The rats weighed 200 - 250 gm each in average. They were obtained from the breeding animal house of Faculty of Pharmacy, Al-Azhar University. Under the suitable and standard conditions of temperature and feeding, the rats were housed in the animal house at Faculty of Pharmacy, Al-Azhar University from 15th Jan., 2021 till 14th Feb., 2021.

Drugs:

"Tamol -X" is the commercial form of tramadol hydrochloride, containing 225

mg of tramadol hydrochloride and obtained from the Royal National Pharmaceutical Company. Tramadol was used at a dose of 15.5 mg /kg body weight orally by intra gastric tube every day for one month in the therapeutic dose group (Group II) (Subedi et al., 2018; Nair & Jacob, 2016) and in a dose of 30 mg/kg body weight orally by intra gastric tube every day for one month in the toxic dose group (Group III) (Matthiesen et al., 2010). N-acetyl cysteine 100 mg/ 5 ml was obtained in the form of "Solvimyst" which is the commercially used syrup of N- acetyl cysteine and obtained from Menapharm Company for Pharmaceutical and Chemical Industries. N- acetyl cysteine was used at a dose of 100 mg/kg body weight orally by intra gastric tube every day for one month (Adikwu & Bokolo, 2017; Borgström et al., 2014).

Experimental design:

The rats were divided into four groups equally as follows:

Group (I) (Control group): A normal saline in a dose of 30 ml /kg body weight was given to the rats of this group every day for thirty days orally via the intra gastric tube.

Group (II) (Tramadol - treated group) (Therapeutic dose group): Tramadol hydrochloride was given to the rats of this group in a dose of 15.5 mg /kg body weight and given orally via the intra gastric tube every day for thirty days (Subedi et al., 2018; Nair & Jacob, 2016).

Group (III) (Tramadol - treated group) (Overdose group): The rats of this group were given also tramadol hydrochloride but in a higher dose than that in group II.

The dose was 30 mg /kg body weight which was given orally via the intra gastric tube daily for thirty days (Matthiesen et al., 2010).

Group (IV) (Tramadol - N-acetyl cysteine treated group): The rats of this group were given tramadol Hydrochloride in a dose of 30 mg/kg body weight and N-acetyl cysteine in a dose of 100 mg/kg body weight orally via the intra gastric tube every day for thirty days (Adikwu & Bokolo, 2017; Borgström et al., 2014). At the end of the experiment, inhalation of ether was used to anesthetize the rats, then the rats were sacrificed and the kidneys were extracted carefully.

II. Methods:

A. Light and Electron microscope examination:

For examination by the light microscope, samples from the kidneys were taken from each kidney and cut into small pieces (about 2-4 mm³), put in 10 % formaldehyde (for 24 hours) for fixation and then proceeded to be embedded in paraffin wax, sectioned at 5 microns thin sections and were stained with hematoxylin and eosin (H& E) and Masson's trichrome (MT) stains and then examined under light microscope.

For examination by the electron microscope, small pieces (about 1mm³) were obtained from the cortices of each kidney, put in 2.5% glutaraldehyde for fixation (for 45 min), and then prepared for embedding in epon resin. The ultrathin sections of (50 nm) thickness was obtained and mounted on grids of copper and stained with lead citrate and uranyl acetate. The examination of the sections was carried out by using a transmission

electron microscope. Photomicrographs were taken to detect the ultra-structure of the kidneys.

B. Biochemical analysis: Blood samples were taken for measurement of urea and creatinine.

C. Statistical analysis: The data obtained from biochemical results for all groups were expressed as means and subjected to statistical analysis using one-way analysis of variance (ANOVA) for comparison between the different groups.

RESULTS

A. Light and electron microscopic results:

Light microscopic findings:

1. Concerning the control (group I) & tramadol (therapeutic dose) treated group (group II): Normal general architecture of the renal cortex was obvious in the sections stained with haematoxylin and eosin and manifested as follows **Figures (1A & 1B)**:

- a. The renal corpuscles appeared with normal glomerular and interstitial capillaries, Visceral and parietal layers of Bowman's capsule were seen separated by average Bowman's space.
- b. Proximal and distal convoluted tubules were also observed in their normal pattern.

Sections stained with Masson's trichrome stain revealed few collagen fibers with normal distribution among the glomerular capillaries and in the interstitium **Figures (2A & 2B)**.

2. Concerning the tramadol treated group (over dose group, group III), haematoxylin and eosin-stained sections revealed the following findings **Figure (1C)**:

- a. Various degrees of renal cortex affection. Focal areas were observed containing renal corpuscles with congested glomerular capillaries. Most renal corpuscles appeared with shrunken

glomeruli and widened Bowman's space.

b) Proximal tubules had apoptotic epithelial lining. Intra luminal hyaline casts were also seen.

C. Distal tubules were seen with irregular outline and widened lumen.

Sections stained with Masson's trichrome stain showed increased collagen fibers among the glomerular capillaries and in the interstitium as compared to the control group **Figure (2C)**.

3. On the other hand, sections from tramadol and N- acetyl cysteine treated group (group IV) stained with haematoxylin and eosin showed decrease in the changes seen in group III denoting incomplete recovery. The glomeruli appeared with normal size. The proximal tubules appeared with normal epithelial lining and partial loss of brush borders. Most of distal tubules appeared normal. Mildly congested interstitial blood vessels were also seen **Figure (1D)**.

Sections stained with Masson's trichrome stain showed incomplete recovery in the form of focal areas with moderate amount of collagen fibers in the interstitium **Figure (2D)**.

Electron microscopic findings:

1. Ultra structurally, sections from the renal cortex of adult male albino rats of: 1. The control group (group I) and tramadol treated (therapeutic dose) group (group II) showed normal

picture in the form of: Normal glomerular blood capillaries with average endothelial cells and regular basement membrane. The podocytes appeared with thin and separated foot processes and patent filtration slits **Figures (3A&4A)**. The proximal tubular cells were seen with rounded euchromatic nucleus and closely-packed microvilli. Numerous mitochondria and average basal lamina were also seen Figs. (3B&4B). The distal tubular cells revealed with few short microvilli and rounded centrally located nuclei with central nucleoli. Numerous mitochondria were also seen scattered in the cytoplasm **Figures (3C&4C)**.

2. Tramadol (over dose) treated group (group III) showed dilated glomerular capillaries. The glomerular filtration barrier appeared with irregular and thick glomerular basement membrane. The foot processes of the podocyte

appeared fused and thick with narrow filtration slits **Figure (5A)**. The proximal convoluted tubular cells contained pyknotic nuclei with clumped chromatin in the periphery and intra-nuclear inclusion bodies. Numerous swollen mitochondria, irregular and destructed basal lamina and multiple cytoplasmic vacuoles were also observed **Figure (5B)**. The distal convoluted tubular cells appeared with rounded centrally located nuclei with clumped chromatin in the periphery. Numerous mitochondria were seen within irregular basal infoldings. Irregular basal lamina and cytoplasmic edema were also observed **Figure (5C)**.

3. Tramadol and N-acetyl cysteine treated group (group IV) showed slightly decrease in the changes noted in the tramadol (over dose) treated group (group III) denoting partial recovery **Figure (6)**.

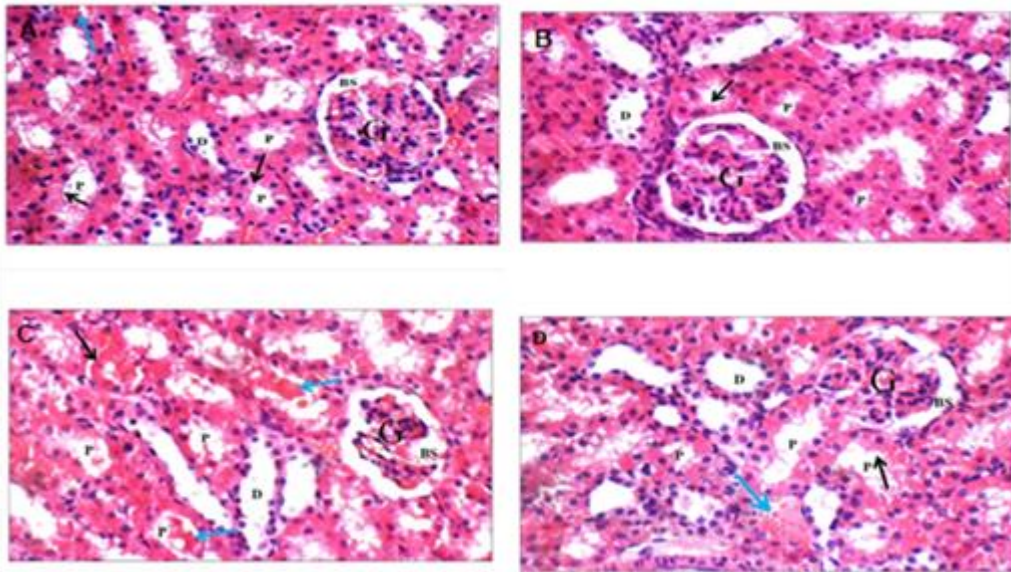


Figure (1): A photomicrograph of hematoxylin and eosin sections of the kidney showing:

A) Average glomeruli (G) with average Bowman's spaces (BS), average proximal tubules (P) with preserved brush borders (black arrows), average distal tubules (D) and average interstitial blood vessels (blue arrow) in group I. **B)** Average glomerulus (G) with average Bowman's space (BS), average proximal tubules (P) with preserved brush borders (black arrows), and average distal tubules (D) in group II. **C)** Small-sized congested glomerulus (G) with widened and irregular Bowman's space (BS), distal tubules (D) with irregular outline and widened lumen and wide proximal tubules (P) with apoptotic epithelial lining containing pyknotic nuclei (black arrow) and intra-tubular casts (blue arrow) in group III. **D)** Average glomerulus (G) with average Bowman's space (BS), average distal tubules (D), proximal tubules (P) with partial loss of brush borders (black arrow) and mildly congested interstitial blood vessels (blue arrow) in group IV (H &E X 400).

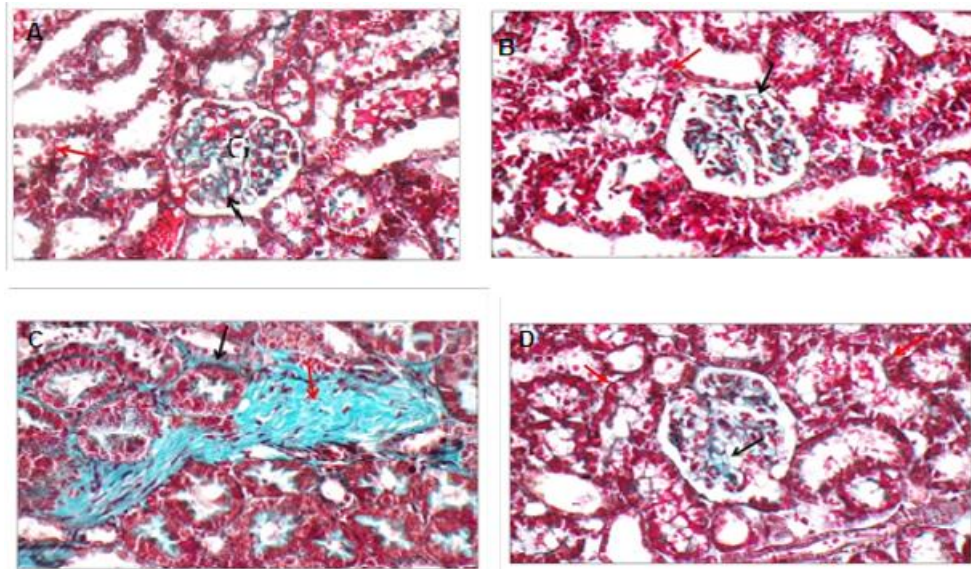


Figure (2): A Photomicrograph of Masson's trichrome sections of the kidney showing: **A)** Average collagen distribution in glomeruli (G) (black arrow) and around tubules (red arrow) in group I. **B)** Average collagen distribution in glomeruli (black arrow) and around tubules (red arrow) in group II. **C)** An excess amount of collagen around tubules (black arrow) and thick fibrous bands (red arrow) in group III. **D)** Average collagen distribution in the glomerulus (black arrow) and around tubules (red arrows) in group IV (Masson's trichrome X 400).

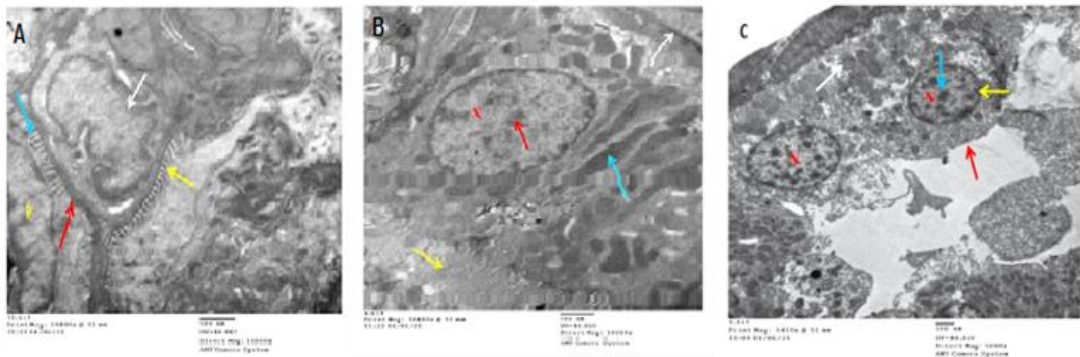


Figure (3): An electron photomicrograph of the renal cortex of adult albino rats of the group I showing: **A)** A part of a glomerulus showing regular basement membrane (red arrow), average podocytes (P) with average foot processes (blue arrow) and patent filtration slits (yellow arrow) and average endothelial cells (white arrow) (TEM x10000). **B)** A part of a proximal convoluted tubule showing cells with basally located euchromatic nucleus (N) and prominent nucleolus (red arrow), numerous mitochondria (blue arrow), dense long microvilli (yellow arrow) and average basal lamina (white arrow). (TEM x 10000). **C)** A part of a distal convoluted tubule showing cells with few short microvilli (red arrow), rounded centrally located nuclei (N) with central nucleoli (blue arrow) and condensed chromatin in the periphery (yellow arrow) and numerous mitochondria (white arrow) (TEM x 5000).

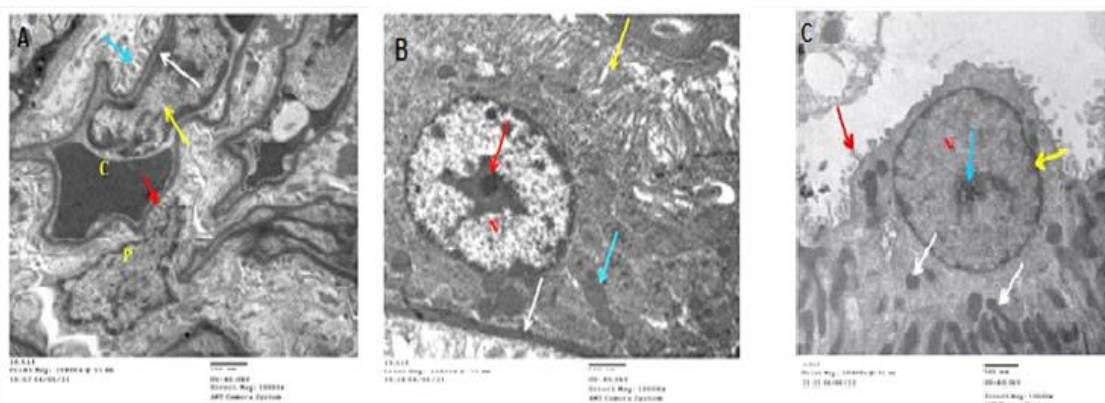


Figure (4): An electron photomicrograph of the renal cortex of adult albino rats of the group II showing: **A)** A part of a glomerulus showing average podocytes (P) with average foot processes (red arrow) and patent filtration slits (blue arrow), regular basement membrane (white arrow) and average capillaries (C) with average endothelial cells (yellow arrow). **B)** A part of a proximal convoluted tubule showing cells with basally located euchromatic nucleus (N) with prominent nucleolus (red arrow), numerous mitochondria (blue arrow), dense long microvilli (brush border) (yellow arrow) and average basal lamina (white arrow). **C)** A part of a distal convoluted tubule showing cells with few short microvilli (red arrow), rounded centrally located nucleus (N) with central nucleolus (blue arrow) and condensed chromatin in the periphery (yellow arrow) and numerous mitochondria (white arrows) (TEM x 10000).

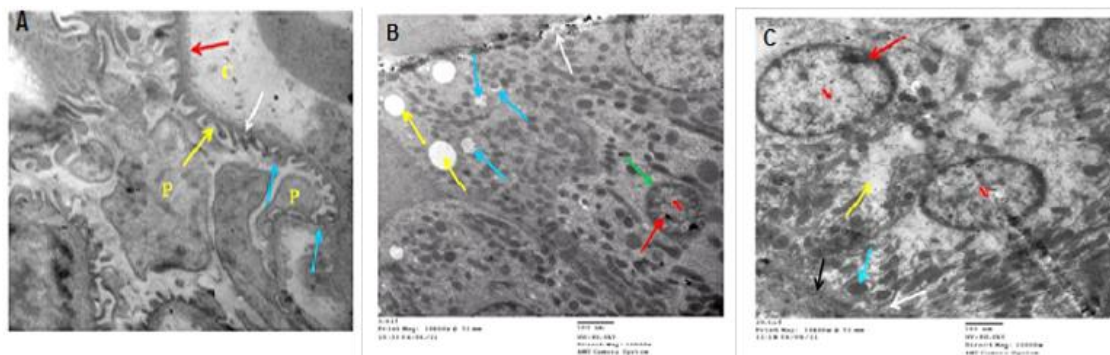


Figure (5): An electron photomicrograph of the renal cortex of adult albino rats of the group III showing: **A)** A part of a glomerulus showing thick irregular basement membrane (red arrow), podocytes (P) with rarefied cytoplasm, thick (blue arrow), fused foot processes (yellow arrow) and narrow filtration slits (white arrow) and dilated capillaries (C). **B)** A part of a proximal convoluted tubule showing cells with markedly pyknotic nuclei (N) with intra-nuclear inclusion bodies (red arrow) and clumped chromatin in the periphery (green arrow), scattered swollen mitochondria (blue arrows), many cytoplasmic vacuoles (yellow arrows) and destructed basal lamina (white arrow). **C)** A part of a distal convoluted tubule showing cells with round centrally located nuclei (N) with clumped chromatin in the periphery (red arrow), numerous mitochondria (blue arrow) within irregular basal infoldings (white arrow), irregular basal lamina (black arrow) and marked cytoplasmic edema (yellow arrow) (TEM x 10000).

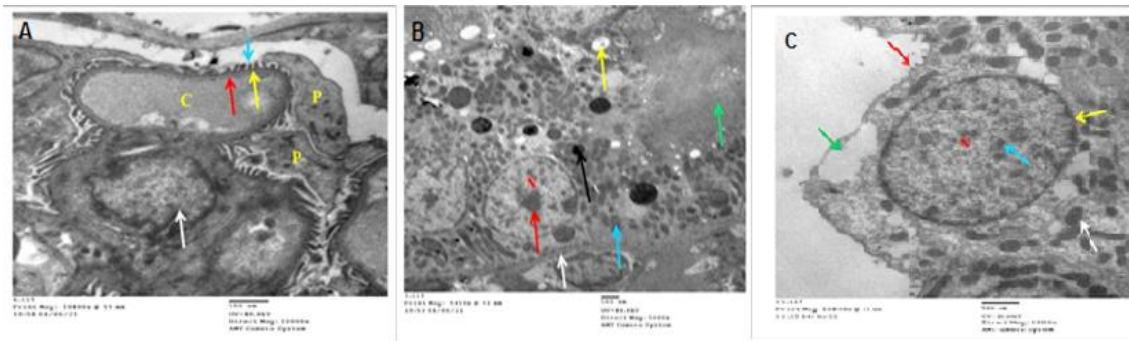


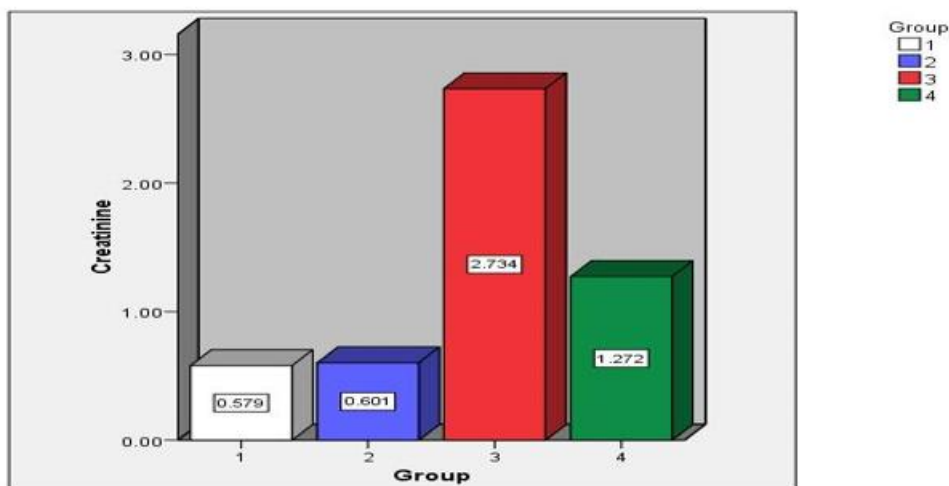
Figure (6): An electron photomicrograph of the renal cortex of adult albino rats of the group IV showing: **A)** A part of a glomerulus showing average basement membrane (red arrow), average podocytes (P) with average foot processes (blue arrow) and patent filtration slits (yellow arrow) and mildly dilated capillaries (C) with average endothelial cells (white arrow) (TEM x 10000). **B)** A part of a proximal convoluted tubule showing cells with basally-located euchromatic nuclei (N) with prominent nucleoli (red arrow), average mitochondria (blue arrow), few scattered cytoplasmic vacuoles (yellow arrow) and inclusion bodies (black arrow), preserved brush borders (green arrow) and average basal lamina (white arrow) (TEM x 5000). **C)** A part of a distal convoluted tubule showing tubular cell with few short microvilli (red arrow), rounded centrally located nucleus (N) with prominent nucleolus (blue arrow) and condensed chromatin in the periphery (yellow arrow), numerous mitochondria (white arrow) and mild cytoplasmic edema (green arrow) (TEM x 10000).

B) Biochemical results and statistical analysis:

The sera of blood samples were taken from the rats of all groups at the end of their experimental period then sent to Royal Lab Tanta for analysis of urea and creatinine. The data were compared to the normal levels of urea and creatinine of the control group (group I). The two parameters were increased significantly in rats of group III while they came toward the normal range in group IV. In group II the results were within normal range. These data have been summarized in tables 1-2 and graphics 1-2.

Table (1): Comparison between the mean values of creatinine in the different studied groups (groups I, II, III, IV) using analysis of variance (ANOVA) test.

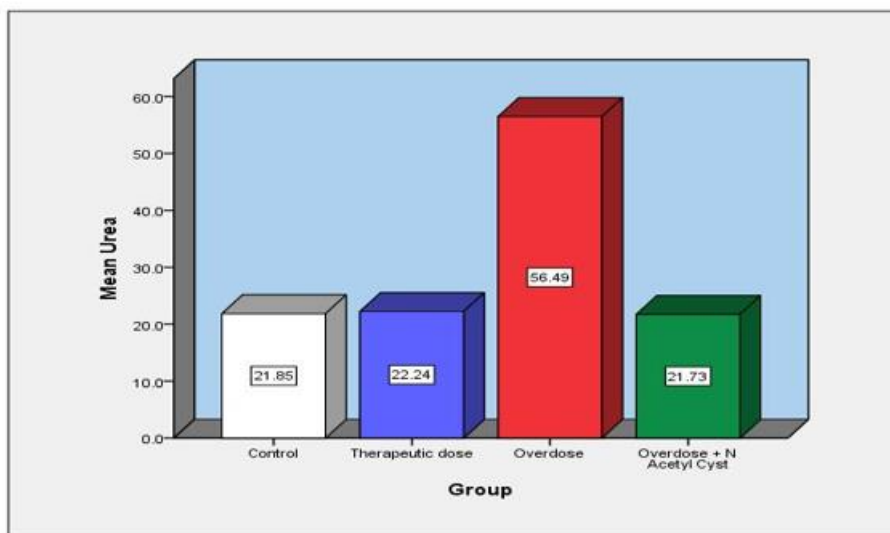
Group	Control (n=10)	Treated with therapeutic dose of tramadol (n=10)	Treated with overdose of tramadol	Treated with over dose of tramadol + N-Acetyl cysteine	F test	P value
Mean ± SD	0.57±0.06	0.6 ± 0.06	2.7 ± 0.26	1.27 ± 0.10	469	0.0001



Graph. (1): Comparison between the mean values of creatinine in the different studied groups (groups I, II, III, IV).

Table (2): Comparison between the mean values of urea in the different studied groups (groups I, II, III, IV) using analysis of variance (ANOVA) test.

Group	Control (n=10)	Treated with therapeutic dose of tramadol (n=10)	Treated with overdose of tramadol	Treated with over dose of tramadol + N-Acetyl cysteine	F test	<i>p</i> value
Mean±SD	21.8±0.5	22.2 ± 0.4	56.48 ± 1.02	21.7 ± 0.4	7426	0.0001



Graph. (2): Comparison between the mean values of urea in the different studied groups (group I, II, III, IV).

Interpretation of Statistical analysis:

Statistical analysis of the biochemical results using one way ANOVA (analysis of variance) test showed a statistically significant difference in tramadol treated (over dose) group (group III) (p value <0.05 was significant) as compared with control group (group I) and tramadol treated (therapeutic dose) group (group II). On the other hand, our findings

showed no statistically significant difference detected in the control group (group I) and tramadol treated (therapeutic dose) group (group II). Also, our results revealed no statistically significant difference detected in the tramadol and N-acetyl cysteine treated group (group IV) as compared with the control group (group I).

DISCUSSION

N-acetyl cysteine (NAC) is a small molecule that can be freely filtered, with easy access to intracellular compartments and is very effective in neutralizing free radicals (oxidants) intra-cellularely (Aruoma *et al.*, 2015; Harrison *et al.*, 2010).

Studies have implicated oxidative stress as one of the possible mechanisms of drug-induced renal toxicity (Santos *et al.*, 2014). Hence, antioxidants (e.g. N-Acetyl cysteine) could have potential as treatment for drug-induced renal toxicity (e.g. Tramadol).

In the current work, examination of haematoxylin and eosin-stained sections from the control and tramadol treated (therapeutic dose) groups revealed that the renal cortex was formed of numerous renal corpuscles and renal tubules with normal interstitium containing peritubular capillaries. Each renal corpuscle consisted of a glomerulus containing tuft of capillaries and surrounded by visceral and parietal layers of Bowman's capsule which were separated by Bowman's space. These results were in accordance with what was reported by previous studies (Rao *et al.*, 2011). They added that the inner visceral layer of Bowman's

capsule was formed of highly differentiated cells called podocytes.

The cortical renal tubules were formed mainly of proximal and distal convoluted tubules. They were lined with simple cuboidal epithelium with central rounded nuclei. The luminae of the proximal tubules were irregular and narrower than the distal ones. Similar results were reported by previous studies (Ross and Pawlina, 2011).

In the present study, the histological picture of tramadol treated (over dose) group showed various degrees of renal cortex affection. Focal areas were observed containing renal corpuscles with congested glomerular capillaries and irregular parietal layer of Bowman's capsule. These results were in concomitant with that reported by other studies (Awadalla and Salah eldin, 2015) who attributed the congested glomerular capillaries and more extensive changes in the form of atrophied glomeruli, wide urinary space and degenerated proximal and distal convoluted tubules to the pro-oxidant effects of the tramadol over dose.

In agreement with other studies (Abd El-aal *et al.*, 2016), some tubular cells had small pyknotic nuclei while others had intracellular vacuolations. Granular hyaline casts in the lumen of some tubules

and abnormal dilatation of others were also seen.

In accordance with other studies (*Masini et al., 2012*), tramadol over dose may induce apoptosis in many cell types. In the current work, tramadol over dose induced histological picture of acute renal failure in the form of small sized (shrunken) glomeruli, degeneration and swelling of the renal tubular cells with necrotic nuclei. Tubular dilatation and intraluminal casts were also observed. These results were in accordance with other studies (*Sayed and Zidan, 2016*).

Toxic effects of tramadol over dose also were explained by previous studies that found that these toxic effects resulted from tramadol-induced lipid peroxidation of renal tissues. Lipid peroxidation of cell membranes leads to loss of membrane fluidity, changes in membrane potential and an increase in membrane permeability, all of which leads to alteration of the chemical compounds of the cells (*Masini et al., 2012*).

In the current work, examination of the haematoxylin and eosin stained sections from tramadol and N-acetyl cysteine treated group revealed nearly normal histological structure of the renal corpuscles. These results were in agreement with other studies (*Aldini et al., 2018*).

In the present study, Masson's trichrome stained sections of the control and tramadol (therapeutic dose) treated groups clarified the presence of few collagen fibers in the renal cortex distributed among glomerular capillaries and in the interstitium.

Compared to the control group, tramadol over dose administration in group III caused an increase in the collagen fibers in the renal cortex distributed among the glomerular capillaries and the interstitium.

On the other hand, Masson's trichrome stained sections of tramadol and N-acetyl cysteine-treated group revealed few collagen fibers among the glomerular capillaries and the interstitium denoting partial recovery.

In the current work, electron microscope examination of ultrathin sections of the renal cortex of the control and tramadol treated (therapeutic dose) groups showed normal glomerular blood capillaries with average endothelial cells. The glomerular basement membrane appeared regular. Podocytes were also noticed with average foot processes and patent filtration slits. These results were in concomitant with other studies (*Tryggvason and Wartiovaara, 2011*).

Proximal tubular cells were seen with rounded euchromatic nucleus and closely-packed microvilli. Numerous mitochondria and average basal lamina were also seen. These results were in agreement with previous studies (*Figueiredo et al., 2018*) who illustrated that the microvilli of the proximal tubule cells provide a large extensive surface area for unidirectional transport of solutes from the lumen to blood.

The lining epithelial cells of the distal convoluted tubules appeared with few short microvilli, rounded centrally located nuclei with central nucleoli and numerous mitochondria which seen scattered in the cytoplasm. These results were in

coinciding with other studies (*Zhai et al., 2016*).

In the present work, ultrathin sections from the renal cortex of tramadol (over dose) treated group showed dilated glomerular capillaries with irregular basement membrane and distorted foot processes of the podocytes. Proximal convoluted tubular cells contained pyknotic nuclei with intra-nuclear inclusion bodies. Numerous swollen mitochondria, irregular and destructed basal lamina and multiple cytoplasmic vacuoles were also observed. The distal tubular cells appeared with rounded centrally located nuclei with clumped chromatin in the periphery. Numerous mitochondria were seen within irregular basal infoldings. Irregular basal lamina and cytoplasmic edema were also observed. These results were in agreement with other studies (*Ali et al., 2015*) who also reported these results.

In the present study, ultrathin sections from the renal cortex of adult male albino rats of the tramadol and N-acetyl cysteine-treated group demonstrated the renal corpuscle containing normal glomerular blood capillaries with regular basement membrane. Podocytes were also seen with average foot processes. Tubular cells containing normal euchromatic nuclei and numerous normal mitochondria were also seen. Normal microvilli appeared at the apical cell membrane of the proximal tubular cells.

Statistical analysis of the biochemical results using one way ANOVA (analysis of variance) test illustrated a statistically significant difference in tramadol treated (over dose) group (group III) (p value <0.05 was significant) as compared with

control group (group I) and tramadol treated (therapeutic dose) group (group II). On the other hand, our findings demonstrated no statistically significant difference detected in the control group (group I) and tramadol treated (therapeutic dose) group (group II). Also, our results exposed no statistically significant difference detected in the tramadol and N-acetyl cysteine treated group (group IV) as compared with the control group (group I).

CONCLUSION

The results of this study clarified that tramadol, when used in over doses causes histological changes in the renal cortex. However, these changes did not present when tramadol was used in low doses. Also, these changes slightly decreased with N-acetyl cysteine usage.

We recommend that renal investigations should be routinely done with tramadol treatment or tramadol abuse. We should be cautious with tramadol a demonstration in patients with renal impairment.

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الدور الوقائي المحتمل لـ "ان-اسيتيل سيستايين" علي التسمم الكلوي المحدث بالترامادول في الفئران البيضاء البالغة (دراسه بالميكروسكوب الضوئي والالكتروني)

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المقدمه: يعد دواء الترامادول من المسكنات الصناعيه التي تعمل مركزيا علي المخ و يستخدم بشكل واسع في علاج الالام المعتدله الي الشديده والالام الحاده والمزمنه كما يعتبر تأثير عقار الترامادول كمسكن مشابه لتأثير عقار الكودايين وعشر مرات اقل من تأثير عقار المورفين. ويعتبر عقار ان-اسيتيل سيستايين مشتق من الحمض الاميني سيستايين وهو لديه عدة خصائص كمضاد للأكسده والالتهابات، وقد تم استخدامه في ابحاث اجريت من قبل علي انسجة الكبد، وقد تبين ان اعطائه يؤدي الي تحسين الانسجه التالفه.

الهدف من البحث: الهدف من هذا البحث هو دراسة هستولوجيه باستخدام الميكروسكوب الضوئي والالكتروني النافذ لبيان التأثيرات السامه لعقار الترامادول علي انسجة الكليه وايضا دراسة الدور الوقائي المحتمل لعقار ان-اسيتيل سيستايين ضد هذه التأثيرات.

الطرق والأدوات المستخدمه في هذا البحث: قد تم استخدام أربعين من الفئران البيضاء البالغه وتم تقسيم هذه الفئران الي اربع مجموعات متساويه كالاتي:

المجموعه الأولي: تمثل هذه المجموعه المجموعه الضابطه وقد تم اعطائها محلول ملح بجرعة 30 مجم /كجم عن طريق انبوبة المعدة يوميا لمدة ثلاثين يوما.

المجموعه الثانيه: اعطيت هذه المجموعه كلوريد الترامادول بجرعه دوائيه تقدر ب 15.5 مجم/كجم عن طريق انبوبة المعدة يوميا لمدة ثلاثين يوما.

المجموعة الثالثة: تم اعطاء هذه المجموعة كلوريد الترامادول بجرعة عالية (سامة) تقدر ب 30 مجم / كجم عن طريق انبوبة المعدة يوميا لمدة ثلاثين يوما.

المجموعة الرابعة: تم اعطاء هذه المجموعة كلوريد الترامادول بجرعة عالية (سامه) تقدر ب 30 مجم / كجم بالاضافه الي عقار ان-اسيتيل سيستايين بجرعة تقدر ب 100 مجم/كجم عن طريق انبوبة المعدة يوميا لمدة ثلاثين يوما.

بعد انتهاء المده المحدده تم تخدير الفئران باستنشاق الاثير واستئصلت الكلي منها ثم تم تحضيرها للفحص بالميكروسكوب الضوئي و الميكروسكوب الالكتروني النافذ، وخضعت النتائج للدراسات التحليليه والاحصائيه.

النتائج: قد اثبتت القطاعات التي صورت بالميكروسكوب الضوئي والالكتروني النافذ النتائج التاليه:

1. بالنسبه للعينات التي عولجت بمحلول ملح فقط (المجموعه الاولي، المجموعه الضابطه): فقد اظهرت الدراسات الهستولوجيه ان هيكل الكريات الكلويه قد ظهر بصورة طبيعيه كما ظهرت الانبيات الكلويه القريبه والبعيده وما حولها من الشعيرات الدمويه بشكل طبيعي.

2. بالنسبه للعينات التي عولجت بجرعات دوائيه من الترامادول (المجموعه الثانيه): فقد اظهرت الدراسات الهستولوجيه ان اعطاء الترامادول بجرعة دوائيه لم يحدث التأثيرات السامة التي احدها الترامادول بجرعة عاليه (سامة) حيث وجد ان:

- قشرة الكلي المصبوغه بصبغة الهيماتوكسالين والايوسين: قد ظهرت فيها الكبيبات الكلويه والاناييب القريبه والبعيده الملتويه بحجم طبيعي ولم يلاحظ اي اتساع في حافظات بومان او تجويف الاناييب البعيده والقريبه الملتويه.

- قشرة الكلي المصبوغه بصبغة ماسون ترايكروم: قد ظهر فيها كميات طبيعيه غير زائده من الالياف الكولاجينيه بين الكبيبات الكلويه وحول اغلفه بومان وبين الانبيات الكلويه.

3. بالنسبة للعينات التي عولجت بجرعات سامه من الترامادول (المجموعه الثالثه) فقد اظهرت الدراسات الهستولوجيه ان:

- قشرة الكلي المصبوغه بصبغة الهيماتوكسلاين والايوسين: قد ظهر فيها كريات كلويه صغيره واتساع في حافظات بومان، كما وجد انبيات كلويه قريبه وبعيده متنسعة التجويف وتحتوي علي اجسام زجاجيه، واحتوت الخلايا المبطنه لها علي انويه داكنه وتجاويف. كما لوحظ ايضا وجود الخلايا منكمشة النواه في بعض الاماكن.

- قشرة الكلي المصبوغه بصبغة ماسون ترايكروم: قد ظهر فيها زيادة في الاليف الكولاجينيه بين الكبيبات الكلويه وحول اغلفه بومان وبين الانبيات الكلويه.

4. بالنسبة للعينات التي عولجت بجرعات عاليه من الترامادول واضيف اليها عقار ان-اسيتيل سيسيتاين (المجموعه الرابعه) فقد اظهرت الدراسات الهستولوجيه ان:

- قشرة الكلي المصبوغه بصبغة الهيماتوكسلاين والايوسين: قد ظهر فيها تحسن جزئي في هيكل الكبيبات الكلويه والاناييب القريبه والبعيده الملتويه حيث عادت الكبيبات الكلويه تقريبا الي حجمها الطبيعي وقل بنسبة ملحوظه الاتساع في حافظات بومان مقارنة بالمجموعه الثالثه، كما لوحظ ايضا ان تجويف الاناييب البعيده والقريبه الملتويه قد عاد تقريبا الي الحجم الطبيعي كما ظهرت تلك الاناييب ايضا بانويه طبيعيه غير داكنه. كما لوحظ ايضا اختفاء وجود الخلايا منكمشة النواه.

- قشرة الكلي المصبوغه بصبغة ماسون ترايكروم: وجود كميات طبيعيه غير زائده من الاليف الكولاجينيه بين الكبيبات الكلويه وحول اغلفه بومان وبين الانبيات الكلويه.

وعلي مستوي الميكروسكوب الالكتروني النافذ فقد وجد الاتي:

1. بالنسبة للعينات التي عولجت بمحلول ملح فقط (المجموعه الاولى، المجموعه الضابطة): فقد اظهرت الدراسات الهستولوجيه ان الكريات الكلويه قد ظهرت بشكل طبيعي محتوية علي غشاء قاعدي منتظم كما ظهرت الانبيات القريبه والبعيده محتوية علي انوية طبيعيه وتجعدات قاعديه منتظمه.
2. بالنسبة للعينات التي عولجت بجرعات دوائيه من الترامادول (المجموعه الثانيه): فقد اثبتت الدراسات والنتائج الهستولوجيه انها تشبه نتائج المجموعه الضابطة وان اعطاء الترامادول بجرعة دوائيه لم يحدث التاثيرات السامة التي احدثها الترامادول بجرعة عاليه (سامة).
3. بالنسبة للعينات التي عولجت بجرعات سامه من الترامادول (المجموعه الثالثه): قد وجد ان الحاجز الكلوي الدموي احتوي علي غشاء قاعدي كبيبي غير منتظم وايضا الخلايا الطلائيه المبطنه للانبيات القريبه والبعيده الملتيه وجد انها احتوت علي انويه منكمشه وميتوكوندريا منتفخه وفجوات صغيره. وشوهت ايضا انتفاخات سيتوبلازميه في بعض خلايا الانبيات البعيده الملتيه.
4. بالنسبة للعينات التي عولجت بجرعات عاليه من الترامادول واضيف اليها عقار ان-اسيتيل سيستايين (المجموعه الرابعه): فقد اظهرت الدراسات الهستولوجيه ان التغيرات الهستولوجيه قلت نسبيا في معظم الحيوانات المستخدمه من خلال تاثير عقار ان-اسيتيل سيستايين المضاد للاكسده.

خلاصة نتائج البحث: استخدام الترامادول بجرعة عاليه (سامة) يؤدي الي الحاق الضرر البالغ في القشرة الكلويه مع توسع ملحوظ في الانابيب وازافة ان-اسيتيل سيستايين حسنت جزئيا من هذه الاثار السامه، في حين ان استخدام الترامادول بجرعة عادية (دوائيه) لا يحدث هذه التاثيرات السامه، لذا نوصي بمتابعة وظائف الكليه للمريض الذي يتناول عقار الترامادول بجرعات عاليه لفترات طويله بشكل دوري، كما يمنع استخدام الترامادول في مرضي الفشل الكلوي.