Original Article

Role of Pomegranate Peel Extract and N-Acetyl cysteine supplementation on Paracetamol-Induced Nephrotoxicity in Adult Male Albino Rats

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ABSTRACT

Background: Paracetamol (PA) is widely spread analgesic and antipyretic drugs. Its overdose can cause severe damage to kidneys representing one of the most common reasons for emergency admissions.

Aim: The study aimed to demonstrate the effects of pomegranate peel extract (PPE), on PA-induced nephrotoxicity, and the possibility of co-administration therapy of PPE and N-acetyl cysteine (NAC) to ameliorate PA toxicity.

Material and Methods: Fifty adult male albino rats were equally divided into 5 groups as follow: Group I (Control animals) received only balanced diet and tap water; Group II received PA a¬t a single dose 400 mg/kg body weight (BW) /day orally; Group III received orally PA at same previous dose and NAC at a single dose 150 mg/kg BW /day orally; Group IV received PA at same previous dose and PPE at a single dose 430 mg/kg BW /day orally and Group V received PA, NAC and PPE at same previous doses. After 28 days of treatment, venous blood samples were collected for assaying blood creatinine (BC) and blood urea nitrogen (BUN) levels. Then, rats were anaesthetized and sacrificed. Kidneys were harvested for histological and immunohistochemical examinations.

Results: PA-induced a highly significant increase in BUN and BC ($p<0.001$). Also all these alterations were confirmed histologically in the renal cortex and medulla where PA induced separated and congested glomerular capillaries with wide Bowman's space. The interstitium infiltrated by the inflammatory cells and vascular congestion were very prominent in-between tubules. Moreover, PA led to apoptotic death in renal tubular cells, manifested by an increase in the expression of caspase-3. Meanwhile, NAC and PPE showed a protective action against PA-toxic effects especially when used in combination.

Conclusion: It is suggested that NAC and PPE could have an ameliorating effect against PA-nephrotoxicity.

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Key Words: kidney, N-acetyl cysteine, paracetamol, pomegranate peel extract, rats.

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INTRODUCTION

Acetaminophen known as paracetamol is widely used as analgesic and antipyretic agent. It is a safe drug when given in therapeutic doses. Acute PA overdose can lead to potentially lethal liver and kidney failure in humans and experimental animals. PA is a phenacetin metabolite representing one of the most nephrotoxic analgesics ***(Slitt et al., 2004)***.

N-acetyl-p-benzoquinone imine (NAPQI) is the end product of acetaminophen metabolism by cytochrome p450 enzyme system which is toxic to kidney and liver. In the case of therapeutic dose, this is inhibited by depleting glutathione, an antioxidant compound in the liver and NAPQI-reduced glutathione is excreted by kidney. In acetaminophen overdose, glucoronidation and sulfation pathways become saturated. The amount and rate of formation of NAPQI is greatly increased. Depleting glutathione stores occurs after conjugation with NAPQI, followed by an increase of NAPQI level, then binds covalently with cells leading to their death and apoptosis appears through oxidative damages ***(Da Silva Melo et al., 2006)***

Increasing level of reactive oxygen species (ROS) in kidney is considered as another explanation to PA induced nephrotoxicity. ROS is the central key in the mechanisms that lead to

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decrease of glomerular filtration rate and tubular necrosis. Nuclear factor kappa B that plays a key role in the inception of inflammatory process is activated by ROS ***(Zhao et al., 2011)***.

Acetylcysteine, also known as N-acetyl-L-cysteine or NAC, is a drug used to overcome acetaminophen overdose. Acetylcysteine acts to protect liver cells from NAPQI toxicity that depletes glutathione reserves in the liver ***(Green et al., 2013)***.

Pomegranate (El-Ruwmon, Punicaceae,. Punica granatum L.) is one of the oldest known drug. It is mentioned in the Ebers .papyrus of Egypt written in about .1550 B.C. Dried fruit peel is used to treat respiratory, urinary tract infections and diarrhea. Also, pomegranate fruit peels showed different pharmacological functions as antifertility effect, antioxidant activity, cytotoxic activity, hypoglycemic activity and hepatoprotective activity. Also, pomegranate peel ethanol extract has ameliorative effect against chlorpyrifos-ethyl-induced oxidative stress in the rats. Pomegranate flower extract has also protective effect against gentamicin induced- nephrotoxicity ***(Ahmed and Zaki, 2009 and Thring et al., 2009)***.

Therefore, the present study was aimed to elucidate the possible ameliorating role of NAC and PPE in alleviating the nephrotoxic effects of PA when administered separately and in combination to adult male albino rats.

MATERIAL AND METHODS

A. Chemicals

PA and NAC were purchased from EIPICO pharmaceutical industries company (10^{th} of Ramadan, Egypt).

B. Preparation of PPE

Pomegranate (Punica Granatum) was obtained from local markets. The peel was manually removed, sun dried and powdered. The powder was mixed with distilled water. The extract was filtered.

C. Experimental Animals

Fifty adult male albino rats, each weighed 150 to 200 gm were used. Animals were housed at the experimental animal house of the Faculty of Medicine, Zagazig University. The animals were kept in controlled environment of temperature, humidity and light. They were fed on tap water ad libitum and a commercial standard diet.

D. Experimental Design

The rats were equally divided into 5 groups (10 rats / each group). Group I (negative control group); the rats were received only regular diet and tap water for 28 days to measure the basic parameters. Group II (PA-treated group): rats were gavaged orally with PA (400 mg/kg body weight) dissolved in 2ml of tap water, once daily for 28 days (Venkatachalam and Muthukrishnan, 2013). Group III (PA and NAC-treated group): each was given PA by the same manner mentioned above and NAC (150 mg/kg body weight) once daily for 28 days ***(Sener et al., 2003)***. Group IV (PA and PPE treated group): rats received orally PA by the same manner mentioned above and PPE (430 mg/kg body weight) once daily for 28 days (Khalil EAM, 2004). Group V (PA, PPE and NAC): rats were gavaged orally with chemicals by the same manner mentioned above once daily for 28 days. At the end of the experiment, venous blood samples were collected from retro orbital plexuses of each rat for assaying BC and BUN levels after that the animals were anaesthetized with intraperitoneal injection of phenobarbitone then sacrificed. Kidneys were collected for histopathological examination and immunohistochemical determination of Caspase 3.

Biochemical Estimation of BUN and BC

Blood samples were collected from the retro–orbital plexus. The collected blood was centrifuged and plasma fraction was separated. Estimation of BUN had been carried out using kit of" bio-merieux France". It was done as reported by ***(Kaplan, 1965)*** according to the pamphlet of Bio-merieux France kit by enzymatic colorimetic method. Estimation of BC had been carried out using kit of Bio-merieux France ***(Bjurosson, 1979)***.

Light Microscopy Technique

Both kidneys were removed and immediately processed for light microscopic study. Each kidney was cut into two halves across the renal pelvis along its longitudinal axis to expose cortex, medulla and papilla. The specimens were immediately immersed in 10% buffered formaldehyde for 48 hours at 4º C. After that, the specimens were processed for preparation of paraffin sections of 5 µm thickness. These sections were stained by Haematoxylin and Eosin (H&E) ***(Bancroft and Gamble, 2008)***.

Immunohistochemical Technique

Caspase 3 (apopain, SCA-1, Yama and CPP32) is one of the members of apoptosis execution functional group of caspases, and is either partially or totally responsible for the proteolytic cleavage of many Key proteins during apoptosis. Immunohistochemical staining of routine histological sections resulted in brown reaction product in antigen-containing cells, whereas the background stained blue ***(Borch et al., 2006)***.

Statistical Data Analysis

Data analysis was done using statistical software package Epi-Info version 6.02 ***(Dean et al., 2000)***. The means and standard deviation (SD) values of variables were calculated. One-way ANOVA test were used for comparison between different groups. The *p-value* ≤ 0.05 was considered statistically significant.

RESULTS

Biochemical Results

Level of BUN: in group I (control group) was 39.53 ± 0.34 mg/dL. It was significantly elevated to 83.25 ±0.43 in group II (PA-treated group). In groups III, IV and V, BUN values were decreased to be 82.88 ± 0.26, 41.23 ± 0.07, and 39.67 ± 0.34 respectively (Table 1; Chart 1). Level of BC: in group I (control group) was 0.76 ± 0.02 mg/dL. It was significantly elevated to 3.76 ± 0.05 in group II (PA-treated group). In groups III, IV and V, BUN values were decreased to 3.68 ± 0.02, 0.81 ± 0.02 and 0.77 ± 0.01 respectively (Table 1). There was found also significant correlation between BUN and BC levels of adult male albino rats (Table 2).

Histological Results

Group I (Control group)

In the renal cortex was consisting of renal corpuscles and renal tubules. Each renal corpuscle consisted of a glomerulus containing tuft of capillaries. The renal corpuscles were surrounded by visceral and parietal layers of Bowman's capsule which were separated by Bowman's space. The cortical renal tubules were formed mainly of proximal and distal convoluted tubules. They were lined by simple cuboidal epithelium with central rounded nuclei. The luminae of the proximal tubules were irregular and Histological Results:

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There was no proapoptotic caspase-3 staining observed in immunohistochemical studies in both cortical and medullary structures of renal tissues taken from control group (Figure 2).

Group II (PA-treated group)

PA-treated group showed some corpuscles appeared with separated and congested glomerular capillaries and wide Bowman's space. Cells of cortical tubules showed extensive vacuolation of their cytoplasm. While other tubules appeared with irregular outlines, disrupted luminal aspect, their epithelial lining loses their normal arrangement and some necrotic cells were seen detaching from the basement membrane and being sloughed into the tubular lumen. Other tubules exhibited intra-luminal eosinophilic homogenous material (Figure 3). The interstitium infiltrated by the inflammatory cells and vascular congestion is very prominent. Other section of the renal cortex showed marked hemorrhage in-between tubules (Figure 4).

Renal medulla showed extensive inflammatory cell infiltration and hemorrhage in the Interstitium. Cells of medullary ducts showed extensive vacuolation of their cytoplasm. Some necrotic cells were seen detaching from the basement membrane and being sloughed into the tubular lumen. Some tubules exhibited intra-luminal eosinophilic homogenous material (Figure 5).

In immunohistochemical examination of renal cortex, caspase 3– positive cells were located in renal corpuscle and the cortical tubules, while in renal medulla caspase 3– positive cells were

located around loop of Henle and medullary tubules (Figure 6).

Group III (PA+ NAC -treated group)

In PA and NAC–treated group, the signs of tubular degeneration were still present, such as epithelial vacuolization in the tubules. Some necrotic cells are seen detaching from the basement membrane and being sloughed into the tubular lumen. The glomeruli maintained better morphology when compared with PA-treated group except widening of Bowman's space. Renal medulla still showed inflammatory cell infiltration in the interstitium and tubular cells showed vacuolation of their cytoplasm with some pyknotic cells in wall of tubule. Hemorrhage in between medullary ducts was also still detected (Figure 7).

In immunohistochemical examination; renal cortex and medulla showed moderate protein expression of caspase- 3 when compared with PA-treated group (Figure 8).

Group IV (PA and PPE- treated group)

In PA- and PPE-treated group, the cortical tubules in renal cortex retained their normal architecture except some necrotic cells were seen detaching from the basement membrane and being sloughed into the tubular lumen. Thereby that tubular injury was not in fact fully reversed. No evidence of vascular congestion and disappearance of intra-luminal eosinophilic homogenous material. Bowman's space was relatively wide. Renal medulla showed more improvement as the tubular lining retained its normal arrangement (Figure 9).

In immunohistochemical examination; renal cortex and medulla showed mild protein expression of caspase- 3 when compared with PA-treated group (Figure 10).

Group V (PA + PPE + NAC-treated group)

PA + PPE + NAC-treated group showed almost normal histology in renal cortex and medulla similar to control rats (Figure 11). There was no detectable proapoptotic caspase-3 staining in immunohistochemical studies was observed in renal cortex and medulla (Figure 12)

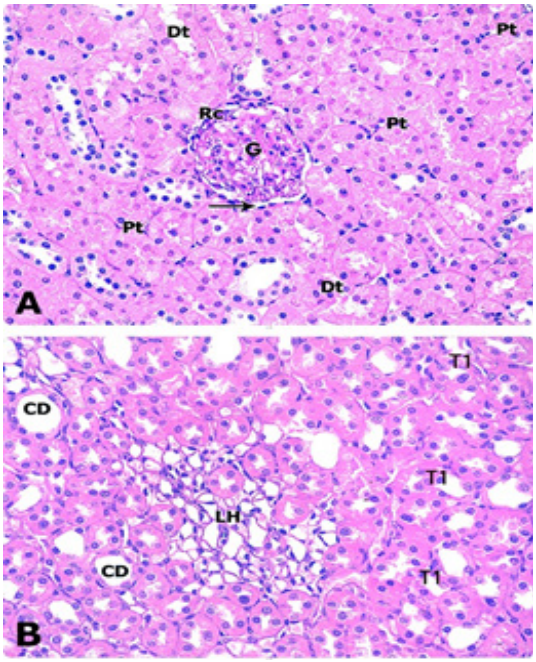

Fig. 1: Photomicrographs of kidney tissues: (A) A transverse section. of the kidney of an adult male albino rat (control group) showing that the renal cortex is forming from renal corpuscles (Rc), consisting of a glomerulus (G) that containing tuft of capillaries, Bowman's space (arrow) and proximal (Pt), and distal convoluted tubules (Dt); (B) A transverse section of the kidney of an adult male albino rat (control group) showing the renal .medulla that formed from loops of Henle (LH), collecting ducts (CD) and different medullary tubules (T1). (H&E ×400)

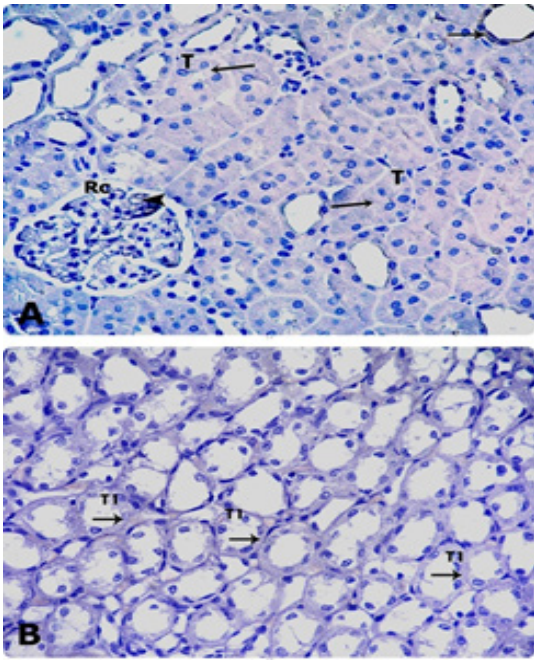

Fig. 2: Immunohistochemical staining photomicrographs of kidney tissues for caspase-3: (A) Renal cortex showing no detectable proapoptotic caspase-3 staining (arrow heads) in the renal corpuscle (Rc) and other caspase 3– negative cells (arrows) around the cortical tubules (T); (B) Renal medulla shows that caspase 3–negative cells (arrows) are located around medullary tubules (T1). (×400)

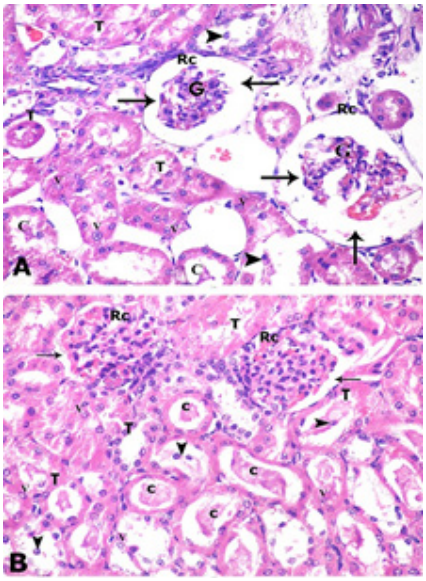

Fig. 3: Photomicrographs of kidney tissues of PA-treated group: (A) A transverse section of the kidney of an adult male albino rat showing the renal cortex with some renal corpuscles (Rc) that appear with separated and congested glomerular capillaries (G) with wide Bowman's space (arrow). Cells of the cortical tubules (T) show extensive vacuolation of their cytoplasm (v). Some tubules exhibit intra-luminal eosinophilic homogenous material (C). Other tubules appear with some necrotic cells are seen detaching from the basement membrane and being sloughed into the tubular lumen (arrow heads); (B) A transverse section of the kidney of an adult male albino rat showing the renal cortex with some renal corpuscles (Rc) with wide Bowman's space (arrow). Many tubules (T) exhibit intra-luminal eosinophilic homogenous material (C). Some necrotic cells are seen detaching from the basement membrane and being sloughed into the tubular lumen (arrow heads). Tubular Cells show vacuolation of their cytoplasm (v). (H&E X 400)

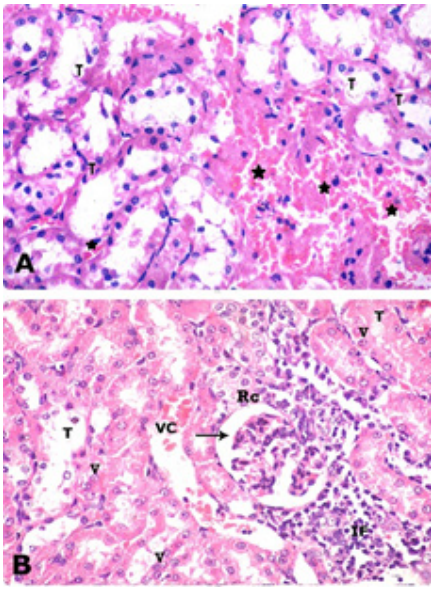

Fig. 4: Photomicrographs of kidney tissues of PA-treated group: (A) A transverse section of the kidney of an adult male albino rat showing the renal cortex with marked hemorrhage (star) in between tubules (T); (B) A transverse section of the kidney of an adult male albino rat) showing the renal cortex infiltrated by the inflammatory cells (IF) and Vascular congestion (VC) is very prominent. Renal corpuscles (Rc) appear with wide Bowman's space (arrow). Cells of the cortical tubules (T) show extensive vacuolation of their cytoplasm (v). (H&E X 400)

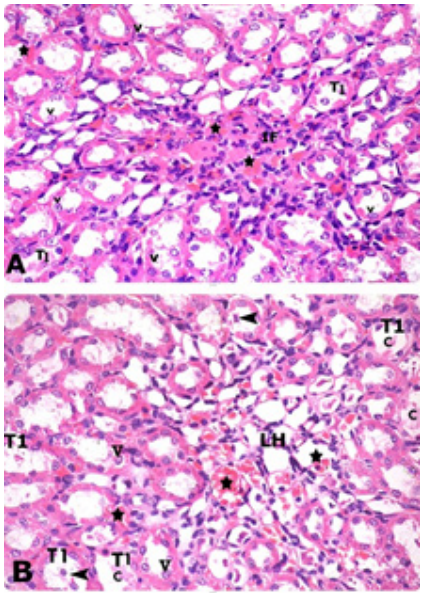

Fig. 5: Photomicrographs of kidney tissues of PA-treated group: (A) A transverse section of the renal medulla of an adult male albino rat (PA treated group) showing extensive inflammatory cell infiltration (IF) and hemorrhage (stars) in between medullary tubules (T1) and extensive vacuolation of cortical cells (v); (B) A transverse section of the renal medulla of an adult male albino rat (PA treated group) showing hemorrhage (stars) within loops of Henle (LH). Cells of medullary ducts show vacuolation in their cytoplasm. Some necrotic cells are seen detaching from the basement membrane and being sloughed into the tubular lumen (arrow heads). Some medullary tubules (T1) exhibit intra-luminal eosinophilic homogenous material (C). (H&E X 400)

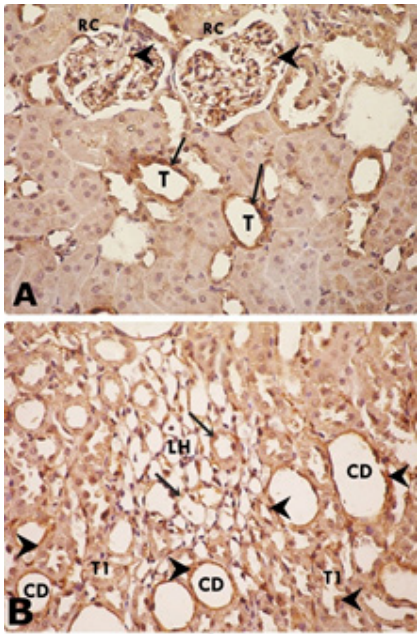

Fig. 6: Immunohistochemical staining photomicrographs of kidney tissues for caspase- 3 in PA -treated group: (A) Renal cortex showing positive brown staining (arrow heads) in the renal corpuscle (Rc) and other positive brown staining (arrows) around the cortical tubules(T); (B) Renal medulla showing that caspase 3– positive cells (arrows) are located around loop of Henle (LH) and other caspase 3– positive cells (arrow heads) around medullary tubules (T1). (X 400)

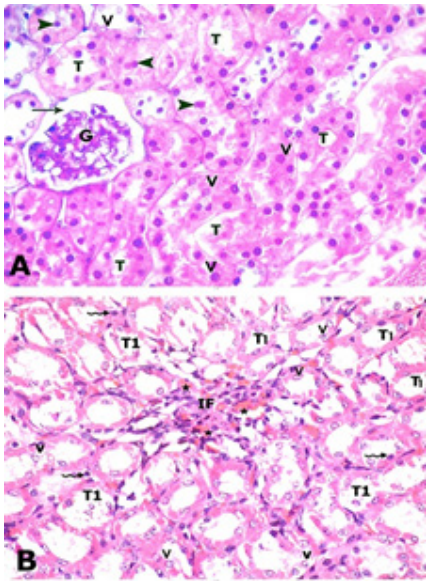

Fig. 7: Photomicrographs of kidney tissues of PA+ NAC -treated group: (A) A transverse section of renal cortex of an adult male albino rat showing epithelial vacuolization in tubules (v), and some necrotic cells (arrow heads) are seen detaching from the basement membrane and being sloughed into the lumen of cortical tubules(T), the glomeruli (G) show widening of Bowman's space (arrow); (B) A transverse section of renal medulla of an adult male albino rat still showing some inflammatory cells infiltration in the interstitium (IF) and cells of medullary tubules (T1) showed vacuolation (V) of their cytoplasm with some pyknotic cells in wall of tubules (zigzag arrow). Hemorrhage in-between medullary ducts are also still detected (star). (H&E ×400)

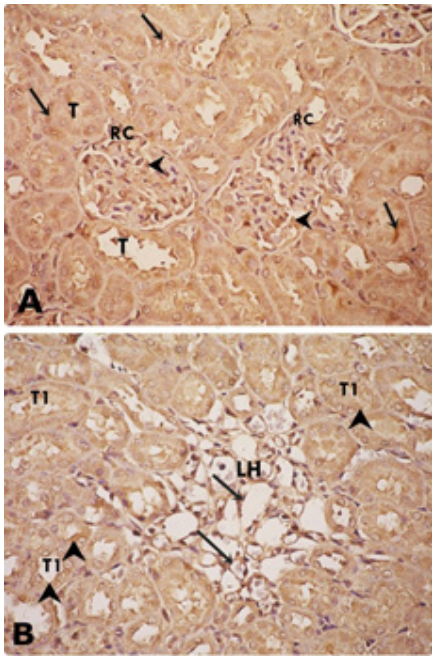

Fig. 8: Immunohistochemical staining photomicrographs of kidney tissues for caspase- 3 in PA+ NAC-treated group: (A) Renal cortex showing moderate positive brown reaction (arrow heads) in the renal corpuscle (Rc) and other positive brown staining (arrows) around the cortical tubules (T); (B) Renal medulla showing that caspase 3– positive cells (arrows) are located around loop of Henle (LH) and other caspase 3– positive cells (arrow heads) around medullary tubules (T1). (X 400)

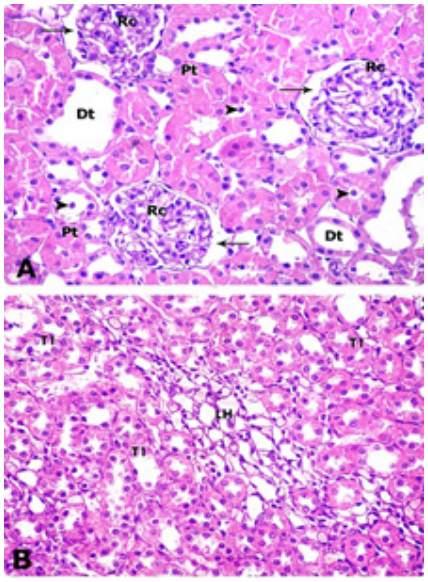

Fig. 9: Photomicrographs of kidney tissues of PA-and PPE- treated group: (A) A transverse section of renal cortex of an adult male albino rat showing nearly normal cortical tubules [proximal tubules (Pt) and distal tubules (Dt) except Some necrotic cells are seen detaching from the basement membrane and being sloughed into the tubular lumen(arrow heads). The renal corpuscle (RC) shows relatively wide Bowman's space (arrow); (B) A transverse section of renal medulla of an adult male albino rat showing nearly normal architecture of loop of Henle (LH) and medullary tubules (T1) and collecting ducts (CD)

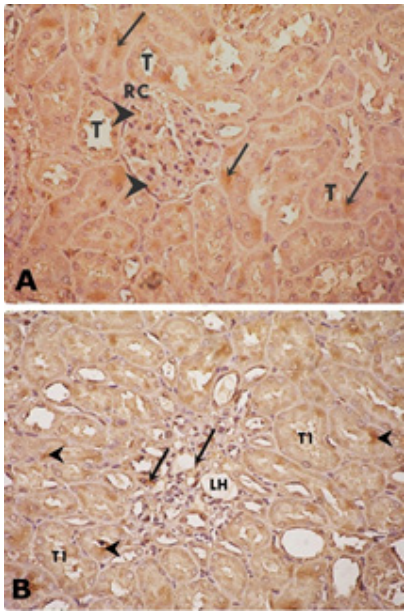

Fig. 10: Immunohistochemical staining photomicrographs of kidney tissues for caspase- 3 in PA and PPE- treated group: (A) Renal cortex showing mild positive brown reaction(arrow heads) in the renal corpuscle (Rc) and other positive brown staining(arrows) around the cortical tubules(T); (B): Renal medulla showing that caspase 3– positive cells (arrows) are located around loop of Henle (LH) and other caspase 3– positive cells (arrow heads) around medullary tubules (T1). (×400)

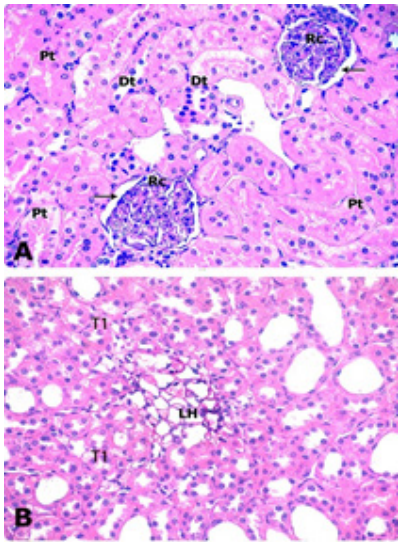

Fig. 11: Photomicrographs of kidney tissues of PA + PPE + NAC- treated group: (A) A transverse section of renal cortex of the adult male albino rat nearly normal proximal tubules (Pt) and distal tubules (Dt). The renal corpuscle (RC) shows relatively normal Bowman's space (arrows); (B) A transverse section of renal medulla of an adult male albino rat showing different types of medullary ducts (T1) and loop of Henle (LH). (H&E ×400)

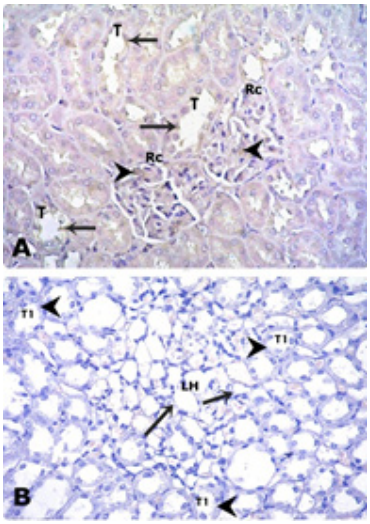

Fig. 12: Immunohistochemical staining photomicrographs of kidney tissues for caspase- 3 in PA + PPE + NAC- treated group: (A) Renal cortex showing no detectable proapoptotic caspase-3 staining (arrow heads) in the renal corpuscle(Rc) and other caspase 3– negative cells (arrows) around the cortical tubules(T); (B) Renal medulla showing that caspase 3–negative cells (arrows) are located around loop of Henle(LH) and other caspase 3– negative cells (arrow heads) around medullary tubules (T1). (×400)

Table 1: Means comparison and P values between each pairs of groups of BUN and BC levels of adult male albino rats in control and other treated groups (±SD)

Groups	Means comparison using one-way ANOVA and LSD		*P values* between each pairs of groups using independent t-test									
			Group I		Group II		Group III		Group IV		Group V	
	BUN (mg/dL)	BC (mg/dL)	BUN	BC	BUN	BC	BUN	BC	BUN	BC	BUN	BC
Group I	39.53(± 0.34 D)	0.76(±0.02 D)			***	***	***	***	*	*	NS	NS
Group II	83.25(± 0.43 A)	3.76(± 0.05 A)					*	*	***	***	***	***
Group III	82.88(± 0.26 B)	3.68(± 0.02 B)							***	***	***	***
Group IV	41.239(± 0.07 C)	0.81(± 0.02 C)									***	***
Group V	39.67(± 0.34 D)	0.77(± 0.01 D)										
LSD at 0.05	0.280 ($P < 0.000$)	0.025 ($P < 0.000$)										

Notes: NS, P, SD and LSD refer to non-significance, probability, standard deviation and least significant difference, respectively. The means with the same letter are not significantly different. If *P-value* is >0.05 indicates non-significant results (NS); *P value* of <0.05 indicates significant results (*); *P value* of <0.01 indicate highly significant results (***).

Table 2: Correlation between BUN and BC levels of adult male albino rats

		BUN	BC
BUN	Pearson Correlation	1.00	0.99**
	Sig. (2-tailed)		0.000
	N	50.00	50.00
BC	Pearson Correlation	0.99**	1.00
	Sig. (2-tailed)	0.000	
	N	50.00	50.00

** Correlation is significant at the 0.01 level (2-tailed).

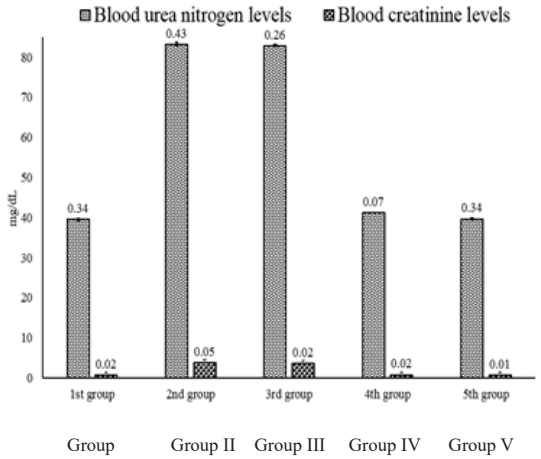

Chart 1: showing comparison between different studied groups regarding BUN and BC levels of adult male albino rats in control and other treated groups

DISCUSSION

The histological examination of the renal cortex and medulla of PA intoxicated rats in the present study demonstrated variable adverse effects of PA. By light microscopic examination, some corpuscles appeared with separated and congested glomerular capillaries and wide Bowman's space. Cortical tubules cells showed extensive vacuolation of their cytoplasm. Some necrotic cells were seen detaching from the basement membrane and being sloughed into the tubular lumen. Other tubules exhibited intra-luminal eosinophilic homogenous material. The interstitium was infiltrated by the inflammatory cells and vascular congestion was very prominent. Renal medulla showed extensive inflammatory cell infiltration and hemorrhage in the Interstitium. These findings are compatible with the study of ***Refaat and Mady (2008)*** who observed proximal tubular distortion and vacuolization after single intraperitoneal dose of 1000 mg/kg of acetaminophen in rats. ***Mehboob and Tahir (2015)*** reported that both proximal and distal convoluted tubules showed vacuoles in .their cells with presence of cellular debris in their lumens after single dose acetaminophen 600 mg/kg body weight to mice.

Gulnaz et al., (2010) also reported many protein casts in distal convoluted tubules and in thick ascending limb of loop of Henle after single intraperitoneal dose of 1000 mg/kg acetaminophen. Also, there was marked interstitial vascular congestion and inflammation. ***Roy et al., (2015)*** reported that severe disorganization of rat kidney after acetaminophen injection of 550 mg/kg BW., that represented by endothelial rupture in capsule, damaged glomeruli and the tubules are dilated with loss of cellular boundary. Our results explained by ***Bessems and Vermeulen (2001)*** that reported acetaminophen at therapeutic doses is metabolized via sulfation and glucuronidation reactions occurring primarily in the liver which produce water-soluble metabolites that are excreted via the kidney. There is more severe

GSH depletion as well as massive production of metabolites when large doses of acetaminophen are ingested.

Immuno-histochemical staining by caspase-3 in PA-treated group showing positive brown staining at renal cortex and medulla. This result has been explained by ***Laughlin et al., (1998)*** who found mild degree of tubular cell apoptosis in renal tissues after PA administration, even at therapeutic dosing. These findings are agreed with the chronic long-term toxicity of the drug.

In the current study, light microscopic examination of PA and NAC-treated group, the signs of tubular degeneration were still present as epithelial vacuolization in the tubules, some necrotic cells are seen detaching from the basement membrane and being sloughed into the tubular lumen, the glomeruli maintained better morphology when compared with PA- treated group except widening of Bowman's space. Renal medulla still showed inflammatory cell infiltration in the interstitium and tubular cells showed vacuolation of their cytoplasm with some pyknotic cells in wall of tubule. Hemorrhage in between medullary ducts was also still detected. In immunohistochemical examination of renal cortex and medulla showed moderate protein expression of caspase- 3 when compared with the PA- treated group.

Ferret et al., (2001) reported that NAC as well as melatonin and vitamin E treatments effectively protected the kidney and the liver tissues against oxidative stress by a significant prevention of MDA production and PO probably in part by scavenging the very reactive hydroxyl (OH_) and lipid peroxyl radical (ROO_). Other results reported incomplete recovery in NAC-treated group as in ***Flanagan and Meredith (1991)*** and ***Kozer and Koren (2001)*** studies those reported the fact that NAC can ameliorate in vivo PA hepatic damage but did not inhibit apoptosis of tubular cells.

On the other hand, in PA and PPE treated–group, the cortical tubules in renal cortex retained their normal architecture except some necrotic cells was seen detaching from the basement membrane and being sloughed into the tubular lumen. Thereby that tubular injury was not in fact fully reversed. No evidence of vascular congestions with disappearance of intra-luminal eosinophilic homogenous material was evident. Bowman's space was relatively wide. Renal medulla showed more improvement as the tubular lining retained its normal arrangement but still somewhat of hemorrhage within loops of Henle. Immunohistochemical examination of renal cortex and medulla showed mild protein expression of caspase- 3 when compared with the PA group. ***Cxayır et al. (2011)*** stated that cisplatin and PPE-treated group showed nearly normal archistructure, except for a minor desquamation of the kidney and liver cells. Also, this group showed improvement in biochemical observations and immune-histochemical findings. So that PPE could be used as a dietary supplement in patients receiving chemotherapy medications by the way of its antioxidant, radical-scavenging and anti-apoptotic effects. The previous studies explained these nearly complete recoveries of PPE -treated group as it had antioxidant, hypoglycemic, antiapoptotic, and peroxidative effects on blood and on tissues such as liver, kidney and prostate. Many natural products detoxify the metabolites of drugs (most formed by cytochrome P450-linked monooxygenase), regulate apoptotic cell death, and, mediate trans-membrane transport of organic solutes ***(Tu¨rkez et al., 2010; Akar et al., 2011)***.

In the current work, the group of rats simultaneously treated with PA and PPE and NAC-treated group showed almost normal histology in renal cortex and medulla in comparison to the control rats. There was no detectable proapoptotic caspase-3 staining in immune-histochemical studies. In consistence with our findings, several authors have reported that combination of antioxidants might be very useful in protection of tissues against drugs toxicity. The thymoquinone and curcumin have the ability to decrease lipid peroxidation and potentiating the antioxidant defense system. So that, concurrent administration of them ameliorates gentamicin induced- nephrotoxicity ***(Mahmoud et al., 2014)***.

PA–treated group expressed a statistically significant increase in the level of creatinine and BUN levels as compared with the control group. These results agree with the study of ***Yousef et al. (2010)***. The previous studies have been reported that an overdose of PA causes many metabolic disturbances including an increase in BUN and creatinine ***(Srinivasan et al., 2014)***.

Co-administration of PA and NAC showed an improvement in the BUN and BC levels as compared with PA- treated rats. This is in agreement with ***Şener et al., (2003)*** reported that the antioxidant agents as melatonin, vitamin E or NAC reduced BUN levels and BC levels. PA and

PPE – treated rats showed very highly significant decrease in the BUN and BC levels. These results agree with the study of ***Boroushaki et al., (2013)*** who reported, pomegranate seed oil has protective effect against acute toxicity of diazinon in rat.

This study revealed that PPE has a better ameliorative effect than NAC against toxic effect of PA and co-administration of NAC and PPE together with PA produced more improvement on the level of the BUN and BC levels. As there is non-significant difference between this group and control group.

CONCLUSION

It can be concluded that, PA can produce toxic effects on the renal tissue of adult male albino rats and the use of NAS and PPE especially in a combination form can ameliorate this toxicity for the first time in the research field.

RECOMMENDATIONS

1. PPE, one of the natural products is suggested as a supplement to overcome the toxicity of PA -exposure.
2. Periodical examination of patients exposed to PA in large doses should be done.
3. Further studies on PPE are recommended using large numbers of animals and different doses to confirm its benefits in this respect.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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دور إضافة مستخلص قشر الرمان مع الإن اسيتيل سيستين على التسمم الكلوي المستحدث من الباراسيتامول في ذكور الجرذان البيضاء البالغة

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ملخص البحث

المقدمة: عقار الباراسيتامول هو مسكن واسع الانتشار كما انه خافض للحرارة وجرعته المفرطة التي بامكانها أن تسبب ضرركلوي هى واحدة من الأسباب الأكثر شيوعا في دخول الطوارىء. في هذه الدراسة نبين التأثيرات لمستخلص قشر الرمان على التسمم الكلوي الناتج من الباراسيتامول, والامكانية لإعطاء علاج مكون من إن اسيتيل سيستين و قشر الرمان معا لتحسين التسمم الناتج عن الجرعة الزائدة للباراسيتامول.

المواد و الطـرق المستخدمة فى البحث: خمسون من ذكور الجرذان البيضاء البالغة تم تقسيمهم الى 5 مجموعات كالتالى:-

- المجموعة الاولى (المجموعة الضابطة) : تم إعطائهم فقط مياه الصنبور مع الغذاء المتوازن بالفم لمدة 28 يوم.
- المجموعة الثانية (المعالجة بالباراسيتامول): تم إعطائهم 400 مجم/كجم يوميا من عقار الباراسيتامول بالفم لمدة 28 يوم.
- المجموعة الثالثة (المعالجة بالباراسيتامول مع ان اسيتيل سيستين): فيها كل فأر تم إعطاؤه الباراسيتامول بنفس الجرعة السابقة مع 150 مجم/كجم من ان اسيتيل سيستين يوميا بالفم لمدة 28 يوم.
- المجموعة الرابعة (المعالجة بالباراسيتامول مع مستخلص قشر الرمان): تم إعطائهم الباراسيتامول بنفس الجرعة السابقة مع 430 مجم/كجم من مستخلص قشر الرمان يوميا بالفم لمدة 28 يوم.
- المجموعة الخامسة (المعالجة بالباراسيتامول مع ان اسيتيل سيستين و مستخلص قشر الرمان معا): تم إعطائهم الباراسيتامول مع إن اسيتيل سيستين مع مستخلص قشر الرمان بنفس الجرعات السابقة يوميا بالفم لمدة 28 يوم.

تم أخذ عينات الدم لتحديد مستوى الكرياتينين و اليوريا. ثم التضحية با لجرذان واستخراج الكلى وإعدادها للفحص بالمجهر الضوئى حيث تم صبغها بالهيماتوكسيلين والأيوسين والصبغة الهيستوكيمائية المناعية لإيضاح نشاط إنزيم الكاسبيس 3 (مؤشرا للموت الخلوي). بالإضافة إلى ذلك تم إجراء دراسة إحصائية.

النتائـــج: أحدث الباراسيتامول ارتفاع كبير جدا في مستوى الكرياتينين واليوريا بالدم. أيضا كل تلك التغيرات تم التأكد منها من خلال بالفحص المجهري (باستخدام المجهر الضوئي) لكل من القشرة الكلوية ولب الكلية حيث ان الباراسيتامول أظهر الشعيرات الكبيبية منفصلة ومحتقنة واتساع في محفظة بومان. كما أن سيتوبلازم (الحشوة) القنوات القشرية والنخاعية اظهر فجوات واسعة النطاق والمادة الأيوسينية المتجانسة . النسيج الخلالي به ارتشاح بالخلايا الالتهابية كما أن هناك احتقان في الأوعية الدموية يظهر بوضوح ما بين الأنابيب. علاوة على ذلك فالباراسيتامول أدى الى الموت الخلوي المبرمج في الخلايا الأنبوبية الكلوية والذي أظهر ذلك التفاعل الايجابي لانزيم الكاسبيس 3في الصبغة الهيستوكيميائية المناعية. وفي الوقت نفسه فإن إعطاء إن اسيتيل سيستين ومستخلص قشر الرمان أظهر تأثير وقائي للآثار السامة الناتجة عن الباراسيتامول وخاصة عند استخدامهما معا.

الخلاصـــة: اتضح من نتائج هذه الدراسة أن اعطاء ان اسيتيل سيستين مع مستخلص قشر الرمان له الأثر المحسن على التسمم الناتج عن اعطاء الباراسيتامول في ذكور الجرذان البالغة.