The effect of paracetamol on the kidney tissues of male albino rats and the protective effect of vitamin C

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Abstract: Vitamin C (VC) is a natural antioxidant found in citrus, soft fruits and leafy green vegetables and has shown protective effects against nephrotoxicity damage caused by different drugs. This study aimed to the possible protective effects of VC against renal damage induced by PCM in rats. Forty male rats were divided into five groups: Group (1) as control; receiving distilled water orally, group (2); receiving 500 mg/kg of VC. Group (3); receiving 500 mg/kg of PCM. Group (4) (protective group); receiving VC for 7 days, and then PCM for another 7 days, and group (5) treated with a combination of VC and PCM for 14 days. Renal tissue of PCM rats showed degeneration of renal corpuscles with the widening of Bowman's space, peritubular capillary dilatation and congestion, desquamation renal tubular epithelium with damage to the brush borders of the cell and presence of debris in the tubular lumen, and pyknotic nuclei of tubular cells. However, protective rats showed the nearly normal renal structure of both cortex and medulla, but VC + PCM rats showed some modifications in kidney structures ranging. In conclusion, VC as an antioxidant can protect kidney from PCM induced tissue damage.

Key Words: Histopathology, Paracetamol, Vitamin C, Kidney, Rats.

Introduction:

Paracetamol (PCM), or acetaminophen (N-acetyl-para-amino-phenol), is widely used of all drugs to treat pain and fever (Lorz *et al*. 2004 and Loh and Ponampalam 2006). An acute paracetamol overdose can lead to potentially lethal liver and kidney failure in humans and experimental animals and in severe cases to death (Majeed *et al.,* 2013 and Tittarelli, *et al.,* 2017). The kidney also possesses drug-metabolizing activity. Mixed function oxidases are present in the proximal tubules and can be selectively induced or inhibited by xenobiotics, where the drug-metabolizing enzymes are not evenly distributed along the nephron (Sheweita, 2000). Acute renal failure occurs in less than 2 % of all PCM poisonings and 10 % of severely poisoned patients, which manifest as acute tubular necrosis in the kidney (Boutis and Shannon, 2001). However, the mode of renal cell death during PCM nephrotoxicity and the mechanisms involved are obscure. Indeed, there is evidence that the molecular basis of nephrotoxicity may differ from those of hepatotoxicity, as N-acetyl-cysteine protects from the latter, but has been shown not to protect from nephrotoxicity (Lorz *et al.*, 2004).

Antioxidants protect key cell components from damage by neutralizing the free radicals. Antioxidants that occur naturally in the body or that are consumed through the diet may block damage to cells (Cherubini *et al.,* 2005). Therefore, supplementation of antioxidants can be considered as the alternative method for chelation therapy. Several studies demonstrated that the cellular antioxidant activity is reinforced by the presence of dietary antioxidants (Prior and Cao, 2000 and Al-Eryani *et al.,* 2014). Accordingly, interest has recently grown in the role of

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natural antioxidants used as a strategy to prevent oxidative damage as a factor in the pathophysiology of various health disorders (Kaniz *et al.,* 2012). Among antioxidants, vitamin C (VC) has the ability to counteract free radicals and protect the structure and function of proteins, DNA, and chromosomes against oxidation injury and they are the most powerful in reducing storage and toxicity of reactive oxygen species (Al-Eryani *et al.*, 2014). Vitamin C (ascorbic acid) is a water-soluble micronutrient required for multiple biological functions (Halliwell, 2001 and Adeneye and Olagunju, 2009). It is found intra- and extracellularly as ascorbate, and is well absorbed from the gastrointestinal tract (Woollard *et al.*, 2002 and Asiley *et al.*, 2004). VC may prevent certain types of hepatic cellular damage (Johnson *et al.,* 2015). VC is a natural antioxidant found in citrus, soft fruits, and leafy green vegetables (Stangeland *et al.,* 2008). It prevents the increased production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues (Johnson *et al.,* 2015). VC is hydrophilic and exerts its antioxidant action by inhibiting lipid peroxidation and oxidative cell damage (Xavier *et al.,* 2007).

Therefore, there is a growing need for exogenous sources of antioxidants such as vitamins, which have various biological activities. In addition, to perform many investigations regarding the nephrotoxicity induced by different drugs and the possible nephroprotective effects of therapeutic strategies from the alternative or complementary medicine. Therefore, the present study examined the possible protective effects of VC on PCM-induced renal oxidative insult in rats.

Material and Methods:

Chemicals:

1-VC (ascorbic acid) ($C_6H_8O_6$) (500 mg) was obtained from the local pharmacy.

2- PCM ($C_8H_9NO_2$) (acetaminophen or N-acetyl-p-amino-phenol) (500 mg) was obtained from local pharmacy.

Experimental animals:

Healthy male albino rats (*Rattus norvegicus*) with an average weight of 200-250 g obtained from the Central Animal House, College of Veterinary, University of Omar Al-Mokhtar, El-Beida, Libya were used in this study. All animals were quarantined two weeks per-experimentation period to acclimatize to laboratory conditions in order to avoid any complications along the course of the experiment. They were housed in cages at room temperature. Rats were fed with laboratory diet and water ad libitum with fresh daily supplies.

Experimental groups:

Forty male rats were distributed randomly into five groups (8 rats in each):

Group **(1):** Normal control group (NC), animals were given distilled water orally by gavage for 14 days.

Group **(2):** VC treated group (VC), animals were given VC (500 mg/kg/b.w) according to Adeneye and Olagunju, (2008) orally by gavage for 14 days.

Group **(3):** PCM treated group (PCM), animals were given PCM (500 mg/kg/b.w.) according to Modo *et al.,* (2015) orally by gavage for 14 days.

Group **(4):** Protective group (PRO), animals were given VC (500 mg/kg/b.w.) for 7 days then given PCM (500 mg/kg/b.w.) orally by gavage for 7 days.

Group **(5):** VC and PCM treated group (VC+PCM), animals were given VC (500 mg/kg/b.w.) before administration of PCM (500 mg/kg/b.w.) orally by gavage for 14 days.

All rats were received treatments six days a week (Khalaf and Mostafa, 2012). At the end of the experimentation and 24 hours after the last dose, all animals were sacrificed, and then the kidney were removed.

Histopathological study:

Kidney tissues specimens from all groups were fixed in formalin and then were embedded in paraffin. Sections of 5µm thickness were stained with hematoxylin and eosin using standard procedures (Lillie, 1954).The stained sections were examined under light microscope.

Changes in the experimental histopathologic parameters for kidney tissues were graded as follows: (-) showing no changes, (+) (++), and (+++) indicating minimum, moderate, and maximum changes, respectively (Aydin *et al.,* 2003 and Moshaie-Nezhad *et al.,* 2021).

Results:

Histopathological examination of kidney tissues:

Microscopically, the renal section showed normal histological structure in both cortex and medulla. Normal histological appearance of the glomerular basement membrane, mesangial cellularity, and matrix was within normal limits with no evidence of shrinkage or swelling. The tubules that were lined by cuboidal epithelial cells had a normal luminous appearance (Figures. 1 and 2). The histological structure of kidney tissue of rats treated with VC alone revealed a normal appearance of cortex with intact renal corpuscles and normal histological architecture of surrounding proximal and distal convoluted tubules, and normal medulla (Figures. 3 and 4).

The histological examination with the light microscope showed more pronounced histopathological changes in animals that were treated with PCM alone when compared with the control. Degeneration of renal corpuscles with the widening of Bowman's space, peritubular capillary dilatation and congestion, desquamation renal tubular epithelium with damage to the brush borders of the cell, and presence of debris in the tubular lumen, and pyknotic nuclei of tubular cells (Figure. 5). Moreover, marked vacuolar degeneration and necrosis of tubular epithelium together with an increased mesangial matrix and hyalinization of glomerular tuft and tubular basement membrane with severe hemorrhage within interstitial tissue were appeared (Figures. 6 and 7). On the other hand, inflammatory cell infiltration with severe hemorrhage in the medulla tubules (Figure. 8) was also reported. The kidney tissue of rats treated with VC for seven days followed by PCM for seven extra days showed the nearly normal renal structure of both cortex and medulla. Improvement in histological appearance of the renal corpuscle, decreases glomerular capillary congestion. Few damage in renal tubular, and less cytoplasmic vacuolation of tubular lining cells were observed (Figures. 9 and 10).

Microscopically, the kidney of rats treated with VC + PCM for 14 days showed some modifications in kidney structures, ranging from shrinkage of the glomeruli and widening of the capsular space, marked vacuolar degeneration and necrosis of tubular epithelium together with an increased mesangial matrix and tubular basement membrane with degenerative changes in the tubules and glomerulus with glomerular capillary congestion. Hemorrhage interstitial tissue and pyknotic nuclei of tubular were observed (Figure. 11). Besides that, the mild tubular epithelial cells degeneration and tubular basement membrane in the medulla was performed (Figure. 12).

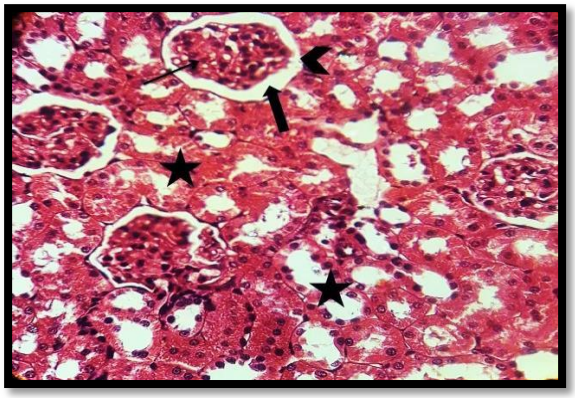

Figure 1: Photomicrograph of the kidney section of control rats showing, normal architectural of renal cortex, normal renal tubules (star), Bowman's capsule (head arrow), Bowman's space (thick arrow) and glomeruli (thin arrow) (H & E stain, X400).

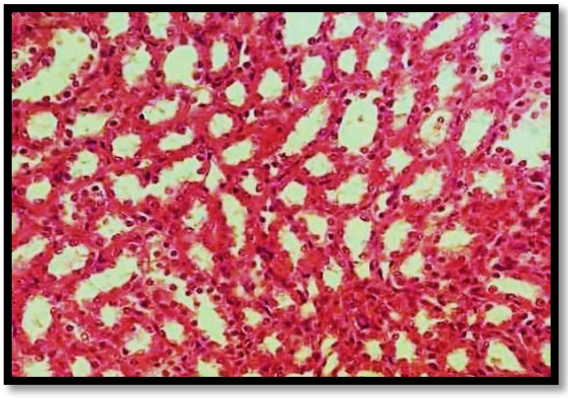

Figure 2: Photomicrograph of the kidney section of control rats showing, normal architectural of renal medulla, collecting tubule is lined with low cubical cells having rounded central nuclei (H & E stain, X400).

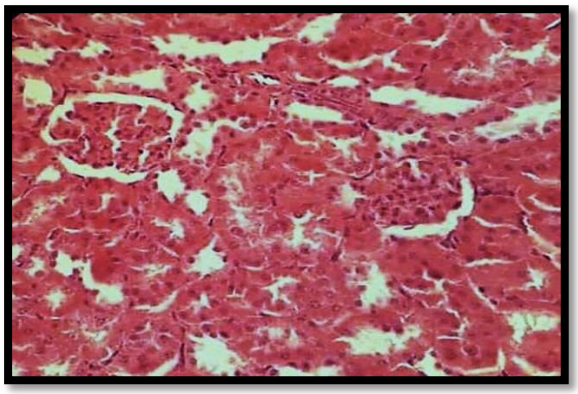

Figure 3: Photomicrograph of the kidney section of treated rats by VC for 14 days showing, normal appearance of cortex with intact renal corpuscles and normal histological architecture of surrounding proximal and distal convoluted tubules (H & E stain, X400).

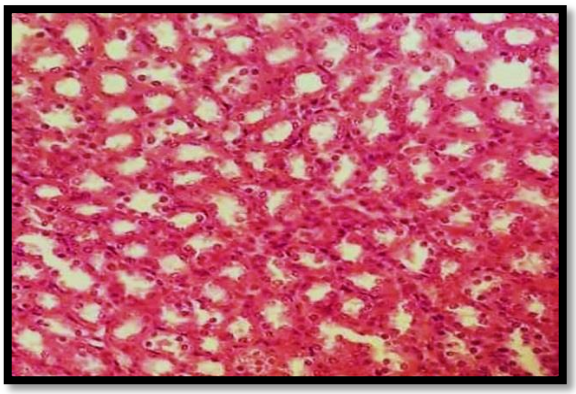

Figure 4: Photomicrograph of the kidney section of treated rats by VC for 14 days showing, normal architectural of renal medulla, normal collecting tubule structure (H & E stain, X400).

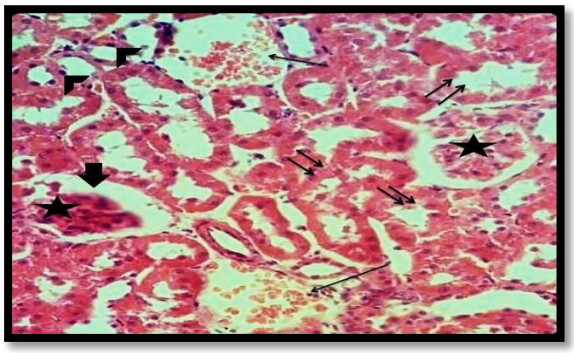

Figure 5: Photomicrograph of the kidney section of treated rats by PCM for 14 days showing, degeneration of renal corpuscles (stars), with widening of Bowman's space (thick arrow),

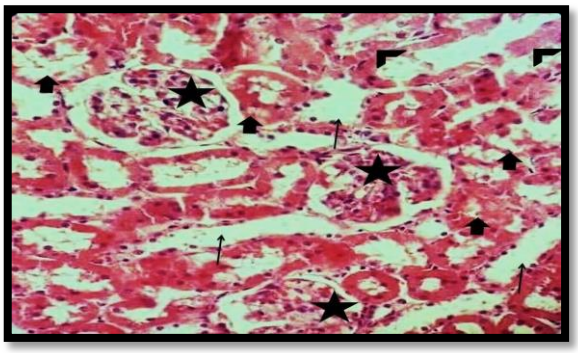

Figure 6: Photomicrograph of the kidney section of treated rats by PCM for 14 days showing, degeneration of renal corpuscles (stars), marked vacuolar degeneration and necrosis of tubular

peritubular capillary dilatation and congestion (long arrows), desquamation renal tubular epithelium with damage to the brush borders of the cell and prescence of debries in tubular lumen (double arrows), and pyknotic nuclei of tubular cells (head arrows) (H & E st ain, X400).

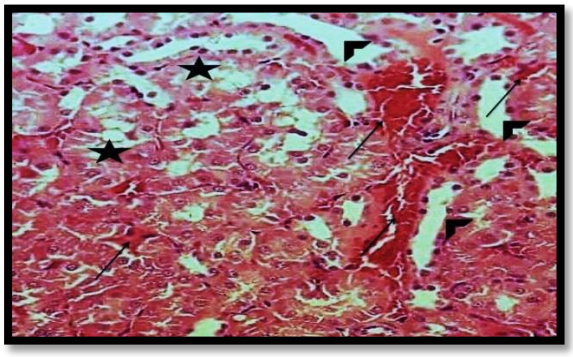

Figure 7: Photomicrograph of the kidney section of treated rats by PCM for 14 days showing, degeneration of renal corpuscles (stars), marked vacuolar degeneration and necrosis of tubular epithelium (stars), desquamation renal tubular epithelium with damage to the brush borders of the cell (head arrows), sever hemorrhage within interstitial tissue (long arrows) (H & E stain, X400).

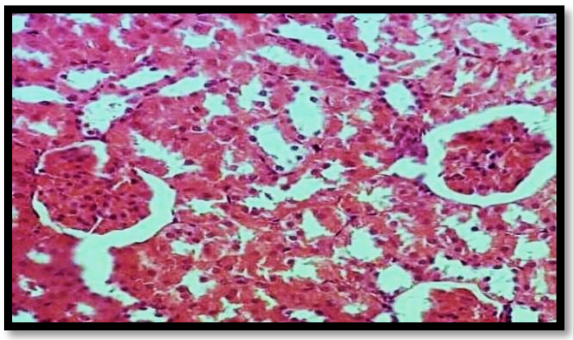

Figure 9: Photomicrograph of the kidney section of PRO rats showing, improvement of histological structure in the cortex. Improvement in histological appearance of renal corpuscle, some damage of renal tubular and less cytoplasmic vacuolation of tubular lining cells (H & E stain, X400).

epithelium (short arrows), hyalinization of tubular basement membrane (thin arrows), and desquamation renal tubular epithelium with damage to the brush borders of the cell (head arrows) (H & E s tain, X400).

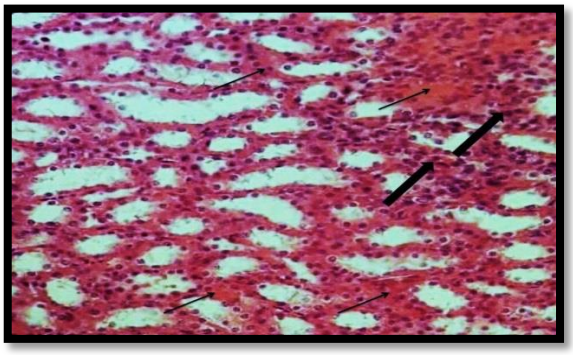

Figure 8: Photomicrograph of the kidney section of treated rats by PCM for 14 days showing, inflammatory cell infiltration (thick arrows), and sever hemorrhage in medulla tubules (thin arrows) (H & E stain, X400).

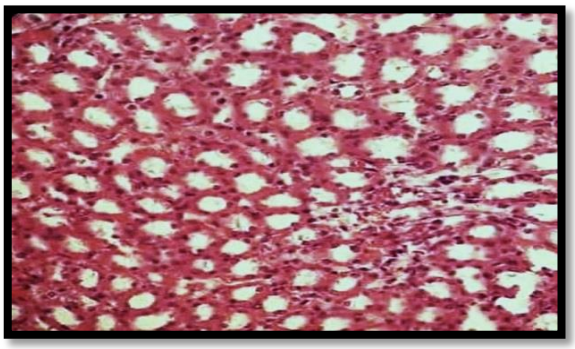

Figure 10: Photomicrograph of the kidney section of PRO rats showing, nearly normal renal collecting tubular of the medulla (H & E stain, X400).

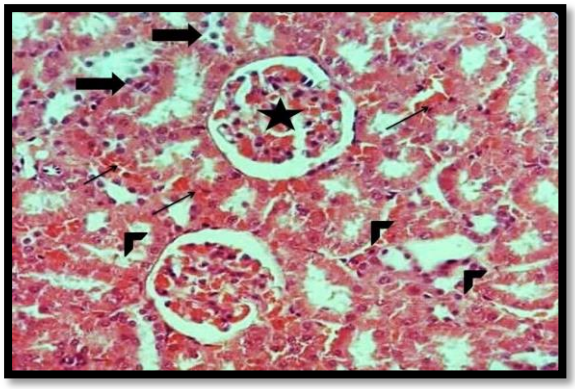

Figure 11: Photomicrograph of the kidney section of treated rats by VC+PCM for 14 days showing, degenerative changes in the tubules (head arrows), degeneration of glomerulus with glomerular capillary congestion (star), hemorrhage interstitial tissue (thin arrows), and pyknotic nuclei of tubular (thick arrows) (H & E stain, X400).

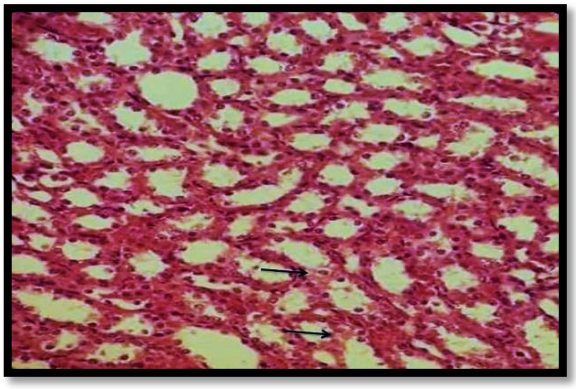

Figure 12: Photomicrograph of the kidney section of treated rats by VC+PCM for 14 days showing, mild tubular epithelial cells degeneration and tubular basement membrane in the medulla (arrows) (H & E stain, X400).

Histopathologic changes in the kidney tissues:

The changes in the histologic structure of the kidney tissues were graded in Table. 1, showing reduction in the inflammatory cells infiltration, congestion, dilation, degeneration, necrosis, and hemorrhage in the kidney tissues in the protective group when compared with the PCM group.

Table 1: Histopathologic changes in the kidney tissues.

Histopathologic changes in kidney tissues	NG	VC	PCM	PRO	VC+PCM
Inflammatory cell infiltration	-	-	+++	-	-
Congestion	-	-	+++	+	+
Dilation	-	-	+++	-	+
Degeneration	-	-	+++	+	++
Necrosis	-	-	+++	-	+
Hemorrhage	-	-	+++	-	++

**(-) showing no changes, (+) (++), and (+++) indicating minimum, moderate, and maximum changes.

* NC = Normal control. PCM= Paracetamol treated group. VC= Vitamin C treated group. PRO =Protective group. VC+PCM=Vitamin C + Paracetamol.

Discussion:

Drugs induced toxicity was reported to be mediated by increased production of reactive oxygen species (ROS) and free radicals. These ROS could interfere with the antioxidant defense system and can cause extensive tissue damage and cell dysfunction by reacting with macromolecules like proteins, membrane lipids and nucleic acids. However, oxidative stress could be a consequence of increased ROS generation and/or decreased antioxidant defense (Farooqui *et al.*, 2016).

The present work showed more pronounced histopathological alterations in treated rats with PCM alone when compared with the control such as degeneration of renal corpuscles. Widening of Bowman's space, peritubular capillary dilatation, congestion, desquamation renal tubular epithelium with damage to the brush borders of the cell, and presence of debris in the tubular lumen were reported. Pyknotic nuclei of tubular cells, marked vacuolar degeneration and necrosis of tubular epithelium together with an increased mesangial matrix and hyalinization of glomerular tuft and tubular basement membrane with severe hemorrhage within interstitial tissue and inflammatory cell infiltration with severe hemorrhage in the medulla tubules. These findings were supported by the findings of Moshaie-Nezhad *et al.* (2021); Al-Asmari *et al.* (2020); Abdullah *et al.* (2017); and Sabiu *et al.* (2016). They were suggested that PCM caused congestion, hypercellularity in Bowman's capsule indicating inflammatory cell infiltration, degeneration concomitant with vacuolization, and exhibited severe tissue damage in the form of focal tubular degeneration for both distal and proximal convoluted tubules with tubular dilation as well as hemorrhage in the glomeruli with glomerular atrophy. These changes may be in response to nephrotoxic by drugs as proximal convoluted tubules are the most common site of toxicant-induced renal injury. The reason for this relates in part to the selective accumulation of xenobiotics into this segment of the nephron.

Maintenance of tubular integrity depends on the cell to cell and cell to matrix adhesions, after a chemical insult adhesion of nonlethally damaged cells to the basement membrane is compromised, leading to their detachment from the basement membrane, and later on, it may lead to sloughing of cells and formation of intratubular casts. Loss of brush border in proximal convoluted tubules can result from toxicant-induced alterations in membrane integrity and cytoskeleton component (Rekha *et al.,* 2013). The significant alterations in glomerular structure, degeneration of renal corpuscles with the widening of Bowman's space, basal in folding patterns with the presence of cytoplasmic vacuolation, and degeneration of the tubules observed in the kidney sections of PCM intoxicated rats could be responsible for their impaired renal function. The consequently decreased glomerular filtration rate with associated elevated serum levels of urea and creatinine was evident in the present study and agreed with a previous report (Sabiu *et al.,* 2016).

Hasan *et al.* (2016) found that the damage due to excessive production of NAPQI and another metabolite p-aminophenol (PAP). PAP after oxidation converted to p-aminophenoxy free radical and subsequently 1,4-benzoquinoneimine, which can covalently bind to renal tissue macromolecules. However, Odigie *et al.* (2015) established that a single nonlethal amount of PCM dosage for rats produces high concentrations of microsomal cytochrome P450 in their kidneys, which develop severe tubular necrosis. These may be due to PCM toxicity that caused glutathione depletion or increased activity of P450 microsomal oxidase enzymes.

In this study infiltration of inflammatory cells was observed in the inter-stitium of kidney of PCM group. This infiltration of lymphocytes was suggestive of interstitial nephritis and may be attributed to hypersensitivity after exposure to toxic drug (Rekha *et al.,* 2013). On the other hand, hypercellularity in glomeruli was suggestive of proliferative glomerular nephritis, which may be due to proliferation of endothelial cells, mesangial cells, or infiltration of inflammatory cells (Kumar *et al.,* 2012). Additionally, tubular epithelial swelling may attribute to the disruption of cell volume and ion homeostasis by toxicants. Thus increasing ion permeability and inhibiting energy production, resulting in ATP depletion. Following ATP depletion Na^+, K^+, and ATP activity decreases resulting in K^+ efflux, Na^+ and Ca^{++} influx, cell swelling, and ultimately cell membrane rupture. This influx may be a trigger for cell swelling and cell death (Rekha *et al.,* 2013).

Another elucidation of nephrotoxicity induced by drugs, is the activation of the inflammatory process shown by elevated pro-inflammatory cytokine renal tumor necrosis factor-α (TNF-α) level. This was proved that some chemicals could upshot ROS generation, induce the protein kinase B (PKB), nuclear factor-kappa B (NF-kB), and mitogen-Activated protein kinase (MAPK) pathways beside elevation of cytokines, including TNF-α and interleukin-1α (IL-1α) levels (Bashandy *et al.*, 2016). In general, most of the tubular and glomerular damages occur during the reperfusion phase following ischemia, and generation of ROS. Reactive oxygen species are capable of reacting with lipids, proteins and nucleic acids leading to lipid peroxidation, impairments of enzymatic processes, and DNA damages. The accumulation of ROS, and reduction in antioxidant enzyme activities lead to damage in cellular components such as lipids and proteins (Caskurlu *et al.*, 2016).

Nevertheless, the kidney tissue of rats treated with VC for 7 days then given PCM for 7 days showed decrease glomerular capillary congestion, few damage in renal tubules, and less cytoplasmic vacuolation of tubular lining cells. Similar results were obtained by Tsvetkova *et al.* (2012); Dortaj *et al.* (2017) and Hassan *et al.* (2020) detected that the VC administered orally produced some defensive effects overall in kidney tissues but with tubular dilation, glomerular degeneration. Adeneye and Olagunju (2009) and Dortaj *et al.* (2017) informed that the VC as an antioxidant agent can prevent chain reactions of free radicals or the reactive oxygen species before reaching their renal targets. So, an antioxidant is able to reduce the incidence of fragmentation and subsequent rearrangement induced by drugs. Where VC's effects on protection in kidneys by the prevention of free radicals generation and/or free-radical cleaning activity. VC as an electron donor to protect the body from radicals and can be water-soluble or lipid-soluble, therefore found in both lipid and water portion of cells, protecting vital biomolecules such as proteins and DNA from being oxidized by the free radicals and ROS (Waheed *et al.*, 2011).

Kidney of rats treated with VC + PCM for 14 days showed some modifications in kidney structures ranging from shrinkage of the glomeruli and widening of the capsular space. Marked vacuolar degeneration and necrosis of tubular epithelium together with an increased mesangial matrix and tubular basement membrane, degenerative changes in the tubules and glomerulus with glomerular capillary congestion, hemorrhage interstitial tissue, and pyknotic nuclei of tubular.

Histopathologic assessments of the experimental parameters consistent with histological findings of the kidney tissues were showed that the VC to be protective against PCM-induced renal damage.

Conclusion:

The present findings clearly demonstrate that paracetamol is capable of inducing histopathological changes in the kidney tissues of the albino male rats. Besides, that VC has a protective effect against nephrotoxicity PCM-induced in male rats. In the near future, VC may found useful as prophylactic agent against drug-induced renal damage.

المستخلص: فيتامين ج هو أحد مضادات الأكسدة الطبيعية الموجودة في الحمضيات والفواكه اللينة والخضروات ذات الأوراق الخضراء وقد أظهر آثارًا وقائية ضد السمية الكلوية التي تسببها الأدوية المختلفة. تهدف الدراسة إلى معرفة التأثير الوقائي المحتمل لفيتامين ج ضد التلف الكلوي الناجم عن الباراسيتامول في الجرذان. 40 من ذكور الجرذان تم تقسيمها إلى خمس مجموعات: المجموعة (1) كمجموعة ضابطة. تلقت الحيوانات الماء المقطر عن طريق الفم، المجموعة (2)؛ تلقت 500 مجم / كجم من فيتامين ج. المجموعة (3)؛ تلقت الجرذان 500 مجم / كجم من الباراسيتامول. المجموعة (4) (مجموعة الحماية)؛ تلقت فيتامين ج لمدة 7 أيام، ثم أعطيت الباراسيتامول لمدة 7 أيام أخرى، عوملت المجموعة (5) بمزيج من فيتامين ج والباراسيتامول لمدة 14 يومًا. أظهرت الأنسجة الكلوية للجرذان المعاملة بالباراسيتامول تنكسًا في الكريات الكلوية مع اتساع مساحة محفظة بومان،

وتوسع الشعيرات الدموية حول الأنبوب، واحتقانها، وتقشر الظهارة الأنبوبية الكلوية مع تلف حدود الفرشاة للخلية ووجود حطام في التجويف الأنبوبي. واظهرت الجرذان في مجموعة الحماية البنية الكلوية الطبيعية تقريبًا لكل من القشرة والنخاع، بينما أظهرت الجرذان المعاملة بفيتامين ج مع الباراسيتامول بعض التغيرات في هيكل الكلية. في الختام، أظهر فيتامين ج كمضاد للأكسدة حماية للكلية من تلف الأنسجة الناجم عن الباراسيتامول.

الكلمات المفتاحية: الأنسجة المرضية ، الباراسيتامول ، فيتامين ج ، الكلية ، الجرذان.

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