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Effects of Methotrexate and Vitamin C on Renal Cortex of Rats

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ABSTRACT

Methotrexate, a well-known cytotoxic chemotherapy, anti-folate, immunosuppressant, effectively treats all major disorders like cancer, psoriasis, refractory rheumatoid arthritis, etc. Abnormal production of reactive oxygen species has been suspected in the pathophysiology of methotrexate-induced renal toxicity. Vitamin C is a water soluble antioxidant that can play a protective role in models of experimentally induced nephropathies. This study aimed to clarify the protective role of vitamin C on the histological and biochemical changes induced by methotrexate in renal cortex. Twenty adult rats were divided into four equal groups. In group I, the rats received a balanced diet for 9 days; in group II, the rats received 250 mg/kg B.w of vitamin C for 9 days by oral gavage dissolved in distilled water; in group III, the rats received a single dose of 20 mg/kg B.w of methotrexate intraperitoneally on the third day of the experiment; in group IV, the rats received a single dose of 20 mg/kg B.w of methotrexate intraperitoneally on the third day of the experiment and 250 mg/kg B.w of vitamin C by oral gavage 2 days before, and 6 days after methotrexate administration. Results: methotrexate-induced renal injury in rats was evidenced biochemically by the significant increase in serum levels of creatinine and urea as well as histopathological alterations. Furthermore, methotrexate administration caused apoptosis by increasing activity of caspase-3. Furthermore, vitamin C reduced renal oxidative stress, and prevented the alterations in renal morphology. Conclusion: methotrexate produces marked degenerative changes in the histological structure of the kidney, and vitamin C is a potent antioxidant agent in preventing kidney injury.

Key words: *Kidney, Methotrexate, Vitamin C, Apoptosis, Histopathology.*

INTRODUCTION

Methotrexate (MTX) is an antifolate drug, and is commonly used in the treatment of malignancy, autoimmune diseases, inflammatory diseases and gestational trophoblast diseases. MTX is also used in inducing abortion, both for the voluntary termination of pregnancy and for the medical management of ectopic pregnancy [1]. More than 90% of a dose of MTX is renally excreted by filtration and active tubular secretion as unchanged drugs or metabolites. An impaired renal function by MTX delays its own excretion. Resulting sustained and elevated plasma concentration causes a marked enhancement of MTX's other toxicities. So, a nephroprotective agent is mandatory for the safe use of this important drug [2]. MTX indirectly prevents protein synthesis and purine bases essential for the synthesis of RNA, DNA and adenosine triphosphate, which leads to the cell death due to a difficult cell regeneration [3].

Clinical use of MTX has been limited due to its side effects that include nephrotoxicity, bone marrow suppression, hepatotoxicity, pulmonary fibrosis and gastrointestinal mucosal damage [4]. MTX-induced nephrotoxicity is one of the most serious side effects of MTX treatment. MTX–induced nephrotoxicity may occur in two general ways. One of them is that MTX and its metabolite 7-hydroxymethotrexate directly lead to toxic effects on tubules. MTX and its metabolites may precipitate in the intratubular area and lead to renal tubular necrosis. MTX can cause renal toxicity with increased serum creatinine levels, blood urea nitrogen and hematuria in both human and animal models. The other mechanism is that, MTX may cause oxidative damage disturbing the balance of oxidant–antioxidant status [3].

The role of vitamin D as well as vitamine C on autoimmune disorders has been investigated by several researchers recently [5]. Despite the poor bioavailability [6], Vitamin C (Vit.C) is a well-known water-soluble antioxidant that scavenges free radicals and other reactive oxygen and nitrogen species that are produced during normal metabolism, by active immune cells, and through exposure to toxins. Also, it inhibits the formation of cytotoxic low density lipoprotein (LDL), on exposure to reactive oxygen species, due to their free radical activity [7, 8]. Vit.C has an inhibitory effect on the expression of pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor alpha (TNF-alpha) in adults' whole blood cells in vitro [9]. And its influential effect on cancer treatment as a disease the burden of which is increasing at tremendous rate worldwide, is noteworthy [10].

MATERIALS & METHODS

Chemicals:

- Methotrexate (MTX) (25 mg/1 mL injectable solution), was purchased from Mylan pharmaceutical company USA.
- Vitamin C **(**Vit.C) was obtained in the form of powder, (L-ascorbic acid) from El Gomhoria Company for Chemical and Medical Trading, Zagazig, Egypt.

Experimental animals:

This study was conducted on 20 adult male albino rats each weighing 200-250 gm. They were obtained from the animal house of the Faculty of Medicine, Zagazig University, and kept under controlled laboratory conditions at 23±2°C, and provided with standard rodent diet and water. They were accommodated to the laboratory conditions for 15 days before being experimented, and were weighed by a digital balance. All the experimental procedures were performed in accordance with the guidelines of Institutional Animal Care and Use Committee, and approved by the Faculty of Medicine; Zagazig University (the protocol approval number: 4046).

Experimental design:

The rats were divided into 4 groups as follows:

Group I: (negative control group), formed of 5 rats, which received a balanced diet for 9 days to measure basic parameters.

Group II: (Positive control group), formed of 5 rats, which received 250 mg/kg B.w of Vit.C for 9 days by oral gavage dissolved in distilled water [11].

Group III: (MTX treated group), formed of 5 rats, which received a single dose of 20 mg/kg B.w of MTX intraperitoneally on the third day of the experiment (9 days) [3].

Group IV: (MTX and Vit.C treated group), formed of 5 rats, which received a single dose of 20 mg/kg B.w of MTX intraperitoneally on the third day of the experiment, and 250 mg/kg B.w of Vit.C by oral gavage 2 days before and 6 days after MTX administration.

By the end of the experiment, the animals were weighed again by the same digital balance, the blood samples were collected for biochemical study (urea and creatinine), and all the animals were anesthetized by ether inhalation. Kidney specimens were processed for light microscope examination using H&E, P.A.S and immunohistochemical stains. In addition, a statistical analysis was performed.

Body weight measurements

Before and at the end of the experiment, the rats of all groups were weighed by a digital electrical balance (Sartorius Goetting type 140/AG, W. Germany).

Biochemical studies

Venous blood samples were obtained from animals by means of capillary glass tubes from the retro-bulbar plexus under light ether anesthesia as described by [12]. About 2 ml of blood was allowed to perculate into a centrifuge tube, and incubated at 37ºC until blood clotted then centrifuged to separate the serum. The samples were maintained at (-20°C) to be used for estimation of creatinine and urea in the serum using a commercially available spectrophotometric enzymatic kit (Thermo Trace BECGMAN, Germany).

Histo-pathological studies

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% formol saline for 48 hours to be processed and embedded in paraffin [13] for:

a) Haematoxylin and eosin stain (H&E).
b) Periodic acid Schiff (P.A.S) reaction.

c) Immuno-histochemical stain for Caspase 3 [14, 15] following the standard avidin-biotin peroxidase method for detection of caspase -3 expression. Reagents used: the primary antibody which was a rabbit monoclonal antibody of IgG type was carried out for localization of caspase 3 (apoptosis marker) in paraffin sections. The kits were delivered from Lab Vision Laboratories (Cat. #: 1475-1). The universal kits used the avidin-biotin peroxidase system which was produced by NeoMarkers Labaratories. The positive results were indicated by brown coloration of antigen-containing cells.

Statistical analysis:

The collected data were computerized, and statistically analyzed using SPSS program (Statistical Package for Social Science) version 18.0. One-way analysis of variance (ANOVA) was used; followed by the least significant difference (LSD). The probability values (P) less than 0.05 were considered significant and highly significant when the P values were less than 0.001.

RESULTS

Histopathological

Results of H&E stain

Both controls (negative and positive) revealed the similar histological structure of the renal cortex, so they were considered as one group. H&E of stained sections of adult male rat kidney of all the control groups (negative and positive) showed that the renal cortex was surrounded by the thin renal capsule. The renal cortex was formed of renal corpuscles and tubules. Each renal corpuscle was consisted of a glomerulus containing a tuft of capillaries. The renal corpuscle was surrounded by visceral and parietal layers of Bowman's capsule which were separated by Bowman's space. The outer parietal layer was formed of flat cells, while the inner visceral layer was closely related to the glomerular capillaries. The cortical renal tubules were formed mainly of proximal and distal convoluted tubules. Distal convoluted tubules had wider lumen than proximal convoluted tubules. They were lined by small cuboidal cells with eosinophilic cytoplasm. Proximal convoluted tubules had narrow lumen, and were lined by cuboidal cells with more eosinophilic cytoplasm (Fig. 1 A, B).

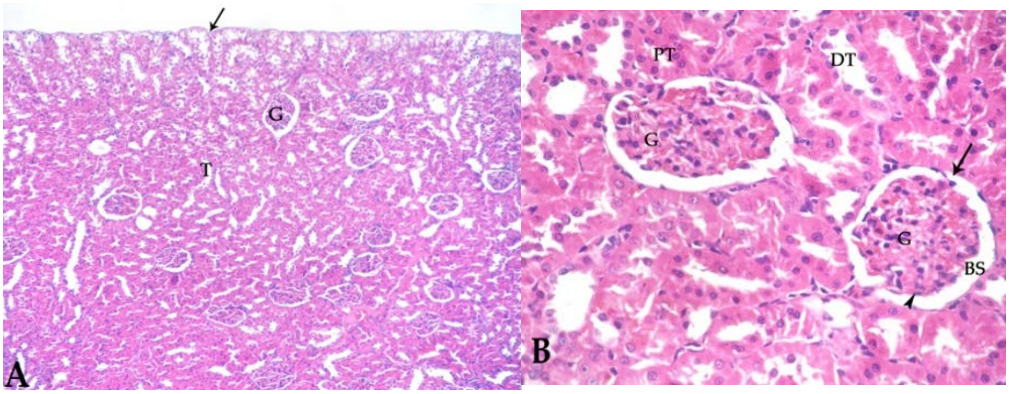
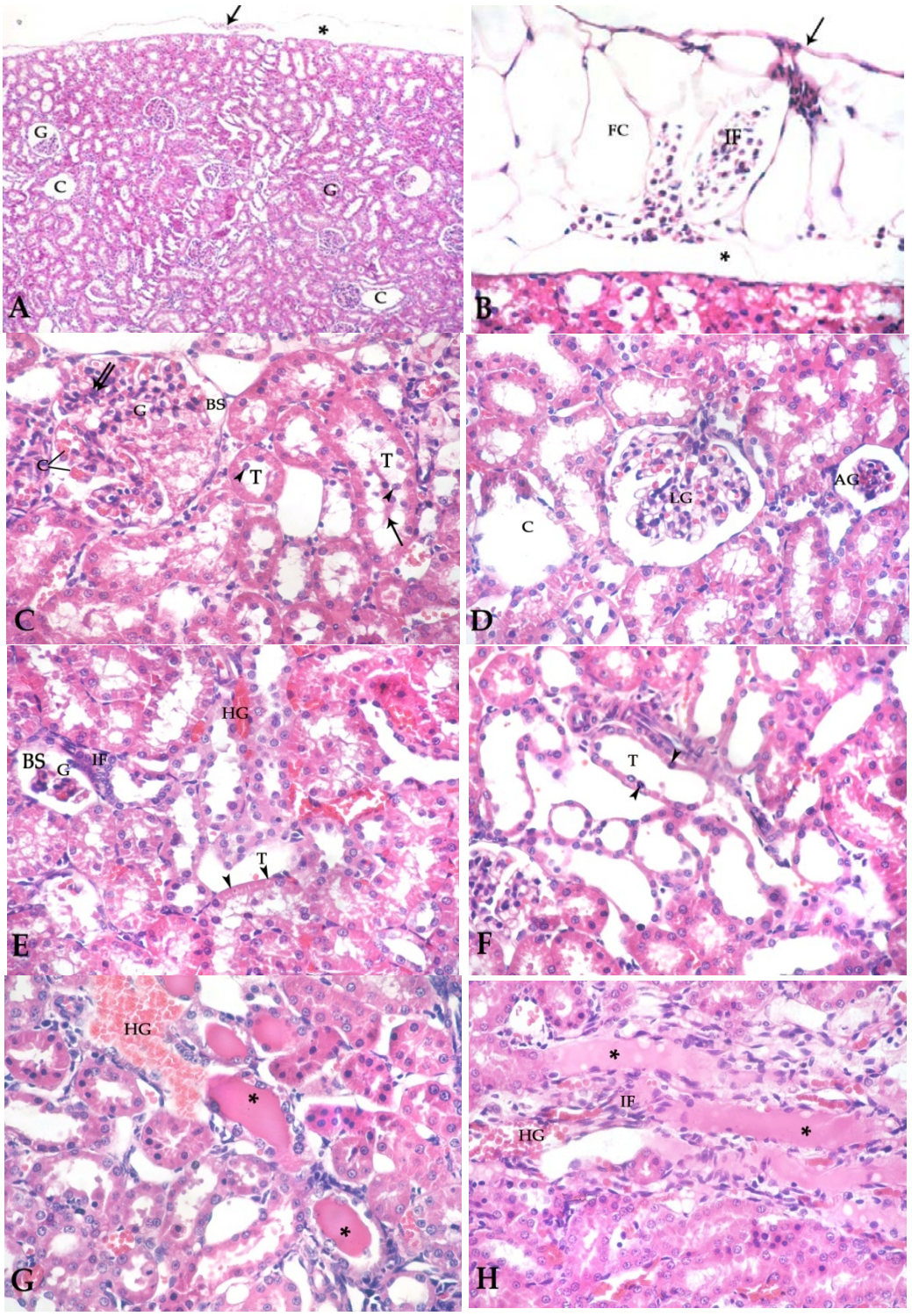


Figure 1. photomicrographs of the section of adult male albino rat kidney of the control group. (**A**) shows the renal cortex surrounded by a thin renal capsule (arrow) and formed of renal glomeruli (G) and cortical convoluted tubules (T) (H&E X 100). **(B)** shows that the glomeruli (G) are surrounded by visceral (arrow head) and parietal (arrow) layers of Bowman's capsule and separated by Bowman's space (B.S). Proximal convoluted tubules with a narrow lumen (PT) and distal (DT) convoluted tubules with wide lumen are also seen (H&EX400).

The 3rdgroup (MTX treated group) showed a massive destructive damage of renal cortex, and thick renal capsule with a fatty cell infiltration and cystic luminal dilatation in some tubules. The capsule was separated from the renal cortex by wide space. Some glomeruli were observed hypertrophied with narrow or greatly obliterated Bowman's space. In some areas, there were shrunken and degenerated glomeruli with dilated Bowman's spaces. However, some glomeruli were lobulated with congested glomerular capillaries, and peri glomerular inflammatory cell infiltrations as well (Fig. 2 A-E). Disorganized dilated tubules with darkly stained nuclei, and the deposition of homogenous acidophilic material in their lumen were also observed (Fig. 2 F, G). There was a loss of architecture of the renal interstitium with the presence of an area of hemorrhage and inflammatory cell infiltration between the renal tubules and massive homogenous acidophilic material (Fig. 2 H). Bundle of collagen fibers could be noticed

(Fig.2I). The blood vessels congested with markedly thickened wall with inflammatory cells around them, were also observed (Fig. 2J).



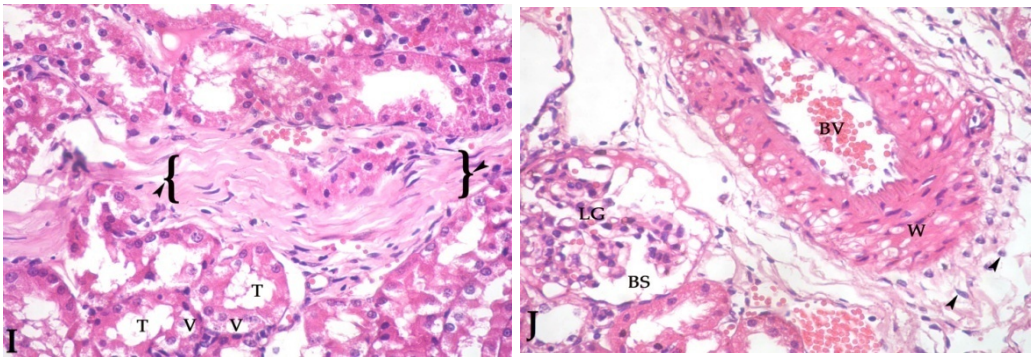
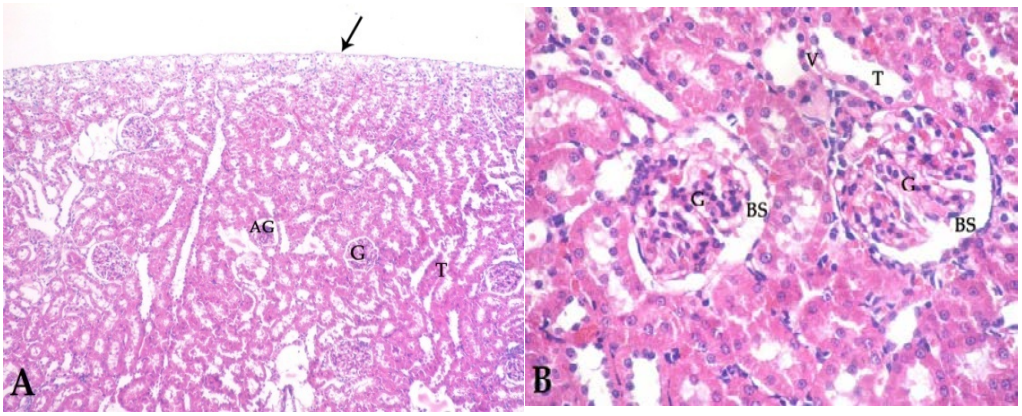


Figure 2. The photomicrographs of the section of adult male albino rat kidney treated with MTX. **(A)** shows the renal capsule (arrows) appeared separated from the renal cortex by wide space (*). Cystic luminal dilatation in some tubules (C). Atrophic renal glomeruli (AG) were observed (H&E X 100). **(B)** shows the thick renal capsule (arrows) with fatty cell infiltration (FC) and inflammatory cell infiltrations (IF). The capsule appeared separated from the renal cortex by wide space (*). **(C)** shows hypertrophied glomerulus (G) with narrow Bowman's space (B.s), glomerular hypercellularity (double arrows) and congested glomerular capillaries (C) can be seen. Some cortical convoluted tubules (T) having cellular debris (arrow) containing pyknotic nuclei (arrowheads) in their lumens. **(D)** shows the different stages of glomerulus: atropic glomerulus (AG) and lobulated glomerulus (LG). Notice cystic luminal dilatation in some tubules (C) which was demonstrated. **(E)** shows atrophic glomerulus(AG) with widening of Bowman's space (B.S), Peri glomerular inflammatory cell infiltrations (IF) could be noticed. Disorganized tubules (T) with desquamation in their epithelial lining (arrowheads) were surrounded by interstitial hemorrhage (HG). **(F)** shows disorganized dilated tubules (T) with darkly stained nuclei (arrowheads). (**G**) shows some renal cortical convoluted tubules containing homogenous acidophilic material (*) in their lumens, and the area of hemorrhage (HG) was also observed. **(H)** shows the loss of architecture of the renal interstitium with the presence of massive homogenous acidophilic material (*), inflammatory cell infiltration (IF) and the areas of hemorrhage (HG). **(I)** shows a bundle of collagen fibers (arrowheads). The convoluted tubules (T) displayed cytoplasmic vacuolation (V). **(J)** shows a congested blood vessel (BV) with markedly thick wall (W). Inflammatory cells (arrowheads) around the blood vessel were also observed. Notice lobulated glomerulus (G) with dilated Bowman's space (B.S) was demonstrated (H&E X 400)

The examination of H&E stained kidney sections of the 4th group (MTX & Vit C treated group) showed a variable degree of improvement when compared to that of MTX treated group. The renal cortex was surrounded by a thin renal capsule, and apparently normal glomeruli were surrounded by visceral and parietal layers of Bowman's capsule with nearly normal Bowman's space. Some cortical convoluted tubules were still showing vacuolation in their cells, while other convoluted proximal and distal began to retain their normal histological architecture. Somewhat, few atrophic renal glomeruli can be seen (Fig. 3A, B). Blood vessel with the normal vascular wall was also observed. Few inflammatory cells around the blood vessel were also observed (Fig. 3C).



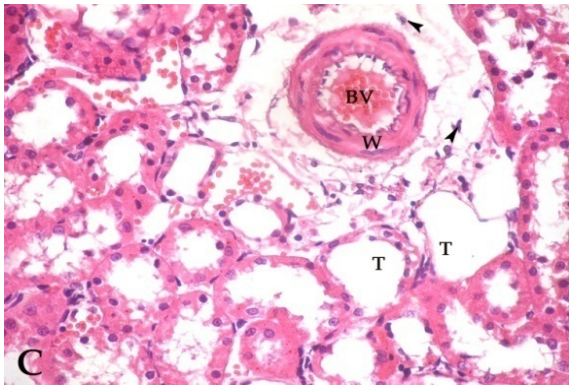


Figure 3. The photomicrographs of a section of adult male albino rat kidney treated with MTX and Vit. C. **(A)** shows the renal cortex surrounded by thin renal capsule (arrow), apparently normal glomeruli (G) and convoluted tubules (T). Atrophic renal glomeruli (AG) were still present (H&E X 100). **(B)** shows the apparently normal renal glomeruli (G) with nearly normal Bowman's space (B.S). Some cortical convoluted tubules (T) with few vacuolated cells (V) were still observed (H&E X 400). **(C)** shows a blood vessel with normal vascular wall (W). Few Inflammatory cells (arrowheads) around the blood vessel were also observed. Notice that the slightly dilated tubules could be seen (T) (H&E X 400).

Results of Periodic acid Schiff reaction

P.A.S-stained kidney sections showed a strong positive reaction in the basement membranes of convoluted tubules and Bowman's capsules. A strong positive reaction in the brush border of the proximal convoluted tubules was seen (Fig.4A). The 3rdgroup showed a weak reaction in basement membranes of tubules and Bowman's capsule. A negative reaction in the brush borders of tubules was also seen (Fig. 4B). While, the 4thgroup showed a strong positive reaction in the basement membrane of tubules and Bowman's capsules. The brush border of the proximal tubule also showed a strong reaction (Fig. 4C).

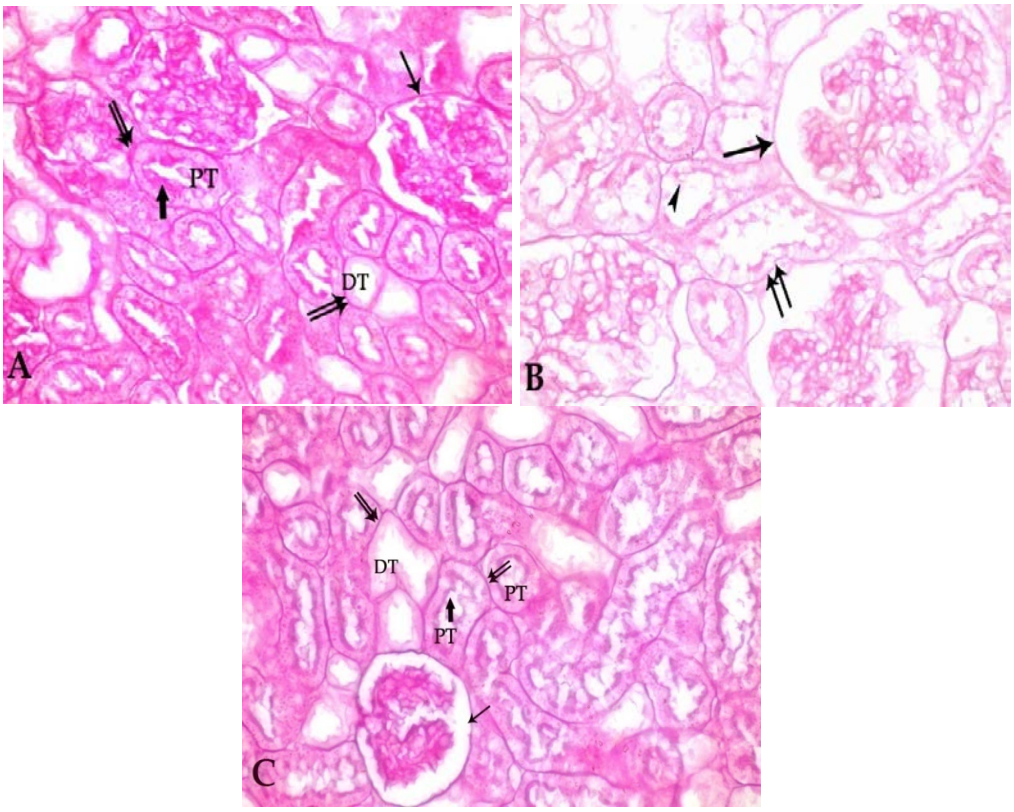


Figure 4. (A) A photomicrograph of a section of control group showed a strong PAS positive reaction of basement membrane (double arrow) of the distal convoluted tubules (DT) and the proximal convoluted tubules (PT) and Bowman's capsule (arrow). The brush border of the proximal tubule also showed a strong reaction (thick arrow). (**B**)

A photomicrograph of a section of kidney treated with MTX showed a weak positive PAS reaction of basement membrane of the convoluted tubules (double arrows) and Bowman's capsule (arrow). A negative PAS reaction at the brush borders of tubular cells (arrowhead) was also observed. **(C)** A photomicrograph of a section of kidney treated with MTX and Vit.C showed a strong PAS positive reaction of basement membrane (double arrow) of the distal convoluted tubules (DT) and the proximal convoluted tubules (PT) and Bowman's capsule (arrow). The brush border of the proximal tubule also showed a strong reaction (thick arrow). (PAS X 400)

Immune histochemical results

Caspase-3 immunohistochemically stained kidney sections of control groups revealed a negative immune reaction of caspase- 3 in the cytoplasm of the most tubular cells and renal glomeruli cytoplasm (Fig. 5A), while a strong positive reaction for Caspase 3 was noticed in the 3rdgroup (Fig. 5B) and the 4thgroup revealed few positive cells for caspase-3 in cytoplasm of the most tubular cells and renal glomeruli in comparison to the treated group (Fig. 5C).

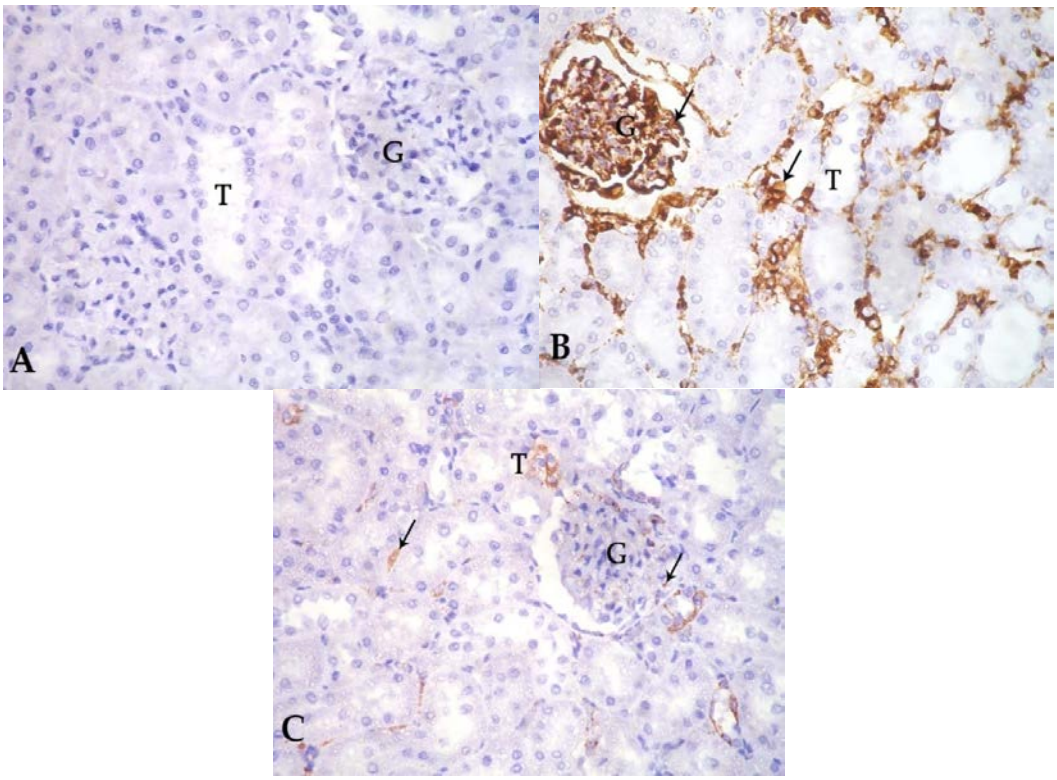


Figure 5. (A) A photomicrograph from a section of the kidney of the control groups showed a negative immune reaction of caspase-3 in the cytoplasm of the most convoluted tubular cells (T) and renal glomeruli (G).**(B)** A photomicrograph of a section of the kidney treated with MTX showed a strongly positive immune reaction (arrows) of caspase -3 in the cytoplasm of renal tubular cells (T) and cytoplasm of glomeruli (G). **(C)** A photomicrograph of a section of the kidney treated with MTX and Vit. C showed a mildly positive immune reaction (arrow) of caspase -3 in the cytoplasm of tubular cells (T) and the cytoplasm of the renal glomerulus (G) (Immunohistochemistry for Caspase-3 X400)

Statistical analysis:

I-Estimation of the body weight of adult male albino rats in the different studied groups:

A) The Results of ANOVA test: The estimation of the body weight (gm) in the different studied groupsz using ANOVA test revealed that there were no statistically significant differences between the studied groups in initial body weight, but there were statistically significant differences between them in the final body weight as in (Table 1) and (Fig. 6).

Table 1. The comparisons of initial and final body weights in the different studied groups using ANOVA (analysis of variance) test:

	1st (Control group)	2nd (Vit C group)	3rd (MTX group)	4th (MTX+Vit.C group)	F	p
Initial BW: Mean ± SD Range	227 ± 5.70 220 – 235	227.8 ± 6.46 220 – 235	229 ± 8.94 220 – 240	228.6 ± 11.61 215 – 245	0.04	0.99 NS
Final BW: Mean ± SD Range	245.6 ±5.13 238 – 252	247 ± 6.96 235 – 253	185 ± 7.91 175 – 195	238.6 ± 12.60 220 – 255	57.38	<0.001**
Paired t	8.03	8.91	7.09	4.88		
P	0.001**	0.001**	0.002**	0.008**		

SD: Standard deviation; F: ANOVA test; Paired t: Paired t test; **: highly significant (p<0.001); Number of rats for each group=5.

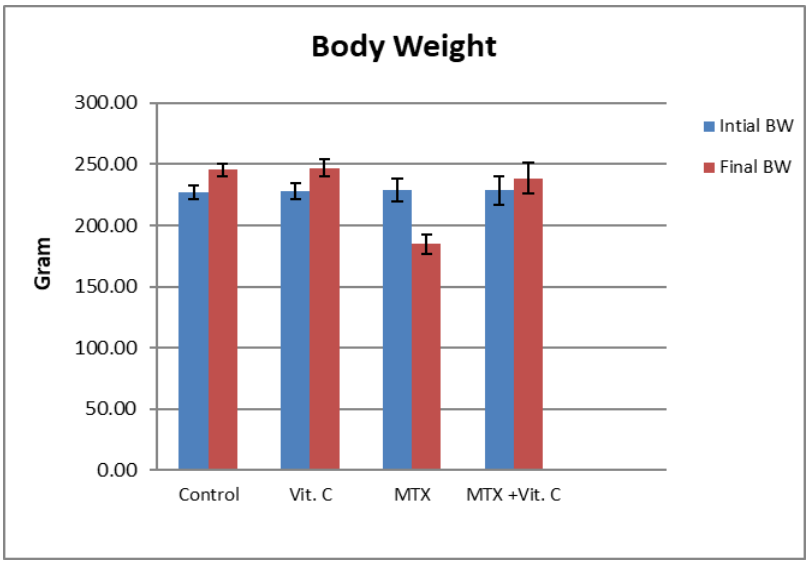


Figure 6. Bar chart showing the comparison between the mean values of body weight (gm) among the different studied groups

B) The Results of The least significant differences (LSD) between the groups: Using LSD to find a relation in-between groups showed that there were no statistically significant differences between the control and the Vit.C and MTX+Vit.C groups, but there were highly statistically significant differences (p<0.001) in the MTX treated group when compared with other groups as in (Table 2).

Table 2. The least significant difference test (LSD) for the comparison of the final body weight in-between groups:

	Vit. C Group	MTX group	MTX+Vit.C group
1st (Control group)	>0.05 NS	<0.001 **	>0.05 NS
2nd (Vit. C group)		<0.001 **	>0.05 NS
3rd (MTX group)			<0.001 **
4th(MTX+Vit.Cgroup)			

NS: Non significant; *: Significant; **: Highly significant; Number of rats for each group=5.

- 1st group vs. 2nd group: >0.05 non-significant.
- 1st group vs. 3rd group: <0.001 highly significant.
- 1st group vs. 4th group: >0.05 non-significant.
- 3rd group vs. 4th group: <0.001 highly significant.

II. The biochemical results and statistical analysis:

A. The Results of ANOVA test: The results of the present study revealed a highly significant increase in both urea and creatinine level (P<0.001) in the MTX of the treated group when compared with other groups as in (Table 3) and (Figs. 7, 8).

Table 3. The Comparisons of Urea and craetinine in the different studied groups using ANOVA (analysis of variance) test:

	Control group	Vit. C Group	MTX group	MTX+Vit.C Group	F	p
Urea Mean ± SD Range	16.5 ± 2.35 14 – 19.8	17.38 ± 2.66 14.2 – 20.4	46.56 ± 2.27 43.8 – 49.7	22.18 ± 2.04 19.6 – 24.8	142.4	<0.001**
Creatinine Mean ± SD Range	0.56 ± 0.14 0.39 – 0.71	0.55 ± 0.10 0.42 – 0.68	1.75 ± 0.15 1.58 – 1.93	0.70 ± 0.10 0.59 – 0.84	85.07	<0.001**

SD: Standard deviation; F: ANOVA test; Paired t: Paired t test; **: highly significant (p<0.001); Number of rats for each group=5.

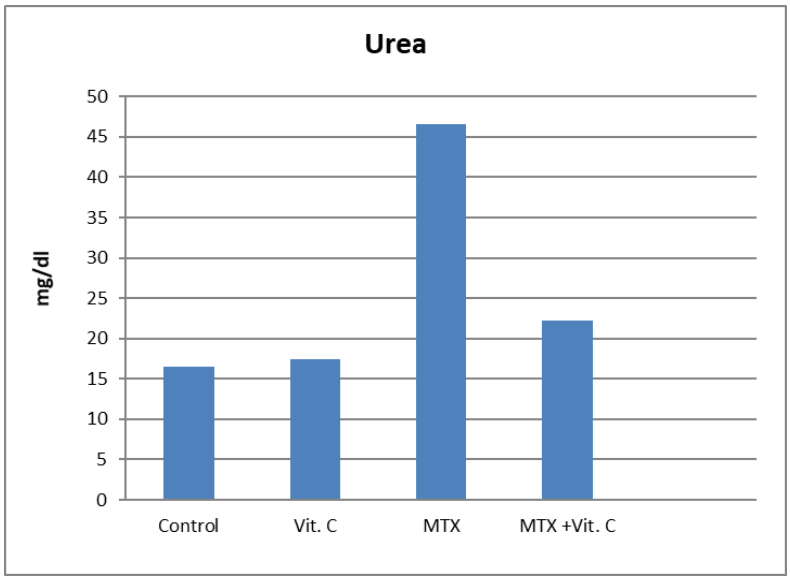


Figure 7: The Bar chart showing the comparison between the mean values of serum urea among the different studied groups.

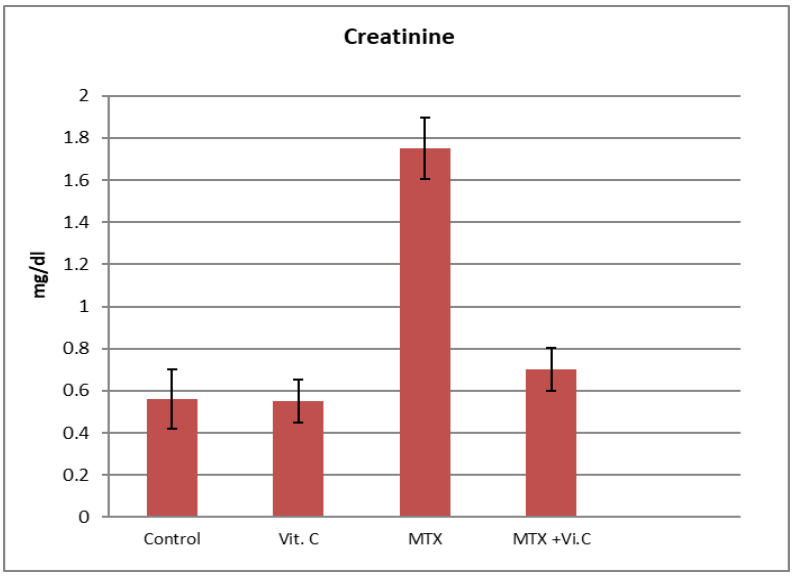


Figure 8. The Bar chart showing the comparison between the mean values of serum creatinine level among the different studied groups.

B. The Results of The least significant differences (LSD): Using LSD to find a relation in-between groups showed that there were no statistically significant differences between the control and the Vit. C and

MTX+Vit.C groups, but there were highly statistically significant differences between MTX in both urea and creatinine as in (Table 4).

Table 4. The Least Significant Difference test (LSD) for the comparison of urea and creatinine in-between groups:

	Vit. C Group	**MTX group**	**MTX+Vit.C Group**
1st (Controlgroup)	>0.05 NS	<0.001 **	>0.05 NS
2nd (Vit. Cgroup)		<0.001 **	>0.05 NS
3rd (MTX group)			<0.001 **
4th(MTX+Vit.Cgroup)			

NS: non-significant; *: Significant; **: Highly significant; Number of rats for each group=5.

- 1st group vs. 2nd group: >0.05 non-significant.
- 1st group vs. 3rd group: <0.001 highly significant.
- 1st group vs. 4th group: >0.05 non-significant.
- 3rd group vs. 4th group: <0.001 highly significant.

DISCUSSION

Experimental studies have demonstrated that there has been an increased production of oxygen-free radicals following MTX treatment, and these free radicals might lead to mitochondrial impairment. In addition, MTX-induced toxicity activated an inflammatory response and significantly increased the production of pro-inflammatory cytokines. Lipid peroxidation by free oxygen radicals has been an important cause of oxidative damage to cell membranes [16].

Nephrotoxicity has been one of the important reasons for restricting its use. High-dose MTX produced a wide clinical range of damages in the kidneys varying from subclinical tubulopathy to an acute renal failure [17].

Many studies in vivo and in vitro have shown the ability of vit.C to prevent and reduce the side effects of chemotherapy. The combination of vit.C and vit. K already given in the chemotherapy increased the survival and the effects of different chemotherapeutic agents in a tumor-ascitic-murine model [18]. Several experimental studies have also highlighted the renoprotective properties of vit.C against nephrotoxicity induced by different drugs such as vancomycin, paracetamol, colistin, and gentamicin [19-21].

In the present work, the results showed a highly statistically significant decrease in final body weight in MTX treated group when compared with the control groups. However, there was a non-significant difference between the control and the vit. C and MTX+vit.C treated groups. This was in line with [22] who reported that weight reduction might be due to the direct toxicity of MTX, gastrointestinal toxicity, and the reduced feed and water intake. However, [23] observed that there was no significant difference in the final body weight between the control and MTX treated groups. On the other hand, [24] reported a significant increase in their body weight in comparison with the normal control group.

In the present study, the kidney histological results were confirmed by the biochemical parameters. The assessment of the kidney functions was made in the present work by estimating the serum urea and creatinine. There was a significant increase in urea and creatinine levels in the MTX treated group when compared with that of the control groups. These results were supported by [25]. [26] reported that MTX induced a renal damage as indicated from the significant increase in creatinine and urea. These measurements were often used as reliable markers of the renal damage, and indicated the loss of a majority of kidney functions. Serum creatinine and urea levels were statistically decreased in MTX +vit C treated group. These results were supported by [11]. Also, [27] reported that the treatment with the antioxidant vitamin C before the experimental renal ischemia reperfusion in adult rats resulted in the marked improvement in the renal functions and were manifested by a significant decrease of plasma urea and creatinine levels.

The present study showed that a single dose of MTX (20 mg/kg) in rats induced different histopathological changes in the renal cortex. Haematoxylin and eosin-stained sections in MTX treated group revealed a massive destructive damage of the renal cortex. Thick renal capsules were with a fatty cell infiltration. [28] reported that they observed fatty infiltration in renal tissue of rats when fed a high-fat diet for 3 months. To the researchers' knowledge, the capsular fatty cell infiltration due to MTX was not encountered previously in other studies. The shrinkage and atrophied renal glomeruli were observed in this study. These results were supported by [29] who demonstrated the

shrunken and atrophied glomeruli with increasing the urinary space. Furthermore, glomerular atrophy might be attributed to a decrease in the glomerular filtration of the drug as a result of capillary constriction. Hypertrophy of renal glomeruli, hypercellularity and congestion of glomerular capillaries observed in this study could be explained as mentioned by [29] who stated that hypertrophy and severe congestion appeared in the glomerular tufts and the renal blood capillaries, with interstitial edema that might be attributed to an increase in the renal blood vessel permeability caused by a high dose of MTX. Also, [30] reported that the increased proliferation of mesangial cells led to the presence of hypercellular glomeruli in the renal cortex.

Considering the renal tubules, they were tubular dilatation, darkly stained nuclei, and tubular cell necrosis, sloughing of necrotic tubular epithelial cells into the lumens of tubules, marked cytoplasmic vacuolization and swelling of tubular cells. These results were supported by [25] who reported that an intensive deformation of epithelial cell structures of both proximal and distal tubules was observed. There were intensive degenerative structures related to the swelling of epithelial cells of the proximal tubules, and there was a cellular shedding of the epithelium of the distal tubules due to the dilatations of the lumen and edematous fluid.

Also, the results of this work demonstrated cytoplasmic vacuolization of the tubular cells. These data could be explained by [31] who demonstrated that cytoplasmic vacuolization occurred as one of the primary responses to all the forms of cell injury. They explained that the increased permeability of cell membranes led to an increase of the intracellular water. As water sufficiently accumulated within the cell, it produced cytoplasmic vacuolization. For cystic luminal dilatation in some tubules found here in the present study, similar findings were proved before by [32] when he examined kidney section of mice treated with MTX 10mg/kg twice weekly for one month, and recorded cystic dilatation of renal convoluted tubules and tubular cell necrosis. He suggested that when MTX level increased in the body, that would cause severe renal toxicity and result in accumulation of MTX crystals in the nephron, resulting in dilatation of renal tubules. Also, he reported that MTX had a lethal effect on the renal tubular epithelium due to its direct toxic damage, or by the precipitation of MTX in these tubules, resulting in renal toxicity and dysfunction.

Marked cellular infiltration was observed in this study. These results were in agreement with [33]. [34] reported that MTX elevated the myeloperoxidase (MPO) activity, pointing to an accumulation of inflammatory cells (neutrophils and monocytes) in the kidney tissue. This observation was in agreement with the histological findings, which revealed interstitial and perirenal inflammation in the renal tissue. In addition, [35] added that kidney tissue showed increased pro-inflammatory cytokines such as renal tumor necrosis factor alpha (TNF-alpha), interleukin 1 beta (IL-1b), and interleukin 6 (IL-6) which could be another explanation of renal injury-induced by MTX.

Also, the results of this work demonstrated interstitial hemorrhage, and congested blood vessels with the thickened wall. These results were supported by [26] who showed dilatation and congestion of renal blood vessel with leukocyte inflammatory cell infiltration. A bundle of collagen fibers was observed in this study. [36] demonstrated significant increases of collagen contents in kidney, liver and ileum after MTX exposure. Also, they reported that the tissue collagen was measured as a free-radical-induced

In the present work, P.A.S stained sections of MTX treated group revealed a weak reaction in Bowman's capsule, basement membranes of tubules, and a negative reaction in the brush borders of tubules. These results were supported by [29] who revealed that the mucopolysaccharides were greatly depleted in the basement membrane of both glomeruli and renal tubules as indicated by decreasing in the stain ability of PAS-positive materials, while the affected degenerated brush borders showed a negative reaction.

One of the aims of this study was to detect caspase-3 expression immune histochemically in the renal cortex. The expression of caspase- 3 was in the cytoplasm of the most of tubular cells and renal glomeruli, and showed a strong positive immune reaction in MTX treated group. These were supported by [3]. Also, [33] mentioned that Caspase-3 activation was used as a marker for cell injury in different diseases.

In the current work, the light microscopic examination of the kidney tissue of rats treated with MTX+vit C revealed some degrees of improvement as compared to MTX treated group, these results were in agreement with that of [37] who reported that the rats which received MTX and vit.C showed a decrease in the severity of histopathological changes in the renal tissue as compared to the control group. They illustrated the antioxidant effect of vit.C which was proved by the significant reduction of malondialdehyde (MDA) level.

These findings were supported by [38]. They stated that the administration of vit. C before MTX injection improved the histological manifestations, and decreased a number of apoptotic cells in the testicular tissue. Furthermore, the results of the current study were consistent with [39] who reported that vit.C showed a protection in a dose-

dependent manner on the cisplatin- induced oxidative damage on the adult rat kidneys due to its antioxidant activity and inhibitory effect on the chain reactions of the cisplatin-generated free radicals before they reached the cell targets damaging the glomerular kidney functions. Moreover, [40] reported that the administration of vit.C may have a protective effect on the chemotherapeutics-induced cell death in their cell culture studies.

Regarding the blood vessel of the same group showed a normal vascular wall with few inflammatory cells around it. Similar findings were proved before by [41] who reported that the rats which received vit. C and vit E showed the decreased vascular hypertrophy of superior mesenteric arteries. Also, they proved that antioxidants improved the integrity of the vascular structure, possibly by preventing the cellular damage induced by oxygen free radicals.

CONCLUSION

Results of this study revealed the injurious effects of MTX on the renal cortex. Co-administration of Vit.C with MTX improved the deleterious changes evidenced by the decrease in the level of serum urea and creatinine, and decreased the caspase activity, in addition to the improvement of the tissues which was observed in this group histologically. According to these results, Vit.C could have protected the effects against MTX-induced renal damage. These data recommended that the administration of Vit.C prevented nephrotoxicity, and might enhance the selectivity of the anticancer, rheumatoid arthritis, psoriasis drugs in patients who required MTX as a treatment.

Conflicts of interest

There were no conflicts of interest.

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