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# EFFECTS OF OXYTOCIN ON CYCLOPHOSPHAMIDE-INDUCED NEPHROTOXICITY IN ADULT MALE ALBINO RATS

By

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## ABSTRACT

**Background:** Cyclophosphamide (CP) is commonly used as anti-cancer drug which causes tissue toxicity by its reactive metabolites. Oxytocin (OT) is a peptide hormone secreted by the hypothalamic paraventricular and supraoptic nuclei. It modulates the immune and inflammatory processes.

**Objective:** Investigating the effects of oxytocin on CP induced-acute renal toxicity in adult male albino rats.

**Materials and Methods:** Seventy adult male albino rats were divided into 5 groups: 0 group served as normal control (20 rats were subdivided into A&B; 10 rats each), group I served as positive control (20 rats injected with single intraperitoneal dose of CP and were subdivided into A&B; 10 rats each), Group-II (10 rats treated with OT for 7 days before CP injection then sacrificed 24 hours later with 0-A and I-A groups), Group III (10 rats treated with OT after CP injection for 10 days then sacrificed with 0-B, I-B & IV groups), and Group IV(10 rats treated with OT for 7 days before and for 10 days after CP injection). By the end of the experimental period, blood samples were collected to measure serum creatinine and urea. Both kidneys of each rat were dissected out carefully. The right kidney was used for measurement of malondialdehyde (MDA), glutathione (GSH) and tumor necrosis factor alpha (TNF-α), while the left kidney was preserved for histological examination.

**Results:** Administration of oxytocin alleviated CP-induced renal toxicity as evident from the decreased levels of kidney toxicity markers (urea, creatinine, MDA and TNF-α) and elevation of GSH levels. No significant differences were found between the groups treated with OT. Administration of oxytocin caused a significant improvement in kidney histopathology with alleviation of tissue inflammation and tissue recovery especially in rats treated with OT pre- and post-CP injection.

**Conclusion**: Oxytocin has a protective and therapeutic role from CP-induced renal toxicity by modulating levels of MAD, GSH and TNF-α.

**Key words**: Cyclophosphamide, oxytocin, renal histology, kidney function, cytokines and oxidative stress.

## INTRODUCTION

Cyclophosphamide (CP) is a highly effective alkylating cytostatic agent **(Perini et al., 2007).** It is used as an immune suppressant in the treatment of cancer and non-malignant disease states like rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis as well as in organ transplantation **(Hamsa & Kuttan, 2012 and Rehman et al., 2012).** The important factor for therapeutic effects of CP is the requirement of metabolic activation by

hepatic microsomal cytochrome P450 mixed functional oxidase system **(Ayala et al., 2014).** Antineoplastic effects of CP are linked with two active metabolites, i.e. phosphoramide and acrolein **(Tripathi and Jena, 2009).**

CP can be nephrotoxic, both in human and animal models. CP treatment results in glomerular and tubular dysfunction, glomerular and tubular proteinuria in addition to reduction of glomerular filtration rate **(Sugumar et al., 2007)** via production of reactive oxygen species (ROS), which cause oxidative damage to kidney and other vital organs **(Alkan et al., 2012 and Said et al., 2015).** Acute kidney injury (AKI) is a serious complication of CP chemotherapy **(Rehman et al., 2012 and Xu et al., 2015**).

Cytokines play pathophysiological roles in acute and chronic renal diseases **(Wu et al., 2009).** Tumor necrosis factor alpha (TNF-α) is a cytokine that expressed in a wide variety of inflammatory conditions. Various studies have been demonstrated that TNF-α plays a key causative role in AKI through its action on renal endothelial cells **(Vielhauer & Mayadas, 2007; Wu et al., 2009 and Xu et al., 2014).**

Oxytocin (OT) is a peptide hormone secreted by the hypothalamic para-ventricular and supraoptic nuclei. It exerts its physiological and biological actions via its G–protein coupled receptor. OT receptors are widespread in the nervous system, vascular, smooth muscle, myocardium, thymus, pancreas, and adipocytes **(Gutkowska and Jankowski, 2009).** OT receptors have also been identified in kidney **(Rashed et al., 2011).** Its beneficial effects against rotenone - induced Parkinson's disease and sepsis-induced polyneuropathy in rats were reported **(Erbas et al., 2012 a & b).** OT was shown to modulate the immune and inflammatory processes. It was found that OT decreases the release of some interleukins and increases the survival of ischemic skin flaps in rats via the activation of the growth factors or anti-inflammatory mechanisms **(Tuğtepe et al., 2007).**

The current study focused on evaluation of the protective and therapeutic efficacy of oxytocin against acute kidney injury induced by cyclophosphamide in adult male albino rats.

## MATERIALS AND METHODS

**Drugs and chemicals:**

- CP (Endoxan - Baxter International Inc). It was freshly prepared and dissolved in sterile water immediately before being injected intraperitoneally.
- OT (Syntocinon vials- Novartis).

**Experimental animals:** Seventy adult male albino rats of local strain, weighing 140–160 g, were used in this study. They were obtained from the Animal House Colony of the National Research Center. Animals were housed in plastic cages (35 x 30 x 35 per 5 rats) in the animal house in the Faculty of Medicine, Al-Azhar University. Rats were kept at room temperature (~25°C) under normal dark/light cycle with free access to food and water. The study was conducted in accordance with ethical procedures and policies outlined in the Canadian Council of Animal Care guidelines **(Zatroch et al., 2017).**

**Experimental design:** After seven days of acclimatization, rats were randomized into 5 groups as follows:

● **Group 0 (control group):** Consisted of 20 rats and served as control group. Each rat received 1ml normal saline (0.9%) by intraperitoneal injection (IP). This group was subdivided into control A and control B.

- **Group I (received CP alone):** Consisted of 20 rats. Each rat received a single intraperitoneal dose of cyclophosphamide (CP) dissolved in saline (150 mg/kg B.WT.) **(Abraham et al., 2009).** This group was subdivided into IA and I-B.
- **Group II (Pre-treated with OT):** Consisted of 10 rats. Rats were treated with OT (80 ?g/kg B.WT.) by IP injection daily for 7 days before receiving a single IP dose of CP (150 mg/kg B.WT.). They were sacrificed 24 hours after CP injection accompanied with control A and I A groups **(Akman et al., 2015).**
- **Group III (Post-treated with OT):** Consisted of 10 rats. Rats received a single IP dose of CP (150 mg/kg B.WT.), then treated with OT (80 ?g/kg B.WT.) by IP injection for 10 days and then sacrificed with control B, I-B and IV groups.
- **Group IV (Pre- and Post-treated with OT):** Consisted of 10 rats. Rats received OT (80 ?g/kg B.WT.) by IP injection for seven days starting with group II, then were injected with a single IP dose of CP (150 mg/kg B.WT.), and OT (80 ?g/kg B.WT.) by IP injection for 10 days, and then sacrificed.

**Serum collection and tissue preparation:** Before scarification, animals were anesthetized and blood samples were collected from retro-orbital sinuses using non heparinized capillary tubes. Blood was immediately centrifuged at 3000 rpm for 20 minutes. Sera were separated and stored at -80˚C until used. Rats were then sacrificed by cervical dislocation and both kidneys were rapidly excised. From each rat, the right kidney was homogenized in 5 ml cold buffer per gram of tissue using a homogenizer (Heidolph Diax 900, Germany) to prepare 10% homogenate. The resultant supernatant was transferred to Eppendorff tubes and stored at -80˚C until used. The left kidney was preserved for histopathological examination.

**Biochemical parameters:**

- **Determination of kidney functions:** Serum levels of creatinine and urea were determined enzymatically using commercially available kits **(Todd et al., 1974).**
- **Measurement of malondialdehyde (MDA) (Wills, 1987).**
- **Measurement of glutathione (GSH) (Ellman, 1959).**
- **Measurement tumor necrosis factor alpha (TNF-α)** by using ELISA kit supplied by quantikine R&D system USA, according to the manufacturer`s instructions **(Maskos et al., 1998).**

**Histological study:** Each kidney was fixed in 10% formalin solution for 48-55h, dehydrated in graded alcohol series, embedded in paraffin wax, then thin sections of 5µm thickness were obtained. Sections were stained with H&E for routine histological examination.

**Statistical analysis:** Data were expressed as means ± standard deviation (SD). Statistical comparison between different groups were done using one-way analysis of variance (ANOVA) followed by Tukey HSD multiple comparison test to judge the difference between various groups. All calculations were performed using the SPSS 16.0 software package. Significance was accepted at $P< 0.05$.

## RESULTS

Group I (group received cyclophosphamide)-A&B showed significantly high serum levels of urea (84.7±20 mg/dl and 106.8±7.8 mg/dl respectively) compared to control group 0-A&B (37.9±9 mg/dl and 35.6±8.1 mg/dl respectively). A significant increase was noticed in group I-B when compared to group I-A, while no significant difference was observed between rats in groups 0-A & B. Oxytocin (OT) administration for 7 days before cyclophosphamide (CP) injection to rats in group II significantly lowered serum levels of urea when compared to group I-A (71±7.2 mg/dl and 84.7±2 mg/dl respectively), but there was still a significant increase in its levels when compared to control group 0-A (71±7.2 mg/dl and 37.9±9 mg/dl respectively). OT treatment for 10 days after CP injection to rats in group III significantly decreased serum levels of urea when compared to group I-B (106.8±7.8 mg/dl and72.8±7.3 mg/dl respectively), but a significant increase was still present when compared to control group 0-B (72.8±7.3 mg/dl and 35.6±8.1 mg/dl respectively). In group IV (treated with OT pre-and post-CP injection), a significant decrease in serum urea was also found when compared to group I-B (74.4±9.3 mg/dl and 106.8±7.8 mg/dl respectively), but a significant increase was still present when compared to control group 0-B (74.4±9.3 mg/dl and 35.6±8.1 mg/dl respectively). However, when group IV was compared with group II and group III (74.4±9.3 mg/dl, 71±7.2 mg/dl and 72.8±7.3 mg/dl respectively), no significant change in urea levels were noticed between them.

CP-treated group (group I) A&B showed significantly high serum levels of creatinine (0.92±0.2 mg/dl and 2.1±1.1 mg/dl respectively) when compared to control groups 0-A&B (0.18±0.06 mg/dl and 0.22±0.1 mg/dl respectively). A significant increase was noticed in group I-B when compared to group I-A, while no significant difference was observed between rats in groups 0-A & B. OT administration before CP-injection in group II significantly lowered creatinine levels when compared to group I-A (0.4±0.08 mg/dl and 0.92±0.2 mg/dl respectively), but a significant increase was still found when compared to control group 0-A (0.4±0.08 mg/dl and 0.16±0.06 mg/dl respectively). On comparing group III to group I-B (0.5±0.1 mg/dl and 2.1±1.1 mg/dl respectively), there was a significant decrease in creatinine levels but a significant increase in its levels was observed when compared with control group 0-B (0.5±0.1 mg/dl and 0.22±0.1 mg/dl respectively). In group IV (treated with OT pre-and post-CP injection), a significant decrease in serum creatinine was also found when compared to group I-B (0.5±0.2 mg/dl and 2.1±1.1 mg/dl respectively), but a significant increase was still present when compared to control group 0-B (0.5±0.2 mg/dl and 0.22±0.1 mg/dl respectively). When group IV was compared with group II and group

III (0.5±0.2 mg/dl, 0.4±0.08 mg/dl and 0.5±0.1 mg/dl), there was no significant difference in creatinine levels.

CP-treated group showed significantly high levels of malondialdehyde (MDA) in group I-A&B (14.1± 2 and16.6±1.7 respectively) when compared to control group 0-A&B (1.2±0.2 nmol/mg tissue and 1.3± 0.3 nmol/mg tissue respectively). A significant increase was noticed in group I-B when compared to group I-A, while no significant difference was observed between rats in groups 0-A & B. Pretreatment with OT in group II significantly lowered MDA levels when compared to group I-A (5.1± 1 nmol/mg tissue and 14.1± 2 nmol/mg tissue respectively) but, these levels were still higher than control group 0-A (5.1±1 nmol/mg tissue and 1.2± 0.2 nmol/mg tissue respectively). Also, a significant decrease in MDA levels was observed in group III when compared to group I-B (5.5±2.1 nmol/mg tissue and 16.6±1.7 nmol/mg tissue respectively), but these levels were significantly higher than those in control group 0-B (5.5±2.1 nmol/mg tissue and 1.3+0.3 nmol/mg tissue respectively). However, on comparison between group IV and group I-B, a significant decrease in MDA levels was observed (5.5±1 nmol/mg tissue and 14.1±2 nmol/mg tissue respectively), while a significant increase was still present when compared to control group 0-B (5.5±1 nmol/mg tissue and 1.3±0.2 nmol/mg tissue respectively). The comparison between groups treated with OT (II, III and IV) revealed a non-significant difference in MDA levels (5.1±1 nmol/mg, 5.5±2.1 nmol/mg tissue and 5.5±1 nmol/mg tissue respectively).

The renal tissue contents of glutathione (GSH) decreased significantly in rats injected with CP (group I) A&B (24.5± 3.5 nmol/mg tissue and 17.6±2.6 nmol/mg tissue respectively) when compared to normal control group 0-A&B (58.0±2.7 nmol/mg tissue and 59.2± 4.8 nmol/mg tissue respectively). A significant increase was noticed in group I-B when compared to group I-A, while no significant difference was observed between rats in groups 0-A & B. OT administration elevated GSH levels significantly in group II when compared to group I-A (53.6±3.8 nmol/mg tissue and 24.5±3.5 nmol/mg tissue respectively), but there was a non-significant difference when compared to control group 0-A (53.6±3.8 nmol/mg tissue and 58±0 nmol/mg tissue respectively). On comparing group III to group I-B, there was a significant increase in levels of GSH (49.5±6.8 nmol/mg tissue and 17± 2.6 nmol/mg tissue respectively), but these levels were significantly lower than those of the control group 0-B (49.5± 6.8 nmol/mg tissue and 59.2± 4.8 nmol/mg tissue respectively). Comparing group IV with control group I-B revealed a significant increase in levels of GSH (51±11 nmol/mg tissue and 17.6±2.6 nmol/mg tissue respectively), but these levels were significantly lower than those of group 0-B (51±11 nmol/mg tissue and 59.2±4.8 nmol/mg tissue respectively). The comparison between groups treated with OT (II, III and IV) revealed a non-significant difference in GSH levels (53.6±3.8 nmol/mg, 49.5±6.8 nmol/mg tissue and 51±11 nmol/mg tissue respectively).

CP-treated group showed a significant increase in renal tissue contents of tumor

necrosis factor alpha (TNF-α) in group I-A&B (109.8±9.7 pg/mg tissue and 127.4±13.4 pg/mg tissue respectively) compared to control group 0-A&B (32.5±2.3 pg/mg tissue and 31.7±2.9 pg/mg tissue respectively). A significant increase was noticed in group I-B when compared to group I-A, while no significant difference was observed between rats in groups 0-A & B. Pretreatment with oxytocin lowered TNF-α levels in group II when compared to group I-A (76.8±15.1 pg/mg tissue and 109.8±9.7 pg/mg tissue respectively), but these levels were still higher than control group 0-A (76.8±15.1 pg/mg tissue and 32.5±2.3 pg/mg tissue respectively). Post-treatment with OT in group III lowered TNF-α levels compared to group I-B (78.6±10 pg/mg tissue and 127.4±13.4 pg/mg tissue respectively), but these levels were still significantly higher than control group 0-A (78.6±10 pg/mg tissue and 32.5±2.3 pg/mg tissue respectively). When group IV was compared to group I-B a significant decrease in TNF-α levels was noticed (66.2±17 pg/mg tissue and 127.4±13.4 pg/mg tissue respectively), but these levels in group IV were significantly higher than control group 0-B (76.2±17 pg/mg tissue and 31.7±2.9 pg/mg tissue respectively). Pre- and post- treatment with OT in group IV lowered the TNF-α levels when compared with group II and group III (66.2±17 pg/mg tissue, 76.8±15.1 pg/mg tissue and 78.6±10 pg/mg tissue respectively), while no significant difference was noticed between groups II and III.

**Table (1):** Serum levels of urea and creatinine as well as renal tissue contents of MDA, GSH and TNF-α in various groups (Mean ± SD)**.**

| Groups / Variables | Group 0-A | Group 0-B | Group I-A | Group I-B | Group II | Group III | Group IV |
|---|---|---|---|---|---|---|---|
| **Urea (mg/dl)** | **37.9±9** | **35.6±8.1** | **84.7±20$^{ab}$** | **106.8±7.8$^{abc}$** | **71±7.2$^{abd}$** | **72.8±7.3$^{abd}$** | **74.4±9.3$^{abd}$** |
| **Creatinine (mg/dl)** | **0.18±0.06** | **0.22±0.1** | **0.92±0.2$^{ab}$** | **2.1±1.1$^{abc}$** | **0.4±0.08$^{d}$** | **0.5±0.1$^{d}$** | **0.5±0.2$^{d}$** |
| **MDA (nmol/mg tissue)** | **1.2±0.2** | **1.3±0.3** | **14.1±2$^{ab}$** | **16.6±1.7$^{abc}$** | **5.1±1$^{abcd}$** | **5.5±2.1$^{abcd}$** | **5.5±1$^{abcd}$** |
| **GSH (nmol/mg tissue)** | **58±2.7** | **59.2±4.8** | **24.5±3.5$^{ab}$** | **17.6±2.6$^{ab}$** | **53.6±3.8$^{cd}$** | **49.5±6.8$^{abcd}$** | **51±11$^{bcd}$** |
| **TNF-α (pg/mg tissue)** | **32.5±2.3** | **31.7±2.9** | **109.8±9.7$^{ab}$** | **127.4±13.4$^{abc}$** | **76±15.1$^{abcd}$** | **78.6±10$^{abcd}$** | **66.2±17$^{abcd}$** |

- Group 0 (A & B): control group.
- Group I (A & B): received CP alone.
- Group II: Treated with OT before CP-injection.
- Group III: Treated with OT after CP-injection.
- Group IV: Treated with OT before & after CP-injection.

(a) Significant values versus control 0-A.
(b) Significant values versus control 0-B.
(c) Significant values versus I-A group.
(d) Significant values versus I-B group.

**Histological results:**

In H&E stained sections, the control groups (0-A & 0-B), showed the normal histological structure of the glomeruli and tubules **(Figures 1&2).** Rats injected with CP (Groups I-A & I-B) showed focal hemorrhage between the degenerated tubules at the cortex **(Figure 3)**, associated with swelling and vacuolization of the endothelial cells lining the tufts of the glomeruli **(Figure 4).** There was focal fibrosis in between the degenerated tubules at the corticomedullary junction **(Figure 5).** In the group II (rats protected by oxytocin before CP injection), the cortical portion showed congestion in the blood vessels **(Figure 6),** while the corticomedullary junction had focal fibrosis **(Figure 7)** in the degenerated tubules. Group III (post-treated with OT) showed focal wide area of hemorrhage as well as congestion in the blood vessels **(Figure 8).** In group IV (pre- and post-treated with OT), mild focal fibrosis was detected in between the tubules **(Figure 9).**

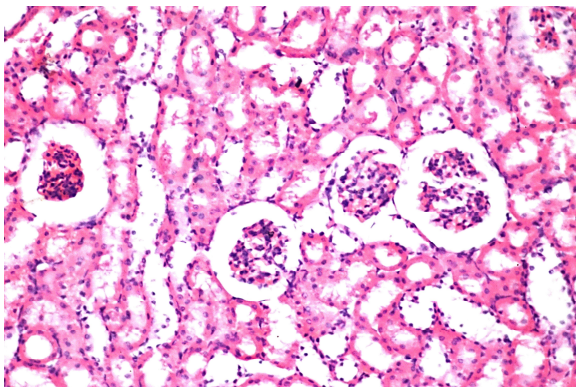

**Figure (1)**: Section of the renal cortex from the control group 0-A showing normal histological structure of the glomeruli and tubules **(H&E x40).**

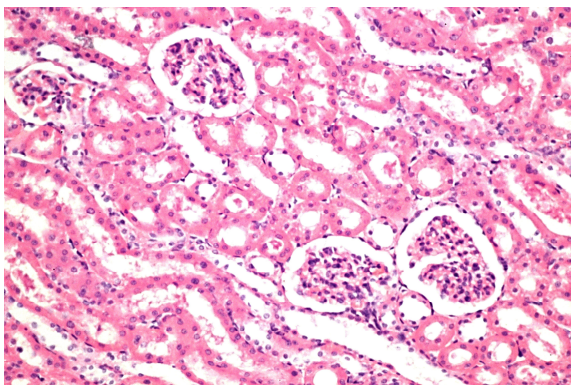

**Figure (2)**: Section of the renal cortex from the control group 0-B showing normal histological structure of the glomeruli and tubules **(H&E x40).**

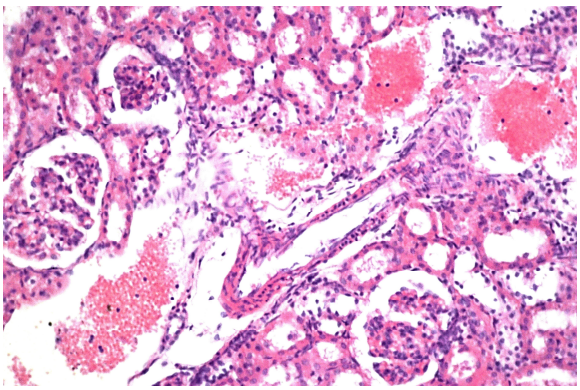

**Figure (3)**: Section of the renal cortex from group I-A showing focal hemorrhage in between the degenerated tubules **(H&E x40).**

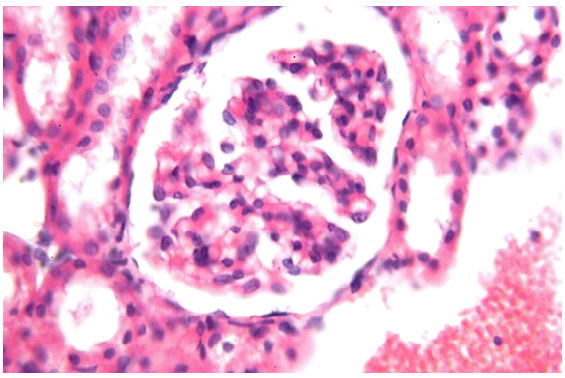

**Figure (4)**: Section of the renal cortex from group I-B showing swelling and vacuolization of the endothelial cells lining the tufts of the glomeruli **(H&E x80).**

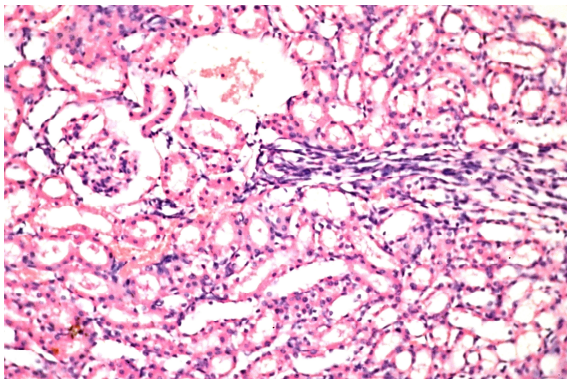

**Figure (5)**: Section of the renal cortex from group II showing focal fibrosis in between the degenerated tubules at the corticomedullary junction **(H&E x40).**

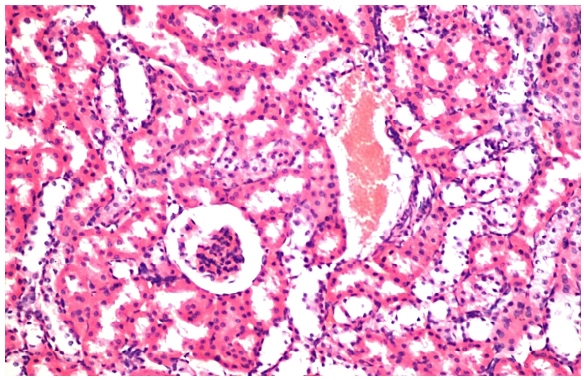

**Figure (6)**: Section of the renal cortex from group II showing congestion in cortical blood vessels **(H&E x40).**

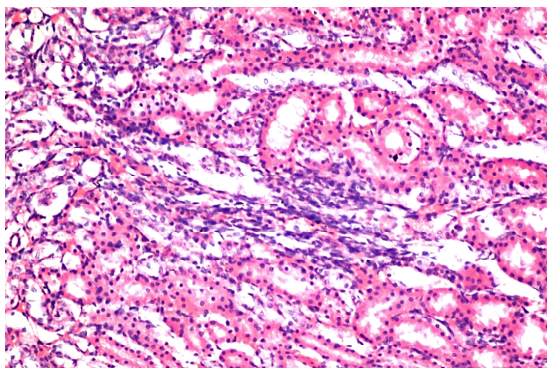

**Figure (7)**: Section of the renal cortex from group II showing focal fibrosis in between the degenerated tubules at the corticomedullary junction **(H&E x40).**

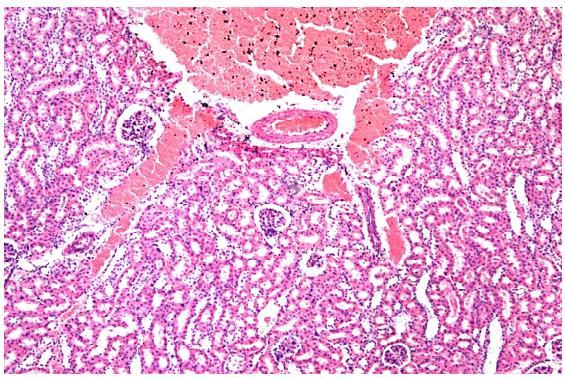

**Figure (8)**: Section of the renal cortex from group III showing focal hemorrhage and congestion in cortical blood vessels **(H&E x16).**

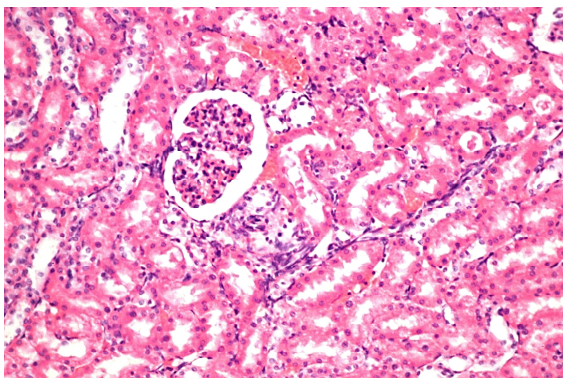

**Figure (9)**: Section of the renal cortex from group IV showing mild focal fibrosis in between the renal tubules **(H&E x40).**

## DISCUSSION

Results from the present study reported that a single dose of CP induced kidney damage as evidenced by increased renal function biomarkers, i.e. serum urea and creatinine. These results were in agreement with **Olayinka et al. (2015)** who reported a significant increase in plasma levels of urea and creatinine after administration of CP to rats, indicating marked damage to functioning nephrons. Oxidative stress and free radical production in renal tubular cells have been suggested to be responsible for CP-induced renal damage **(Abraham et al., 2007).** In the present study, OT ameliorated CP-induced renal toxicity, as indicated by the decrease of serum urea and creatinine levels possibly by maintaining the renal cellular membrane integrity through improving the antioxidant status **(ElBerry et al., 2012).**

Consistent with other studies, this study revealed that CP-induced renal toxicity was associated with marked increase of MDA content in renal tissue. Others reported that single and multiple CP-injections resulted in a significant negative shift in the host oxidant/antioxidant balance system with significant elevation in kidney MDA content, and significant renal tissue injury **(Rehman et al., 2012 and Zarkovic et al., 2013).** MDA is one of the end-products of lipid peroxidation. Lipid peroxidation causes breakage of lipids with formation of reactive compounds leading to changes in the permeability and fluidity of the membrane lipid bilayer and can dramatically alter cell integrity **(Barrera, 2012).**

Depletion of GSH below its basal level promoted the generation of ROS and oxidative stress with a cascade of effects on the functional and structural integrity of cells and organelles membranes **(Nagaraj et al., 2012).** In consistency with the results of this study, other researchers indicated that the free radicals and reactive oxygen species are involved in the CP induced oxidative stress and are reason for possible renal injury because of depletion of the GSH concentration, and decreased antioxidant enzyme activity in renal tissue of rats **(Said et al., 2015).** CP-induced depletion of GSH is primarily mediated by the interaction of its reactive metabolite; acrolein with GSH **(Hamsa and Kuttan, 2012).** Acrolein also interacts with cysteine which is one of the constituent amino acids of GSH. Therefore, a number of sulfhydryl (SH) compounds and cysteine itself have been observed to protect the animal from toxic effect of CP **(Rehman et al., 2012).**

The results of the present study revealed that oxytocin improved the antioxidant status through increased levels of GSH along with decreased levels of MDA. Other studies confirmed the antioxidant properties of OT in cisplatin-induced nephrotoxicity in rats **(ElBerry et al., 2012),** and also in rat models of renal ischemia/reperfusion injury **(Tuğtepe et al., 2007).** In brain membranes, OT displayed antioxidant properties in aqueous medium through scavenging free peroxyl radicals, preventing LDL oxidation and inhibiting lipid peroxidation **(Karelina et al., 2011).** The antioxidant effects of OT may also take place through the inhibition of tissue neutrophil accumulation and associated production of reactive oxygen species. A possible

mediator behind this mechanism could be nitric oxide. Nitric oxide released by oxytocin, may inhibit the adhesion and accumulation of neutrophils **(AlJanabi et al., 2012).** Another possibility to consider is that OT may release atrial natriuretic peptide (ANP), which is a vasodilator and has antioxidative properties (**Vincent and SU, 2008 and Erbas et al., 2014).**

In the current study, the light microscopic examination revealed that rats injected with CP showed focal hemorrhage which was observed in between the degenerated tubules at the cortex, associated with swelling and vacuolization of the endothelial cells lining the tufts of the glomeruli. Moreover, there was focal fibrosis in between the degenerated tubules at the corticomedullary junction. These results were in agreement with **Abraham et al. (2007)** who found that CP-treated rat kidneys showed glomerular nephritis, interstitial edema and cortical tubular vacuolization. The degenerative changes observed in renal tissue might be related to oxidative stress **(Contini et al., 2012).** It has been demonstrated that increased generation of ROS by CP in kidney tissues can cause damage to several cell structures. **(Amien et al., 2015).** Such oxidative stress can activate p38 MAPK (mitogen-activated protein kinases). P38 MAPK has an important role in regulating many apoptotic and inflammatory pathways **(Rashed et al., 2011).**

Administration of oxytocin caused a significant improvement in kidney histopathology with alleviation of tissue inflammation and tissue recovery especially in rats treated with OT pre-and post-injection of CP. These results were in agreement with the results of **Elberry et al. (2012)** who revealed that, in the OT-treated rats, there was a remarkable improvement in the histological features of the kidney. The reduced tubular damage and interstitial inflammation were the features indicating regeneration and improvement **(Erbas et al., 2014).** OT has a powerful antioxidant effect that can alleviate the CP-induced nephrotoxicity through inhibition of P38 MAPK resulting in improvement of kidney structure and functions **(Rashed et al., 2011).**

In the current study, CP-injection resulted in a significant elevation in serum TNF α. Similar results were found by **Said et al. (2015)** who reported that intraperitoneal CP significantly impaired oxidant/anti-oxidant balance and increased tumor necrosis factor levels, with significant impairment of kidney architecture and functions. TNF-α is a multifunctional pro-inflammatory cytokine that belongs to the tumor necrosis factor superfamily. It signals through two distinct cell surface receptors, TNF-R1 and TNF-R2 **(Léia et al., 2010).** TNF-α has been reported to trigger a series of various inflammatory molecules which contribute to the extent of severity of tissue injury, such as IL-8 (interleukin-8), prostaglandins and reactive oxygen species (ROS) **(Giebelen et al., 2007).** Also, TNF-α could induce apoptosis in renal tissue **(Sanz et al., 2008).**

The current study demonstrated that the elevated level of TNF-α, after CP-injection was reduced by OT indicating its protective effect against CP-induced nephrotoxicity. Similar results were found by **ElBerry et al. (2012).** It has been also reported that OT treatment before or

immediately after hepatic ischemia-reperfusion significantly reverses trans-aminase and TNFα elevation in the circulation **(Düşünceli et al., 2008).** Moreover, oxytocin may affect other mediators involved in the pathogenesis of inflammation including increased release of nitric oxide and decreased release of IL-6; both inhibit adhesion and aggregation of neutrophils. Moreover, oxytocin was shown to increase corticosterone levels acutely in rats which is capable of inhibiting neutrophil extravasation in response to different stimuli **(Gutkowska and Jankowski, 2009). Said et al. (2015)** proposed that oxytocin can modulate both early onset and delayed onset CP-induced nephrotoxicity. Thus, oxytocin could be helpful for patients using CP for long periods.

**In conclusion**, the current study provides evidence on the cytoprotective efficacy of oxytocin against CP-induced nephrotoxicity by potentiating the antioxidant defense mechanisms, and by alleviating the inflammatory status. Such hypothesis makes oxytocin an attractive option for cancer patients using CP.

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# تأثير الأوكسيتوسين على التسمم الكلوي الناجم عن السيكلوفوسفاميد في ذكور الجرذان البيضاء البالغة

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**خلفية البحث:** يشيع استخدام سيكلوفوسفاميد كعقار مضاد للسرطان، ويسبب تسمم الأنسجة عن طريق نواتج الأيض النشطة الخاصة به. ويفرز هرمون الأوكسيتوسين من النواتين الوطائية فوق البصرية وجنيب البطين، وله خاصية مناعية وفى حالات الالتهابات.

**الهدف من البحث:** دراسة تأثير الأوكسيتوسين على سمية الكلى الناجمة عن السيكلوفوسفاميد في ذكور الجرذان البيضاء البالغة.

**مواد و طرق البحث:** تم تقسيم ٧٠ من ذكور الجرذان البيضاء البالغة إلي خمس مجموعات : المجموعة صفر وتمثل المجموعة الضابطة الطبيعية (عشرون جرذا قسموا إلى مجموعتين متساويتين صفر: ألف و باء) المجموعة الأولى تمثل المجموعة الضابطة المريضة ( حقنوا عبر الغشاء البريتونى بجرعة واحدة من السيكلوفوسفاميد وقسموا إلى مجموعتين متساويتين ألف و باء) والمجموعة الثانية عولجوا بالأوكسيتوسين لمدة ٧ أيام قبل الحقن بالسيكلوفوسفاميد ثم ذبحوا بعد ٢٤ ساعة مع المجموعات صفر أ والأولى أ و المجموعة الثالثة عولجوا بالأوكسيتوسين بعد الحقن بالسيكلوفوسفاميد لمدة ١٠ أيام ثم ذبحوا مع المجموعات صفر ب والأولى ب والمجموعة الرابعة والمجموعة الرابعة عولجوا بالأوكسيتوسين قبل وبعد الحقن بالسيكلوفوسفاميد. وفى نهاية مدة التجربة تم جمع عينات الدم لقياس نسبة اليوريا والكرياتينين في مصل الدم. وقد تم استخراج كلتا الكليتين لكل فأر بعناية. واستخدمت الكلية اليمنى لقياس المالونديالدهيد والجلوتاثيون وعامل نخر الورم- ألفا. أما الكلية اليسرى فقد تم الاحتفاظ بها للفحص النسيجي.

**نتائج البحث**: خفف إعطاء الأوكسيتوسين من سمية الكلى الناجمة عن السيكلوفوسفاميد، واستدل على ذلك من انخفاض دلالات سمية الكلى مثل اليوريا والكرياتينين المالونديالدهيد وعامل نخر الورم- ألفا، بينما تحسنت مستويات الجلوتاثيون التي انخفضت بعد إعطاء السيكلوفوسفاميد بعد العلاج بالأوكسيتوسين. ولم تلاحظ فروق ذات دلالة إحصائية بين المجموعات المعالجة بالأوكسيتوسين. وقد أدى إعطاء الأوكسيتوسين إلى تحسن ذي دلالة إحصائية في التغيرات النسيجية المرضية بالكلى، وتخفيف التهاب الأنسجة، واستعادة الأنسجة خاصة في الجرذان المعالجة بالأوكسيتوسين قبل وبعد الحقن بالسيكلوفوسفاميد.

**الإستنتاج:** للأوكسيتوسين دور وقائي من تسمم الكلى الناجم عن السيكلوفوسفاميد بواسطة تعديل مستويات المالونديالدهيد والجلوتاثيون وعامل نخر الورم- ألفا.