Cisplatin (CP) is an inorganic platinum complex that is highly effective against many tumors.\(^1\) Although higher doses of CP are more effective than lower ones, high-dose therapy is associated with irreversible renal dysfunction and cumulative nephrotoxicity.\(^2\) Irreversible renal damage occurs in about one third of CP-treated patients.\(^3\) Thus, prevention of the dose-limiting side effects of CP is one of the major issues in treating cancer patients.\(^4\) Oxidative stress process was reported to contribute to CP-induced nephrotoxicity.\(^5,6\) CP generates reactive oxygen species (ROS) and consequently, depletes glutathione and inhibits the activity of antioxidant enzymes in renal tissues.\(^7,8\) Some antioxidants were tested for their ability to protect against CP-induced nephrotoxicity in experimental animals.\(^8,9\)

Oxytocin (OT) is a neurohypophysial peptide synthesized in the paraventricular and supraoptical nuclei of the hypothalamus. The well-known effect of OT is the stimulation of the uterine contractions at parturition and myoepithelial contractions in the mammary gland during suckling.\(^10\) OT receptors have been identified in many tissues, including the central nervous system, kidney, heart, thymus, pancreas, adipocytes as

### Background and Objectives

The clinical use of cisplatin (CP) is highly limited because of its renal toxicity and the production of reactive oxygen species (ROS) that intensify the cytotoxic effects. Oxytocin (OT) was previously shown to have antioxidant activity.

### Design and Setting

Experimental study on male Wistar albino rats performed in the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia.

### Methods

Forty-eight male Wistar albino rats were classified into four equal groups: a control group, OT only-treated group which received OT twice (500 µg/kg intraperitoneally (ip) 30 minutes and just before saline administration), a CP-induced nephrotoxicity group that received a single dose of CP (7.5 mg/kg ip) and treated with saline, and CP+OT group treated with the same previous doses. Seventy-two hours after CP administration, the rats were sacrificed and blood was withdrawn for determination of urea, creatinine, albumin and lactate dehydrogenase (LDH). The kidneys were extracted for histopathological examination and determination of the tissue levels of reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS) and nitric oxide end product nitrite (NO\(_2\)). Glutathione S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and myeloperoxidase (MPO) activities were assessed.

### Results

CP-induced renal injury was evidenced histopathologically and manifested by a significant increase in serum LDH activity as well as urea and creatinine levels. Moreover, renal injury was associated with decreased renal tissue activities of CAT, SOD, GPx and GST as well as GSH level. On the other hand, renal tissue content of TBARS and NO\(_2\) as well as the activity of MPO were increased. Alterations in these biochemical and histopathological indices due to CP were attenuated by OT.

### Conclusion

OT protected rats from CP-induced nephrotoxicity. Such protection is attributed, at least in part, to its antioxidant activity.
well as endothelial and smooth muscle cells of blood vessels. Houshmand et al. showed that OT possesses a dose-dependent cardioprotective effect against ischemia/reperfusion injury. Moreover, it was shown in brain membranes that secretory peptides, including OT, have antioxidant properties, scavenging free peroxyl radicals, preventing LDL oxidation and inhibiting lipid peroxidation. Recently, Rashed et al. showed that OT has a powerful antioxidant effect through inhibition of NADPH oxidase. The current study was designed to investigate the possible protective effect of OT against CP-induced nephrotoxicity.

METHODS
CP was purchased from Bristol-Myers Squibb (New York City, NY, USA) as solution (2mg/mL). OT and all other chemicals used were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Forty-eight male Wistar albino rats (250-300 g) were used in the experiment in accordance with the guidelines of the Biochemical and Research Ethical Committee at King Abdulaziz University, Jeddah, Saudi Arabia. Animals were housed in a well-ventilated temperature-controlled room at 22±3°C with 12 hours light and dark cycles. Food consisted of standard laboratory rat chow with free access to water. All experimental procedures were performed between 08:00 to 10:00 and care was taken to avoid all stressful conditions.

Rats were divided into four equal groups in separate cages. Group I (control group) received saline (1 mL/kg; intraperitoneally (ip)). Group II (OT group) received OT alone (500 μg/kg; ip) twice; 30 minutes and immediately before saline administration (instead of CP). The dose of OT was based on our preliminary study and a previous study by Düşünceli et al. Group III (CP group) received saline (1 mL/kg) 30 minutes and immediately before CP administration. CP was given in a dose of 7.5 mg/kg once ip, the dose which is documented to induce nephrotoxicity in rats. Group IV (OT + CP group) received both OT and CP in the same previous doses.

Seventy-two hours after CP administration, rats were sacrificed using ether anesthesia and blood was collected directly by a cardiac puncture from the heart of each animal. The blood was centrifuged at 3000×g for 10 minutes to obtain clear sera which was stored at –70°C for further analysis. The abdomen of each rat was opened and kidneys were rapidly dissected out, washed in ice-cold isotonic saline and blotted between two filter papers. The right kidneys from each animal were fixed in 10% formalin for histopathological examination. For subsequent analysis, a 10% homogenate was prepared from a part of the left kidney in phosphate buffer (0.05mol/L, pH 7.0) using a polytron homogenizer (PT 10/35) obtained from Brinkmann Instruments Inc. (Westbury, NY, USA) at 4°C. The homogenate was centrifuged at 1000×g for 10 minutes at 4°C to remove the cell debris.

Serum albumin concentrations were determined by a spectrophotometric bromocresol green method described by Doumas et al. Serum lactate dehydrogenase activity (LDH), blood urea nitrogen (BUN) and serum creatinine levels were determined spectrophotometrically by the methods described by Martinek, respectively. Reduced glutathione (GSH) was determined according to the method of Moron et al.22 based on the formation of a yellow-colored complex with Ellman reagent. Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) in tissue homogenates referring to the malondialdehyde (MDA) standard calibration curve according to the method of Uchiyama and Mihara. Nitric oxide (NO) end product nitrite production (NO₂⁻) was used to assess relative values of the NO. Nitrites assay was determined by a colorimetric method based on the Greiss Reaction.

The activity of glutathione S-transferase (GST) was measured according to the method of Habig et al. P-nitrobenzylchloride was used as a substrate. The absorbance was measured spectrophotometrically at 310 nm using a UV-double beam spectrophotometer obtained from Labomed Inc. (Culver City, CA, USA). Catalase (CAT) activity was determined from the rate of decomposition of H₂O₂, monitored by a decrease of 240 nm following the addition of tissue homogenate. Superoxide dismutase (SOD) levels in the kidney tissue were determined according to the modified method of Kakkar et al. Glutathione peroxidase (GPx) activity was determined by the method of Habig et al. based on the degradation of H₂O₂ in the presence of GSH. Myeloperoxidase (MPO) activity was determined using a 4-aminopyrrole/phenol solution as a substrate for MPO-mediated oxidation by H₂O₂ and changes in absorbance at 510 nm (A510) were recorded. The protein content of tissue homogenates was determined by the Lowry protein assay using bovine serum albumin as a standard.

Histopathological sections of kidney from all groups were evaluated using Meiji light microscope model 109-L (Meiji Techno. Co., Ltd., Saitama, Japan). Tissues were fixed in 10% formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin.

The GraphPad InStat 3 (graphPad Software Inc, San Diego, CA, USA) was used for statistical analysis. Two-tailed Student’s t-test was carried out for comparison of two groups and one-way analysis of variance (ANOVA) followed by Tukey’s test was used for multiple comparisons among groups. A p-value of ≤ 0.05 was considered to be statistically significant.
La Jolla, CA, USA) computer program was used to conduct regression analysis and to plot the collected data. All data were expressed as means and standard deviation. Assessment of these results was performed using one-way ANOVA procedure followed by Tukey-Kramer multiple comparisons tests using Software GraphPad Prism, Version 4. Statistical significance was determined as a $P$ value $<.05$.

**RESULTS**

**Effect on serum markers**
Administration of CP to rats significantly increased BUN and serum creatinine levels and significantly reduced serum albumin compared to the control group ($P<.05$). Pretreatment with OT significantly reduced ($P<.05$) BUN and nearly normalized serum creatinine and albumin levels (Figures 1 and 2 and Table 1). Moreover, serum LDH activity significantly increased in CP group compared to the control group. Pretreatment with OT significantly inhibited CP-induced increase in serum LDH activity (Figure 2).

**Effect on tissue markers**
In CP rats, significant ($P<.05$) depletion of GSH and an increase in TBARS and NO$_2$ levels were observed compared to the control group. Pretreatment with OT significantly attenuated these changes in OT+CP group compared to CP group (Table 2).

**Effect on tissue enzymatic activity**
Renal tissue activities of the antioxidant enzymes (GST, SOD, CAT and GPx) were significantly decreased ($P<.05$) in CP group compared to control group. Pretreatment with OT significantly elevated these levels (Table 3). MPO activity, an indicator of neutrophil infiltration, was significantly higher in the renal tissue of CP group compared to control group. On the other hand, OT pretreatment significantly decreased the renal tissue activity of MPO approximating the control group activity (Figure 3).

**Histopathological results**
The light microscopic study of the control and OT groups revealed a regular morphology of renal parenchyma with well-designated glomeruli and tubules (Figure 4A and 4B). In the CP group, CP caused severe widespread tubular and interstitial damage evidenced by tubular lumen dilatation due to flattening of tubular cells with brush border loss, cast formation and inflammatory cell infiltration (Figure 4C). Pretreatment with OT significantly improved the histological features of the CP-induced nephrotoxicity. Kidneys of rats pretreated with OT showed reduced tubular damage and interstitial inflammation indicating regeneration and improvement (Figure 4D).

**DISCUSSION**
Nephrotoxicity limits the clinical use of CP as a chemotherapeutic agent in 25% to 30% of patients, even after the first dose. The current study investigated the
possible protective effect of OT against CP-induced nephrotoxicity. CP induced renal injury as evidenced by the histopathological features as well as serum and tissue parameter changes. Townsend et al\(^3\) showed that renal toxicity from CP is usually localized to the proximal tubular cells. This is consistent with the findings of the present study that showed tubular necrosis with interstitial inflammation.

BUN and serum creatinine were elevated with a significant decrease in serum albumin, indicating renal impairment in CP-treated rats. Similarly, LDH was significantly elevated following CP administration, indicating nonspecific cellular injury. The exact mechanism of CP-induced nephrotoxicity is not completely understood. However, oxidative stress and free radical production in renal tubular cells have been suggested as reasons for the oxidative renal damage.\(^3\) Mansour et al\(^3\) demonstrated that CP induces ROS by depleting the antioxidant system whether enzymatic as GST, GPx, SOD and CAT or non-enzymatic as intracellular GSH. These findings are in accordance with the results of the present study. Pretreatment with OT obviously ameliorated these alterations in the antioxidant system.

The present study supports the hypothesis that the mechanism of CP toxicity is related to exhaustion of the antioxidant defense system. A significant increase in renal TBARS and a decrease in the activities of antioxidant enzymes were reported by many researchers following CP treatment of rats.\(^8\) Mansour et al\(^3\) demonstrated that CP induces ROS by depleting the antioxidant system whether enzymatic as GST, GPx, SOD and CAT or non-enzymatic as intracellular GSH. These findings are in accordance with the results of the present study. Pretreatment with OT obviously ameliorated these alterations in the antioxidant system.
verified the oxidative damage in the renal tissue. OT pretreatment inhibited the increase in MPO activity and TBARS levels as well as the inflammatory cell infiltration induced by CP. These data are in accordance with Çetinel et al., who reported similar effects of OT on both MPO and MDA in stress-induced colitis.

Previous studies indicated that CP undergoes metabolic activation in the kidney to a more potent toxin with highly reactive thiols. This process begins with the formation of glutathione conjugates in the circulation, perhaps mediated by GST. In the current study, pretreatment with OT ameliorated the decrease in the GST induced by CP. Moreover, OT prevented both oxidative renal injury and tissue neutrophil accumulation. A possible mediator behind the decrease in MPO activity in response to OT could be NO. It has been reported that CP-induced nephrotoxicity is associated with higher kidney NO levels due to overexpression of inducible nitric oxide synthase activity. Inhibition of NO was reported to decrease CP-induced lipid peroxidation and organ toxicity. In the current study, OT reduced the elevation of NO and the lipid peroxidation marker TBARS, which was increased by CP.

Some studies confirmed the antioxidant property of OT in colitis, pyelonephritis, and sepsis-induced inflammatory models. It was also shown that in brain membranes, OT displayed antioxidant properties in aqueous medium, scavenging free peroxyl radicals, preventing LDL oxidation and inhibiting lipid peroxidation. OT has a protective effect against CP-induced renal toxicity in rats. This protective effect is probably mediated, at least in part, through its antioxidant effect.

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