Role of curcumin in prevention of cisplatin-induced nephrotoxicity in rat

Adeeb A. Al-Zubaidy*
Dalia Abd Al Kader Al-Salihy**[1]
* College of pharmacy, Kerbala University
** College of Pharmacy, Al Mustansiriya Universit

Abstrac
Cisplatin is a platinum anticancer drug approved by the FDA in 1978. It is used to treat a broad spectrum of malignancies but the amount of drug necessary to produce a significant reduction in tumor burden usually produces significant nephrotoxicity. the purpose of this study was to evaluate the role of curcumin in the prevention of cisplatin-induced nephrotoxicity in rats. Thirty male albino rats weighing (200-250 gm), they were equally divided into three groups: Normal group (Isotonic saline group**)**, control group "Cisplatin group" (12 mg/kg cisplatin, single dose I.P.) and curcumin group (50 mg/kg I.P.) prophylactically one day prior to cisplatin administration and continued for further 3 days. Cisplatin administration resulted in a significant increase in serum creatinine, BUN and MDA while serum glutathione level was significantly decrease compared to that of normal group. The Sections of kidneys rats treated by cisplatin were showed degenerative and necrosis of the proximal and distal convoluted tubules. Curcumin administration resulted in a significant decrease in serum creatinine, BUN and serum MDA while serum glutathione level was to be significantly increased. There were very minimal degenerative changes in kidney tissues of rats comparing to that of control group.

الخلاصة
السيسبلاتين هو علاج كيمياوي فعال يستخدم في معالجة طيف واسع من الاورام السرطانية لكن المشكلة في استخدامه تكمن في ان الجرعة العلاجية قريبة من الجرعة التي تسبب التسمم وان كمية الدواء الضرورية لاحداث تاثير علاجي عادة تؤدي الى حدوث تلف كلوي. اجريت هذه الدراسة على الجرذان لتقييم دور الكركمين في حماية الكلى من التلف الكلوي الذي يسببه دواء السيسبلاتين. تم استخدام 30 جرذ اوزناهم تتراوح بين (200-250غم) وقسموا بالتساوي على ثلاث مجاميع: مجموعة النورمال سلاين اعطيت عن طريق البريتون (0,2 مليلتر نورمال سلاين) و مجموعة السسبلاتين (اعطيت جرعة واحدة 12 ملغم/ كغم) من السيسبلاتين
عن طريق البريتون ومجموعة الكركمين والتي حقنت لاربع ايام بالكركمين وحقنت بجرعة واحدة من السيسبلاتين في اليوم الثاني (12 ملغم/كغم) عن طريق البريتون . اظهرت النتائج ان اعطاء السيسبلاتين نتج عنه زيادة ملحوظة في مستوى الكرياتنين واليوريا والمالوندايالديهايد وحصول نقصان ملحوظ في مستوى الكلوتاثيون وأظهرت النتائج عدم حدوث تغيير ملموس في مستوى البروتين (الالبومين و الكلوبيولين) في المصل بالمقارنة بمجموعة النورمال سلاين، كما ان المقاطع النسيجية لكلية الجرذان اظهرت تلف كلوي ناتج عن اعطاء السيسبلاتين. كما اظهرت النتائج ان اعطاءالكركمين نتج عنه نقصان ملحوظ في مستوى الكرياتنين واليوريا والمالوندايالديهايد وحصول زيادة ملحوظة في مستوى الكلوتاثيون. بالاضافة لذلك فان اعطاء الكركمين قلل التلف الكلوي الذي يسببه السيسبلاتين بشكل ملحوظ وكبير. وأظهرت النتائج عدم حدوث تغيير ملموس في مستوى البروتين (الالبومين و الكلوبيولين) في المصل بالمقارنة بمجموعة السيسبلاتين. يمكن الاستنتاج ان الكركمين ضمن الجرع المستخدمة له دور في حماية الكلى من التلف الكلوي الذي يسسببه السيسبلاتين.

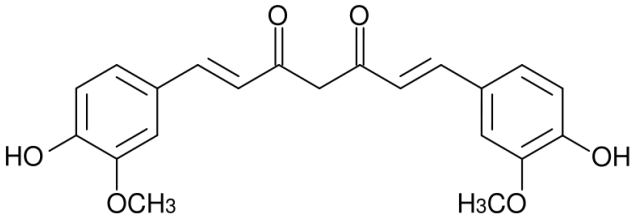
[1] Corresponding author E- mail: dalya_al_salihy @ yahoo.com

Introduction

One of the great success stories in the field of cancer chemotherapy is that of cisplatin a curative treatment for testicular tumors, in testicular cancer, the drug reaches greater than 90% cure rates, approaching 100% in early stage cases [(1)]. Cisplatin usually in combination with other drugs is commonly used as first line chemotherapy against cancers of the testis, lung, head-and-neck, esophagus, stomach, colon, bladder, ovaries, cervix, uterus and as second line treatment against most other advanced cancers, such as cancers of the breast, pancreas, liver, kidney, prostate as well as against glioblastomas, metastatic melanomas, and peritoneal or pleural mesotheliomas [(2)] and it has synergistic cytotoxicity with radiation and other chemotherapeutic agents [(3)]. The therapeutic effects of cisplatin are significantly improved by dose escalation. However high-dose therapy with cisplatin is limited by its cumulative nephrotoxicity and neurotoxicity [(4)].The mechanism of action of cisplatin is similar to that of the alkylating agents; cisplatin enters cells by diffusion and by an active Cu^{2+} transporter and inside the cell, the chloride atoms of cisplatin are replaced by water, yielding a positively charged molecule that reacts with nucleophilic sites on DNA and proteins. Aquation is favored at the low concentrations of Cl^- inside the cell and in the urine. The platinum complexes can react with DNA, forming both intrastrand and interstrand cross-links [(5)]. The resulting cytotoxic lesion inhibits both DNA replication and RNA synthesis [(3)]. Severe and irreversible damage to the kidney remains the most important complication of cisplatin treatment as it may limit further treatment or even threaten life [(6)]. The efficacy of cisplatin is dose dependent, but the significant risk of nephrotoxicity frequently hinders the use of higher doses to maximize its antineoplastic effects [(2,4and 7)]. Cisplatin gets accumulated in the tubular epithelial cells of proximal kidney tubule, causing nephrotoxicity, characterized by morphological destruction of intracellular organelles, cellular necrosis, loss of microvilli, alterations in the number and size of the lysosomes and mitochondrial vacuolization, followed by functional alterations including inhibition of protein synthesis, glutathione depletion, lipid peroxidation and mitochondrial damage[(8)].

Proximal tubular damage appears acutely after administration of cisplatin as the result of impairment of cell energy production, possibly by binding to proximal tubular cellular proteins and sulfhydryl groups with disruption of cell enzyme activity and uncoupling of oxidative phosphorylation[(9)]. The initial proximal tubular damage is followed by a progressive loss of glomerular filtration and impaired distal tubular function[(10)] .

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is the principal curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae) [(11)].



Curcumin structure [12]

Curcumin has been shown to have multiple anticancer effects, including inhibition of proliferation[13,14], induction of apoptosis, inhibition of angiogenesis[13,15], and inhibition of DNA topoisomerase II [13].

Materials and methods

Thirty male rats (Rattus norvegicus) weighing (200-250 gm), were selected. The animals were housed in the animal house of Institute of embryo researches and infertility treatment / Al-Nahrain University under condition of controlled temperature, allowed free access to water and food. The rats were equally divided into three groups (10 rats/ group). Normal group (Isotonic saline group) rats were received 0.2 ml of isotonic saline intraperitoneally (I.P.) for four days and sacrificed one day after. Control group (Cisplatin- induce nephrotoxicity group) rats were received 0.2 ml of isotonic saline (I.P.) one day prior to cisplatin administration (single I.P. injection 12mg/kg for induction of nephrotoxicity) and repeated isotonic saline administration daily, then rats were sacrificed after 3 days of cisplatin administration. curcumin group rats received curcumin (50 mg/ kg I.P.) prophylactically one day prior to cisplatin administration (single I.P. injection 12mg/kg) and repeated curcumin administration daily for further three days. The levels of serum creatinine[16] , BUN [17], serum total protein [18], serum albumin [19], serum glutathione [20], serum Malondialdehyde (MDA) [21] were measured and histopathological study by staining with hematoxylin and eosin [22], then examined under light microscope were done.

Statistical methods:

In this study, the obtained quantitative data were presented as [mean ± standard error of mean (S.E.M.)]. Student (unpaired) t-test for independent data was used to test the significance of the differences between the results of the any two groups [23].

Results

Cisplatin administration resulted in a significant increase in serum creatinine ($P = 1.923 \times 10^{-07}$) (fig. 1), serum BUN ($P = 3.9 \times 10^{-09}$) (fig. 2) and serum MDA ($P = 0.000339$) (fig. 4) while serum glutathione level was significantly decrease ($p = 5.314 \times 10^{-11}$) (fig. 5) compared to that of normal group. There were no significant changes in serum total protein, albumin and globulin when

compared with normal group (fig. 3). Section of the kidneys rats treated by Cisplatin showing degenerative and necrosis of the proximal and distal convoluted tubules (fig. 7) when compared with normal group (fig. 6). Curcumin administration resulted in a significant decrease in serum creatinine ($p = 9.97\times 10^{-07}$), serum BUN ($p = 4.1\times 10^{-08}$) and serum MDA ($p = 0.00025$) while serum glutathione level was to be significantly increased ($p = 2.61\times 10^{-6}$). There were very minimal degenerative changes in kidney tissues of rats comparing to that of control group (fig. 8)

Discussion:

The usefulness of cisplatin is limited by its toxicity to normal tissues, including cells of the kidney proximal tubules. It has been reported that cisplatin induced nephrotoxicity is closely associated with an increase in lipid peroxidation in the kidney. In addition, cisplatin has been found to lower the activities of antioxidant enzymes and to induce depletion of GSH [8]. Since the induction of nephrotoxicity by cisplatin is assumed to be a rapid process and renal damage occurs within 1 hour so it is important that the protective agent is present in renal tissue before damage occurs[24] thus, in this study, curcumin was administered one day before cisplatin injection. The most common manifestation of cisplatin induced nephrotoxicity is a decline in glomerular filtration rate (GFR) leading to a rise in serum creatinine and BUN. The onset of toxicity in hospitalized, acutely ill patients is most often recognized by routine laboratory monitoring of these two chemistries [25; 26]. Serum creatinine is considered as a marker of acute nephrotoxicity [6]. In the present study, using an experimental model of cisplatin-induced nephrotoxicity in rats (single dose of 12mg/kg I.P.) was characterized by alterations in renal function as a significant increase in serum creatinine and BUN levels compared to normal group and this result is compatible with those observed by many others [8,26, 27] .

In all groups of this study; there were no significant changes in serum total protein, serum albumin and serum globulin comparing to that of normal group. Yao and co-workers (2007) [28] mentioned that in cisplatin induced nephrotoxicity there is a little change in protein being excreted in urine. In the present study, cisplatin caused elevation of MDA, reduction of glutathione which indicated severe kidney damage. Histological results of this study indicated that severe degenerations at the proximal tubular cells could be correlated with the harmful effects of cisplatin parallel to high MDA and low GSH levels. The increase in thickness of the glomeruli basement membrane could be a result of membrane disturbance due to cisplatin administration. Lipid peroxidation mediated by oxygen free radicals causes destruction and damage to cell membranes [29] .

Curcumin (50 mg/kg I.P.) administration resulted in highly significant decrease in serum creatinine and BUN levels comparing to that of control group.

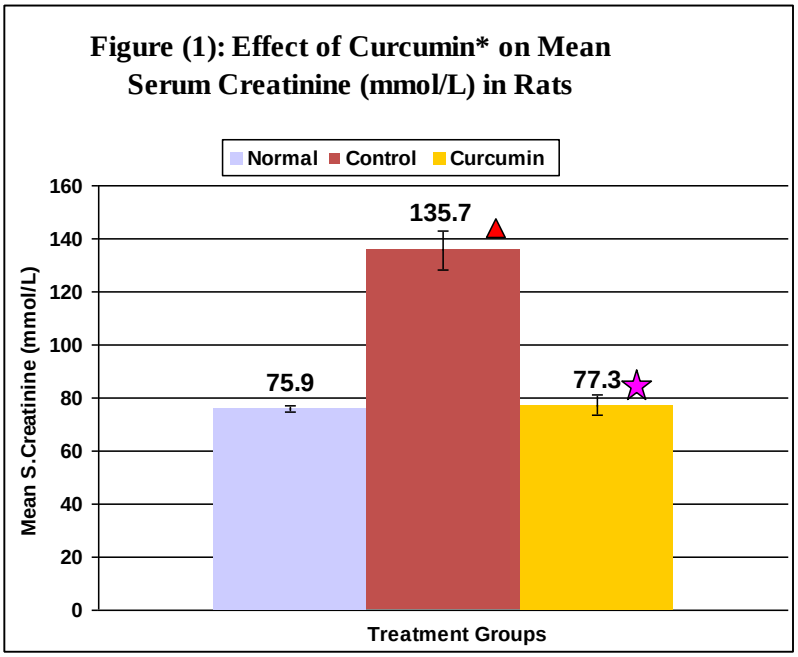
There was high significant decrease in serum MDA and high significant increase in serum glutathione comparing to that of control group.

Curcumin has strong antioxidant and anti-inflammatory properties [30, 31]. Extensive scientific researches on curcumin demonstrated its antiinflammatory action. Curcumin was found to inhibit arachidonic acid metabolism, cyclooxygenase, lipoxygenase, cytokines (Interleukins and tumor necrosis factor) and release of steroidal hormones. Curcumin was reported to stabilize lysosomal membrane and cause uncoupling of oxidative phosphorylation besides having strong oxygen radical scavenging activity, which was responsible for its anti-inflammatory property. Also curcumin was found to be a potent scavenger of superoxide [32].

The role of curcumin is supported by a number of scientific evidences that have confirmed its anti-inflammatory and antioxidant actions both in vivo and in vitro. Many activities of curcumin can be also explained by its ability to suppress acute and chronic inflammation by scavenging reactive oxygen and reactive nitrogen species and enhancing antioxidant defense (i.e. by increasing glutathione level). However, curcumin is not only a simple antioxidant, but it plays a key role in activating antioxidative enzymes [33]. It has been proposed that antioxidants which maintain the concentration of reduced glutathione may restore the cellular defence mechanisms, block lipid peroxidation and thus protect against the toxicity of a wide variety of nephrotoxic chemicals [34]. Priyadarsini et al., (2003) [12] tested the antioxidant activity of curcumin by radiation-induced lipid peroxidation in rat liver microsomes and found that the efficiency to inhibit lipid peroxidation is 82%. P53 is a key regulatory protein in the cell cycle and initiator of apoptosis [35]. Jiang et al. (2007) [36] suggested a role of p53 in renal cell injury by cisplatin and it is activated during cisplatin treatment. Curcumin has an unprecedented number of molecular targets justifying its chemopreventive, antioxidant and anti-inflammatory activities; one of these targets is p53 [33].

Histological result of the rat treated with curcumin showed very mild degenerative changes of the epithelial cells of the proximal tubules. The glomeruli look like normal, this correlated to strong antioxidant and anti-inflammatory properties of curcumin. One of cisplatin- induced nephrotoxicity mechanisms is elevated renal expression of TNF-α [37], and as mentioned above curcumin has the ability to inhibit TNF-α [32] and this may be one of the protective mechanism of curcumin against cisplatin nephrotoxicity.

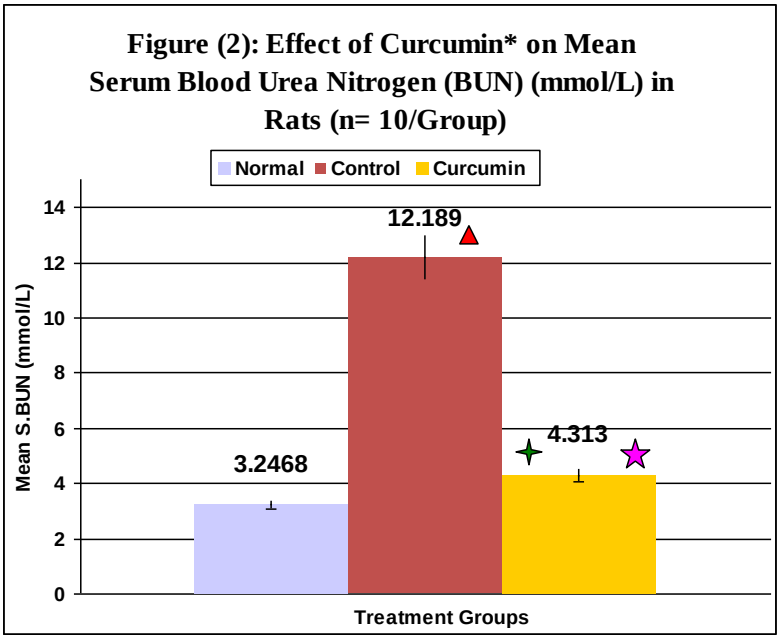
Conclusion: curcumin (50 mg/ kg, I.P.) had protective effects against cisplatin induced nephrotoxicity in rats.



* = Curcumin (50mg/kg) I.P.

▲ = Significant difference (P =1.923× 10^{-07}) when compared with corresponding value in normal group.

★ =Significant difference (p =9.97× 10^{-07}) when compared to corresponding value in control group.

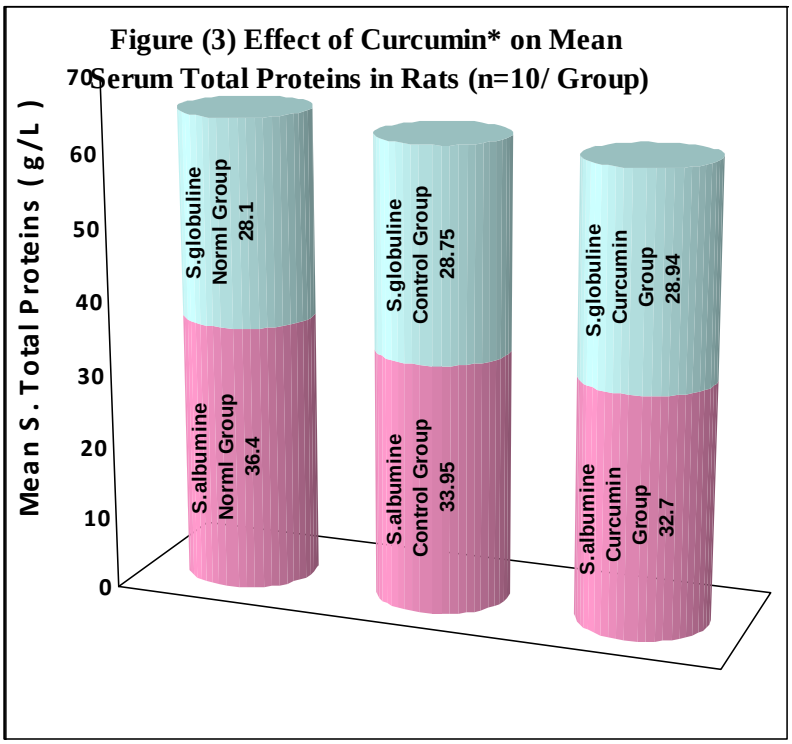


* = Curcumin (50mg/kg) I.P.

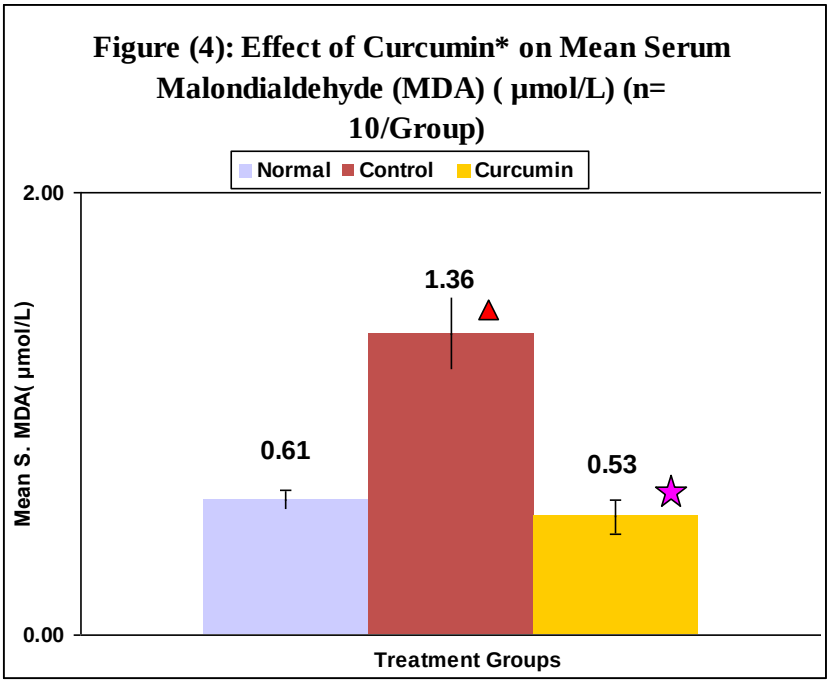
▲ = Significant difference (P =3.9× 10^{-09}) when compared to corresponding value in normal group.

★ =Significant difference (p =4.1× 10^{-08}) when compared to corresponding value in control group.

✦ =Significant difference (p =0.0029) when compared to corresponding value in normal group.



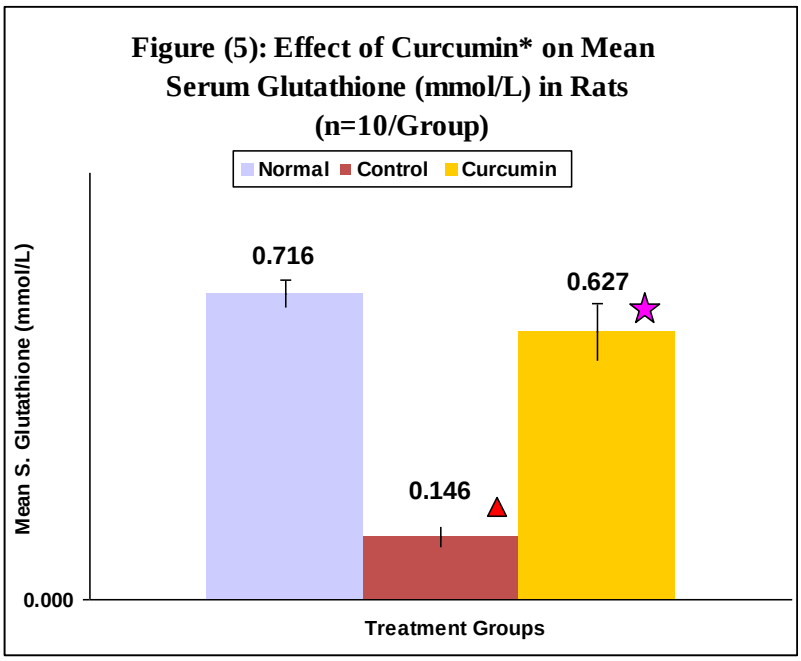
* = Curcumin (50mg/kg) I.P.



*** = Curcumin (50mg/kg) I.P.**

▲ = Significant difference (P =0.000339) when compared to corresponding value in normal group.

★ =Significant difference (p =0.00025) when compared to corresponding value in control group.



* = **Curcumin (50mg/kg) I.P**.

▲ **=Significant difference ($p = 5.314 \times 10^{-11}$) when** compared to corresponding value in normal group

★ =Significant difference ($p = 2.61 \times 10^{-6}$) when compared to corresponding value in control group.

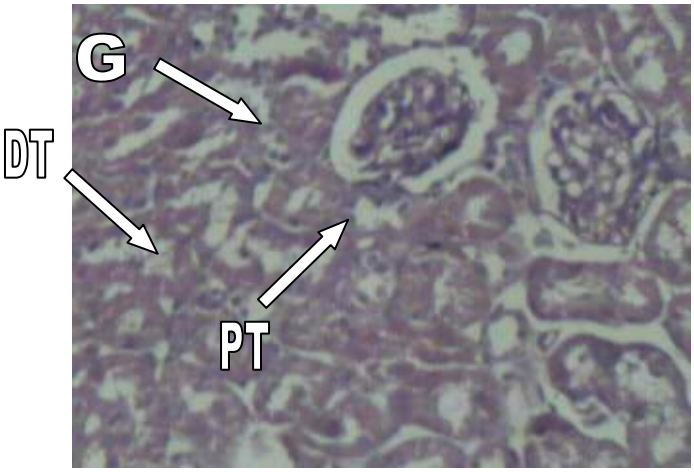


Figure (6): Normal appearance of glomeruli, proximal and distal convoluted tubules of rat's kidney.
(Magnification: 200 X, staining; haematoxylline and eosin)

G: glomerulus , PT : proximal tubule , DT : distal tubule

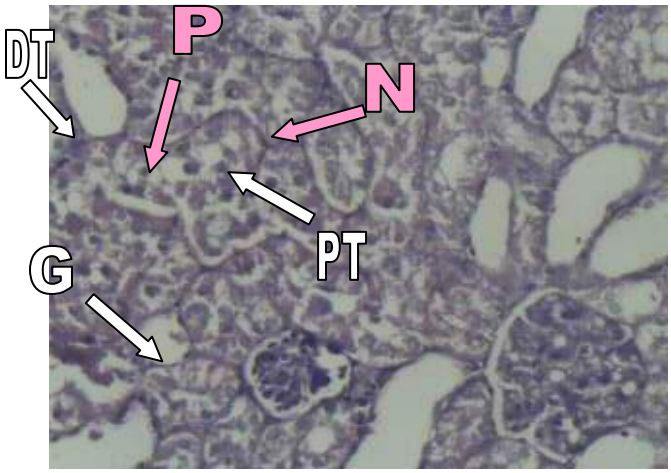


Figure (7): Nephrotoxic effect of cisplatin on rat's kidney
Magnification: 200 X, staining; haematoxylline and eosin.

G: glomerulus , PT : proximal tubule , DT : distal tubule

N: Necrosis, P: Pyknosis

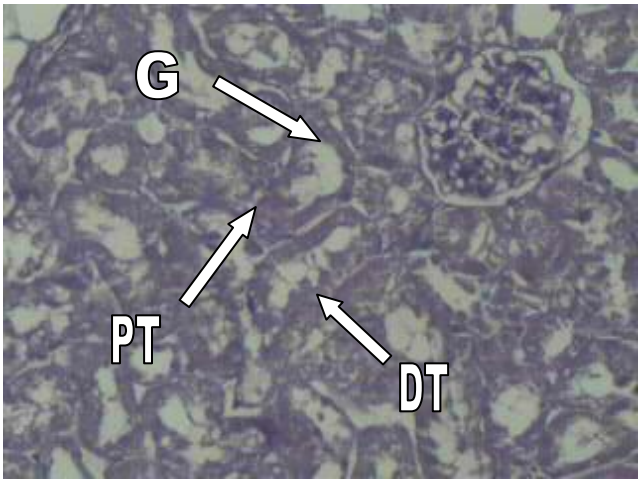


Figure (8): Protective Effect of Curcumin on Cisplatin-Induced Nephrotoxicity in Rats

Magnification: 200 X, staining; haematoxylline and eosin
(Very mild degenerative changes of the epithelial cells of the proximal tubules with accumulation of glycoprotein inside the renal tubules. The glomeruli look like normal)

G: glomerulus , PT : proximal tubule , DT : distal tubule

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