

Effect of Vitamin C on gentamicin induced nephrotoxicity in broiler chicks

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Abstract

This study aimed to evaluating the effect of vitamin C on gentamicin induced nephrotoxicity in broiler chicks. A total of 80 one-day old broiler chicks were used for this study. chicks were divided into 4 groups each of 20 chicks. First group was given gentamicin 50 mg/kg bw daily for 4 successive days . Second group was given Vitamin C 250 mg/kg bw. +. Gentamicin 50 mg/kg.bw. daily for 4 successive days Third group was given vitamin C only and forth group was left as control group. Gentamicin was administered by intramuscular injection and vit. C were orally administered in drinking water for 4 successive days. At the 7th and 14th days of experimental period , blood samples were collected from 5 chicks in each group for biochemical analysis and oxidative stress, chicks were slaughtered and kidneys were taken for pathological examination. The result of biochemical analysis of group 1 revealed a significant increase in serum creatinine and uric acid. However , reveled significant decrease in Ca .It also induce a gradual significant increase in lipid peroxide malondialdehyde MDA with gradual significant decrease in glutathione GSH. Histopathological examination of group 1 reveled vacuolar degeneration and necrosis of the renal tubular epithelium associated with cellular casts. Administration of vitamin C improve all biochemical parameters levels and oxidative stress as compared with control and improves the histopathological picture of kidneys .These improvement were prominent at 14 days . In conclusion, vitamin C has beneficial effects and could ameliorate gentamicin nephrotoxicity .

Introduction

Gentamicin is an aminoglycoside and is used for treating gram-negative bacillary infections (Goodman and Gillman, 2005). Side effects of gentamicin usage involve nephrotoxicity that is typically illustrated by following observations: decrease in glomerular filtration rate, diminished urine concentrating capacity and proteinuria (Cojocel et al., 1984). A single intramuscular dose of gentamicin 50mg or greter/kg body weight of one day old broiler chicks is found to be the most common origin of nephrotoxicity. Kidneys were congested, enlarged and had hemorrhages on the surfaces. Microscopically kidneys exhibited acute tubular necrosis (Saleemi et al., 2009).

It has been observed that gentamicin causes renal toxicity by disturbing the proximal tubular cells. The mode of renal toxicity by gentamicin involves its binding to the cell wall phospholipids resulting in the blockage of chain reactions of phosphatidyl inositol, and ultimately impairment of cell integrity (Kadkhodae et al., 2004).

One of the previous studies presents that free radicals were increased with gentamicin treatment (Varzi et al., 2007). Gentamicin-induced renal toxicity arises as a result of membrane lipid peroxidation due to the stimulation of reactive oxygen entities like H_2O_2 and O_2 which potentially induce the contraction of mesangial cells. As a result, filtration surface area and ultrafiltration coefficient changes, which causes the retardation in glomerular filtration rate (Derakhshanfar et al., 2007).

Vitamin C is a hydrophilic substance that acts as a nutritional supplement and is considered to be very essential in preventing scurvy (Belin et al., 2009). It is known to be an outstanding chain-breaking antioxidant as well as a very good free radical scavenger (Premila, 2005).

Despite its nephrotoxic potential, the aminoglycoside antibiotic gentamicin (GM) is still widely used against infections by Gram-positive and Gram-negative aerobic bacteria due to its excellent antibacterial profile and efficacy (Chen and Kaye., 2009) and considered to be an important agent against early mortality in broiler chicks. The goal of reducing nephrotoxicity has attracted much effort and attention during the last decade.

Therefore, Present study aims to assess various biochemical, oxidative activity and pathological parameters for investigating the nephroprotective activity of vitamin C against gentamicin-caused renal toxicity in one day old broiler chicks.

Materials and Methods

Chicks:- Eighty one-day old commercial broiler chicks obtained from private company for poultry were used for the experiment. Chicks were kept under hygienic conditions and maintained on a commercial balanced ration and water ad-libitum

Experimental design:- Eighty chicks, one-day old were divided into four groups each of twenty chicks. The 1st group were received gentamycin 50 mg/kg. bw. 2nd group were administered vitamin C 250 mg/kg.bw. + gentamycin 50 mg/kg.bw. 3rd group were received vitamin C 250 mg/kg.bw. The 4th group were left as control group. Gentamycin were taken by im injection and vitamin C were orally administered in drinking water. All doses were received daily for 4 consecutive day

Sampling:- Two blood samples were collected at 7 and 14 days from 5 chicks in each group. The first blood sample was collected on EDTA for estimation of antioxidant stress (lipid peroxide malondialdehyde MDA and glutathione GSH). The second blood sample was collected in a centrifuge tube and was used for separation of clear serum and assay of serum kidney function tests (creatinine, urea, uric acid, calcium and phosphorus). After blood sampling chicks were sacrificed and samples from kidneys were taken and immersed immediately in 10% neutral formalin for histopathological examination.

Methods:-

lipid peroxidation was estimated in terms of thiobarbituric acid reactive substances (TBARS), using malondialdehyde (MDA) as standard by the method of Beuge and Aust (1978). Glutathione (GSH) concentration was determined in samples according to the method of Mates et al. (2000). Serum urea, uric acid and creatinine were determined according to the method described by Tabacco *et al.* (1979) and Todd and Henry (1984), respectively. Serum calcium were measured according to Baginski (1973). Serum inorganic phosphate were measured, according to the method described by Plummer (1978)

Pathological examination: After careful postmortem examination of kidneys of all groups, tissue specimens were collected from kidneys and fixed in 10% neutral buffered formalin, processed for paraffin section and stained with haematoxylin and eosin for light microscopic examination (Bancroft et al., 1996)

Statistical analysis:-

The obtained data were statically analyzed using Student's t-test according to Petrie and Watson (1999).

Results and discussion

The current results in table (1) shows gradual increase in serum creatinine and uric acid of group 1 administered gentamycin daily for 4 successive days these increases were significant ($p < 0.05$) at 7, 14 days of experiment when compared to control group. Administration of vitamin C and gentamicin improved the levels of creatinine, urea and uric acid as compared with group 1.

This increase in serum creatinine levels was similar with the finding of (Saleemi et al., 2009). Also, (Varzi et al., 2007) reported markedly increase in these biochemical parameters after receiving high doses of aminoglycosides

The marked increase in the values of serum creatinine indicate reduction in glomerular filtration rate as well as impairment of renal blood flow. In addition uric acid is the primary catabolic product of protein, and non protein nitrogen . Birds kidney excretes uric acid primarily by tubular excretion. Uric acid increase in massive tissue destruction and renal disease (Sonnenwirth and Jarett, 1980)

The results of group of chicks received Gentamicin showed decrease in Ca level ,this was agreed with Stojiljkovic *et al.*, 2008) who reported that gentamicin increases the entry of Ca⁺² in the mesangial cells leading to reduced glomerular filtration rate . . Gentamicin administration is associated with severe necrosis, desquamation in proximal tubules and dysfunction of co-transport systems and channels, leading to decrease absorption of electrolytes (Chen *et al.*, 2009) . The decrease in electrolyte level may be due to increased wasting or reduced absorption of electrolytes resulting from kidney damage. Histopathological finding in the current work confirmed these results. The mechanism by which gentamicin induces nephrotoxicity is unknown; however, gentamicin has been shown, by both *in vitro* and *in vivo* studies, to enhance the generation of reactive oxygen species (ROS). Abnormal production of ROS may result in cellular injury and necrosis through peroxidation of membrane lipids, protein denaturation and DNA damage (Parlakpinar *et al.*, 2005) .

The result of this work revealed significant increase in ca and normal phosphorus level in group administrated vit C and this finding attributed to enhancement absorption of intestinal calcium as the binding capacity of the calcium-binding protein significantly improved with VC supplementation Orban et al ., (1993) . Doan (2000) found significantly higher serum Ca levels with the VC-supplemented diet compared with the unsupplemented diet But did not find any significant effect on serum P levels in broilers fed VC. While Chen et al. (1997) did not find any significant difference in the plasma calcium levels of 6- wk-old but plasma phosphorus levels were significantly lower in the un supplemented group. These results confirmed by The histopathological examination of kidneys from the group of chickens received vitamin C at 7 and 14 days of the experiment.

Table (2) Lipid peroxide, MDA showed a gradual significant ($P<0.05$) elevation in gentamicin intoxicated chicks at 7 and 14 day of experiment when compared to the control group . treatment the nephrotoxic chick with vitamin C was able to lower the raised MDA.

GSH showed a gradual significant decrease ($P<0.05$) in chicks treated with gentamicin alone at 7 and 14 days of experiment . Chicks receiving vitamin C at 250 mg/kg. bw. in drinking water daily for 4 consecutive days showed gradual improvement in GSH toward normal (Kim et al., 2006) reported that oxidative stress means an

imbalance between ROS and antioxidants, thus body antioxidant activity decreases on the production of free radical species which is further augmented by increasing the antioxidant levels in the body. Vitamin C has been proposed as an agent that is capable to uptake the ROS in plasma and thus play a role in the prevention of their entrance and subsequently damaging the membrane (Ajith et al., 2007).

Gentamicin-induced renal toxicity arises as a result of membrane lipid peroxidation due to the stimulation of reactive oxygen entities like H_2O_2 and O_2 which potentially induce the contraction of mesangial cells. As a result, filtration surface area and ultrafiltration coefficient changes, which causes the retardation in glomerular filtration rate (Derakhshanfar et al., 2007)

Mechanism of antioxidant activity involves the conversion of vitamin C into its oxidized form (dehydro-ascorbic acid) by donating two electrons to reactive oxygen species (Fischer et al., 2004) while the oxidized forms of ascorbate are relatively stable and unreactive and do not cause cellular damage (Carcamo et al., 2004) however, these reactive oxygen species are then reduced to water.

The macroscopical finding of kidney of chicks in group 1 revealed multiple areas of pale discoloration in both renal cortex and medulla with occasional hemorrhagic patches in the renal cortex especially at 7 days of treatment. While the gross appearance of kidneys from chicks received both gentamicin and vit. C revealed few areas of pale whitish discoloration in renal cortex at 7 & 14 days

The histopathological examination of kidneys from the control negative group and the groups of chicks received vitamin C at 7 and 14 days of the experiment revealed normal histological structures.

Regarding to the group of chicks received gentamicin at 7 days, the kidneys exhibiting vacuolar degeneration and necrosis of the renal tubular epithelium associated with cellular casts and dilatation of some convoluted renal tubules (fig.1), other renal tubules showed severe vacuolar degeneration with occlusion of the tubular lumen (fig.2). The glomeruli showed hypercellularity of the tuft capillaries with narrowing of the lumen (fig.3). Some cases showed dilatation of the renal convoluted tubules with hyperplasia and vacuolation of the lining tubular epithelium with interstitial haemorrhage (fig.4).

While the microscopical pictures of the group of chicks received gentamycin at 14 days showed small multifocal interstitial haemorrhage and moderate vacuolar degeneration of the renal tubular epithelium, other tubules showed occlusion of their lumen (fig.5). Some lining tubular epithelium revealed pyknotic nuclei, other

epithelium were detached (fig.6). Also dilatation and congestion of the renal blood vessels with few interstitial mononuclear cells aggregation (fig.7).

Regarding the group of chicks received gentamycin and vitamin C at 7 day of treatment showed vacuolar degeneration of renal tubular epithelium as well as vacuolation of some glomerular tuft capillaries (fig.8). While at 14 days of treatment examination of kidneys revealed mild degenerative changes in the renal tubular epithelium. (fig.9&10).

These alterations in damaged kidneys can supported by a previous author (Edwards et al ., 2007) who proposes that the treatment of nephrons with toxic substances causes the cell disruption which results in the sloughing of these injured cells. Thus nephrotoxicity can be attributed to the buildup of drug in tubular epithelial cells followed by the production of reactive oxygen species (ROS). This condition produces the oxidative stress and causes a fall in renal function; due to which the diminished (as compared with the levels of control animals) level of total serum antioxidant activity in gentamicin-treated animal is detected.

Gentamicin is thought to augment the production of reactive oxygen species (ROS) such as hydrogen peroxide, super oxide anions, hydroxyl radicals and reactive nitrogen species in the kidney (Tavafi et al., 2012). Since ROS are relatively unstable, they have a tendency to cause irreparable damage to cells and tissues. A dynamic equilibrium exists between the quantity of ROS and endogenous antioxidant enzymes including superoxide dismutase, catalase and glutathione peroxidase etc. which detoxify and scavenge ROS and thereby protects the body against their damaging effect. This delicate balance is sometimes disturbed due to ROS build-up caused by gentamicin resulting in lipid peroxidation and depletion of endogenous antioxidants (Kim et al., 2006).

Regarding the histopathological changes in chicks treated with gentamicin alone or in combined with vitamin C, they were closely correlated well with the biochemical changes in blood and plasma oxidative enzyme and reflect the nephrotoxic effect of gentamicin also the protective role of vitamin C against that toxicity these finding were similar to the finding of (Ali Abdil Razzaq., 2013)

CONCLUSION-

Administration of vitamin C is effective in preventing gentamicin-induced nephrotoxicity in one day old broiler chicks.

AKNOLDGMENT

Many thanks for professor dr. Mahmmod Arafa Biochem. Dep. Animal Health Research Institute ,Dokki for helping in preparation and estimation of MDA and GSH.

Table (1): Some serum biochemical parameters of chicks after administration of gentamicin with or without vitamin C for 4 consecutive days (n=5)

Days after treatment	Treatment	Creatinine (mg/dl)	Urea mg/dl	Uric acid mg/dl	Ca mg/dl	Ph mg/dl
7	Gentamicin	0.84±0.04*	14±0.4	14.2±0.43*	8.05±0.16*	5.0±0.1
	Gentamicin+ vit C	0.72±0.02*	14±0.24	12.8±0.31*	8.6±0.34	4.5±0.23
	Vit C	0.63±0.03	14.5±0.004	11.14±0.66	9.61±0.23*	4.9±0.12
	Control	0.69±0.03	15±0.001	11.5±0.56	8.7±0.1	4.4±0.16
14	Gentamicin	1.12±0.45*	14.5±0.6	15.7±0.14*	7.9±0.1*	4.7±0.12
	Gentamicin+ vit C	0.95±0.041	14.5±0.56	13.4±0.53	8.2±0.2	4.5±0.23
	Vit C	0.65±0.03	15.0±0.005	12.2±0.11	9.7±0.01*	3.8±0.54
	Control	0.74±0.02	15.0±0.001	11.9±0.76	9.2±0.001	4.3±0.26

*Significant difference at ($P \leq 0.05$)

Results are represented as mean ± standard error

Table (2) : MDA and GSH of chicks after administration of gentamicin with or without vitamin C for 4 consecutive days (n=5)

Days of treatment	Treatment	MDA(nmol/ml)	GSH(mg/dl)

7	Gentamicin	19.03±0.22*	12.17±0.45*
	Gentamicin+ Vit C	13.93±0.95*	14.53±0.45*
	Vit C	11.73±0.17	16.7±0.44
	Control	11.36±0.12	16.04±0.09
14	Gentamicin	30.1±2.57*	11.1±2.21*
	Gentamicin+ Vit C	13.97±0.75	15.87±0.41
	Vit C	11.47±0.09	17.37±0.29
	Control	11.5±0.12	16.2±0.06

*Significant difference at ($P \leq 0.05$)

Results are represented as mean ± standard error

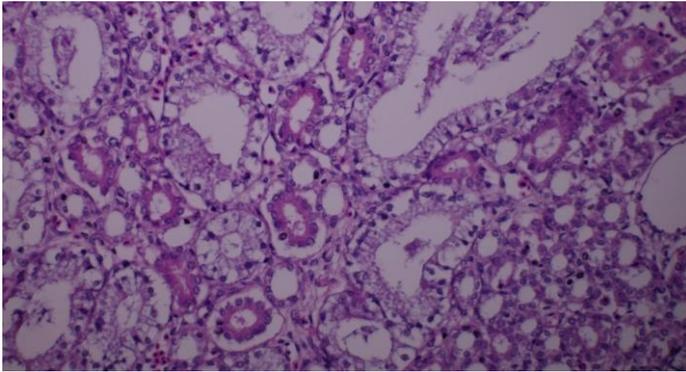


Fig.1: Kidney from chicks received gentamicin at 7 day exhibiting vacuolar degeneration and necrosis of the renal tubular epithelium associated with cellular casts and dilatation of some convoluted renal tubules. H&E X200

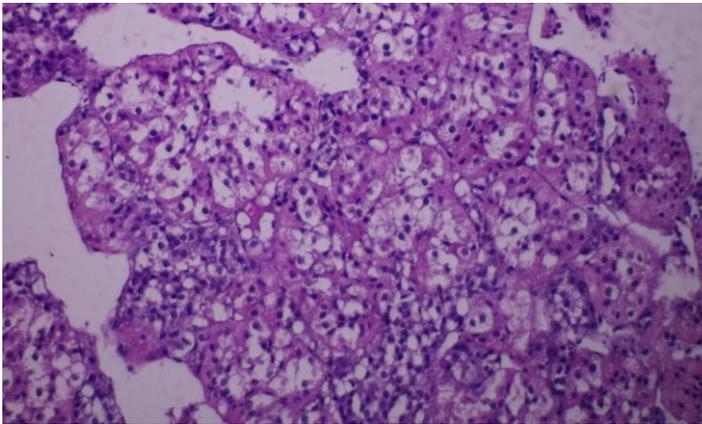


Fig.2: Kidney from the previous group revealing severe vacuolar degeneration with occlusion of the tubular lumen. H&E X400

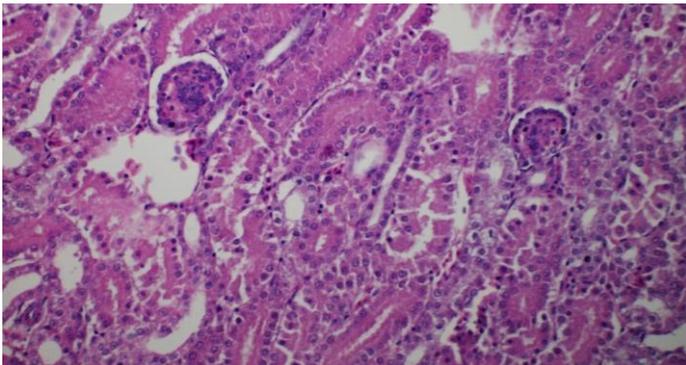


Fig.3: Kidney of the previous group exhibiting hypercellularity of the glomerular tuft capillaries with narrowing of the lumen. H&E 200

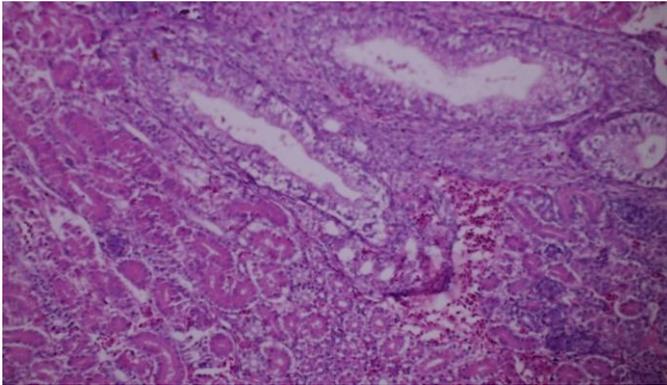


Fig.4: Photomicrograph of the same previous case revealing dilatation of the renal convoluted tubules with hyperplasia and vacuolation of the lining tubular epithelium with interstitial haemorrhage. H&E X400

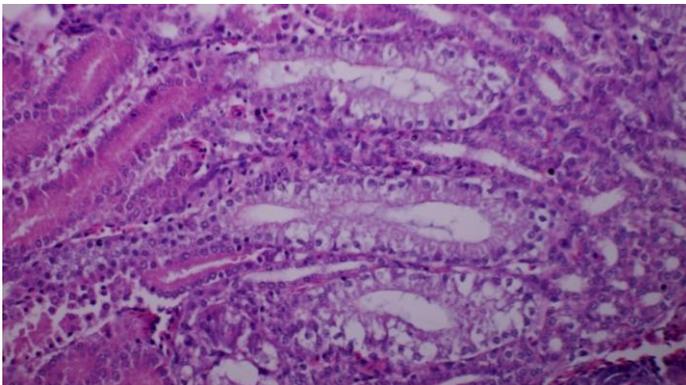


Fig.5: Kidney from the group of rat received gentamicin at 14 day of treatment showing multifocal interstitial haemorrhage and moderate vacuolar degeneration of the lining tubular epithelium, other tubules showing occlusion of the tubular lumen. H&E X400

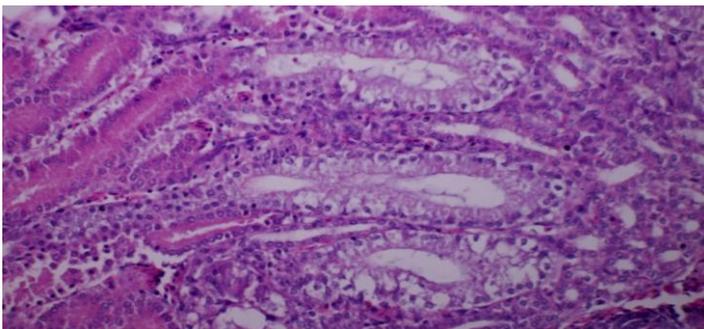


Fig.6: Photomicrograph of the previous group the renal tubules appear with multiple pyknotic nuclei and detached renal epithelium. H&E X400

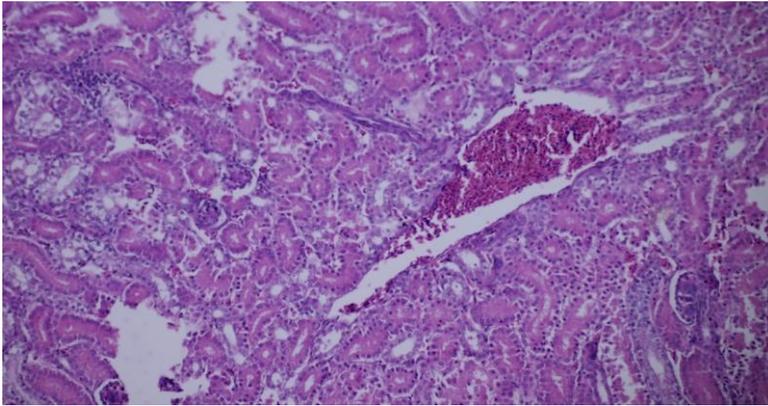


Fig.7: Kidney of the previous group exhibiting dilatation and congestion of the renal blood vessel with interstitial mononuclear cells aggregation. H&E X200

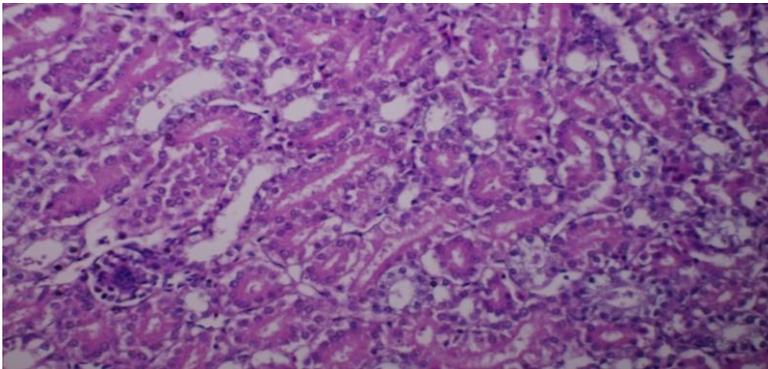


Fig.8: Kidney from the group of rat received gentamicin and vitamin C at 7 day of treatment, some renal convoluted tubules exhibiting vacuolation of their lining epithelium. H&E X200

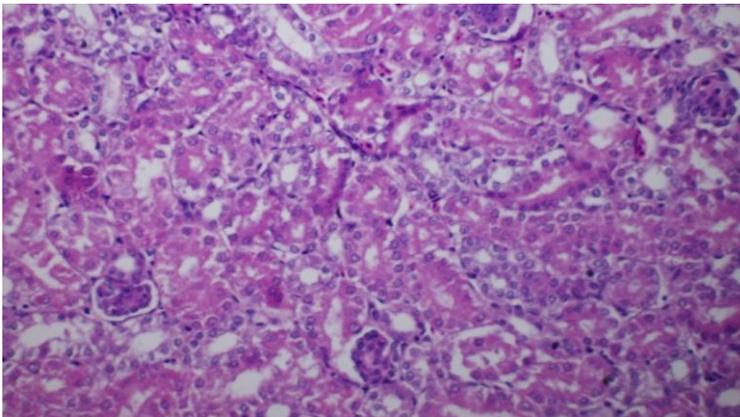


Fig.9 : Kidneys from the group of rat received both gentamicin and vitamin C at 14 day of treatment revealing mild degenerative changes in the lining epithelium of some renal tubules. H&E X200

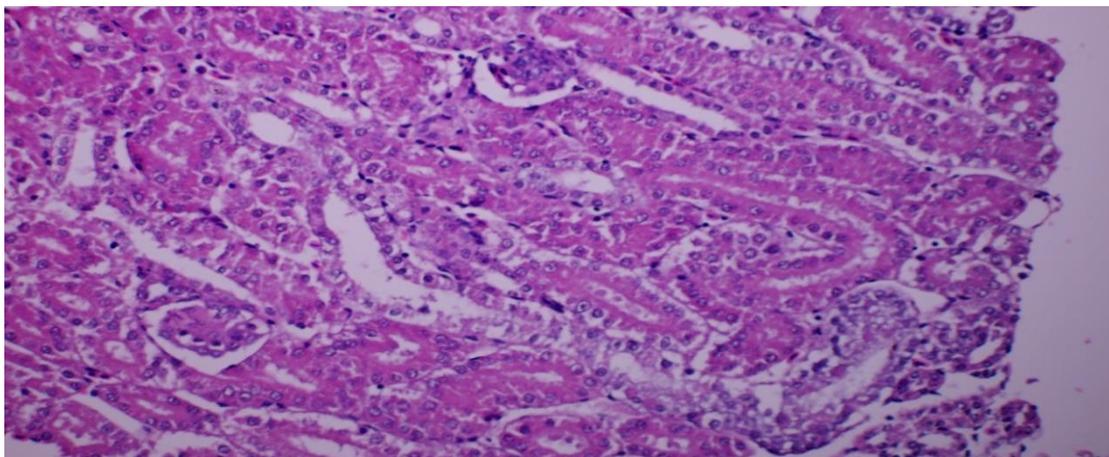


Fig.10: Kidney : high power view of the previous photomicrograph . H&E X400

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