Metabolic, Biochemical and Histopathological Studies to Explore the Calcium Role in Antagonizing Gentamicin Toxic Side Effect in Rats

Duraid Abdulhadi Abbass
Dept. of Physiology & Pharmcology
College of Vet Medicine – Baghdad Univ.

Khalid Ali Ubaid
Quality control laboratory
Vet. service Company

Summary

Twenty rats were divided equally into four groups, housed separately in special metabolic cages. Animals of first, second and third group injected S.C. with gentamicin sulphate alone at a dose of 5mg/Kg (T_1) or as a mixture with calcium at a dose of 7.5 mg/Kg injected S.C. (T_2) or separately with calcium given orally at a dose of 22.5 mg/Kg (T_3). The fourth group considered as a control and injected S.C. with distilled water (C). The experiment includes samples analysis of sample of one week pre and post treatment and three weeks daily treatment.

The results showed significant changes in studied parameters proportional with treatment period in animals of first group (T_1) in comparison with other groups expressed metabolically as a decline in body weight, increase in daily urine volume, biochemical as increase in serum creatinine, BUN, AP, AST, as well as histopathological changes in kidney nephrons and tubules.

Calcium therapy whether as a mixture (T_2) or alone (T_3) gave nearly complete protection against gentamicin toxicity in the first and second week and incomplete one in the third week of treatment that could be attributed to the competitive interaction between calcium and gentamicin due to the similarity in charges and binding site that may cause a decline in gentamicin disposition in the target organ and tissue cell and so reduce its toxic side effect.

دراسة أيضية وكيموحياتية ونسيجية لبيان دور الكالسيوم في معاكسة التأثيرات الجانبية السمية للجنتاماسين في الجرذان

دريد عبد الهادي عباس *خالد علي عبيد

جامعة بغداد/كلية الطب البيطري/فرع الفسلجة والأدوية /شعبة الأدوية و السموم
*وزارة الزراعة/ الشركة العامة للبيطرة/مختبر السيطرة النوعية

الخلاصة

تم تقسيم عشرين جرذ إلى أربعة مجاميع متساوية وإيوائها بصورة مفردة في أقفاص أيض خاصة. حيوانات المجموعة الأولى والثانية والثالثة تم حقنها تحت الجلد بالجنتاماسين بجرعة 5ملغم/كغم أما بصورة مفردة T_1 أو بصورة خليط مع الكالسيوم بجرعة 7.5 ملغم/كغم T_2 بحقن تحت الجلد أو بصورة مفردة مع الكالسيوم المجرع فمويا بجرعة 22.5 ملغم/كغم، المجموعة الرابعة حقنت تحت الجلد بالماء المقطر واعتبرت مجموعة سيطرة.

اشتملت التجربة على أسبوع – ما قبل وبعد العلاج وثلاثة أسابيع علاج يومية. النتائج أظهرت وجود تغيرات إحصائية تتناسب مع طول فترة العلاج بالنسبة للمجموعة الأولى بالمقارنة مع المجاميع الأخرى توضحت أيضياً من خلال انخفاض وزن الجسم، زيادة في حجم البول اليومي وكيموحياتياً كزيادة في كل من الكرياتين ونايتروجين يوريا الدم (BUN) ومستوى فعالية خميرة الفوسفاتيز القاعدية والاسبارتيت ترانز امينيز في مصل دم حيوانات المجموعة الأولى وكذلك نسيجياً حيث ظهرت تغيرات النسيجية في كيبيات ونبيبات الكلية تم الاستنتاج بأن إعطاء الكالسيوم كمخلوط T_2 أو لوحده T_3 يعطي حماية شبه تامة ضد الأعراض الجانبية السمية للجنتامايسين في الأسبوع الأول والثاني وحماية غير تامة في الأسبوع الثالث من العلاج وقد يعزى ذلك الى التنافس بين الجنتامايسين والكالسيوم بسبب الشحنة الموجبة المتشابهة والتنافس على موقع الإرتباط الموحد مما يؤدي الى انخفاض ارتباط الجنتامايسين وتراكمه في خلايا الأعضاء والأنسجة المستهدفة وبالتالي الى تقليل سمية فيها.

Introduction

Nephrotoxicity, ototoxicity and neuromuscular junction blockade are the major limiting factors in the use of aminoglycoside antibiotic for treatment of bacterial infection.

Kahmater and Dahlager [1] have reported that the average frequency of gentamicin – induced nephrotoxicity is 14% while Su-Hua and Schacht [2] reported incidence of ototoxicity due to gentamicin between 6-16% hearing loss and 9-15% impairment of vestibular function. Aminoglycoside nephrotoxicity is manifested functionally by decreased urine concentrating capacity, tubular proteinenurea, mild glucosurea, decreased ammonium excretion depression of GFR and electrolyte imbalance for cations + (Ca^{+2} , Mg^{+2}, Na^{+}, K^{+}) in urine and blood serum. [3].

Histopathologic lesions are confined primarily to the proximal tubule and consist of increase in the number and size of secondary lysosomes and cytosegresomes containing myeloid bodies, disruption of brush border membranes, mitoctcondral swelling and tubular cell necrosis, [4].

It is well reported that the use of gentamicin whether in therapeutic or toxic doses caused hypercalcuria and hypocalcemia proportional with the dose [5,6] and also it was noticed that calcium channel blocker drugs increase the toxicity of gentamicin when used together. [7].

Recent studies indicate that calcium intake by ingestion [8] or injection [9] offer protection for aminoglycoside toxicity.

The aim of the present study is to explore the possibility of using calcium separately or as new formulating mixture with gentamicin in order to minimize toxicity offer or complete protection from gentamicin toxic side effect after prolonged therapentic use.

Materials And Methods

The experiment was designed to study changes in metabolic, biochemical as well as histological parameters in Swiss Sprague rats

housed individually in special metabolic cages with food and water provided ad. libitum under controlled light and temperature.

Twenty albino rats with average weight (180-200)g were divided into four groups each of five rats. The first group T_1 injected S.C. with 5mg/Kg B.W. of gentamicin sulphate alone (Arab Pharma Co. 80mg/2ml). The second group T_2 was injected S.C. with the same dose of gentamicin formulated as mixture with calcium gluconate (Mino Pharm Ind, Netherland, 10%) at a dose of 7.5mg/Kg. The third group T_3 was injected S.C. with 5mg/Kg B.W. gentamicin + 22.5 mg/Kg calcium gluconate given orally. The fourth group was injected with distilled water S.C. and served as a control group (C).

The experiment included samples analysis to studied parameters during one week pre and post treatment and three weeks of daily treatment. The daily assessment of metabolic parameters measured that include, animal body weight, food and water intake as well as measuring urine volume. The biochemical parameters were measured weekly in blood serum at different experimental periods included creatinene according to Jaffe method [10], blood urea nitrogen (BUN) by using diacetyl monoxime method [11], alkaline phosphatase (AP) according to two point Colormetric assay and aspartate trasaminase by using Reitman and Frankel method [10].

One animal from each group at the end of the second, third and post treatment week was killed and its kidney removed and fixed for histological examination.

Two ways analysis of variance (ANOVA) and least significant difference (LSD) were used to compare the results of all groups statistically.

Results

The results showed that animal's body weight of first group T_1 declined significantly ($P<0.05$) during treatment period while in the second and third groups (T_2, T_3), body weight main tend without any increase in comparison with that of the control group that conserved its normal gradual increase during this period (Figure 1).

The food and water intake showed no significant increase among the different groups during different experimental periods (Figures 2 & 3).

Urine volume increased significantly ($P<0.05$) in the first group (T_1) from the second week treatment and returned to normal at the end of the experiment in comparison with the other groups that showed no significant changes in all experimental periods (Figure 4).

A significant gradual increase ($P<0.05$-0.01) in serum levels of creatinine and BUN were seen in the animals of the first group in the second week of treatment and in post treatment in comparison with other groups.

However, there was an increase in serum creatinine and BUN levels of T_2 and T_3 groups in the second and third week of treatment, but they were not beyond the reported normal range [10] and they returned to their normal levels after stopping treatment (Figure 5 & 6).

A significant gradual increases ($P<0.05$-0.01) in AST and AP enzymes was recorded in animals of 1st group from the second week of treatment. These levels did not return to normal after stopping treatment in comparison with other groups (T_2, T_3, and C) that showed normal levels during all experimental periods (Figure 7 & 8).

The histological changes observed in kidney of T_1 group in the second week of treatment included hydrobic degeneration in most of renal tubular epithelium with different sizes of vacuolization and cytoplasmic granulosis while the bowman capsule showed some enlargement (Figure 9). All these observed changes were severely observed in the third week (Figure 10), while no obvious histological changes observed in kidneys of T_2 and T_3 group during all treatment periods (Figure 11 & 12) in comparison with control group.

Figure1:Rates of animals body weights (gram) of different experimental groups

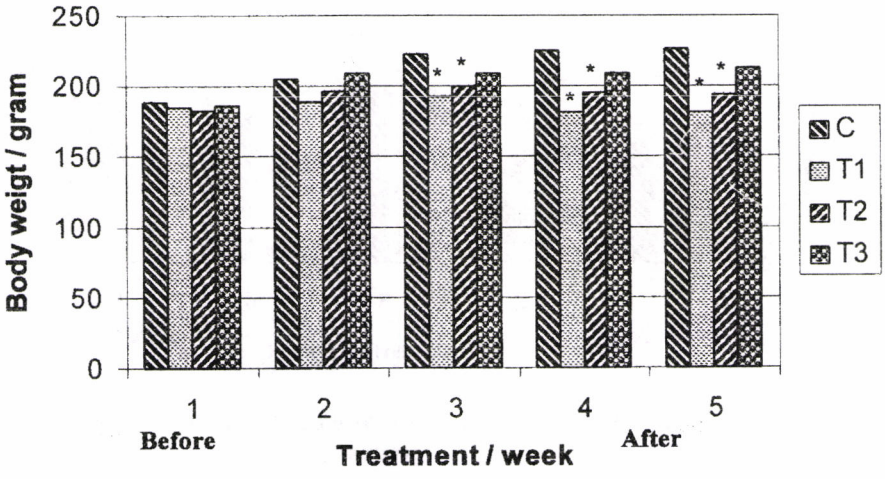
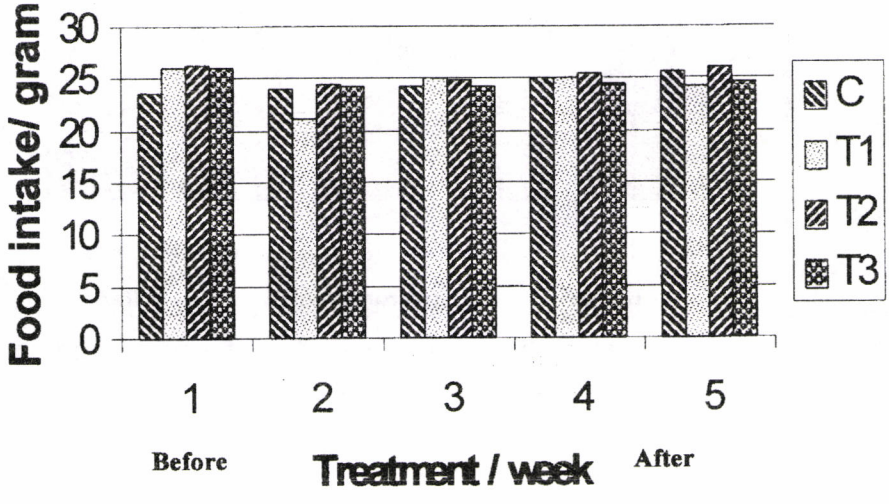


figure2: Daily food intake (gram) of different experimental groups



Figur3:Daily water intake (ml)/wk of different experimental groups

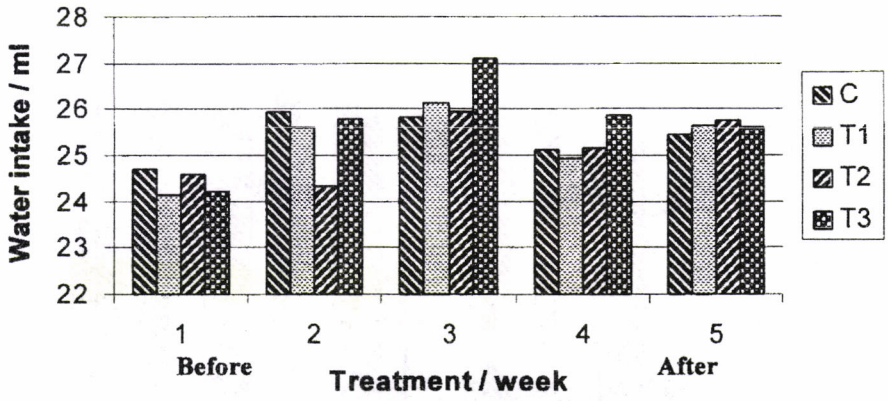
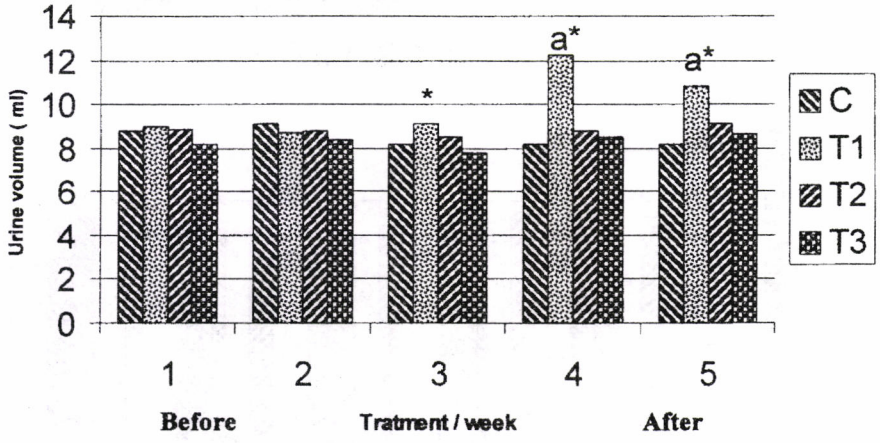


Figure 4: Daily urine volume(ml/) week of different experimental groups



* Statistical difference from the control group
a : Statistical differences from T_2 and T_3 group

Figure5: Serum creatinine levels of different experimental groups

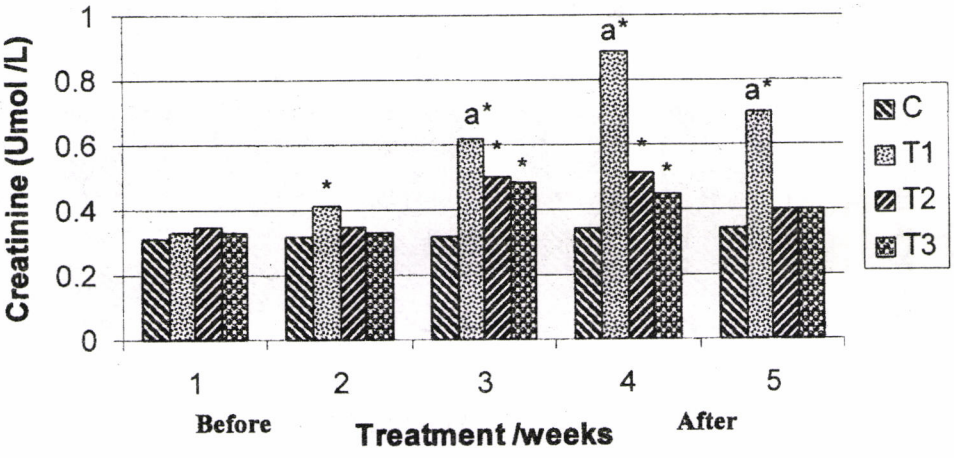
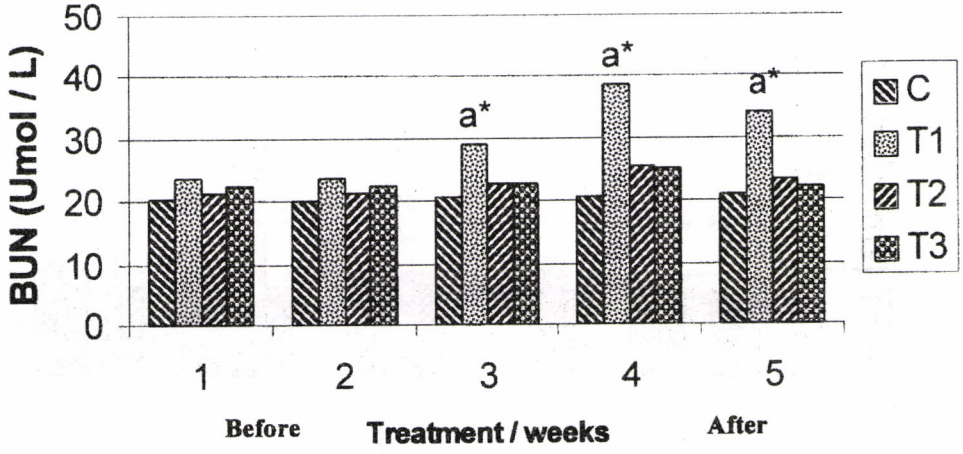


Figure6: Serum BUN levels of different experimental groups



* Statistical difference from the control group
a : Statistical differences from T_2 and T_3 group

Figure7: Serum alkaline phosphatase Ap levels of differents experimental groups

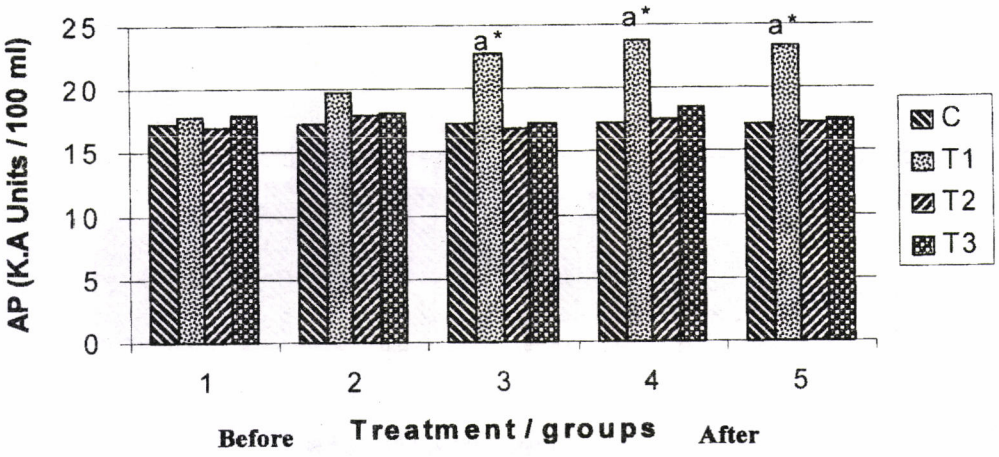
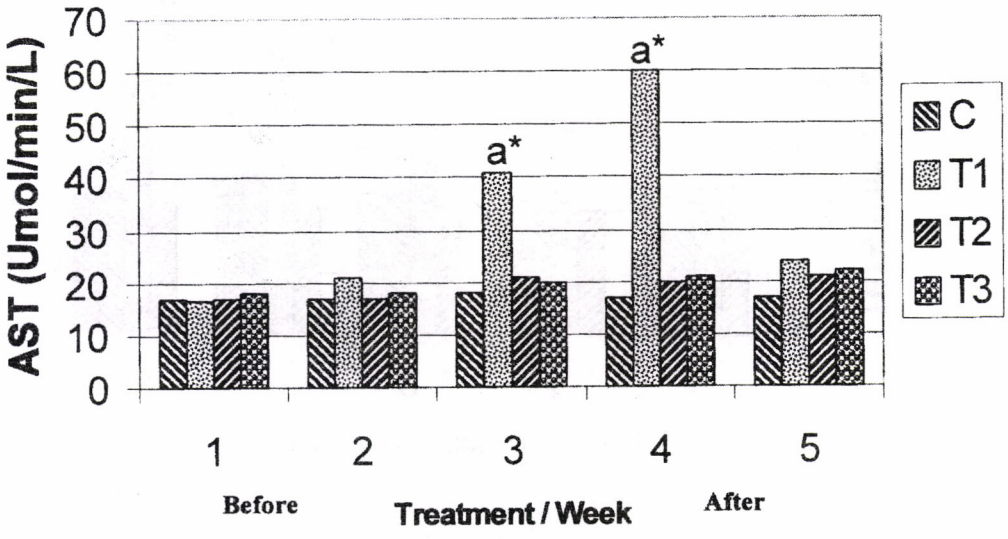


figure8: Serum aspartate transaminase (AST) levels of different experimental groups



* Statistical difference from the control group
a : Statistical differences from T_2 and T_3 group

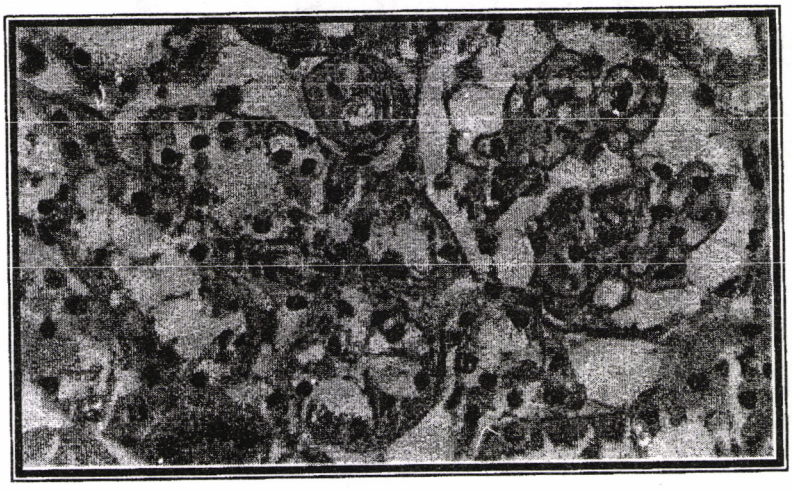


Figure 9: Nephrotubules after 2 weeks treatment with gentamicin (T_1) (X-130)

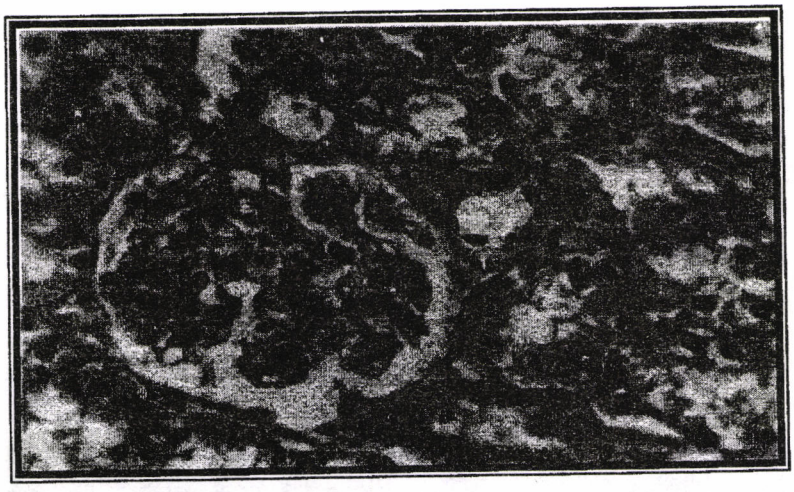


Figure 10: Nephrotubules after 3 weeks treatment with gentamicin (T1) (X-130)

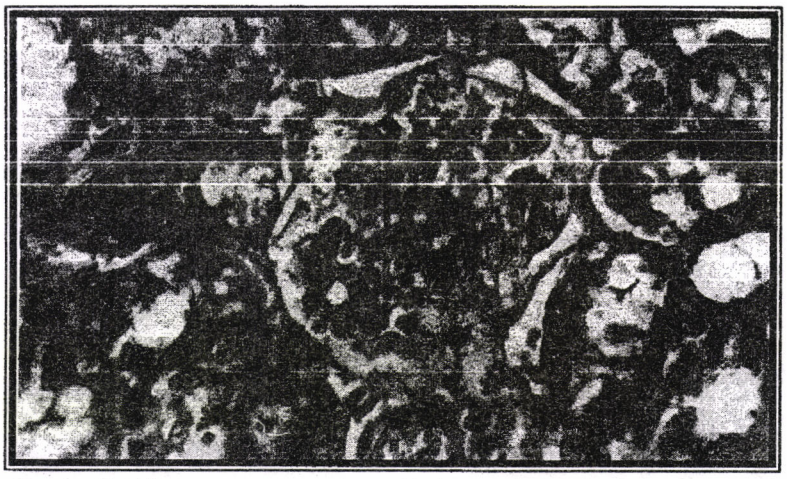


Figure 11: Nephrotubules of (T_2) group after 3 weeks treatment with gentamicin (X-130)

Figure 12: Nephrotubules of (T_3) group after 3 weeks treatment with gentamicin (X-130)

Discussion

The results of the present study showed a decline in animal's body weight of the first group only T_1 during treatment period which may represent a decline in food conversion ability since there was no changes in their daily food consumption.

Previous studies reported an increase in proteinuria ([12]) and decrease in protein synthesis ([13]) due to the toxic effect of gentamicin that noticed in our study in the animals of the first group which may influence their ability to gain weight.

There was also a significant increase in urine volume of the first group T_1 only, which my attributed to their lower ability to concentrate urine as a result of inhibition of water tubular re-absorption as reported by ([12]) or due to interference of gentamicin with vasopression effect on the collecting duct ([14]).

The biochemical parameters showed a significant gradual increase in serum creatinine and BUN in animals of T_1 group during treatment period which reflect the change in the kidney excretory function due to the damage in the nephron after exposing to long gentamicin therapy.

This was supported by the histopathological and enzymatic results of animals of the first group only that showed a gradual but significant increase for both AP and AST during treatment and post treatment periods.

Alkaline phosphatase is considered as one of the nephrotubular brush border enzymes so its increase reflects damage in these tubules while the increase in AST could be extrapolated by the reported gentamicin toxic effect in other organs and tissues. ([15]).

One can conclude from these results that calcium treatment whether alone T_3 or as a mixture T_2 with gentamicin gives nearly a complete protection against gentamicin toxic effect in the first and second week and incomplete protection in the third week of treatment supported by the metabolic, biochemical and histological results that

showed either a little change that did not reach the abnormal level or no change at all in comparison with the control group.

The antagonizing effect of calcium to gentamicin toxicity could be attributed to the reported competitive inhibitory interaction between calcium and aminoglycoside ([16]) due to the similarity in charges and binding site that may lead to decrease in gentamicin disposition in the cells of target organs and tissues and thus reduce its toxic side effect.

Calcium therapy may also compensate the reported decline in its level in serum and cellular site aiding inrepair of the damage caused by its deficiency and regaining the normal cellular function of cells of treated organs and tissues.

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