HISTOPATHOLOGICAL AND BIOCHEMICAL STUDY OF THALLIUM SULFATE TOXICITY ON KIDNEYS OF WHITE LABORATORY RATS (*RATTUS NORVEGICUS*)

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Keywords; Thallium sulfate**,** Creatinine, Glomeruli**.**

ABSTRACT

In this study 48 rats were used and divided into four groups :- A,B,C and D each group contains 12 rats . All these groups were administrated orally with aqueous solution of thallium sulfate as 0.4 mg/kg b.w. , 0.8 mg/kg b.w. , 1.6 mg/kg b.w. respectively while the control group(A) was administrated with normal saline . This experiment was continued for 90 days . After this period all animals were anesthetized with chloroform by inhalation and sacrificed to collect blood samples for obtaining the serum which used for estimation of serum urea and creatinine , and to study histopathological changes caused by thallium sulfate toxicity on kidney tissue . Compared with control group, all treated groups showed markedly dose –dependent elevation in serum urea and creatinine, and pathological changes as dilatation and vacoulation of cortical tubules and atrophy of glomeruli and also necrosis of proximal convoluted tubules in high dose group . .

Keywords : Thallium sulfate toxicity , kidneys histopathology , rats.

INTRODUCTION

Thallium (Tl) has been identified to be an environmentally significant element because of its toxic effects. It is a heavy metal available in a number of soluble salts such as acetate, sulfate, and carbonate. A part from Thallium there were a lot of studies about heavy metals being exposed to the aquatic ecosystem [1, 2, 3 , 4, 5] . Thallium is extremely toxic in a aqueous solution [6]. It is also known that this metal is still employed for many purposes such as optical, costume jewelry, cement,

photographic, and the electronic industries, along with high-tech industries including semi conductors, scintillation counters, low-temperature thermometers ,and special glasses [7]. Thallium was extensively used for medical purpose. It was given to children to produce hair loss in the treatment of ringworm of the scalp, and widely used in the treatment of venereal diseases, tuberculosis and malaria [8]. In some parts of the world it is still used for killing rodents and this may lead to inadvertent ingestion by humans and animals [9] . Its toxic effect is due to its ability to inhibit a number of intracellular potassium-mediated processes and legends formation with protein sulfhydril groups, inhibition of cellular respiration, interaction with riboflavin and riboflavin-based cofactors, and distribution of calcium homeostasis [10 ,11] . Acute and chronic exposures to Tl produce damage to central and peripheral nervous system, and these events are likely to be related with substitution of potassium in vital processes; mitochondrial dysfunction, reactive oxygen species (ROS) formation and oxidative stress [12] .The highest thallium concentrations have typically been found in the kidney and the lowest concentrations in the brain, with none being detected in fat tissue. Thallium also has been demonstrated to cross the placenta in humans [13] . and experimental animals [14] .

MATERIALS AND METHODS

The experimental animals were divided into four groups (each group contains 6 males and 6 females). These four groups were given daily orally a single dose of thallium sulfate for 90 days as following : Group(A): Administered orally 0.9 % normal saline (N.S) daily , Group (B) : Administered orally 0.4 mg/kg b.w. of thallium sulfate daily , Group (C) : Administered orally 0.8 mg/kg b.w. of thallium sulfate daily, and Group (D) :Administered orally 1.6 mg/kg b.w. of thallium sulfate daily

At the end of the period of experiment, the animals of each group were sacrificed, after rats were anesthetized by the chloroform inhalation. Blood samples were collected from the abdominal vein by disposable syringes of 5cc capacity. Blood was put into test tubes free from anticoagulant to separate blood serum and allowed to clot at room temperature then the tubes were centrifuged at 3000 rpm for 15 minutes

and the serum samples were stored at 20 ºC until used to estimate the biochemical parameters including activity of kidney enzymes (urea and creatinine) .

Laboratory assays

All groups were sacrificed on days 90 after treatment. The blood collected from each rat from Abdominal vein after deep inhalation of chloroform. Serum was taken stored at -20 ̊c until biochemical assays were done.

Test procedure for Urea

More than 90% of urea is excreted through the kidneys in urines. measurement of plasma or serum urea concentration is widely regarded as a test of renal function (15);(16).

Test procedure for creatinine

Phosphocreatine and creatine interconversion is a particular feature of the metabolism process of muscle contraction .creatine and phosphocreatine partially converted to a waste product, creatinine .because creatinine is endogenously produced and released in to body fluids at a constant rate and its clearance can be measured as an indicator of glomerular filtration rate.

Test procedure for urea and creatinine were done according to manual of (biolabo reagent, france) kit .

Histopathological parameters

Procedure of Tissue Processing

In brief the routine sequence of events according to [17 and 18] is as follows:- After the kidney tissue was obtained .It was fixed for 24 hours or more in an appropriate fixative buffered formalin 10%. And then dehydrated through graded alcohol (increasingly higher concentration) overnight. Then they replaced with xylol for clearing . The tissue infiltrated with paraffin and embedded in a block of paraffin. The block was cut into thin sections on the microtome (5µm- thick) and the section was mounted by Mayer albumin on glass slide . Dissolve the embedding medium by putting the slides on hot plate overnight .Rehydrate the sections in descending alcohols and Stain the section with an appropriate staining sequence haematoxylin and eosin (H&E).

In the staining procedures used haematoxylin and eosin stains according to [19] .

Statistical Analysis

The data of serum urea and creatinine were subjected to analysis of variance and the significance differences at ($p<0.05$) which were determined by (ANOVA), one-way by using the statistical softwares sigmastat statistical (Version 19.0,SPSS Inc., Chicago, Illinois, USA, 2010).

RESULTS

Biochemical examination

In table (1) the results showed significant increase ($P\leq 0.05$) in the kidney enzyme creatinine in the groups (B,C, and D) in comparison with the group (A) .but there was no significant differences among groups (B,C ,and D).while the urea was significantly increased in groups (C,D) compared with control group (A) but no significant differences between groups (B) and (A).

Table (1) shows results of creatinine and urea levels in different groups of animals.

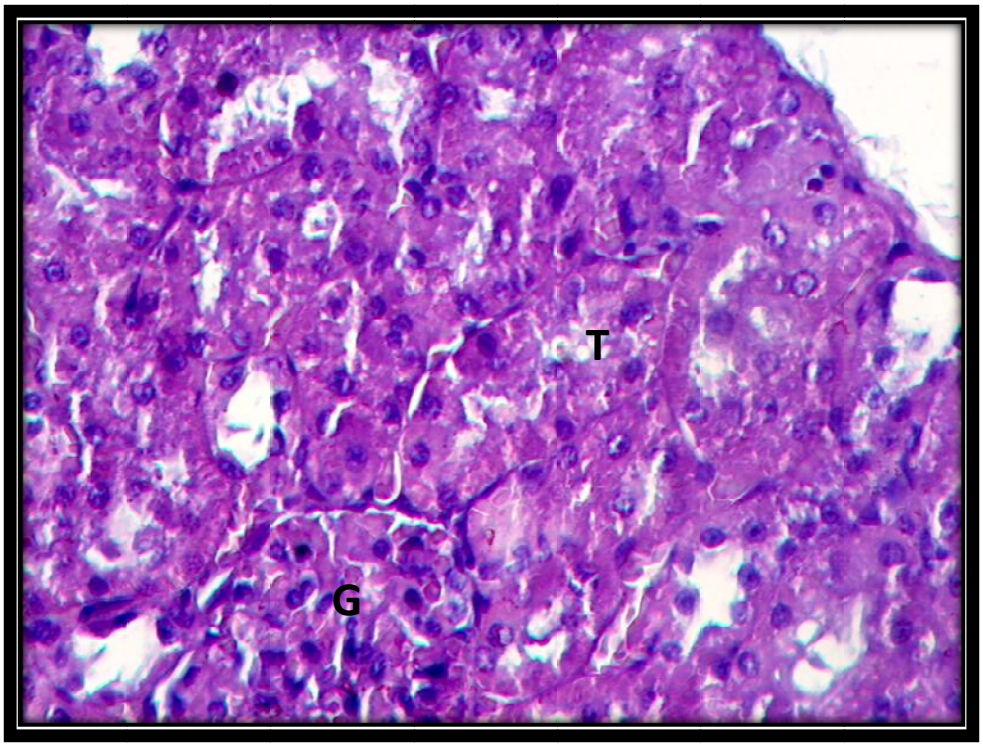
Parameter / Group	Creatinine Mean ± SE	Urea Mean ± SE
Group (A) Normal saline	00.2623 ± 00.09 A	24.93 ± 02.62 A
Group (B) 0.4 mg/kg	00.3723 ± 00.09 B	37.88 ± 05.90 A
Group (C) 0.8 mg/kg	00.4684 ± 00.09 B	66.77 ± 13.53 B
Group (D) 1.6 mg/kg	00.5329 ± 00.07 B	90.30 ± 13.68 B

N=12

Different letters indicate to significant differences ($P<0.05$) between different groups.

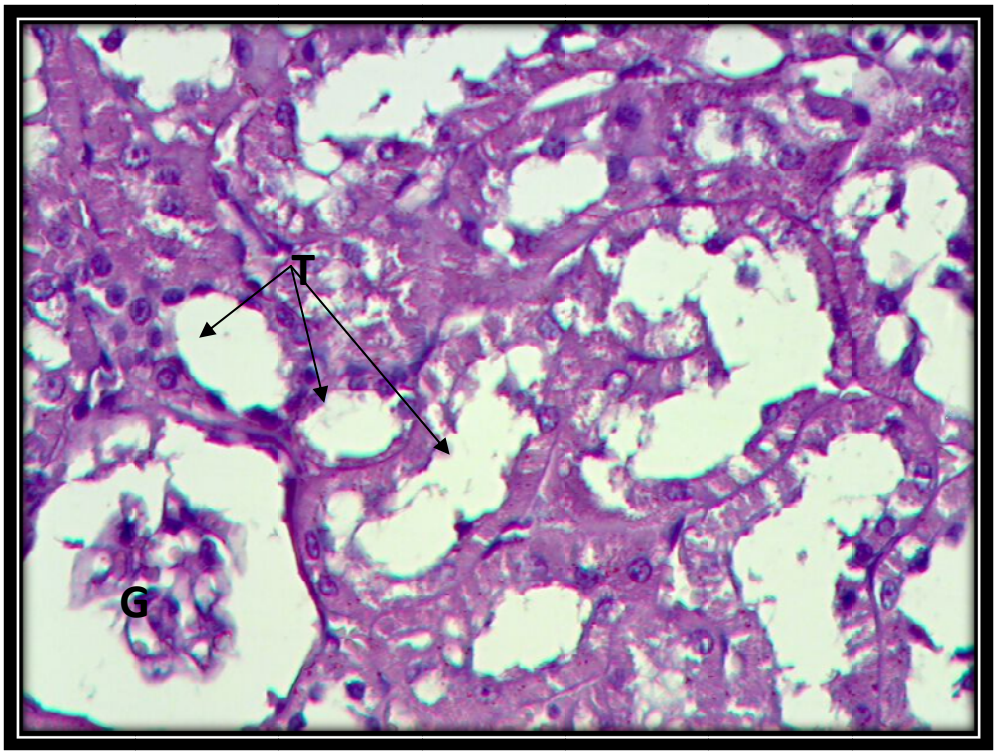
Histological examination

The microscopic examination of the kidneys (especially cortical tubules and glomeruli) in the control group was within the normal histological limits (Fig.1).

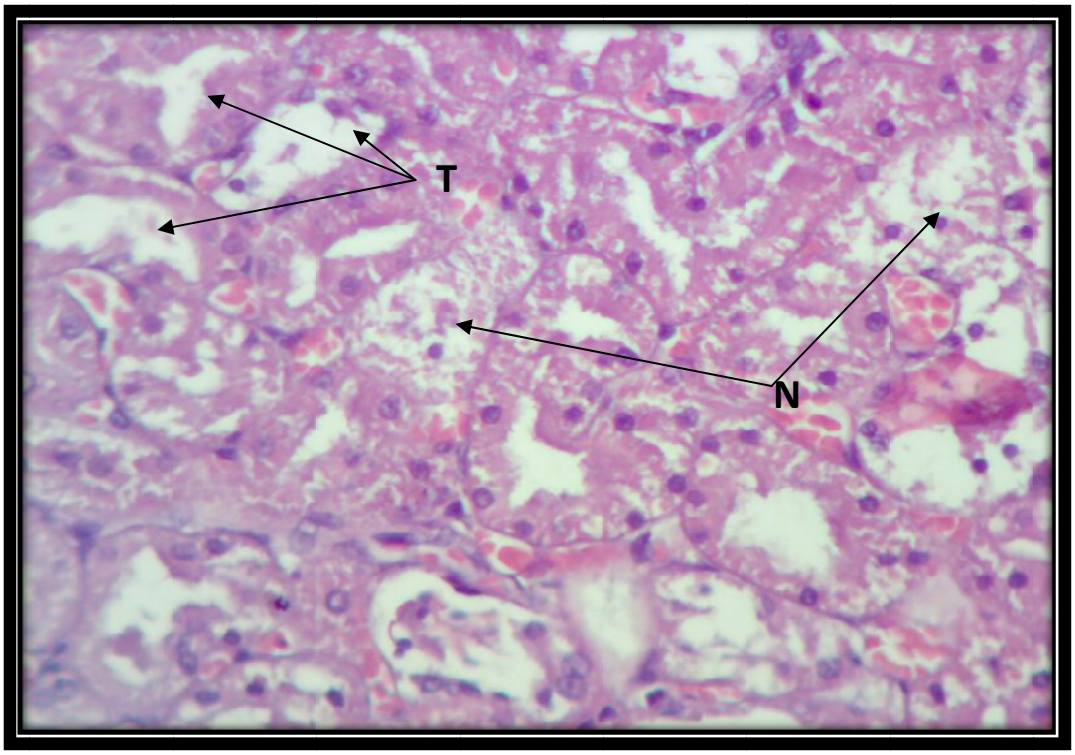


(Fig.1) Transverse section of kidney of control group shows cortical tubules T and glomeruli G within normal limits.H&E.400 X.

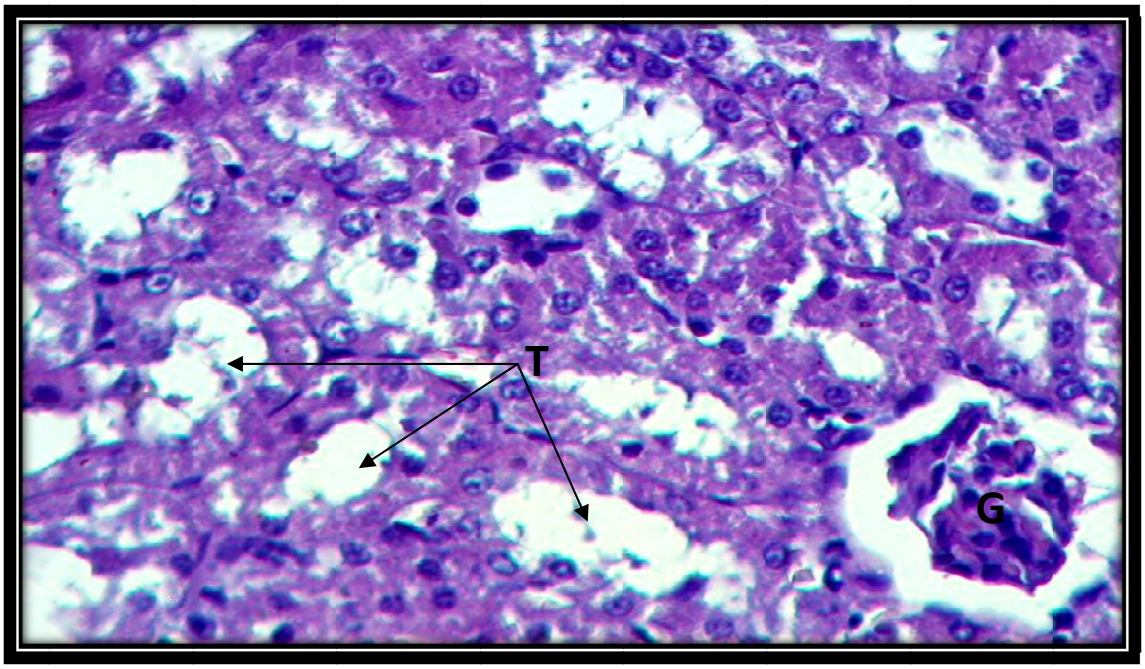
The histological examinations of the kidney in the animals that were treated with (1.6 mg/kg bw) of thallium sulfate revealed sever dilatation of renal cortical tubules and atrophy of glomeruli (Fig.2) and necrosis of proximal convoluted tubules (Fig.3) compared with control animals, while, minimum dilatation and atrophy of glomeruli were found in animals administrated with 0.8 mg/kg bw (Fig.4), but less vacuolation of cortical tubules and atrophy of glomeruli were observed in the group which gavaged by 0.4 mg/kg bw Fig.(5).



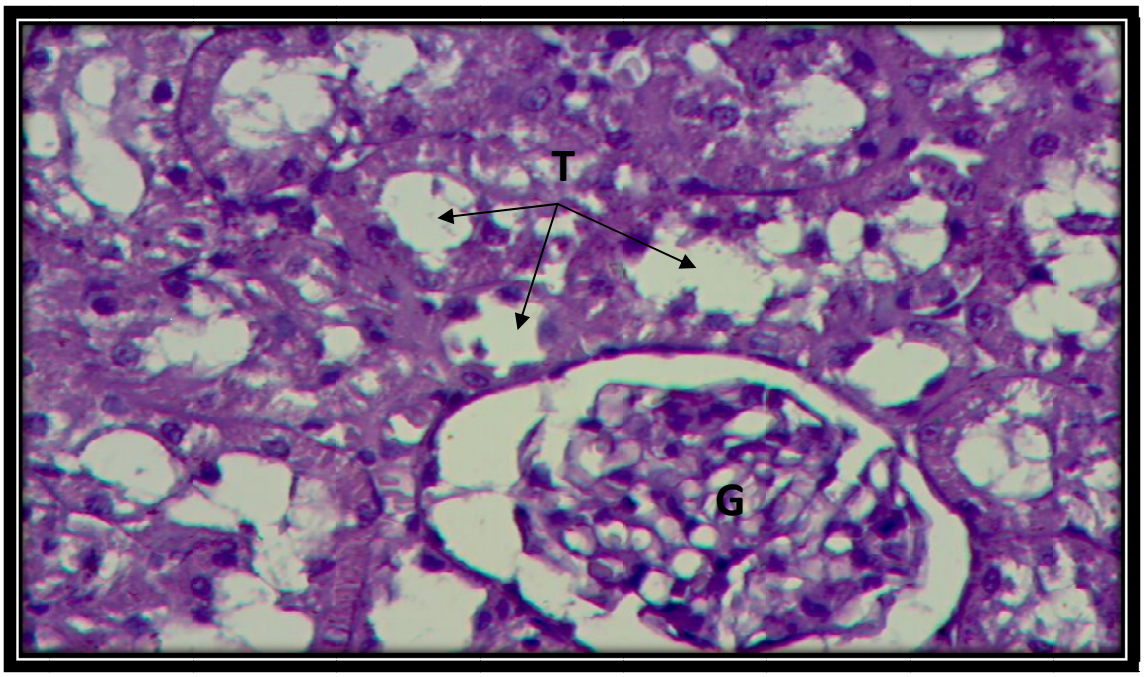
(Fig.2) .Transverse section through the kidney in high dose shows : Dilated cortical tubules and vacuolation of sub capsular cortical tubules T and atrophy of glomeruli G . H&E.400 X



(Fig.3) . Transverse section through the kidney in high dose shows: Dilated cortical tubules T and necrosis of proximal convoluted tubules N .H&E.400 X .



(Fig.4) Transverse section through the kidney in intermediate dose shows : minimal vacuolation of cortical tubules T and atrophy of glomeruli G . H&E.400 X.



(Fig.5) Transverse section through the kidney in low dose shows : less vacuolation of cortical tubules T and atrophy of glomeruli G . H&E.400 X .

DISCUSSION

The present study shows significant dose –dependent increase (P≤0.05) in serum creatinine in all treated groups (20; 21; 22;and23 who showed a thickening of ascending limb of the loop of Henle and Na+/K+-ATPase activity was increased in the medulla .In the present study, the level of creatinine was increased significantly (P≤0.05) in the all intoxicated groups which was probably corresponding to the cell death of epithelial cells of the renal tubules in comparisons with control group this result was agree with (21;22; 24) in rats. this increase in serum creatinine was confirmed with histological study which showed that the kidney was focally damaged. This result of increment in serum creatinine was in agree with the result of (20; 25) Who's mentioned that the diminished in creatinine clearance indicate to renal involvement in human, and (26) who demonstrated that the decrease in creatinine clearance indicate renal function impairment in human .

Also the results revealed that there is significant increase (P≤0.05) in blood urea in treated groups (C and E) which administrated with 0.8 ,1.6 mg/kg bw respectively in comparisons with control .but there is no significant differences between treated group (B) (0.4 mg /kg bw) and control group (A) . this may be due to the thallium replace potassium in Na / K ATPase and inhibit its function . the inhibition of this enzyme cause osmotic imbalance ,and the organelles' in renal cells become disrupt .

The abundance in blood urea was corresponded with : (20; 21 ; 22 ; 28 ; and 23) who showed a thickening of ascending limb of the loop of Henle and Na+/K+-ATPase activity was increased in the medulla .

The degree of histological changes was increased with increasing of dose in all treated groups in comparisons with control group. These findings were look like of (24) who showed that The tubules from the kidney in the intoxicated rats appeared to undergo hydropic degeneration . The tubular cells became atrophied and vacuolated this due to the kidney of the treated rats was found to accumulate the greatest amount of thallium .also the histopathological changes in the glomeruli were disagree with (27) who reported that the acutely poisoned rat showed swelling of the epithelial cells of the glomeruli after injection of thallium this may be due to the dose which is injected , period or route of exposure of animal to thallium .

دراسة الامراضيه النسيجية والكيموحيويه للتسمم بكبريتات الثاليوم على الكلى في الجرذان ألمختبريه البيضاء (*Rattus norvegicus*)

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الخلاصة

في هذه التجربة تم استخدام ٤٨ جرذ مختبر حيث قسمت هذه الحيوانات الى أربع مجاميع احتوت كل مجموعه على ١٢ حيوان (٦ ذكور و ٦ إناث). كل هذه الحيوانات جرعت فمويا بمحلول مائي لكبريتات الثاليوم وكالاتي : ٠,٤ . ٠,٨ . ١,٦ ملغ /كغم وزن الجسم بالترتيب بينما مجموعة السيطرة تم اعطاؤها ماء الملح الفسيولوجي .استمرت هذه التجربة ٩٠ يوما ، بعد نهاية هذه الفترة تم تخدير كل الحيوانات بالكلوروفورم عن طريق الاستنشاق و قتلها للحصول على مصل الدم لغرض حساب تركيز اليوريا والكرياتنين فيه وكذلك لدراسة الامراضيه ألنسيجية التي حدثت بسبب التسمم بسلفات الثاليوم على نسيج الكلى .بالمقارنة مع مجموعة السيطرة . جميع الحيوانات ألمعاملة أظهرت ارتفاع في مستوى اليوريا و الكرياتنين معتمدا على زيادة الجرعة وكذلك اظهرت تغيرات نسيجية في نبيبات ا لكلية والكبيبة.

مفاتيح البحث : التسمم بكبريتات الثاليوم ،الامراضية النسيجية ،الكلى،الجرذان ألمختبرية

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