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Histopathological and Biochemical Study on the Kidneys of Male Mice Injected Intraperitoneally with Thioacetamide

Hadeer Ahmed Abed, Hind Kareem Kadhem, DuhaMuhanad Bayram*
Department of Medical Laboratory Techniques, Al-Esraa University College, Baghdad, Iraq

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Abstract

Thioacetamide (TAA) is a thiono-sulfur containing compound with a wide manufacturing application. Humans and animals exposure to TAA may occur in different ways and may cause nephrotoxicity. So, in this study serum creatinine concentration and urea, in addition to renal pathological changes, were examined in mice treated with TAA/P (100 mg/kg B.W). One hundred twenty male albino (BALB/c) mice were used. They were randomly divided into 2 main groups. In the control group 30 mice were fed water only and normal mice pellet.The other TAA-treated group of mice were divided into 3 subgroups as follow: 1st group (G1) was injectsed with TAA for 2 months (injected twice a month), while the 2nd(G2) and 3rd(G3) groups were injected with TAA for 4 and 6 months respectively (single injection monthly). Subsequently, serum urea and creatinine were measured in the control and the treated mice. The pathological changes in renal tissue were studied by examiningstained Hematoxylin and eosin (H and E). The results showed a significant increase ($P<0.05$) in blood urea and creatinine levels of all studied groups with diverse pathological changes in kidney, including degeneration, necrosis, hemorrhage, nephritis, abscess and granulomatous lesions due to TAA toxicity.

Keywords: TAA, Urea, Creatinine, Necrosis, Renal failure.

دراسة نسيجية مرضية و كيميائية حيوية على كلى ذكور الفئران المحقونة داخل الصفاق بالثيواسيتامايد

هدير احمد عبد, هند كريم كاظم, ضحى مهند بيرم*
قسم تقنيات المختبرات الطبية, كلية الاسراء الجامعة, بغداد, العراق

الخلاصة

الثيواسيتامايد هو مركب الكبريت العضوي والذي يدخل في مجال الصناعة وله العديد من التطبيقات. لذلك من الممكن ان يتعرض الإنسان والحيوان لهذه المادة بطرق مختلفة وقد ينتج عن ذلك تسمم في الكلى.تناولت هذه الدراسة قياس مستوى تركيز الكرياتينين واليوريا في دم الفئران التي تم معاملتها بالمركب بتركيز ١٠٠ ملغم لكل كغم من وزن الفأر بالإضافة إلى دراسة التغيرات التي حدثت في الانسجة الكلوية. قسمت الفئران والبالغ عددها ١٢٠ بشكل عشوائي إلى مجموعتين رئيسيتين: مجموعة السيطرة والبالغ عددها 30 فأراً تلقت الماء و الغذاء الاعتيادي فقط ، المجموعة الأخرى والتي تم معاملتها بالثيواسيتامايد مكونة من 90 فأرًا و مقسمة إلى 3 مجموعات و على النحو التالي: المجموعة الأولى (G1) والتيتم حقنها بـ TAA لمدة

*Email : duha.bayram@yahoo.com

شهرين (مرتين في الشهر) ، بينما المجموعة الثانية (G2) والمجموعة الثالثة (G3) تم حقنها بـ TAA لمدة 4 و 6 أشهر على التوالي (حقنة واحدة في الشهر). بعد ذلك ، تم جمع عينات المصل في الشهر الثاني والرابع والسادس عن طريق ثقب القلب لغرض قياس مستوى اليوريا والكرياتينين قبل وبعد المعاملة. كذلك تمت دراسة التغيرات المرضية في النسج الكلوية من خلال فحص عينات الأنسجة المصبغة بصبغةالهيماتوكسيلين والايوسين. أظهرت النتائج زيادة معنوية ($P < 0.05$) في مستوى اليوريا والكرياتينين في الدم لجميع المجموعات التي خضعت للدراسة مع تغيرات مرضية متنوعة بما في ذلك التنكس،النخر، والنزيف، التهاب الكلية، الخراج وأخيرا الآفات الحبيبية ويعود السبب الرئيسي في هذه السميه الى الثيواسيتامايد.

Introduction

Thioacetamide (TAA) is a thiono-sulfur containing compound with yellow or colorless solid crystalline.It produces sulfide ions which are used in the inorganic and organic compounds industry. Because of sulfide ions production, TAA has been used as a fungicide where it ceases the germination of fungal spores [1]. Its other applications include stabilizing motor oil, chemical reagent, textile dye, organic solvent [2, 3],leather industry, textile, paper and as a preservative of motor fuel. Thioacetamide is now used in metal salt nanoparticles production [4-6] and as a substitute for hydrogen sulfide in qualitative investigation [7, 8].

Humans can be exposed to TAA in a variety of ways [1]. It can be swallowed, inhaled or absorbed through skin. When thioacetamide is bioactivated, it produces thioacetamide S-oxide which produces peroxide radicals, subsequently causing reactive oxygen species (ROS) to develop. ROS initiates oxidation events, such as lipid peroxidation to unsaturated lipids or starting interactions with sulfhydryl molecules which results in liver damage [9, 10]. Eventually, the produced metabolites are dispersed throughout the body, including plasma, liver, bone marrow, adrenals and kidneys [11].

Exposure to TAA for longer periods causes more damage to the renal cortex than medulla due to its unequal metabolites distribution in the renal tissue where about ninety percent of the total renal blood flow arrives in the cortex via the blood stream. Therefore, relatively high concentrations of TAA metabolites can reach the cortex through blood stream before entering the medulla [12-14]. High volume of blood flow through kidney can cause damage by filtering large amount of toxins that have concentrated in the renal lobules [15]. Thioacetamide induces cells death and affects the termination of the proximal renal tubule [2]. The kidney is very susceptible to toxicants because of high blood volume that runs through it. So a large amount of toxins are filtered through it and can be concentrated in the renal tubules. It can result in systemic toxicity, thus causing a decrease in body wastes excretion ability, inability to maintain body fluid and electrolytes balance [15, 16]. Therefore, this study aimed to confirm the toxic effect of TAA in the kidneys of albino mice (BALB/c).

Materials and Methods

Chemicals

Thioacetamide was purchased from Qualikems, India and was administered to the mice intraperitoneally with a dose of 100 mg/kg body weight, according to [17].

Animals

Around 3 months old one hundred twenty male albino mice with a body weight ranging 30-35g, were used to accomplish the current study. The animals were obtained from the animal house of Al-Razi Center,the Ministry of Industry, Baghdad Iraq.

For adaptation mice were held in plastic cages of 60*10*10 cms dimensions and were kept at animal house of veterinary directorate,Ministry of Agriculture for two weeks. They were fed

on water and commercial feed pellets (standard rodent diet). Housing conditions were kept at 22± 25°C, plus daily organized lighting using automatic electrical timer that provided a twelve hours light (07.00am- 19.00) and a twelve-hour night cycle. The cages litter was removed every 7 days.

Study Design

Animals were randomly divided into two main groups.A control group consisted of 30 mice uptaking the normal diet only. Whereas the second treated group of ninety mice was divided into 3 subgroups as follow:

a) 1st subgroup (G1): 30 mice treated intraperitoneally, monthly (one dose a month) for 2 months with TAA of 0.5 mg/ 100 g B.W.

b) 2nd subgroup (G2): 30 mice treated intraperitoneally, monthly (one dose a month) for 4 months with TAA 0.5 ml/100 g B.W.

c) 3rd subgroup (G3): 30 mice treatedintraperitoneally, monthly (one dose a month) for 6 months with TAA 0.5 ml/100 g B.W.

Blood Collection

Blood was collected for biochemical tests in different periods,2, 4 and 6 months of experiment via cardiac puncture technique. Blood samples were collected in test tubes without using any anticoagulant and waiting for 15 minuntes till clot was formed. The serum was then separated from coagulated blood samples by centrifugation at 5000 rpm for 15 minutes. Finally the serum was collected in plain tubes and was frozen at -20°C until used [18].

Biochemical Tests

Blood urea was measured by colorimetric-enzymatic method which is based on the urease action that hydrolyses urea in ammonium ions carbon dioxide complex. This coloration was measured at 600 nm which was relative to urea concentration in serum sample [17].

Serum creatinine evolution was based on alterationin the original reaction of picrate. Under alkaline state the creatinine reacted with picrate ions that lead to form a reddish complex. The concentration of creatinine on samples depended on color complex density and was determined through the increase of absorbance in a pre-fixed interval of time as described by Cannon *et al*.[19].

Histological Evaluation

The animals were killed by chloroform and postmortem inhalation. Subsequently kidneys were macroscopically examined to document any abnormalities. Tissue specimens were collected from the organs and were fixed in 10% formalin. The specimens were later processed during routine procedure by using the histokinette [20, 21].

Statistical Analysis

Resulting data wase analyzed using statistical analysis system (SAS) by means of a complete randomized design (C.R.D.), using two way analysis of variance (ANOVA) in factorial experiment design to compare the studied parameters mean between control and the treated groups.Duncan's multiple range tests were applied [22].

Results and Discussion

After treatment of mice with TAA, the results revealed that the mean of blood urea level in G3 (55.36 ± 0.20mg/dl) had significantly increased ($P<0.05$) in comparison with

concentration of G2 and G1 which were 47.43 ± 0.25 mg/dl and 40.03 ± 0.17mg/dl respectively.
Regarding creatinine level in G1, G2 and G3, they were 2.7 ± 0.15, 3.2 ± 0.35 and 4.7 ± 0.54 mg/dl respectively. This means there was a significant increase in the three treated groups, especially G3 group in comparison with the control.

Table 1: The serum concentration of blood urea and creatinine of G1, G2, G3 and control show at different period

Groups	Blood Urea mg/dl	Creatinine mg/dl
Control	23 ± 0.20	**0.6 ± 0.03**
G1 2 months	40.03 ± 0.17*	**2.7 ± 0.15***
Control	23.03 ± 0.42	**0.60 ± 0.05**
G2 4 months	47.43 ± 0.25**	**3.2 ± 0.35****
Control	23.05 ± 0.25	**0.6 ± 0.09**
G3 6 months	55.36 ± 0.20***	**4.7 ± 0.54*****

Mean ± S.E*significant at P<0.05, **significant at P<0.01 and ***significant at P<0.001

The histological evaluation of albino mice kidney tissue after H and E staining, showed that the kidney of control group had normal architecture. Histological changes in kidney of albino mice that were administrated with TAA I/P (100 mg/kg B.W) for the period 2 months had acute cellular swelling of renal tubules, necrosis of glomeruli and renal tubules with interstitial hemorrhage (Figure1). Whereas, after 4 months of TAA treatment, kidney showed a necrosis in renal tubules, large glomerular tuft due to an increase of mesenchymal cells and PMNCs infiltration of the interstitial (Figure 2).

Finally, after 6 months of TAA treatment, kidneys showed granuloma with center of necrosis and MNCs infiltration that was surrounded by capsule, necrotic glomeruli, increase in the space of bowman and interstitial necrosis (Figure 3).

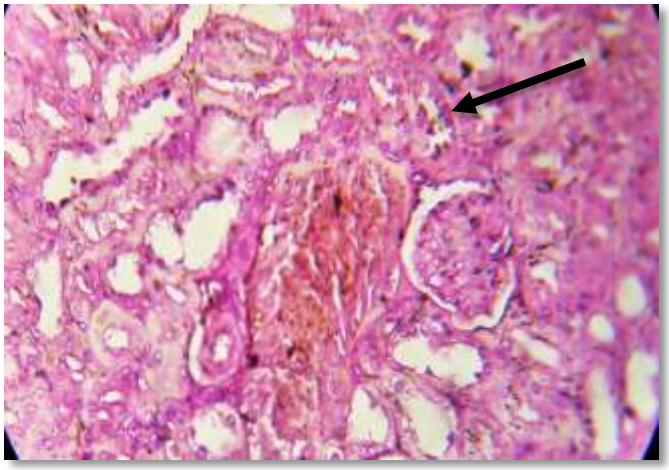

Figure 1: Kidney of albino mice administrated with TAA I/P for two months: →acute cellular swelling of renal tubules, and necrosis of glomeruli and renal tubules interstitial hemorrhage (H&E, X400).

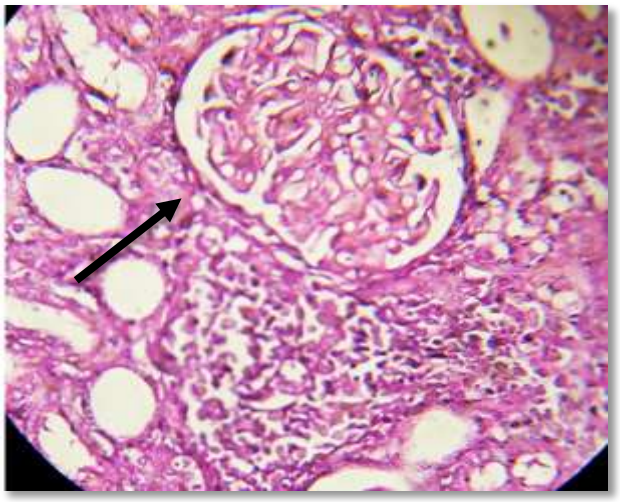

Figure 2: Kidney of albino mice administrated with TAA I/P for 4 months: →necrosis of renal tubules, large glomerular tuft due to increase of mesenchymal cells and PMNCs infiltration in the interstitial (H&E, X400).

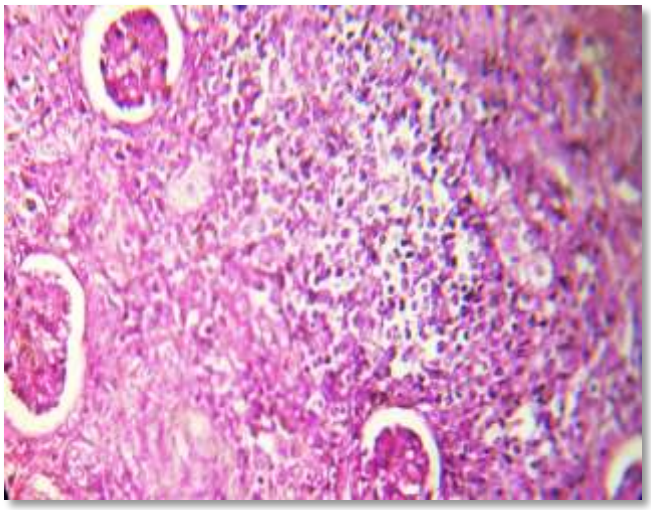

Figure 3: Kidney of albino mice administrated with TAA I/P for 6 months: →granuloma with center of necrosis with MNCs infiltration and surround by capsule, necrotic glomeruli, increase in the space of bowman and interstitial necrosis (H&E, X400).

The present study revealed that mice exposed to TAA exhibited histopathological changes and apparent impairment of renal function, manifested by the enhancement of urea and serum creatinine levels. Blood urea and creatinine are waste products of metabolism that are excreted by the kidney elevation of these parameters, indicating insufficiency of renal function [23]. Many previous studies have reported an increase in urea and creatinine levels in mice due to damaged kidney, causing acute renal failure [24, 25].

In addition, the toxic effect of thioacetamide has also been reported in many studies that when applied on different experimental animals, it may lead to an increase in serum creatinine levels [26-28]. This increase may occur due to a disturbance in Na^+-K^+ pump of nephron and detachment of epithelial cells from renal tubules which causes nephropathy while damaging and destroying renal parenchyma [29]. Also, a study by Serag [12] reported an increase inurea

and creatinine levels in mice after getting treated with TAA. While another study by Abdou *et al.*[30] mentions that the urea level may change due to inhibition of urea cycle by inhibiting 2-oxoglutarate formation that increases ammonia which may lead to a decrease in urea concentration.

Histopathological changes, including the cortical area of kidney which was damaged more than in the medulla, were reported. The glomerular necrosis, areas of hemorrhages, cuffing, proliferation of mesangial cells and granuloma were observed. This abnormality may be due to high toxins filteration rate as a result of high blood volume flowing through kidney lobules which can concentrate in it. The renal morphology is impaired, with severe and generalized necrosis of tubular epithelial cell, diffused tubular swelling, glomerular congestion, and inflammatory cell infiltration [15].
Tubular damage and necrosis may occur as a result of decreased glomerular filtration. Tubular abnormalities may also result in tubule obstruction which causes glomerular filtrate to flow backward [1].

A study by Al-Attar *et al.*[27] mentioned that administration of TAA100mg/kg intraperitonially for 12 weeks leads to several changes in the renal corpuscles structure, involving a high rate of necrosis and degeneration of glomeruli and Bowman's capsules due to the high volume of blood flow through them, and the filtering big amounts of toxins that can concentrate in kidney tubules. In conclusion, The TAA had a series of toxic effects on kidneys of the mice, leading to nephrotoxicity which finally leads renal failure.

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