Hepatoprotective effects of zinc sulphate and silymarin against thallium-induced poisoning in rats

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Abstract

Background: Thallium (Tl) is a heavy metal with its salts are highly toxic. This element is widely used in manufacture, and still being used as a rodenticide in some countries. Many occupational, accidental and criminal cases present to clinics all over the world. Thallium toxicity affects all body compartments including hepatic tissue.

Objective: to study the hepatoprotective effects of zinc sulphate and silymarin therapy in rats poisoned with thallium.

Methods: 48 albino rats of both sex were classified into 4 groups, each group contains 12 rats, first group animals were given only distilled water orally for 5 successive days, second group were given a single oral dose of thallium acetate (16 mg/ kg) followed by a daily oral dose of distilled water. Third group given the same oral dose of thallium followed by a daily dose of zinc sulphate solution (20 mg/kg) for 5 successive days. Fourth group rats were given the same oral dose of thallium acetate followed by a daily oral dose of silymarin solution (25 mg/kg) for 5 successive days. Serum transaminases (ALT, AST) and alkaline phosphatase were measured and hepatic tissue sections were taken for histopathological study.

Results: rats treated with zinc sulphate and silymarin had shown significant changes in serological and histopathological results in comparison to normal and thallium groups.

Conclusion: zinc sulphate and silymarin have hepatoprotective effects against induced thallium poisoning in rats.

Key words: thallium, zinc sulphate, silymarin, hepatoprotective.

التأثير الواقي للكبد لكل من كبريتات الزنك والسليمارين المضاد لتسمم الثاليوم المحدث في الجرذان

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الخلاصة

الخلفية العلمية : الثاليوم من المعادن الثقيلة حيث يعتبر مع املاحه ذا سمية عالية كما استعمل سابقا كمبيد للقوارض ، ان سمية الثاليوم يمكن ان تؤثر على اعضاء الجسم المختلفة ومنها الانسجة الكبدية وهناك حالات سريرية متعددة تخص سمية الثاليوم وترتبط بالحرف والحوادث والجريمة.

أغراض البحث: لدراسة التأثير الواقي لكبريتات الزنك والسليمارين في الجرذان المسممة بالثاليوم.

الطرائق: وزع ٤٨ جرذيا ابيضا من كلا الجنسين على اربع مجاميع ، تحوي كل مجموعة ١٢ جرذيا ، أعطيت المجموعة الأولى : الماء المقطر فمويا لمدة ٥ أيام متتالية في حين أعطيت المجموعة الثانية جرعة واحدة من خلات الثاليوم ١،٦ ملغم/ كغم فمويا أعقبها جرع يومية من الماء المقطر ، أما المجموعة الثالثة فأعطيت نفس الجرعة من الثاليوم المعطاة للمجموعة الثانية اعقبها جرع يومية من محلول كبريتات الزنك ٢٠ ملغم / كغم اعطيت فمويا لمدة ٥ أيام متتالية، كذلك المجموعة الرابعة فلقد اعطيت نفس الجرعة من الثاليوم تبعتها جرعة فموية يومية من محلول السليمارين ٢٥ ملغم/كغم لمدة ٥ أيام متتالية . وتم قياس انزيمي الترانسامينيز (ALT, AST) وكذلك الفوسفوتيز القلوي في المصل . كما اخذت مقاطع من انسجة الكبد لغرض الدراسة النسيجية المرضية.

النتائج : اظهرت الجرذان المعالجة بكبريتات الزنك والسليمارين متغيرات معنّدة في النتائج المصلية والانسجة المرضية في مقارنة لها مع المجاميع الطبيعية او المسممة بالثاليوم.

الإستنتاج: لقد وجد ان كبريتات الزنك والسليمارين تمتلك تأثيرات واقية للكبد وفي تضاد للتسمم المحدث بالثاليوم.

الكلمة المفتاح: الثاليوم ، كبريتات الزنك ، السليمارين ، وقاية الكبد.

Introduction :

Thallium is a chemical element referred to by the symbol (Tl) . It was discovered in 1861 by the English chemist William Crookes and metallic thallium was first prepared by a French scientist Lamy in 1862[1]. The discovery of thallium occurred incidentally by spectroscope when the English chemist Crookes was examining a sludge left over from the production of sulfuric acid (H_2SO_4) in which he was looking for Selenium. He observed a bright green line that no one had ever seen before. He named the new element that was producing the green line thallium, after the Greek word for 'green twig', thallos [2] . Thallium is a natural constituent of earth crust, it presents in nearly all environmental media [3].

Thallium is present in mainly industrial wastewaters. Information on specific waste streams is not difficult to find; however, individual mines often do not want their thallium concentrations published (Mine Waste Treatment Program [4]

Absorption of thallium compounds is rapid after ingestion, inhalation, or skin contact and may be complete after ingestion.[5] Thallium is widely distributed in the body after its rapid absorption in animals. Acute and chronic studies agree that the highest concentration is found in the kidneys.[6] Thallium is mainly excreted in the faeces though this may be decreased significantly by paralysis of the small intestine, a characteristic feature of thallium poisoning. [7] Thallium is also excreted in the urine, but about half the amount in the glomerular filtrate is reabsorbed in the tubules. The ratio of faecal to urinary elimination is approximately 2:1.[8]

The exact mechanisms that mediate thallium toxicity are still poorly understood and not known since thallium interacts with cells at different levels. [9]

There are similarities between the ions of thallium and those of potassium: they have similar ionic radii, and there is experimental evidence to suggest that the biological handling of thallium and potassium ions is interrelated [10]. Thallium can mimic potassium ion in most biological processes because of the same ionic radius and inability of the cell membrane to differentiate between these two cations. Moreover, thallium follows potassium distribution pathways and in this way alters many of potassium-dependent processes. For example, thallium may substitute potassium in the Na+/K+-ATPase. Interference with potassium transport has been demonstrated in rabbits, where thallium had a 10 times higher affinity for Na+/K+-ATPase than potassium [11].

Thallium has a high affinity for the sulfhydryl groups present in many enzymes and other proteins. Due to the presence of empty d-orbitals in electronic configuration, thallium has a high affinity for sulphur ligands. It can form complexes with sulfhydryl groups of proteins which are usually involved in reactions catalysed by enzymes leading to their inactivation[12]

When bound to membrane phospholipids, especially to the anionic head groups, thallium changes membrane rheology, lipid packing, lipid arrangement in the lateral phase of the bi-layer, and the hydration of the polar head groups. [13]

A study had described some pathological findings in several organs including livers of patients intoxicated with thallium. Histopathological findings showed centrilobular necrosis with fatty change present in most liver lobules, as well as the presence of abnormal amounts of porphyrins in the urine. Several other secondary pathological changes were described in the organs of these patients.[14]

Methods :

The present study was conducted in the animal house / Baghdad college of medicine, between June 2012 and March 2013.

48 healthy albino rats of both sexes with body weight between 150 - 200 gm were divided into 4 groups, each group contains 12 rats. They were supplied by animal house of Baghdad college of medicine. They were kept in a well controlled hygienic environment,

every rat was housed in a single cage . Rats had taken food and water ad libitum.

The groups were arranged as the following:

group A in which 12 rats were given only distilled water orally for 5 successive days, group B in which 12 rats were given a single oral dose of thallium acetate (16 mg/ kg) dissolved in distilled water followed after 2 days by a daily oral dose of 3 ml of distilled water.

Group C in which the same oral dose of thallium was given followed after 2 days by a daily dose of zinc sulphate solution (20 mg/kg) for 5 successive days. Group D in which 12 rats were given the same oral dose of thallium acetate followed after 2 days by a daily oral dose of silymarin solution (25 mg/kg) for 5 successive days. Serum transaminases (ALT and AST) and alkaline phosphatase were measured and hepatic tissue sections were taken for histopathological study.

The results are expressed as means ± standard deviation (SD). Data were analyzed by paired t- test to elucidate differences between each 2 groups of the study. First, to compare between normal group and thallium group and to study the effects of thallium on normal parameters. Then, to compare the effects of each drug group with thallium group. Results considered significant when P value < 0.05.

Results :

Thallium caused an extremely statistically significant increase in serum transaminases ALT & AST (P value = 0.001 for both) in comparison with normal group. Also, ALP increased significantly (P value = 0.02). (Table 1)

Table (1) : comparison of liver function tests (ALT, AST & ALP) serum values between thallium group and normal group of rats.

Biochemical Test	Thallium M ± SD No.=12	Normal group M ± SD No.=12	P value
ALT (U/L)	45.33* ± 9.21	30.75 ± 9.11	**0.001***
AST (U/L)	218.2 *± 46.5	133.1 ± 45.2	**0.001***
ALP (U/L)	212.9* ± 30.0	168.8 ± 40.6	**0.02***

Values represent M ± SD

Results were considered statistically significant when ($P < 0.05$)

Zinc sulphate treatment had shown a statistically significant reduction in both transaminases (P value =0.033 and 0.023 for ALT and AST respectively) and non- statistically significant reduction in ALP (P value = 0.315) . (Table 2)

Table (2): comparison of liver function biochemical tests (ALT, AST & ALP) serum values between thallium group and zinc sulphate treatment values in poisoned rats.

Parameters	thallium (M ± SD) No.=12	Zinc sulphate treatment (M ± SD) No.=12	P value
ALT (U/L)	45.33 ± 9.21	34.75* ± 11.07	**0.033***
AST (U/L)	218.2 ± 46.5	180.8* ± 29.1	**0.023***
ALP (U/L)	212.9 ± 30.0	199.5 ± 25.33	0.315

Values represent M ± SD

Results were considered statistically significant when ($P < 0.05$

Silymarin produced a general reduction in all parameters. The statistically significant reduction of both transaminases ALT & AST (P values = 0.027 and 0.037 respectively) resulted in silymarin treatment group in comparison with thallium group. (Table 3)

Table (3): comparison of liver function biochemical tests (ALT, AST & ALP) serum values between thallium group and both silymarin treatment group.

Parameters	thallium (M ± SD) No.=12	Silymarin Post-treatment (M ± SD) No.=12	P value
ALT (U/L)	45.33 ± 9.21	37.33* ± 5.45	**0.027***
AST (U/L)	218.2 ± 46.5	176.7* ± 27.6.1	**0.037***
ALP (U/L)	212.9 ± 30.0	204.5 ± 33.24	0.512

Values represent M ± SD

Results were considered statistically significant when ($P < 0.05$)

Histopathological results :

Liver sections of normal group showed normal, well defined histological structures without any signs of vascular or inflammatory changes. (figure 1)

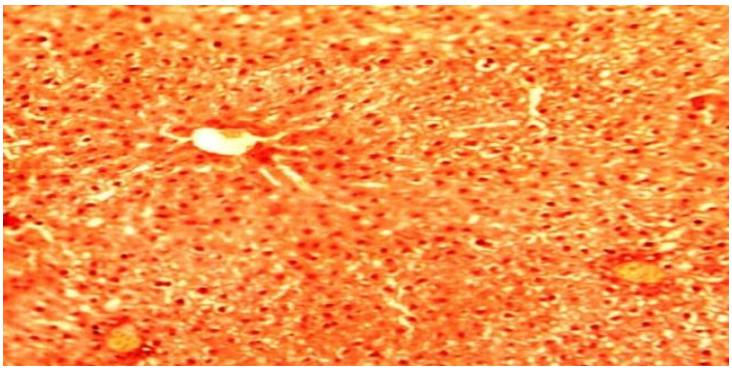

Figure (1) : **normal liver histology : A** photomicrograph of normal rat liver showing typical lobular structure and hepatocytes around the central vein in a cord –like arrangement.

Liver sections from rats of thallium group revealed several histopathological changes. There is loss of normal hepatic lobular architecture, the normal cord –like arrangement of liver cells was disorganized. Extensive vascular congestion with red blood cells was seen in all sections of thallium group also, RBC extravasations was found pooling in sinusoids. leukocytes infiltrations was present in different areas of many sections.

Higher microscopical magnification power revealed necrotic changes including cellular degeneration features like cellular coagulative necrosis, pyknosis (condensed nucleus), dark eosinophilic cytoplasm of hepatocytes and Vacuolated

hepatocytes were present in varying degrees of intensity (mostly intense) .
(figure 2)

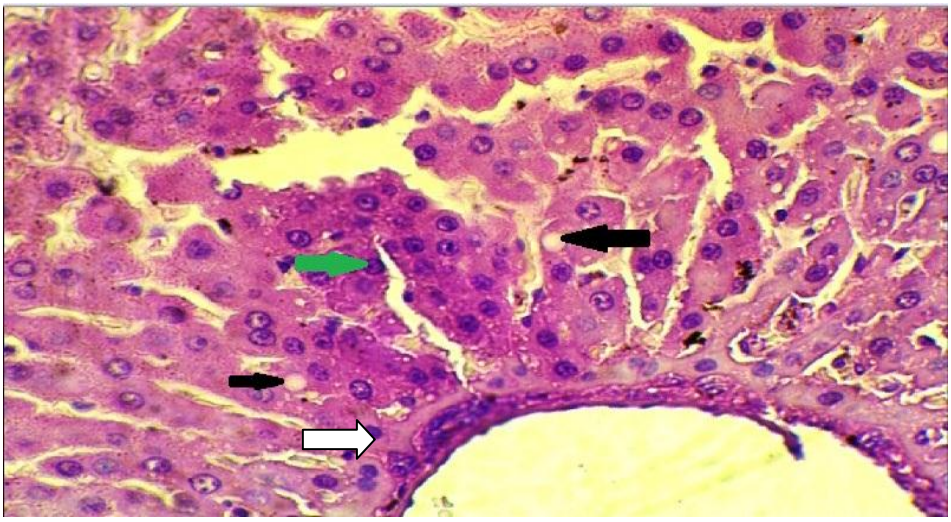

Figure (2) Histopathological changes of liver section in thallium group. Showing features of cellular degeneration, nuclear pyknotic changes and dark eosinophilic cytoplasm (green arrow) . vacuolated hepatocytes (black arrow) and perivascular layer of necrotic cells (white arrow) **(H&E staining, 400x)**

Liver sections of zinc treated rats revealed preservation of architecture and mild congestion in a number of vessels. Degenerative changes were infrequent in most sections. Mild to moderate RBC extravasations were present.

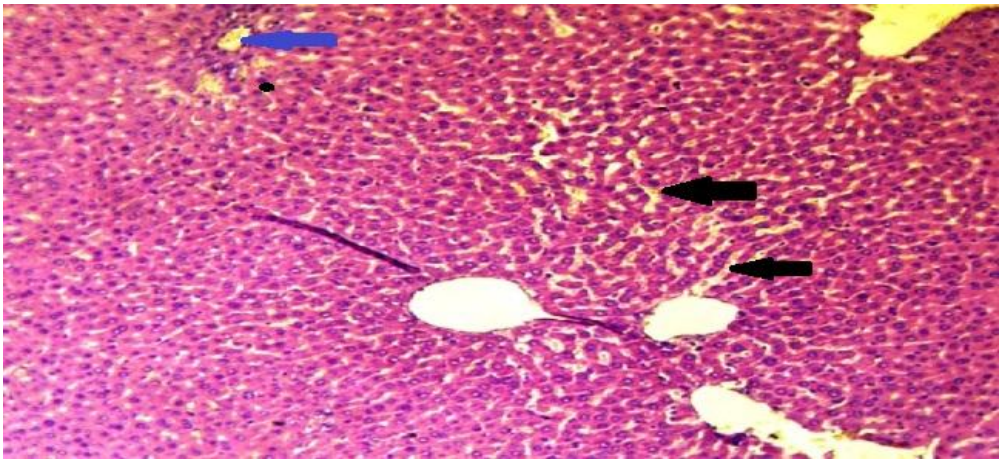

Figure (3) : histopathological changes of rat liver from zinc sulphate post-treatment group. Preservation of lobular architecture. Mild vascular congestion (blue arrow). and mild RBC extravasation (black arrows). **(H&E staining, 100x)**

Liver micrographs of silymarin group of animals had shown well preserved architecture and mild infrequent degenerative changes, mild to moderate vascular congestions and frequent moderate RBCs extravasation.

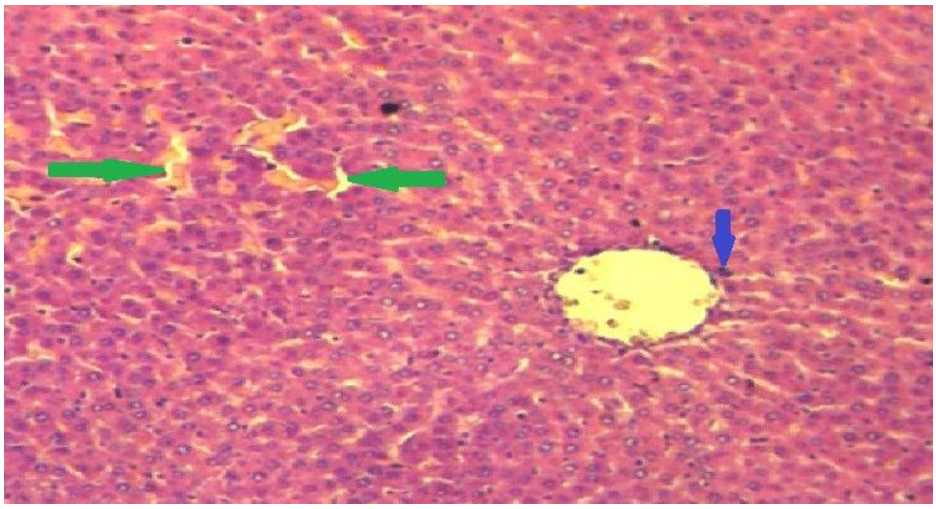

Figure (4) : histopathological liver changes of a rat from silymarin post-treatment group. Showing RBCs extravasation (green arrows) and pyknotic nuclei indicating mild perivascular necrosis (blue arrow), preserved architecture . **(H&E staining, 100x)**

Discussion:

In comparison to normal group, thallium had shown extremely significant increase in liver enzymes (ALT, AST and ALP). This increase was reported by many authors. Leung and Ooi revealed that male albino rats receiving a single i.p. injection of 30, 60, or 120 mg/kg thallium sulfate had statistically significant increased levels of AST and ALT above controls 16 hours after treatment, regardless of dose[15]. It is well known that thallium causes a significant increase in these enzymes in human [16].

Increased serum values of liver enzymes are usually regarded as expressions of cellular necrosis, especially in hepatocytes. Hepatocellular damage with the subsequent disruption of the plasma membrane allows leakage of intracellular enzymes such as ALT or AST into the bloodstream. [17]

Zinc sulphate had shown a whole liver enzyme reduction. ALT and AST levels were reduced significantly revealing a hepatoprotective effect of zinc at the cellular level against thallium

hepatotoxicity. Bandhu et al study revealed the antioxidative role of zinc in hepatotoxic conditions induced by subjecting the rats to protein-deficient diet and lead treatment[18].

silymarin reduced all liver enzymes particularly ALT which was reduced significantly . ALT is more specifically indicative for liver injury since it is synthesized mainly by hepatocytes[19]. This fact suggests a stronger hepatoprotective effect of silymarin since it caused a significant ALT reduction . Reduction of liver enzymes strongly consolidate the proposed silymarin mechanism of stabilizing cellular membrane.

Liver histopathological features were less prominent in sections taken from rats treated with zinc sulphate . General architecture was preserved indicating less cellular damage and degenerative changes, extravasation was less frequent indicating lower vascular damage and there were less frequent congestion and RBCs extravasation indicating less venous obstruction. These observations confirm the hepatoprotective effects of zinc via its different mechanisms of action.

Silymarin treatment group sections had shown no prominent histopathological improvement than those of thallium group since all features of typical thallium picture were present but to a lesser extent in some of them (e.g. RBCs extravasation and vascular congestion).

Silymarin protective effects were more prominent since architecture was preserved in most parts of sections, less centrilobular necrotic changes, less cellular degenerative changes and less congestion. All these changes indicate a hepatoprotective effect of silymarin mediated by its antioxidant effects. Muriel and Mourelle had shown that silibinin preserves the functional and structural integrity of hepatocyte membranes by preventing alterations of their phospholipid structure produced by carbon tetrachloride.[20]

Conclusion:

Oral zinc sulphate and silymarin have hepatoprotective effects against hepatic damage caused by thallium poisoning.

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