Anti-oxidant effect of silymarin against DDT-induced nephrotoxicity in rats

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Abstract

Background: Oxidative stress is a common mechanism contributing for initiation and propagation of renal damage induced by several chemicals such as DDT. Silymarin, the dried extract of the ripe seeds of the plant *Silybum marianum* is found to be a powerful protective agent against toxin-induced tissue injury in many organs especially the liver by its antioxidant property; accordingly, the intended property needs to be clarified in other organ subjected to toxic chemicals.

Objective: The present study was designed to evaluate the possible protective effect of silymarin on the status of oxidative stress by measuring the levels of (MDA) and glutathione (GSH) in renal tissue in addition to assessment of the serum levels of urea and creatinine and examination of possible histological renal changes induced in rats by a toxic dose of DDT.

Methods: White albino rats were administered a single oral dose of DDT (100mg/kg) to induce renal toxicity. Silymarin was orally administered twice daily dose (500 mg/kg) for 7-days prior to DDT administration, then the animals were sacrificed 24-hours after DDT-treatment. The parameters of oxidative stress, MDA contents and GSH levels were measured in renal tissue homogenate. Blood was collected for measuring serum urea and creatinine levels, in addition to the histological examination of the kidneys.

Results: Treatment of rats with silymarin for 7-days prior to DDT administration caused a significant reduction in the contents of the lipid peroxidation end product, MDA down to (61%) with the increasing in the levels of GSH levels up to (82%) in renal tissue homogenate compared to DDT-treated animals. Furthermore, silymarin was able to counteract significantly the elevation in the levels of serum urea and creatinine by about 38% and 34%, respectively compared to DDT-treated rats. Sections of rats' kidney treated with silymarin 7 days prior to DDT administration, elicited improvement in the histopathological changes induced by DDT characterized by inhibition of cloudy swelling, inflammation and necrosis.

Conclusion: According to the results obtained from this study, it is conclude that silymarin have antioxidant property through direct and/or indirect mechanism that provide protective effects against DDT-induced nephrotoxicity, and makes it a good candidate to be tried clinically in this respect.

التأثير الوقائي المحتمل للسليمارين ضد التسمم الكلوي المستحدث بواسطة دايكلورودايفنيل ترايكلوروايثان (DDT) في الجرذان

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مفتاح الكلمات: دي دي تي، سليمارين، التسمم الكلوي، الجرذان

الخلاصة:

يعد الاجهاد التأكسدي من الاليات الشائعة في نشوء وتقدم انواع متعددة من الاعتلالات الكلوية المستحدثة بواسطة مواد كيميائية ومنها دايكلورودايفنيل ترايكلوروايثان (DDT). تعد عصارة البذور الناضجة silymarin لنبات *Silybum marianum* عامل وقائي قوي ضد الضرر النسيجي المستحدث باسموم في العديد من الاعضاء وخاصة الكبد حيث يعمل كعامل مضاد للاكسدة، وطبقا لذلك فان هذه الخاصية قد تمتد لتشمل اعضاء اخرى والتي تحتاج الى توضيح.

الهدف من الدراسة: لقد تم تصميم هذه الدراسة لتقييم التأثير الوقائي المحتمل لمادة السليمارين على حالة الاجهاد التأكسدي من خلال قياس مستوى مالون داي الديهايد(MDA) ومستوى الكلوتاثايون (GSH) في انسجة الكلى مع قياس وظائف الكلى من خلال قياس مستوى اليوريا والكرياتينين في مصل الدم عند الجرذان المعطاة جرعة سامة من مادة الDDT بالإضافة إلى التغييرات المحتملة في انسجة الكلى.

طرق العمل: تم معاملة مجموعة من الجرذان بجرعة واحدة من ال دي دي تي (100 ملغم لكل كيلوغرام من وزن الجسم) عن طريق الفم لغرض استحداث تنخر كلوي. مجموعة اخرى من الجرذان عوملت بمادة السليمارين مرتان يوميا (500 ملغم لكل كيلوغرام من وزن الجسم) عن طريق الفم لمدة 7 أيام قبل معاملتها بجرعة ال دي دي تي، ثم تم قتل الحيوانات بعد 24 ساعة من اعطاء جرعة ال دي دي تي.

تم قياس الادلة الخاصة بظاهرة فرط الاكسدة مثل مستويات مالون داي الديهايد (MDA) والكلوتاثايون (GSH) في نسيج الكلى و تم تحضير مصل الدم لقياس مستويات اليوريا والكرياتينين لاختبار وظائف الكلى. اضافة الى ذلك تم تحضيرمقاطع نسيجية من الكلى لاختبارها تحت المجهر الضوئي .

النتائج: دلت نتائج البحث ان اعطاء الجرذان مادة السيليمارين لمدة 7 أيام قبل اعطاءها DDT قد احدثت تناقص معنوي لمحتويات ال MDA بمقدار (61%) وزيادة معنوية في مستوى ال GSH بمقدار (82%) في النسيج الكلوي مقارنة بالجرذان المعاملة بمادة ال DDT. اضافة الى ذلك فان لمادة السيليمارين القابلية على عكس التاثير الكلوي السام لمادة DDT من خلال التقليل الواضح لمستويات اليوريا (38%) والكرياتينين (34%) في مصل الدم مقارنة بالجرذان المعاملة بمادة ال DDT .

كما أكدت المقاطع النسيجية نتائج التحاليل المختبرية حول الحماية الكلوية لمادة السيليمارين من خلال تحسن الصورة النسيجية العامة مع منع عملية تجمع الدهون والتهاب وتنخر انسجة الكلى الذي تسببه مادة الDDT .

الاستنتاجات: من خلال النتائج التي تم التوصل اليها يمكن الاستنتاج بان لمادة السيليمارين خاصية مضادة للأكسدة بواسطة الية مباشرة أو غير مباشرة من حيث توفير الحماية الخلوية للنسيج الكلوي ضد التلف المستحدث بمادة ال دي دي تي ، ومن الممكن ان يصبح مرشح جيد للاستعمال السريري فيما يتعلق بهذا الغرض.

Introduction.

DDT (1, 1, 1,-trichloro-2, 2-bis (*p*-chlorophenyl)-ethane), a well-known organochlorine pesticide, is still present in the environment [1]. Although some persistent organochlorine pesticides have been banned from agricultural and public health use during the past few decades, high concentrations of DDT and its metabolites have been found in soil, water, and sediment samples [2].

The intended pesticide is considered as an enzyme inducing agent in a dose-dependent manner, including its own metabolism in rats and hamsters [3] and showed similar metabolic pathways in both humans and rats [1]. Its major urinary metabolite is DDA-Cl and the minor DDMU-epoxide metabolite may contribute to the known tumorigenicity of DDT via the formation of covalent DDA adducts in the mouse [4]. Furthermore, DDT also uncouples oxidative phosphorylation in

addition for its binding to protein complexes and submitochondrial fractions, altering mitochondrial morphology and function [5]. The disposition of DDT occurs mainly in the adipose tissues [6]. However, when lipids are mobilized to meet energy demands, accumulated organochlorine pesticide become available and may reach sensitive tissues like liver and kidney.
Silymarin is a mixture of polyphenolic flavonoids extract obtained from seeds and fruits of the milk thistle (*Silybum marianum* L. Asteraceae) [7]. It has been used since 4th century BC for the treatment of plague and congestive conditions of the liver, spleen, gall bladder and kidney disorders [8].
Thus, this study was designed to evaluate the possible protective effects of silymarin on the status of oxidative stress in renal tissue with measuring the serum levels of urea and creatinine in rats induced by a toxic dose of DDT in addition to the histopathological changes that may occur in the renal tissue.

Materials and Methods:
Thirty white Albino male rats weighing (200-250 gm) were obtained from and maintained in the Animal House of the College of Pharmacy /University of Baghdad, under conditions of controlled temperature, humidity and light/dark cycle. They were fed a standard commercial pellets and allowed free access to tap water.

They were divided into three groups (ten animals each)**. Group I** (normal control) rats received single oral dose of corn oil by gavage tube then the animals were sacrificed on the second day. **Group II**- rats received single oral DDT dose (100 mg/kg) by gavage tube and then they were sacrificed on the second day. **Group III-** rats received silymarin orally by gavage tube (500 mg/kg) twice daily for 7-days prior to DDT administration. The animals were sacrificed after 24 hours of DDT treatment.

After euthanization of the animal by diethyl ether, one kidney was quickly excised, homogenated and utilized for the estimation of MDA contents [9] and GSH levels [10]. The blood was allowed to clot for 30 min; serum was separated by centrifuging at rate of 3000rpm for 15min which was utilized for biochemical estimations serum urea level [11] and serum creatinine level [12]. The second kidney was quickly removed after autopsy and fixed in 10% formalin and utilized for histological examination [13].

The significance of differences between the mean values was calculated using unpaired Students't-test. P-values less than 0.05 were considered significant for all data showed in our results.

Results

Rats treated with DDT 100 mg/kg alone produced a significant increase in the contents of MDA in renal homogenate ($P<0.05$) with consequent significant decrease in the levels of GSH ($P<0.05$) in the renal tissue homogenate compared to control group as shown in figures 1 and 2. Additionally, there were non-significant differences ($P>0.05$) concerning both renal MDA contents and GSH levels in group of animals treated with silymarin 7 days prior to DDT administration in comparison with the levels of control group. (Figures 1 and 2).
Concerning the levels of serum urea and creatinine, figures 3 and 4 showed significant increase in both levels ($P<0.05$) in group of rats treated with DDT compared to control group.
Moreover, both serum urea and creatinine levels were non-significantly different ($P>0.05$) in groups of animals treated with silymarin 7 days prior to DDT administration compared to the corresponding levels of control group as shown in figures 3 and 4.
Concerning kidney histopathology, renal sections of animals treated with DDT showed cloudy swelling with narrowing of the tubular lumen of the proximal convoluted tubules (PCTs) and distal convoluted tubules (DCTs), interstitial tissue edema with inflammatory cell infiltration.

Necrotic changes mainly in the proximal and distal convoluted tubules. In addition to the tubular epithelial loss, there were a granular casts in PCTs and blood vessels congestion were observed (Fig. 6) compared to control groups (figure 5). Sections of the rat's kidney treated with oral dose of 500mg.kg^{-1}.day^{-1} silymarin given twice daily for 7-days prior to DDT-administration showed an improved histological picture of kidney tissue with mild inflammatory cells infiltrations (Fig. 7) compared to DDT-treated animals.

Discussion

It has been demonstrated that, organochlorine pesticides have the ability to induce oxidative stress in different organs through the generation of free radicals and induction of peroxidative degradation of membrane poly-unsaturated fatty acids of endoplasmic reticulum, resulting in the formation of lipid peroxides, with further damage to the membrane, cellular protein and alter cellular function [14] with the reduction of activities of antioxidant defense mechanisms [15]. Furthermore, changing in levels of enzymatic and non enzymatic antioxidants (biomarkers of contaminant mediated pro-oxidant challenge) were found to be associated with renal lesions causing some form of impairment in the nephron function [16] [17].

The reactive oxygen species (ROSs) generated from DDT and its metabolites including epoxides may be responsible for the marked decrease in renal GSH levels, as much as more GSH were consumed for conjugation of metabolites, leading to disturbances in antioxidant enzyme systems [18] so the redox potential of the tissue was impaired. The results of this study confirmed that, DDT at a dose of 100mg.kg^{-1} day^{-1} produced significant nephrotoxicity as evidenced by elevation of percent of renal tissue homogenate MDA contents 159% and depletion of GSH levels 46% which is compatible with other studies [14, 19]. The basis of pesticide toxicity is the generation of free radicals and production of reactive oxygen species that alter the normal homeostasis of the body resulting in oxidative stress. If the requirement of continuous antioxidants is not maintained [20], oxidative stress is possibly involved in the pathophysiology of renal diseases, renal failure, renal interstitial fibrosis and nephropathy. The data presented in this work clearly demonstrated the significant elevation in serum urea (90%) and serum creatinine levels (55%) in DDT-treated animals compared to controls, which reflect impairment in renal function that can be attributed to oxidative stress concerning DDT-induced tubular changes, calcification, and necrosis of the kidneys [18]. This result is compatible with those observed by others [21].

The present work showed that, oral administration of 500mg.kg^{-1} .day^{1} silymarin twice daily for 7- days prior to orally-administered DDT reversed the increase in MDA (61%) and depletion of GSH (75%) in renal tissue homogenate. These results are consistent with others [14]. Treatment of animals with silymarin one week orally prior to DDT treatment protects against DDT-induced nephrotoxicity manifested by significant lowering of both serum urea level (38%) and creatinine level (34%) compared to DDT- treated rats. Additionally, silymarin was able to improve the state of oxidative stress emerged due to DDT administration, which seems to be a direct way of interfering with lipid peroxidation process, and can be considered as a secondary consequence for other toxic events. The results of this study were consistent with others [22]. Furthermore, by comparing the present work with other study, which revealed that, infusion of the active constituent of silymarin (silybinin) before treatment with cisplatin, the cytotoxic drug that produce nephrotoxicity, silybinin significantly reduces glomerular toxicity [23], resulted in normalization of the serum levels of both urea and creatinine.

Regarding histological features of kidney tissues of DDT-treated rats, there was necrosis of tubular epithelial cells, whereas it was nearly comparable to control when DDT is given to rats pretreated with silymarin. The protective effect observed for silymarin against DDT-induced nephrotoxicity may be attributed to its powerful anti-oxidant activity and may also be related to enhancement in the intracellular anti-oxidant enzymes, the superoxide dismutase and glutathione peroxidase in kidney tissues [17].

Furthermore, silymarin, by interacting with the lipid component of the cell membrane, can influence their chemical and physical properties, and renders cell membrane more resistant to lesions [24].

Conclusion

This study confirmed the protective effect of silymarin against nephrotoxic effect induced by DDT in rats which is manifested by improving the parameters of oxidative stress and the levels of serum urea and creatinine. This is in accord with histological finding of silymarin in decreasing morphological alteration of rats' kidney histological sections. The cytoprotective mechanism of silymarin is attributed to its antioxidant property against the production of free radical and ROSs.

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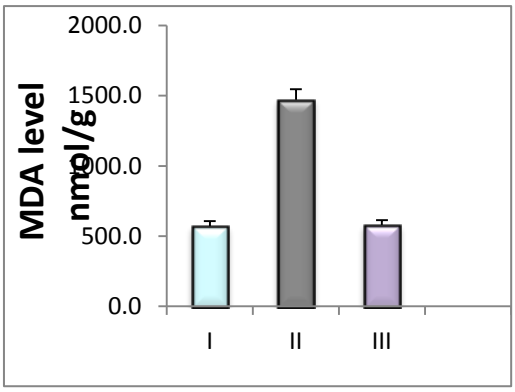

Figure 1: The effects of DDT (group II) and treatment with silymarin prior to DDT administration (group III) on renal MDA contents compared to control (group I).

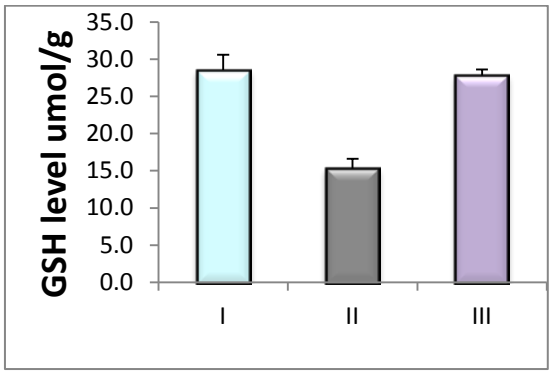

Figure 2: The effects of DDT (group II) and treatment with silymarin prior to DDT administration (group III) on renal GSH levels compared to control (group I).

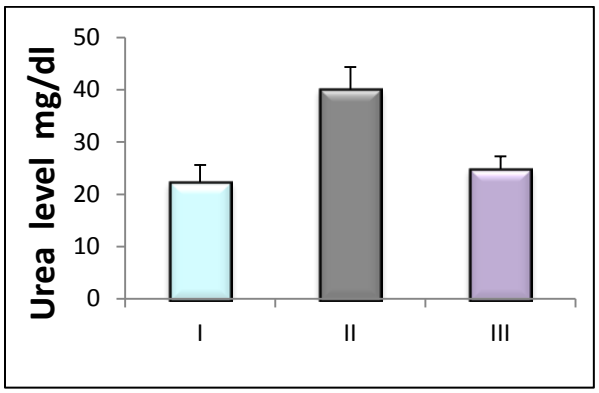

Figure 3: The effects of DDT (group II) and treatment with silymarin prior to DDT administration (group III) on serum urea levels compared to control (group I).

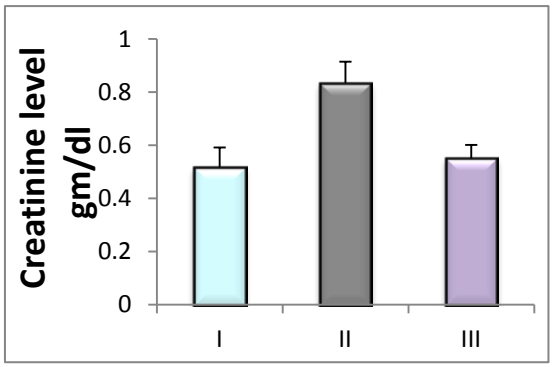

Figure 4: The effects of DDT (group II) and treatment with silymarin prior to DDT administration (group III) on serum creatinine levels compared to control (group I).

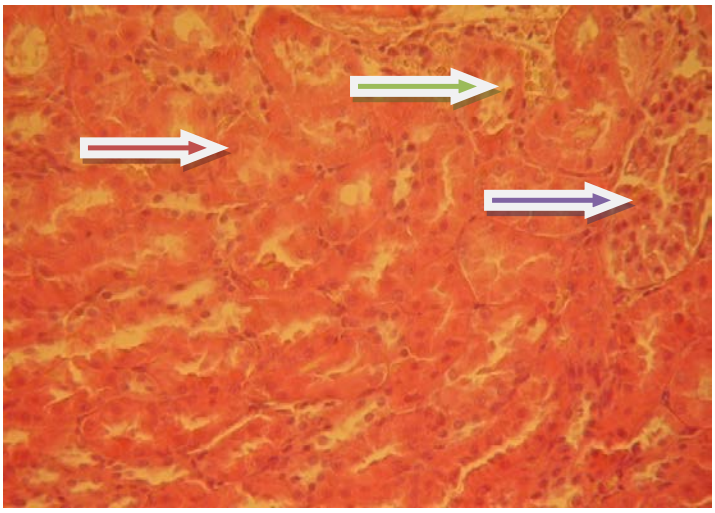

Figure 5: Cross section of normal rat's kidney stained with H and E, 100X.

Red arrow: Normal proximal convoluted tubules; **Green Arrow:** Normal distal convoluted tubules; **Violet arrow:** normal glomerulus.

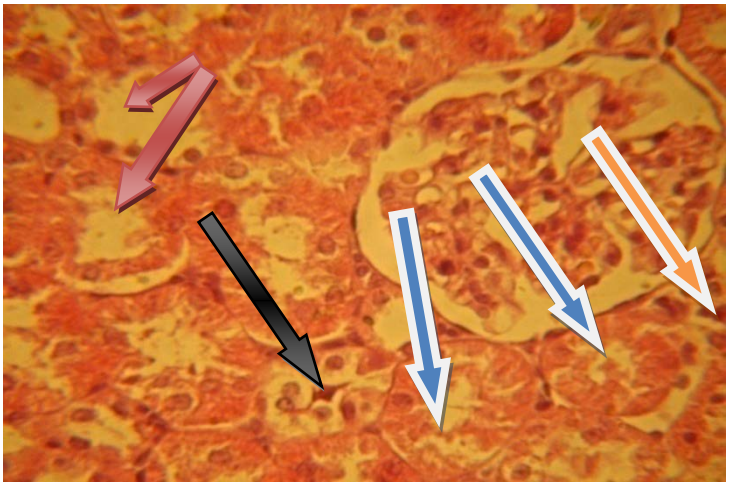

Figure 6: Cross section of morphological alteration of rat's kidney treated by a single dose of DDT (100mg.kg^{-1}). (H and E), 400X.

Blue arrows: Cloudy swelling; **Black arrow:** Narrowing of the lumen; **Violet arrow:** Necrosis.

Orange arrow: Inflammatory cell infiltration**.**

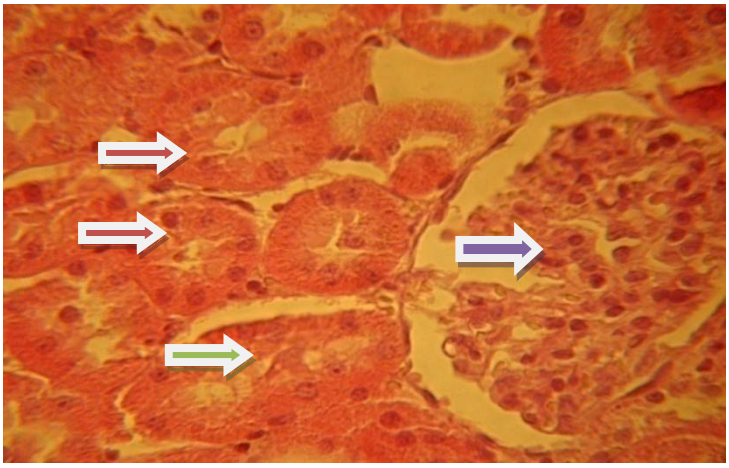

Figure 7: Cross section of rat's kidney treated with 500mg.kg^{-1} twice daily silymarin for 7-days prior to DDT administration. (H and E), 400X

Red arrow: Normal proximal convoluted tubules, **Green arrow:** Normal distal convoluted tubules, **Violet arrow:** Normal glomerulus.