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RESEARCH ARTICLE

The Possible Protective Effects of Candesartan cilexetil Against Methoteraxte Induced Nephrototoxicity In Rabbits

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Abstract

This study was designed to evaluate the possible protective effects of candesartan cilexetil against methoteraxte induced nephrotoxicity in rabbits . The animal groups were divided into a control group received tap water these animals were anesthetized by ether and then will be sacrificed on day 10. Methoteraxte (MTX) group (Induction group) received (100 mg /kg intraperitonealy a single dose at day 4 to induced nephrotoxicity. The animals were anesthetized by ether & then will be sacrificed on day 10 ,this group served as second positive control of renal damage . Candesartan + Methoteraxte (MTX) group (pre & post-group) in which rabbits treated with Candesartan10 mg /kg orally for 10 days & MTX 100 mg/kg a single dose intraperitonealy at day 4 , the animals were anesthetized by ether and then will be sacrificed on day 10. then estimation of serum levels of urea , creatinine ,albumin and total serum protein (TSP) . Also the kidney tissue homogenate was prepared to evaluate tissue levels of malondialdehyde (MDA) and glutathione (GSH) . Finally, kidney tissue sections were prepared by using paraffin sections technique and stained with hematoxylin and eiosin for histological evaluation .

Introduction

Cancer chemotherapy remains an intriguing area of pharmacology. On one hand, the use of anticancer drugs produces high rates of cure of the disease in which without chemotherapy, result in extremely high mortality rates. On the other hand, some types of cancer are barely affected by currently available drugs[1]. Methotrexate (MTX) is an anti-metabolite anti-folate chemotherapeutic agent that introduced for therapeutic application in the1950s , It is commonly prescribed for various cancers and autoimmune diseases that is widely used as a cytotoxic chemo-therapeutic agent for treatment of leukemia and other malignancies and as anti-inflammatory and immunosupressive agent in non-neoplastic diseases such as psoriasis, arthritis and SLE [2]. In the liver the conversion of MTX to its major extracellular metabolite (7-hydroxy methotrexate) occurred where it is oxidized by a soluble enzymatic system, the exact mechanism of methotrexate by which it induces nephrotoxicity remains obscure , several mechanisms for its nephrotoxicity were caused by firstly, the drug or its metabolite (7-hydroxy methotrexate) may precipitate within the tubular lumen in an acidic environment which may interfere with the tubular cell function causing intra tubular obstruction and further decline in glomerular filtration rate (GFR) [3]. Secondly; methotrexate may be toxic to renal tissues as it induces cell swelling and cell death in renal tubular cells in time-dependent manner ; thus indicating that the Na^+/H^+ ant porter and possibly other volume regulatory factors in renal tubular cells are involved in methotrexate induced renal failure [4]. Finally; the drug increases the activities of purine catabolizing enzymes xanthine oxidase(XO) and adenosine deaminase (ADA) where the former enzyme catalyzes the conversion of hypoxanthine to uric acid and the later catalyzes the conversion of adenosine to inosine and deoxyadenosine to deoxyinosine following dephosphorylation and seubsequent generation of superoxide anion

(O_2^-) and indirectly hydroxyl radical formation causes oxidative stress and lipid peroxidation in renal tissue [5,6]. Candesartan Cilexetil (CC) is an orally active and selective angiotensin II (Ang II) type one receptor blocker and it is widely used for the treatment of hypertension and heart failure [7]. Ang II is the main effector molecule of the rennin-angiotensin system (RAS) in which its binding to the AT1 receptor leads to activation of several classic second messenger systems that induce the following actions (Pro-oxidative effects of Ang II and Aldosterone, Proinflammatory and Profibrogenic actions, Ang II induce vasoconstrictor effect, Stimulation of cell proliferation) [8] .

Aim of study:

The present study aimed to investigate and evaluate the possible protective effects of candesartan cilexetil against methoteraxte induced nephrotoxicity in rabbits .

Materials and Methods

Thirty domestic rabbits of both sexes weighing 1.5-2 kg were used in this study will be randomly divided into five groups each of six animals . The animals were fed commercial pellets and tap water . Groups of animals utilized in this study were kept in separate cages .The experimental protocol for this study are :

Group-I : (control group) apparent healthy rabbits were received tap water and the animals were anesthetized by ether and then were sacrificed on day 10.

Group-II : (Induction group) rabbits received at day 4 a single parenteral dose of methotrexate (MTX100mg/kg i.p) in order to induce hepatotoxicity . The animals were anesthetized by ether and then were sacrificed on day 10 . This group served as positive control of liver .

Group-III : (treatment group) in which rabbits received a single daily dose of candesartan10 mg/kg orally for 10 days and received at day 4 a single parenteral dose of methotrexate (MTX 100 mg/kg i.p) . The animals were anesthetized by ether and then were sacrificed on day 10.

Before the animals have been anesthetized by ether, the blood is collected by intracardiac puncture then centrifuged at 3000 rpm for 15 minute the supernatant was used for the estimation of serum levels of urea , creatinine, albumin and total serum protein (TSP) . Also kidney tissue homogenate will be prepared to evaluate tissue levels of malondialdehyde (MDA) and glutathione (GSH) . Finally, kidney tissue sections were prepared according to the method of Junqueira et al in 1995 by using paraffin sections technique and stained with hematoxylin and eiosin for histological evaluation [9]. The slides were coded and semi-quantitative analysis of the kidney sections was performed without knowledge of the treatment protocol . The changes seen were limited to the tubule interstitial areas and graded [10], as follows:[(0) : normal , (1) : mild (include areas of tubular epithelial cell swelling ,cellular vacuolization, glomerular congestion, necrosis, hyaline cast deposition and desquamation involving (25%) of cortical tubules), (2) : moderate (similar changes involving (25%) but less than (50%) of cortical tubules), (3) : sever (similar changes involving (50%) but less than (75%) of cortical tubules), (4) : very sever (similar changes involving (75% and more) of cortical tubules).

Statistical Analysis:

Statistical analysis was performed with the SPSS 20 statistical package for social sciences and Excel 2013. Descriptive statistics for the numerical data were formulated as mean and standard error mean(Mean ± SEM) . Numerical data were analyzed using independent Student's (t-test) for comparison between two groups. Mann-Whitney U test for measuring of histopathological changes scoring . The difference was considered significant when p value was equal to or below 0.05 [11] .

Results

Data of all groups are expressed as (Mean±SEM) , in group-II(GP-II) a single parenteral dose of methotrexate (100mg/kg) administered to rabbits produced a significant elevation in the serum levels of urea and creatinine [(40.4±2.38),(0.89±0.07)] with a significant reduction in the serum levels of total protein (TP) and albumin [(4.38±0.13), (1.88±0.19)] respectively compared to control group (GP-I) at ($p<0.05$) . Also produced a significant increment in the levels of lipid peroxidation end product (MDA) (57.66±4.02) and a significant reduction in (GSH) levels (2.06±0.31) in renal tissue homogenate compared to control group (GP-I) at ($p<0.05$). These results are showed in table (1) and figures (1,2,3,4,5,6) . While in group-III(GP-III) treatment of rabbits with oral dose of candesartan cilexetil (10mg/kg/day) for 10 days prior to and during parenteral administered methotrexate

(100mg/kg) produced a significant reduction in the serum levels of urea and creatinine [(28.15±1.81),(0.68±0.05)] with a significant elevation in the serum levels total protein (TP) and albumin [(5.04±0.12),(2.51±0.07)] respectively compared to that observed in group-II(GP-II) at ($p<0.05$) , but all results showed anon significant difference compared to control group (GP-I) at ($p>0.05$) . Also produced a significant reduction in (MDA) levels (26.25±3.88) and a significant elevation in (GSH) levels (6.72±0.61) in renal tissue homogenate compared to that observed in group-II(GP-II) at ($p<0.05$) . The value showed a non significant difference compared to control group (GP-I) ($p>0.05$) These results are showed in table (1) and figures (1,2,3,4,5,6)

GROUP	Mean Urea Level mg/dl	Mean Creatinine Level mg/dl	Mean TSP Level gm/dl	Mean Albumin Level gm/dl	Mean MDA Kidney Level Mmol /g. tissue	Mean GSH Kidney Level Mmol /g. tissue
CONTROL (N= 6)	25.56 ± 1.96	0.61 ± 0.03	5.11 ± 0.11	2.72 ± 0.15	24.63 ± 3.23	8.53 ± 0.99
MTX 100mg/kg (N= 6)	40.4 ± 2.38 a	0.89 ± 0.07 a	4.38 ± 0.13 a	1.88 ± 0.19 a	57.66 ± 4.02 a	2.06 ± 0.31 a
MTX 100mg/kg + CAND.10mg/kg (N= 6)	28.15 ± 1.81 NS , c	0.68 ± 0.05 NS , c	5.04 ± 0.12 NS , c	2.51 ± 0.07 NS , c	26.25 ± 3.88 NS , c	6.72 ± 0.61 NS , c

Table (1) : the Effects of candesartan treatment on the serum levels of urea , creatinine, albumin and total serum protein (TSP) , MDA levels, GSH levels in rabbits' kidney homogenate compared to control and MTX-treated groups . Data are presented as Mean ± SD. , N = number of animals. , a : $p < 0.05$ significant in compare to control group , c : Significant to MTX 100 mg/kg , N.S. : Non- Significant to control group

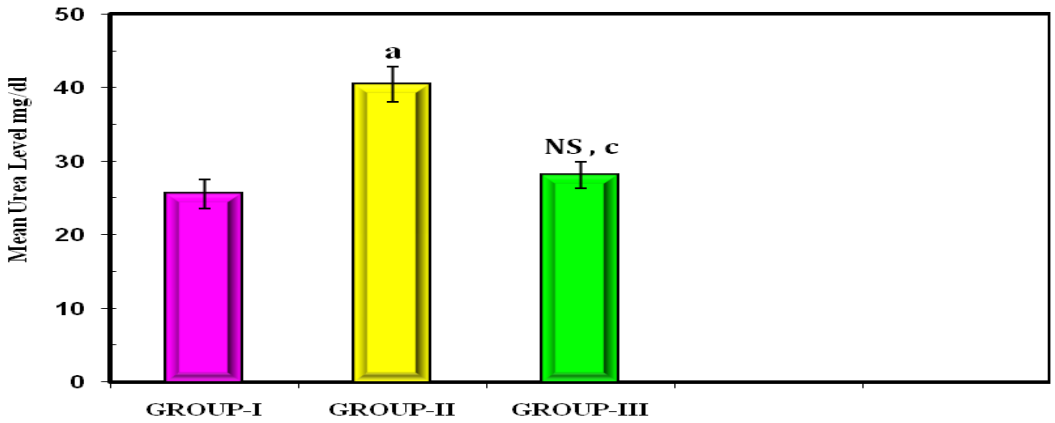


Figure (1) : Effects of candesartan treatment on the serum level of urea compared to control and methotrexate treated groups . Group-I : control group , Group-II : MTX 100mg/kg , Group-III : (MTX 100 mg/kg + CAND. 10mg/kg) , a : $p < 0.05$ significant in compare to control group , c : Significant to MTX 100 mg/kg , NS : Non Significant to control group

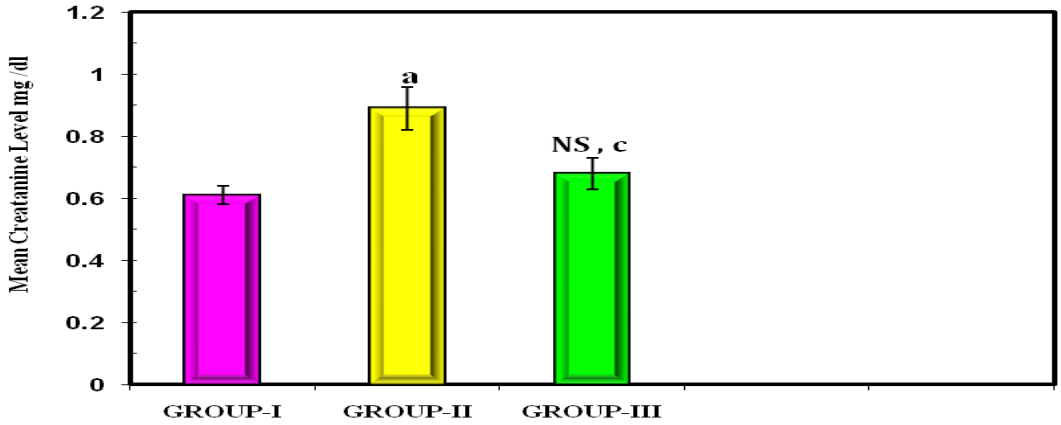


Figure (2) : Effects of candesartan treatment on the serum level of creatinine compared to control and methotrexate treated groups . Group-I : control group , Group-II : MTX 100mg/kg , Group-III : (MTX 100 mg/kg + CAND. 10mg/kg) , a : p < 0.05 significant in compare to control group , c : Significant to MTX 100 mg/kg , NS : Non Significant to control group

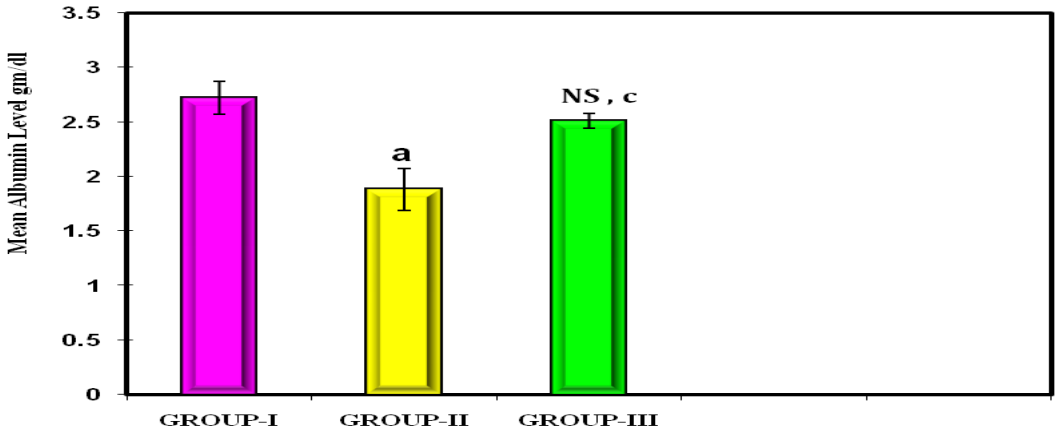


Figure (3) : Effects of candesartan treatment on the serum level of albumin compared to control and methotrexate treated groups . Group-I : control group , Group-II : MTX 100mg/kg , Group-III : (MTX 100 mg/kg + CAND. 10mg/kg) , a : p < 0.05 significant in compare to control group , c : Significant to MTX 100 mg/kg , NS : Non Significant to control group

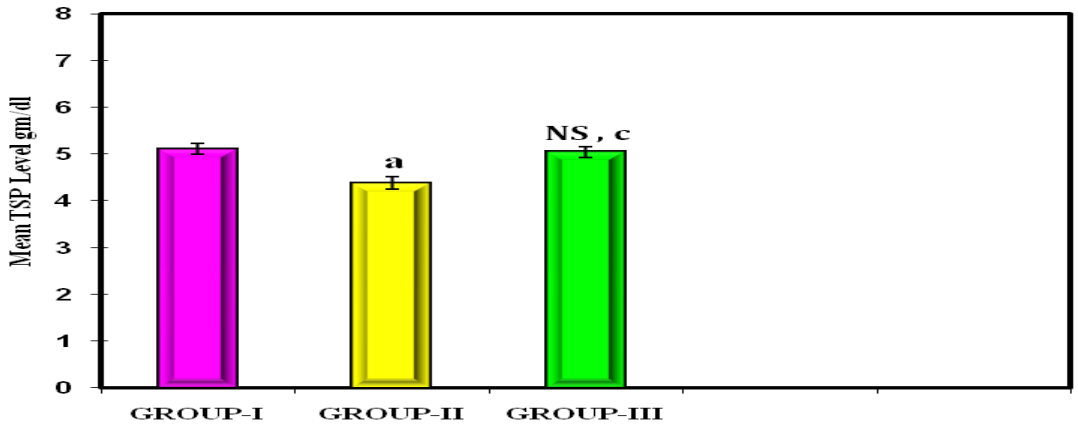


Figure (4) : Effects of candesartan treatment on the level of total serum protein (TSP) compared to control and methotrexate treated groups . Group-I : control group , Group-II : MTX 100mg/kg , Group-III : (MTX

100 mg/kg + CAND. 10mg/kg) , a : p < 0.05 significant in compare to control group , c : Significant to MTX 100 mg/kg , NS : Non Significant to control group

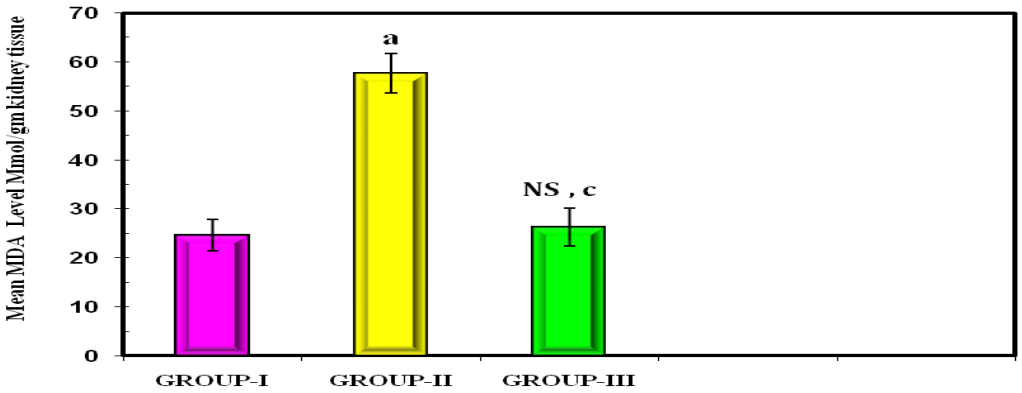


Figure (5) : Effects of candesartan treatment on the MDA levels in rabbits' kidney homogenate compared to control and methotrexate treated groups . Group-I : control group , Group-II : MTX 100mg/kg , Group-III : (MTX 100 mg/kg + CAND. 10mg/kg) , a : p < 0.05 significant in compare to control group , c : Significant to MTX 100 mg/kg , NS : Non Significant to control group

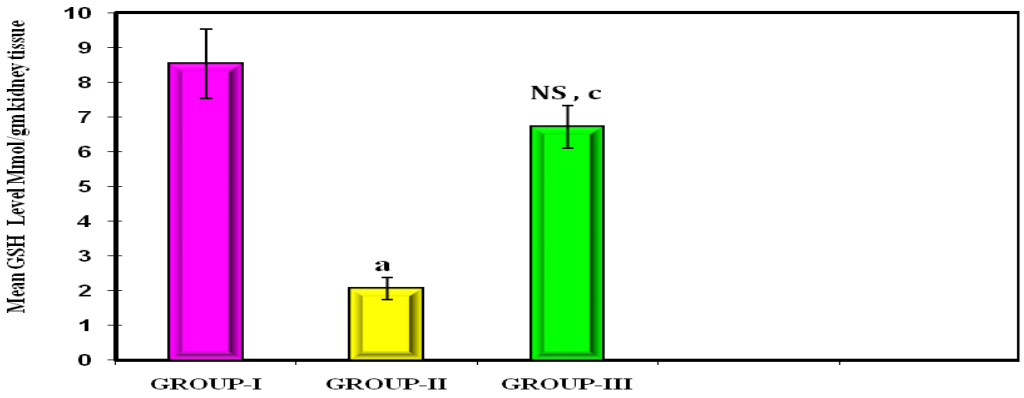


Figure (6) : Effects of candesartan treatment on the GSH levels in rabbits' kidney homogenate compared to control and methotrexate treated groups . Group-I : control group , Group-II : MTX 100mg/kg , Group-III : (MTX 100 mg/kg + CAND. 10mg/kg) , a : p < 0.05 significant in compare to control group , c : Significant to MTX 100 mg/kg , NS : Non Significant to control group

The histopathology changes of in group-II for rabbits treated with a single parenteral dose of MTX (100mg/kg) showed sever to a very sever hydropic degeneration of tubules with loss of central lumen and vascular congestion of glomeruli with sever tubular vacuolization and desquamation [score number (3,4)] , these histopathological changes showed in (Figure-2) and they are significantly different in comparison with control group (GP-I) at ($p<0.05$) ,(Figure-1).

In last group-III (GP-III) the mean of score level for rabbits received oral dose of candesartan cilexetil (10mg/kg/day) for 10 days prior to and during parenteral administered methotrexate (100mg/kg) showed a mild score level which evaluated as mild hydropic degeneration of tubules with mild glomerular vascular congestion [score number (1)] and it is significantly differ in compared to (GP-II) at ($p<0.05$) and a non significantly different as compared to control group (GP-I) , these histopathological changes are shown in Figures (1,2,3) .

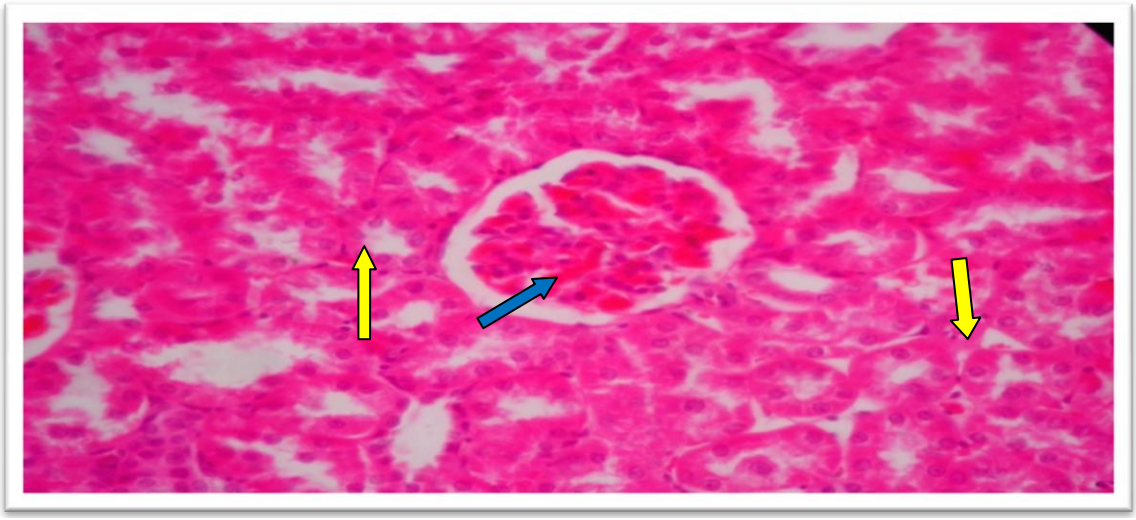


Figure (1) : Section of kidney of group-I (control group) shows normal glomerulus (blue arrow) surrounded by normal proximal tubules (yellow arrows) -score number (0) . H&E (40X).

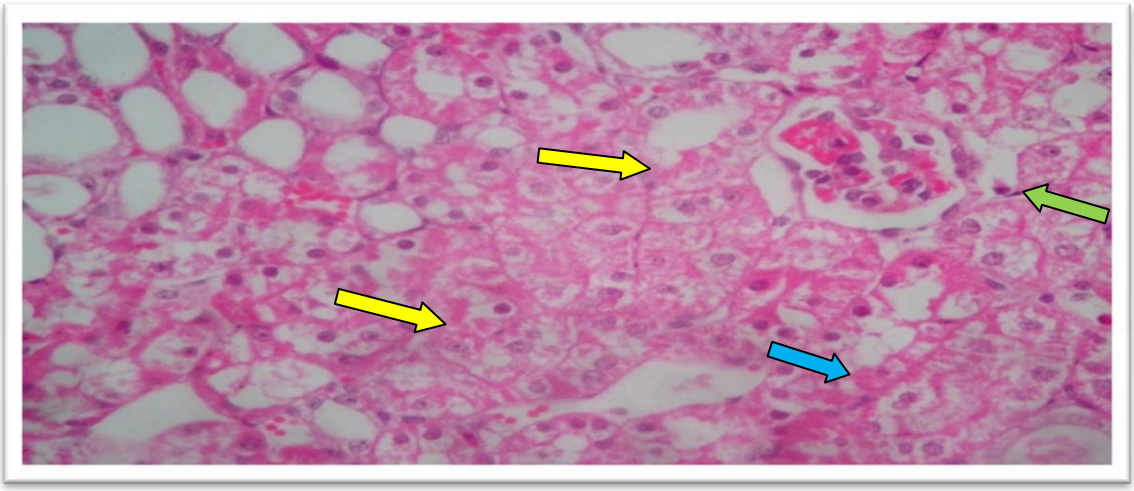
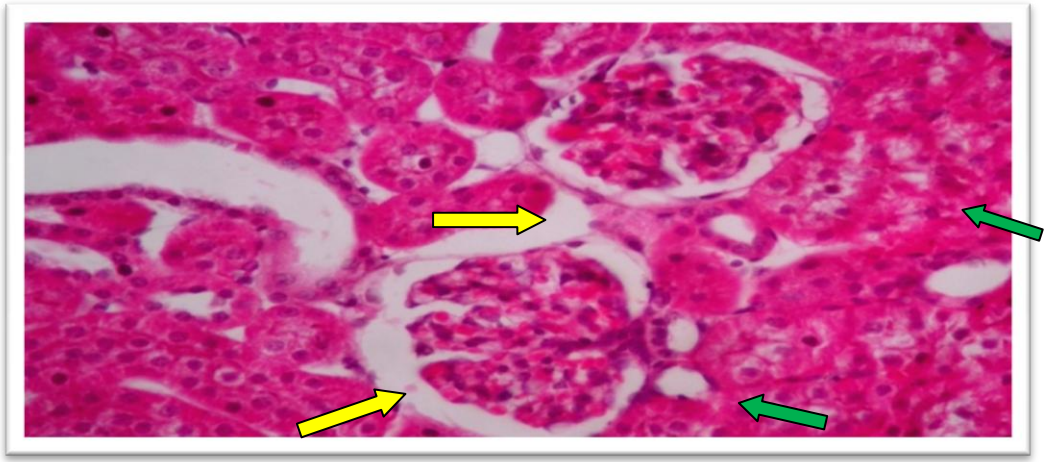


Figure (2) : Section of kidney of group-II (MTX 100 mg/kg) shows sever hydropic degeneration of tubules with loss of central lumen (yellow arrow) and vascular congestion of g



lomeruli (green arrow) with sever tubular vacuolization and desquamation (blue arrow) - score number (3,4) . H&E (40X) .

Figure (3) : Section of kidney of group-III (Candesartan10mg/kg/day for 10days with Methotrexate100mg/kg) shows mild hydropic degeneration of tubules (yallow arrow) with mild glomerular vascular congestion (green arrow) score number (1) . H&E (40X) .

Discussion

Antitumor drugs are being increasingly utilized as adjuvant therapy for patients at high risk for recurrent disease [12]. Further, drugs used for cancer chemotherapy are well known to produce side effects in multiple organ systems the most common target organs are tissues that contain self renewing cell populations such as gastrointestinal tract, bone marrow, kidney, mucosal membranes, and hair follicles [13].

Methotrexate a folic acid antagonist is widely used anti-metabolite cancer chemotherapy or in many rheumatologic, dermatologic, and hematologic diseases successfully. It is known to have the major toxic effects due to oxidative reactions that take place during its metabolism in the liver [14,15] . There is substantial evidence for a role of reactive oxygen metabolites in mediating nephrotoxicity of some xenobiotics and the pathogenesis of organ failure [16]. A previous study showed that oxygen free radicals and hydrogen peroxides are linked with the development of several pathological processes associated with chemotherapy [17]. The severity of methotrexate associated renal injury is related to both the dose and duration of the treatment [18] . In the present study, administration of a high-dose of methotrexate may also results in acute renal damage possibly due to precipitation of MTX and/or 7-OH-MTX in the renal tubules that leads to delayed MTX elimination which consequently leads to toxicities of different tissues including myelosuppression, gastrointestinal toxicity and mucositis [19,20] . At this dose of methotrexate (100mg/kg i.p.) in group-II (GP-II) the changes that occurred in the renal tissue were produced a marked significant elevation in the serum levels of urea and creatinine respectively compared to control group (GP-II) ($P<0.05$) , these results reflecting impaired renal function which indicate that the reduction in renal filtration rate can be due to toxic effect of methotrexate on kidney that is supported by histopathological observation on kidney tissue revealing tubular degeneration and necrosis in MTX treatment group (GP-II) [21].

Also this dose of methotrexate in (GP-II) provoked a significant reduction in the serum levels of total protein and albumin respectively compared to control group (GP-I) ($P<0.05$) and this reduction in serum total protein and serum albumin induced by this dose of methotrexate (100mg/kg) could be due to several factors like damage to liver, increased intestinal protein loss, protein losing nephropathy which in turn is associated with cell death, damage or necrosis of renal tubular cells and dietary protein deficiency as there was decrease in feed intake these results related with previous studies [22,23].

Furthermore, the oxidative tissue damage in the kidney of rabbits that received a single (i.p.) dose of methotrexate 100mg/kg in group-II also has been altered the oxidant/antioxidant balance that produced a significant elevation in levels of lipid peroxidation end product (MDA) in renal tissue homogenate compared to control group (GP-I) . The mechanism of methotrexate induce nephrotoxicity is not fully understood and damage to glomeruli and tubules occurred because of precipitation of methotrexate and/or 7-hydroxy-metabolite in the renal tubules that induced free radicals production causing oxidative stress on the kidney tissue , so that administration of antioxidant as adjuvant therapy may be promising in alleviating the renal side effect of methotrexate [24]. Other explanation included methotrexate bind to dihydrofolate reductase with greater affinity than folic acid that limits the conversion of folic acid to tetrahydrofolate, a molecule necessary for the synthesis of DNA[25] that cause inhibition in the synthesis of purine and pyrimidine thymidilate results in improper DNA synthesis and subsequent apoptosis and cell death [26] .

It was reported that under normal condition glutathione (GSH) could have a role in maintaining activity of the pentose phosphate cycle at a level which is appropriate for the severity of the oxidative challenge, as well as for the capacity of the cellular antioxidant defenses [27], which in its reduced form (reduced GSH) is necessary for the detoxification of xenobiotics . So that ; decline in the constitutive GSH levels and capacity for GSH synthesis adversely affects cellular thiol redox balance and potentially sensitize the cells and made them susceptible to a number of internal and environmental stresses [28] . As a result at this dose (MTX 100mg/kg) in group-II produced a significant depletion in the levels of GSH in the kidney. The reduction in GSH levels promoted by MTX represents an alteration in the cellular redox state in which under normal conditions NADPH is used by glutathione reductase to maintain the reduced state of cellular glutathione an important cytosolic antioxidant ; previous study demonstrated that the cytosolic nicotinamide adenosine diphosphate (NADP) dependent dehydrogenases and NADP malic enzyme are inhibited by MTX suggesting that the drug could decrease the availability of NADPH (nicotinamide adenosine

diphosphate hydrogen) in cells that lead to inhibition of glutathione reductase activity and finally an inhibition of GSH cycle [29], and this lead to the cells could be more sensitive to reactive oxygen metabolites and leads to a reduction of effectiveness of the antioxidant enzyme defense system [30,31].

In the present study administration of oral candesartan cilexetil (10mg/kg/day) for 10 days prior to and during parenteral administered methotrexate (100mg/kg) to the rabbits in group-III provoked a significant reduction in serum levels of urea, creatinine which reflecting improvement in the renal function and produced a significant elevation in the serum levels of total protein and albumin, this elevation could be explained by the antiproteinuric effect of candesartan that cause blockade of angiotensin receptor and inhibition of Ang-II that lead to decrease efferent arteriolar tone and consequently glomerular capillary pressure which in turn results in a reduction in the glomerular lesions that leading to proteinuria [32] . This antiproteinuric effect of candesartan also seems linked to an inhibition of the direct and indirect trophic effects of intrarenal angiotensin II [33] as well as to a modulation of the glomerular size barrier function [34] .

In the same group (GP-III) the effect of candesartan in renal tissues provoked a significant reduction in the levels of lipid peroxidation end product (MDA) and a significant elevation in the levels of glutathione (GSH) in compared with methotrexate treated animals (MTX100 mg/kg) in (GP-II) .

These events are briefly discussed in which the antioxidant and free radical scavenging activity of candesartan may reduce the toxic effects of MTX. The antioxidant and anti-inflammatory effect of candesartan was attributed to blockade of angiotensin receptor and inhibition of Ang-II induced generation of ROS via activation of NADPH-oxidase which is a major source of ROS and oxidative stress in different tissues that mediates tissue damage [35,36] . Other explanation include candesartan attenuated tissue damage by suppressing transforming growth factor-b1(TGF-β1) mRNA and decreasing production of extracellular matrix proteins , Similar antioxidant effects of candesartan were reported by previous study [37]. But here the nephroprotective actions of candesartan seem to relate to the inhibition of the renin-angiotensin-aldosterone system (RAAS) [38]. In particular, angiotensin II and aldosterone in the RAAS system generate active oxygen and oxidative stress in renal tissue there by promoting interstitial fibrosis and tubular damage as well as vascular endothelial disorders [39]. Controlling renal dysfunction factors is thought to be attributable to ARB actions [40]. These findings demonstrated the cytoprotective and anti-lipid oxidation activities of candesartan that suggested it acts as a free radical scavenger and thus attenuates the cytotoxic and genotoxic effect of MTX and protects cellular DNA from oxidative damage, similar antioxidant effects of candesartan were reported by previous study [41] .

Finally, the biochemical changes induced by MTX treatment were confirmed histopathologically [42]. The histopathological changes in rabbits received a single (i.p.) of MTX (100mg/kg) in group II showed sever hydropic degeneration of tubules with loss of central lumen and vascular congestion of glomeruli with sever tubular vacuolization and desquamation . These histopathological findings in kidneys are in agreement with the biochemical findings like increased levels of serum urea, creatinine due to decrease in glomerular filtration rate of methotrexate and its metabolites from kidneys which may lead to kidney lesions because of irritation caused by deposition of methotrexate crystal in nephrons causing the nephrotoxicity [43].

While rabbits treated with oral candesartan cilexetil (10mg/kg/day) for 10 days prior to and during (i.p.) administration of methotrexate (MTX100mg/kg) in group-III showed mild hydropic degeneration of tubules with mild glomerular vascular congestion. These histopathological findings in kidneys are in agreement with the biochemical findings like decreased the serum levels of urea, creatinine and increase the serum levels of total protein and albumin , these changes occurred firstly due to candesartan cause inhibition of the renin-angiotensin-aldosterone system (RAAS) [38] . In particular, angiotensin II and aldosterone in the (RAAS) system generate active oxygen and oxidative stress in renal tissue there by promoting interstitial fibrosis and tubular damage as well as vascular endothelial disorders [39]. Controlling renal dysfunction factors is thought to be attributable to ARB actions [40] .

Conclusion:

The results of this study suggest that exogenously administered candesartan cilexetil is capable of minimizing and reversing the oxidative toxic effects of methotrexate in the kidney in the dose of (100mg/kg) and protect them from further side effect ; these data suggest that candesartan may enhance the selectivity of antitumor drugs in the patients who require high doses of methotrexate.

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