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Inhibition of NF-κB Pathway by Guggulsterone in the Protective Effects of Cyclophosphamide-Induced Renal Toxicity

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Abstract

Cyclophosphamide which acts as cytotoxic alkylating agent can induce a renal damage through the toxic metabolites which result from metabolic activation of Cyclophosphamide by cytochrome P-450 inside hepatocyte and develop renal toxicity by direct binding with cellular organelles in the urinary tract cells. Guggulsterone is a sterol derived from plant has ability to bind to farsenoid X receptor, mineral corticosteroid receptor, androgen receptor, glucocorticoid receptor and estrogen receptor. It efficiently decreases the pro-inflammatory response by downregulate some of genes that implicate in production and regulation of interleukins (IL-2, IL-4, IL-6 and TNF-α). In this study, the albino rats were divided to four groups: control group treated with vehicle, cyclophosphamide group, cyclophosphamide with guggulsterone group and guggulsterone alone group. Guggulsterone doses were administered orally (25 mg/kg/day) for 8 consecutive days while the cyclophosphamide (150 mg/kg) was administered intraperitoneal at day 5 of experiment. At the end of experiment, the kidney index and the serum level of tumor necrosis factor (TNF-α), urea and creatinine were determined, also the NF-κB P65 and catalase tissue activities were measured with histopathological assessment. The daily dose of guggulsterone produced a significant reduction in the serum level of TNF-a, urea, creatinine, and tissue activities of NF-κB, and catalase enzyme. The histological feature was also improved. These data represent the protective effect of guggulsterone against the cyclophosphamide renal toxicity.

Key words: Guggulsterone, NF-κB, Cyclophosphamide, Renal toxicity.

تثبيط الـ NF-κB بواسطة الكوكيلستيرون والتاثير الوقائي للسمية الكلوية للسيكلوفوسفاميد

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الخلاصة

سيكلوفوسفاميد ، عامل ألكيل نشط سامة للخلايا ، له سمية كلوية بسبب الأيضات السامة الناتجة عن تنشيط السيكلوفوسفاميد بواسطة السيتوكروم ميكروسوم P-450 في الكبد ومن ثم إفراز الكلى لهذه الأيضات السامة عن طريق الربط المباشر لعامل ألكيل مثل الأكورولين في المسالك البولية. غوغولستيرون هو ستيرول نباتي لديه القدرة على الربط بمستقبلات الفارسويد X ومستقبلات الكورتيكوستيرويد المعدنية ومستقبلات الأندروجين ومستقبلات الجلوكوكورتيكويد ومستقبلات الإستروجين. انه قلل بشكل فعال من الاستجابة المؤدية للالتهابات عن طريق الحد من مجموعة متنوعة من الجينات التي تنطوي على تنظيم إنتاج الانترلوكينات مثل (IL-2, IL-4، IL-6 و TNF-α). أن في هذه الدراسة تم تقسيم الفئران البيضاء إلى أربع مجموعات: المجموعة الضابطة التي عولجت بمركبة ، ومجموعة سيكلوفوسفاميد ، وسيكلوفوسفاميد مع مجموعة غوغلسترون ومجموعة غوغلسترون وحدها. سلعت جرعات غوغلسترون عن طريق الفم (٢٥ مغ / كغ / يوم) لمدة ٨ أيام متتالية بينما كان سيكلوفوسفاميد (١٥٠ مغ / كغ) يزرق داخل الصفاق في اليوم ٥ من التجربة. في نهاية التجربة ، تم تحديد مؤشر الكلى ومستوى المصل لعامل نخر الورم (TNF-α) ، واليوريا والكرياتينين ، كما تم قياس أنشطة NF-κB P65 والكاتلاز في النسيج الكلوي مع تقييم التشريح المرضي. تنتج الجرعة اليومية من غوغلسترون انخفاضًا كبيرًا في مستوى مصل TNF-α ، واليوريا ، والكرياتينين ، وأنشطة ال NF-κB ، وأنزيم الكاتلاز في النسيج الكلوي. كذلك تم تحسين الميزة النسيجية أيضًا. تمثل هذه البيانات التأثير الوقائي للغلغلسترون ضد سمية السيكلوفوسفاميد في الكلى.

الكلمات المفتاحية: الكوكيلستيرون، NF-κB ، للسيكلوفوسفاميد ، سمية كلوية .

Introduction

Cyclophosphamide, a strong cytotoxic alkylating agent, is unique member of Nitrogen mustards and broadly used to treat a variety of malignant and nonmalignant conditions. It is somewhat inactive in vitro and transformed to the active form in vivo[1]. The drawback of medical usages of Cyclophosphamide owing to non-specific cytotoxicity which produce several adverse effects in multi-organs primarily in bone marrow, liver, testes, heart, kidney, and urinary bladder (1). The main mechanism of Cyp induce renal injury is the formation of reactive toxic metabolites subsequent to metabolism of parent drug by hepatic cytochrome P-450 which followed by the renal excretion of these toxic metabolites that act as an alkylating agent (e.g. acrolein) with bind to several cellular organelles of the urinary tract. The acrolein has high reactivity to react with numerous target sites in cells of kidney and liberation of free radical which result in diminution of cellular thiols or stimulation of NF-κB(2).

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Cyclophosphamide administration can prompt tubular fibrosis, tubular necrosis, glomerular nephritis, interstitial edema, cortical tubular vacuolization, mild hemorrhagic cortex and then renal dysfunction. Similarly, it has been demonstrated that administration of Cyp result in an acute inflammation in the urinary bladder [3]. It has also been identified as a product and also an initiator of lipid peroxidation[2]. Guggul resin take out from guggul tree named (*Commifora mukul*) of family Burseraceae. It has been used in Ayurvedic remedy for many years[4]. The key active constituent of guggul is guggulsterone (GS) which is a plant sterol with two isomers, E- and Z-guggulsterone, that accountable for hypolipidemic action[5]. The two isomers of plant sterol has capability to bind and activate several nuclear receptors such as FXR, androgen receptor, mineral corticosteroid receptor, estrogen receptor, and glucocorticoid receptor .GS efficiently reduce the pro-inflammatory response by downregulation of variety of genes that involve in regulation and production of IL-2, IL-4, IL-6 and TNF-alpha [6–8]. Also, the suppression of NF-κB and NF-κB regulated gene may define the effects of GS against atherosclerosis, diabetes, osteoarthritis, psoriasis and other inflammatory diseases [9, 10]. This study was designed to evaluate the protective effect of GS against renal toxicity of Cyclophosphamide.

Material and Method

Drug and chemical

Cyclophosphamide was purchased from Baxter Oncology (Frankfurt, Germany). Guggulsterone was purchased from Xi'an geekee biotech, China. Rat NF-kB/p65 ELISA kit and TNF-alpha (ELISA) kit were purchased from Elabscience biotechnology Co. Ltd. Creatinine kit and Urea kit for rats were purchased from Biolabo SAS, Les Hautes Rives, Maizy, France. Measurement of catalase performed by manual method affording by M. H. Hadwan and H.N. Abed. (2016)[11]

Animal

In a randomized, controlled animal study plan, 32 Wistar albino adult male rats weighing (150-250) were obtained from the animal house of department of pharmacology and toxicology /collage of pharmacy of Baghdad University. All animals were reserved in a area controlled for temperature at 25 ± 2, humidity 50-70 % and 12h lighting cycle followed a possible hygienic condition and permitted to eat and drink freely throughout the experiments.

After 2 weeks of acclimatization, animals were weighed and their initial weights were recorded. The rats were divided equally to four groups, group I (control), group II (cyclophosphamide-treated group Cyp) where the rats treated with an oral dose of the blank suspension for 8 consecutive days, at day 5, a single dose of 150 mg/kg of cyclophosphamide injected intraperitoneal (I.P) and group III (cyclophosphamide with guggulsterone Cyp+ GS group) the rats were treated with an oral dose of 25 mg/kg/day of guggulsterone suspension for 8 consecutive days, at day 5, a single dose of 150 mg/kg of cyclophosphamide injected intraperitoneal (I.P), and group IV (guggulsterone GS) where the animal were treated with an oral dose of 25 mg/kg/day of guggulsterone suspension for 8 consecutive days. All the animals were euthanized at day nine after record the body weight.

Determination of organ index

In the start of experiment, altogether Wister albino rats have been weighed and when rats have been euthanized by cervical decapitation. Kidney were collected and kidney index being measured by dividing kidney weight by entire body weight [12].

Biochemical analysis

At the end of experiment, the animals were anesthetized with ethyl ether and the blood samples were collected from the heart puncture and allow all blood samples to clot for 1 hour at room temperature and the serum were separated by centrifugation for 15 minute on 10000 g speediness in a cold centrifuge. All samples are kept at -20°C for subsequent measuring the serum level of tumor necrosis factor (TNF-α), urea and creatinine.

Measuring the level of NF-κB P65 and catalase activity in tissue homogenate:

A 10% of kidney tissue homogenate was prepared and utilized to determine the concentration of NF-κB P65 by ELISA kits by following to Sandwich-ELISA principle. The concentration of Rat NF-κB in the samples were calculated by comparing the OD of the samples to the standard curve[13]. Catalase activity was measured by incubating the enzyme sample in 1.0 ml substrate (65 mmol/ml hydrogen peroxide in 60 mmol/l sodium–potassium phosphate buffer, pH 7.4) at 37 °C for three minutes. The reaction was terminated with ammonium molybdate. Absorbance of the yellow complex of molybdate and hydrogen peroxide is measured at 374 nm against the blank.

Histological assessment

Tissues sample from the kidney was collected and fixed in 10% buffer formalin for histological examination by staining with hematoxylin and eosin (H&E) and investigated under light microscope (using 40X magnification).

Statistical analysis

All results are presented as mean ± SD. The P-value < 0.05 are considered a significant for all data. The unpaired student's t-test was obtained to determine significance of differences between the mean values of each group [14].

Results

As presented in table 1, the nephrotoxic effect of cyclophosphamide represent a significant elevation in renal level of NF-κB in comparison to control group. Also, this effect produced a significant reduction in antioxidant activity of

catalase enzyme in the kidney tissue. These toxic effects of cyclophosphamide were reduced significantly by the oral doses of guggulsterone by decreasing the renal level of NF-κB and increases in the action of catalase in the renal tissue. As well as, the serum level of urea and creatinine were significantly elevated in the Cyp group compared to control group. However, the administration of the oral guggulsterone with Cyp (Cyp+GS) significantly reduce the urea and creatinine levels ($P<0.05$) compared to Cyp treatment group.

Table 1. Effect of Guggulsterone on the kidney parameters in the cyclophosphamide-treated rats.

Parameter	Group I (Control)	Group II (Cyp)	Group III (Cyp+GS)	Group IV (GS)
Kidney NFκB level (ng/gm)	6.76 ± 0.65	1.36 ± 0.53 *	8.66 ± 1.73#	6.98 ± 0.52#
Kidney Catalase activity (U/g)	2.51 ± 0.12	2.06 ± 0.24 *	2.305 ± 0.22 #	2.54 ± 0.06 #
Serum TNF-α (pg/ml)	93.25 ± 40.66	722.7 ± 186.6 *	462.38 ± 197.91 #	101.85 ± 45.97 #
Serum Urea (mg/dl)	23.60 ± 7.15	33.24 ± 7.74 *	24.40 ± 7.54 #	21.88 ± 5.79#
Serum Creatinine (mg/dl)	0.80 ± 0.27	1.797 ± 0.50 *	0.93 ± 0.69 #	0.66 ± 0.53 #
Kidney index	6.19 ± 0.44	8.06 ± 1.04*	7.05 ± 0.74#	6.35 ± 0.64#

* is significantly different compared with control group ($P<0.05$).
# is significantly different compared with Cyp group ($P<0.05$).

Table 1 shows that the kidney index in the Cyp treated animals was significantly increase compared to the control group ($P<0.05$). The administration of GS with Cyp cause a significant reduction in the kidney index ($P<0.05$) compared to Cyp treated group. All parameters were not significantly altered in the GS treated rats (group IV) compared to control group.

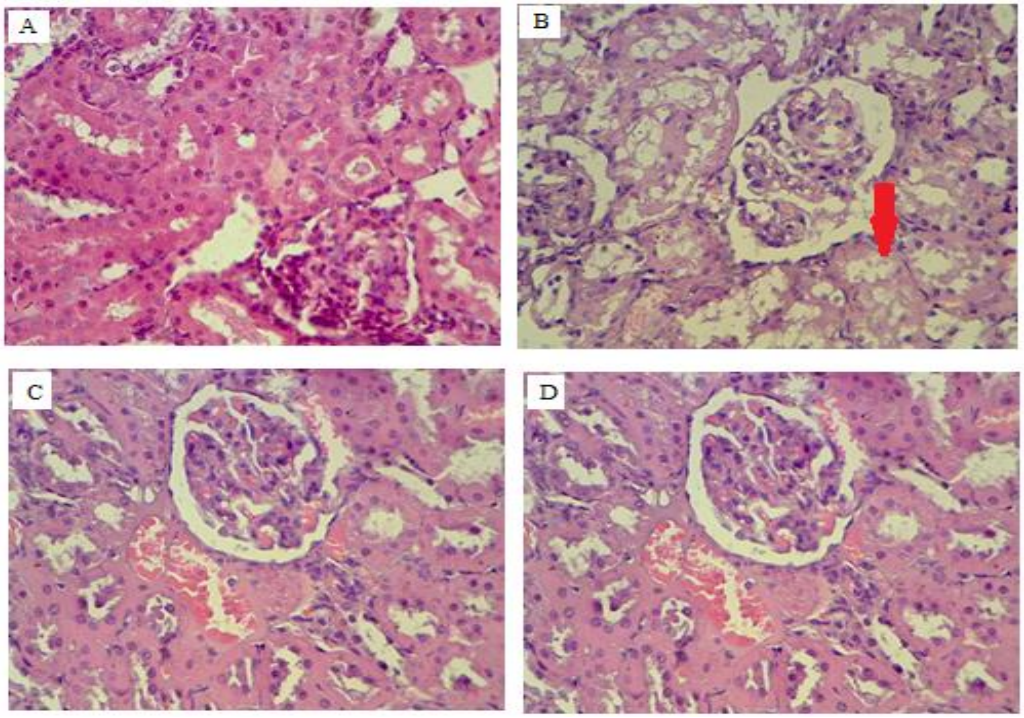

Figure 1. Influence of guggulsterone (GS) administration on patho-histological structure of kidney in Cyp-induced toxicity in rats. Photo-micrographs of hematoxylin and eosin-dyed kidney sections (400X) viewing: (A) control, (B) Cyclophosphamide group, (C) Cyclophosphamide + guggulsterone administration group and (D) guggulsterone (GS) only groups.

In figure 1A, the control group displays a normal kidney tissue with normal glomeruli and tubules. However, cyclophosphamide treatment group as in figure 1B the image was revealed a widespread degenerative damage (red arrow) and cell death of epithelium lining of renal tubules. Figure 1C, the Cyclophosphamide + guggulsterone treatment showed narrow degenerative area and

little area of cellular death in the proximal tubules cells. Whereas the GS only group (Figure 1D), showed an extensive matching view to the normal tissue of normal glomeruli and normal renal tubules but there is infrequent of degenerative region of renal tubules.

Discussion

The effect of cyclophosphamide induced significant increase in the NF-κB amount in renal tissue as compared to the control group. Cyp prompted nephrotoxicity particularly at the proximal renal tubules in the rats due to oxidative stress and production of pro inflammatory cytokines which are controlled by stimulation of transcription factor NF-κB which is accountable for the kidney damage[15,16]. The renal tubular cells also have uptake and efflux transporters that implicated in the magnification the concentrations of Cyp and /or its metabolites in the cells[17]. The administration of guggulsterone with a Cyp causes significant decreases in the activity of the transcription factors NF-κB compared to Cyp only treated group owing to GS down regulate the expression of constitutive NF-kB activity and blocked IkBα phosphorylation besides it enhanced separation of inhibitory retained protein by inhibition of IkB kinase activation, therefore reducing p65 phosphorylation and don't permit the nuclear translocation. The outcomes of this study come to an agreement with other studies[18-20]. Therefore, GS decreased inflammation by suppression NF-κB signaling and has a brilliant impact on protection against Cyp induced renal toxicity .

The administration of Cyp induce an oxidative damage and lipid peroxidation that result in a decrease in the catalase enzyme level. At high dose of cytotoxic Cyp, it generates toxic metabolites with additional amount of reactive oxygen specie s(ROS) for example super oxide, hydroxyl radical and hydrogen peroxide[21]. Intracellular oxidant/antioxidant equilibrium system was interrupted due to high level of ROS and decline of anti-oxidant enzyme activity. Oxidative stress (OS) might also cause catalase expenditure which consider antioxidant enzymes[22]. Table 1 shows that Cyp can produce a significant decrease in catalase activity compared to control group and significantly increase when GS added with Cyp. In this study, the role of GS improves the structure and function of kidney, as a minimum in part by anti-oxidant effect that is mediated by increase renal tissue catalase activity. The suppression of NF-κB signaling and decrease release of pro-inflammatory cytokine which induce the inflammation, reduce generation of ROS and its effects. Also, previous studies showed that guggulsterone has an anti-oxidant activity[23,24].

Similarly, Cyp treatment could also result in cellular injury by interfering with mitochondrial electron transport system which may interrupted oxidant/antioxidant balance through buildup of ROS and induce the release of TNF-α [2,25,26]. Numerous studies supposed that the formation of reactive oxygen species and OS were the key intermediaries for initiation of inflammatory pathways [27,28]. In the present study, a significant surge in TNF-α level in serum after treatment with Cyp compared to control group and significant reduction in the serum level of TNF-α when GS is administered (Table 1). The induction of TNF-α level was decresed in GS treatment due to their protective consequence against Cyp- promoted inflammation. This is consistent with numerous previous studies reported the anti-oxidant and anti-inflammatory action of GS [7,8,29].

The serum creatinine and urea is the typical serum markers being used to diagnose acute kidney injury[30]. Treatment with Cyp prompted acute kidney injury that was proven by the rise in renal biomarkers, serum creatinine and urea and the reasonable histopathological injuries in kidney [30]. In our study, the treatment of Cyclophosphamide to rats prompted renal injuries and cause a significant increase of urea and creatinine serum level as compare to the control group (Table 1). The intraperitoneal administration of guggulsterone (25 mg/kg) to rats was prevent the elevation of urea and creatinine levels which represent the biomarkers for the renal function. These outcomes might approve the protecting consequence of GS against Cyp induced kidney damage and consistent in with the previous studies that reported the protective effect of guggulsterone from toxicant in kidney[32,33]. The protection mechanism of guggulsterone demonstrated by anti-oxidant properties as potent scavenger of free radical and inhibition of NF-κB pathway.

The histo-pathological testing also brought further endorsement for the protective action of guggulsterone against Cyclophosphamide induced renal toxicity. Renal tissue (Fig. 1) of Cyp treated rats display extensive region of degenerative and apoptosis of epithelium lining with dilatation of renal tubules. This consistent with previous researches which stated Cyclophosphamide induced histological and pathological changes, OS and lipid peroxidation [31,34,35]. The oral guggulsterone at dose of 25 mg/kg to rat for 8 days with intra-peritoneal injection of Cyclophosphamide had revealed less histological alterations (Figure1C), over narrow region of degenerative area and little zone of apoptosis in the cells of proximal renal tubules. This effect approve the protective consequence of guggulsterone treatment against the Cyclophosphamide prompted renal injury. Histological interpretation is in agreement with preceding reports that stated the protective action of

guggulsterone against other kidney toxicants [32,33].

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