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Research Article

Protective Effects of Irigenin against Cyclophosphamide-induced Nephrotoxicity in Male Rats: Comparative Study with Vitamin E

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Abstract

Background: Cyclophosphamide is an alkylating agent that is effective against a broad spectrum of tumors, with nephrotoxicity as a side effect. Irigenin is a natural isoflavonoid isolated from the rhizome of *Belamcanda chinensis* that has been reported to exert antioxidant activities. ***Objective***: Evaluating the possible protective effects of irigenin on cyclophosphamide-induced nephrotoxicity in male rats. ***Methods***: Fifty apparently healthy male albino rats were divided into five groups: (control, induction, irigenin, irigenin with cyclophosphamide, and vitamin E with cyclophosphamide. At the end of the experiment (day 29), all rats were sacrificed. Different parameters were evaluated, including urea and creatinine serum concentration, antioxidant markers reduced glutathione, glutathione peroxidase enzyme, and malondialdehyde level in kidney tissue homogenate, and kidney histological examination. ***Results***: Upon cyclophosphamide administration, malondialdehyde, creatinine, and urea were increased, while their levels were reduced when irigenin was used as pretreatment. On the other hand, the reduced glutathione and glutathione peroxidase enzyme showed a reverse behavior. Additionally, the histological examination confirmed the nephroprotective effect of irigenin. ***Conclusions***: Irigenin has a protective effect against renal damage induced by cyclophosphamide by amelioration of biochemical markers and oxidative stress parameters.

Keywords: Cyclophosphamide, Irigenin, Nephrotoxicity, Vitamin E.

أثر الإريجنين على السمية الكلوية المستحثة بواسطة السيكلوفوسفاميد في ذكور الفئران : دراسة تشمل المقارنة مع تأثير فيتامين هـ

الخلاصة

الخلفية: السيكلوفوسفاميد علاج فعال ضد طيف واسع من الأورام، مع سمية كلوية كأثر جانبي. وقد أُفيد بأن الإريجينين، وهو مركب أيزوفلفونويد طبيعي معزول من جذمور نبات بيلامكاندا تشينينسيس، يمارس أنشطة مضادة للأكسدة. **الهدف**: تقييم التأثيرات الوقائية المحتملة للإريجينين على السمية الكلوية المُستحثة بالسيكلوفوسفاميد لدى ذكور الجرذان. **الطرائق**: قُسِّم خمسون جرذًا ذكرًا من الجرذان البيضاء سليمة ظاهريًا إلى خمس مجموعات: (مجموعة التحكم، مجموعة الحث، مجموعة الإريجينين، مجموعة الإريجينين مع السيكلوفوسفاميد، مجموعة فيتامين هـ مع السيكلوفوسفاميد). في نهاية مدة التجربة (اليوم التاسع والعشرين)، تم التضحية بجميع الجرذان. تم تقييم معايير مختلفة، بما في ذلك تركيز اليوريا والكرياتينين في مصل الدم، وعلامات مضادات الأكسدة التي خفضت مستوى الجلوتاثيون، الجلوتاثيون بيروكسيدي، والمالونديالدهيد في متجانس أنسجة الكلى، والفحص النسيجي للكلى. **النتائج**: عند إعطاء السيكلوفوسفاميد، ارتفعت مستويات المالونديالدهيد والكرياتينين واليوريا، بينما انخفضت مستوياتها عند استخدام الإريجينين كعلاج أولي. من ناحية أخرى، أظهر الجلوتاثيون وإنزيم الجلوتاثيون بيروكسيديز المختزل سلوكًا معاكسًا. بالإضافة إلى ذلك، أكد الفحص النسيجي التأثير الوقائي للكلى للإريجينين. **الخلاصة**: للإريجينين تأثير وقائي ضد تلف الكلى الناجم عن السيكلوفوسفاميد من خلال تحسين العلامات البيوكيميائية ومعايير الإجهاد التأكسدي.

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INTRODUCTION

The kidneys are vital organs that play a key role in maintaining homeostasis and regulating the extracellular environment. They are responsible for detoxification and the elimination of toxic metabolites and drugs from the body [1]. Nephrotoxicity is defined as the detrimental effect of various substances on renal function [2]. Nephrotoxicity caused by anticancer drugs is a significant and increasingly prevalent adverse drug event that limits the effectiveness of cancer treatment. The kidneys serve as a critical elimination route for many antineoplastic drugs and their metabolites through processes such as glomerular filtration and tubular secretion [3]. Cancer patients can develop various renal lesions that not only compromise their immediate survival but also hinder the effective treatment of the underlying malignancy [4]. Cyclophosphamide (CPA) is widely used as an anticancer drug; however, its application in cancer therapy is constrained by severe

toxicities, primarily caused by oxidative stress in normal cells. Reactive oxygen species (ROS) are responsible for inducing damage to multiple organs, including the kidneys. The metabolic activation of CPA results in the production of two cytotoxic metabolites: phosphoramide mustard and acrolein. Phosphoramide mustard is thought to exhibit antineoplastic activity, whereas acrolein, a highly reactive metabolite with a short biological half-life, is likely implicated in CPA-induced kidney injury [5]. Irigenin (Iri), a flavonoid derived from natural plants, has anti-inflammatory and antioxidant activities [6-9], showing promise in alleviating inflammation and oxidative stress in several disorders. Iri demonstrated antioxidant properties by reducing intracellular ROS levels. The decreased ROS levels may be ascribed to the ability of Iri to enhance the activity of essential antioxidant enzymes, such as GSH, GPX, and MDA [6]. Iri, an isoflavone derived from the rhizomes of *Belamcanda chinensis*, has shown significant biological activity in cancer and diabetes problems. Inflammation and heart damage. Iri also decreased the synthesis of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and interleukin-18 (IL-18), as well as neutrophil infiltration [9]. Vitamin E, as one of the four fat-soluble vitamins, is an essential nutrient vital for maintaining overall human health and plays an important role in scavenging free radicals [10-13]. As a potent antioxidant, it protects polyunsaturated fatty acids within cellular membranes from oxidative damage, regulates the production of reactive oxygen and nitrogen species, and plays a key role in modulating signal transduction pathways [14-17].

METHODS

Materials

Irigenin and Vitamin E, purchased from Baoji Guokang Bio-Technology Co., Ltd., China. Cyclophosphamide "Endoxan®" 1 g (GmbH, Germany), purchased from Baghdad Pharmacy. Biochemical kits for urea and creatinine, purchased from Linear Chemicals, Spain. Eliza kits for (GSH), (GPx), and (MDA) were obtained from Elabscience (USA). Fifty male albino rats weighing 150-250 gm at 8–10 weeks were supplied from the College of Pharmacy, Baghdad University.

Study design

The animals were divided into five groups, each with ten rats. Group I (control) received 1% Tween 20 [18] *via* rats' oral gavage for 28 days, and group II (the induction group) orally administered 1% Tween 20 *via* an oral gavage for 28 days and a single intraperitoneal (IP) dose of CPA (150 mg/kg/day) [19-21] to be injected on day 28. Group III, orally administered IRI at a dose of 20 mg/kg/day [22,23] *via* an oral gavage for 28 days. Group IV, orally administered IRI at a dose (20 mg/kg/day) *via* an oral gavage for 28 days; and a single IP injection of CPA (150 mg/kg/day) to be given on day 28. Group V orally administered vitamin E at a dose of 50 mg/kg/day [24] *via* an oral gavage for 28 days and a single IP injection of CPA (150 mg/kg/day) to be given on day 28. After (24 hrs) from the end of the treatment duration (i.e., on day 29), all rats were euthanized. Then, blood samples were collected to determine serum concentration of urea and creatinine level, and the oxidative stress (OS) parameters (MDA), antioxidant markers such as (GSH), (GPX) enzyme levels in the kidney tissue homogenate. A histological examination of rat's kidney architecture was performed, also.

Outcome measurements

Biochemical tests, including urea and creatinine serum levels, were evaluated in each animal of the five groups [25,26]. In addition, Enzyme-Linked Immunosorbent Assay (ELISA) tests were done to measure the MDA, GSH, and GPX in the kidney tissue homogenate according to the manufacturer's instruction manual [27-29] after euthanization of each rat. Moreover, a histopathological examination of the kidney tissues was done.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 24 and GraphPad Prism 10. Data were presented as the significance among experimental groups and were performed using Tukey's Test One-way ANOVA (mean ± SD: $p<0.05$).

RESULTS

For serum urea and creatinine levels, there was a significant increase ($p< 0.05$) in group II rats (induction) and a non-significant difference ($p> 0.05$) in group III (Iri alone) compared to their serum level in group I (negative control). On the other hand, there was a significant decrease ($p< 0.01$) in group IV (Iri with CPA) and group V (Vit E with CPA) in their serum level compared to group II. Finally, there was a non-significant difference ($p> 0.05$) in serum urea and creatinine levels between groups IV and V, as shown in Table 1.

Table 1: Effect of Iri alone and with CPA on serum urea and creatinine levels in comparison to vitamin E in male rats (n=10 in each group)

Animal Groups	Urea (mg/dL)	Cr (mg/dL)
Negative Control (Group I)	25.12±3.961[a]	0.667± 0.053[a]
CPA (Group II)	35.96±14.99[b]	1.131±0.417[b]
Iri (Group III)	16.68±1.848[c]	0.683±0.056[a]
Iri and CPA (Group IV)	16.6±1.346[c]	0.624±0.080[a]
Vit. E and CPA (Group V)	16.04±2.388[c]	0.650±0.082[a]

Data was expressed as mean±SD. Values with different superscripts (a,b,c) are significantly different within the same parameter (ANOVA and Tukey post hoc test at $p<0.05$).

For the effect on oxidative stress parameters (GSH, GPX, and MDA) levels, in group II, there was a significant decrease ($p< 0.01$) in GSH and GPX levels and an increase in MDA level compared to corresponding levels in the negative control rats (group I). Furthermore, in group III, there was a non-significant difference ($p> 0.05$) in the GSH and MDA levels and a significant decrease ($p< 0.01$) in the GPX level compared to the corresponding level in rats of group I. Moreover, in Groups IV and V, there was a significant increase ($p< 0.01$) in the GSH and GPX levels and a decrease in MDA level, compared to the aforementioned parameter in group II rats. In addition, in group V, there was a non-significant difference ($p> 0.05$) in the GSH level compared to the corresponding level in group IV. While there was a highly significant increase ($p< 0.01$) in the GPX level and a decrease in MDA compared to the aforementioned level in group IV, as shown in Table 2. The histopathological results of kidney tissues were illustrated in Figure 1.

Table 2: Effect of Iri alone and with CPA on GSH, GPX, and MDA levels in comparison to vitamin E in male rats (n=10 in each group)

Animal groups	MDA (ng/ml)	GSH (µg/ml)	GPx (pg/ml)
Negative control (Group I)	34.56±7.485[a]	24.11±4.184[a]	1698±146.6[a]
CPA (Group II)	308.6±65.4[b]	2.974±0.545[b]	215±79.22[b]
Iri (Group III)	15.24±7.092[c]	23.21±1.259[a]	1317±169.9[c]
Iri and CPA (Group IV)	78.53±7.802[d]	19.52±6.095[c]	1244±210.1[c]
Vit. E and CPA (Group V)	15.57±8.816[c]	23.02±1.728[a]	1577±164.9[a]

Data was expressed as mean±SD. Values with different superscripts (a,b,c) are significantly different within the same parameter (ANOVA and Tukey *post hoc* test at $p<0.05$).

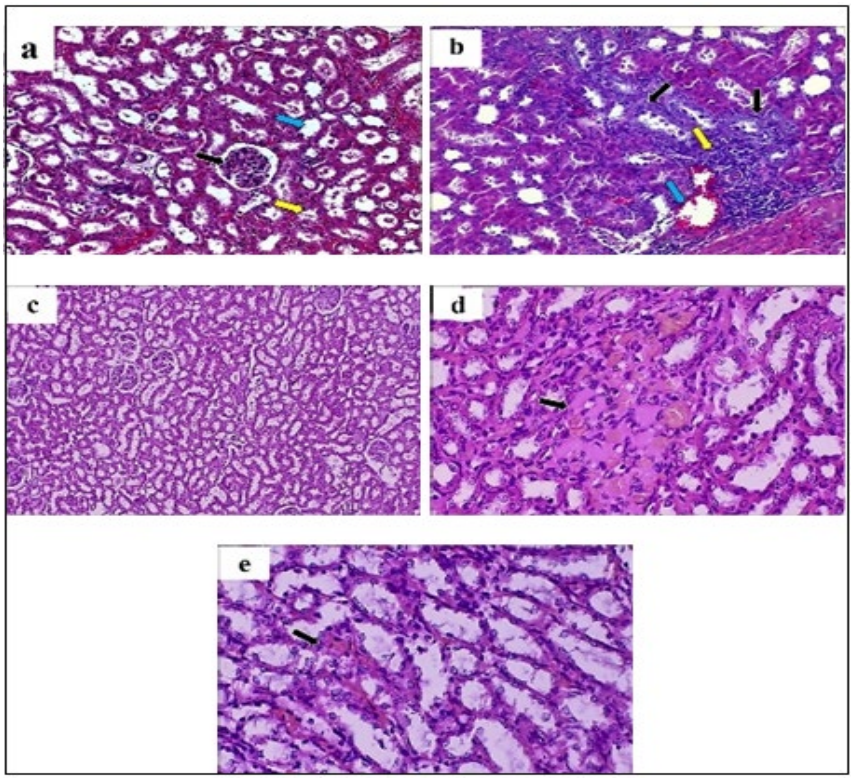

Figure 1: Histopathological images of the rat's kidney (**a**) Group I "Control", (**b**) Group II "CPA", (**c**) Group III "Iri", (**d**) Group IV "Iri with CPA", (**e**) Group V "Vit. E with CPA". The Proximal convoluted renal tubules (yellow arrow), Distal convoluted renal tubules (blue arrow), and glomerulus (black arrow).

Images 1a and 1b showed that CPA induced damage in the medulla and cortex areas that appeared as a necrosis of the epithelial cells of Henle loops tubules with infiltration of inflammatory cells and hemorrhage (Group II) compared to control (Group I). Images 1c and 1d showed that Iri did not affect the kidney tissues (Group III), while the necrosis effect, induced by CPA, was decreased when Iri was administered together (Group IV). On the other hand, image 1e showed that vitamin E had a similar effect to Iri in reducing the necrosis effects induced by CPA on kidney tissues (Group V).

DISCUSSION

The results of this study were similar to results that were published in previous studies. For urea and creatinine serum levels, a single dose of CPA caused an elevation of their levels after 24 hrs, indicating a renal functional deterioration [19,20]. The possible mechanism for this

effect could be attributed to the renal impairment (damage in the renal tubules and altering the membrane permeability), causing urea and creatinine leakage to the systemic circulation [30-32]. Other probable mechanisms could be related to the inflammatory effect of CPA, which results in disruptions of the urea cycle (a metabolic pathway that normally converts ammonia (NH_3), a toxic byproduct of protein metabolism, into urea, a less toxic compound that can be excreted by the kidneys), thus leading to hyperammonemia (elevated serum urea level) [33]. For Iri (an isoflavone derivative), upon administration, the kidney damage effect of CPA was reduced, and the possible mechanism for this nephroprotective effect could be related to the preserved effect of isoflavone on the renal cellular membrane integrity compared with the CPA-intoxicated group [32]. On the other hand, for vitamin E, the results of this study were in tune with other published studies, but on the model of hepatotoxicity [34]. In addition, CPA-induced renal toxicity *via* its most active toxic metabolite, acrolein (electrophilic compound) [35], which can react with nucleophilic sites of renal cell proteins (such as GSH), causes alkylation of the cysteine sulfhydryl group, thus impairing protein function in eliminating the CPA metabolite from the kidney, and this can lead to an increase in the tubular reabsorption of CPA [36]. Acrolein could interact with the antioxidant defense system, including GSH protein and GPX enzyme, reducing their renal cell levels through increasing the free radicals with the lipid peroxidation mechanism; thus, more free radicals can be generated, which are responsible for the oxidative stress and kidney cell damage [37,38], as reported by Ijaz *et al.* (2022) [39]. Previously published studies on Iri as a nephroprotective agent were founded, but in another model of toxicity with doxorubicin (DOX-induced cardiotoxicity), it was found to suppress oxidative stress, apoptosis, and inflammation [40] by acting as a free radical scavenger. The reduction in MDA level could be related to the Iri effect as a direct antioxidant (as an effective hydroxyl radical scavenger) by trapping activity towards the lipid-derived aldehydes such as MDA; in addition, Iri restores the antioxidants in the tissues [41] and possesses an anti-inflammatory effect [34,41]. For vitamin E, as a free radical scavenger, it protects cells and tissues against the harmful effects caused by CPA-free radicals and protects the lipid membranes against oxidative stress [42], a function similar to that of Iri.

Conclusion

Irigenin has a nephroprotective effect against cyclophosphamide-induced cytotoxicity similar to vitamin E as an antioxidant (free-radical scavenger).

Conflict of interests

The authors declared no conflict of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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