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EFFECT OF DIETARY FLAXSEED OIL SUPPLEMENTATION ON CISPLATIN INDUCED RENAL AFFECTIONS IN RATS

Hend Samy Kheira, Shimaa Abd El-Salam El-Sayed, Gehad Ramadan Elsayed

Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, Mansoura University

ABSTRACT

Cisplatin (CP) is an effective chemotherapy that is used widely in treatment of malignant tumors. However due to its toxic side effects both CP dose and duration are limited. Nephrotoxicity is one of the major side effects caused by CP. Several strategies and agents were studied in attempt to protect against CP harmful effect on kidney but not proved useful and safe for clinical use. Dietary flaxseed oil (FXO) is considered the richest plant source of omega 3 (PUSFA) which is proven for its anti-oxidant and anti- inflammatory properties. The protective effect of FXO on CP induced nephrotoxic and other deleterious effects were investigated. Rats were prefed experimental diet for 10 days and then injected with a single dose of CP in two different concentrations (3 mg/kg and 5 mg/kg body weight) intraperitoneally while still on diet. Serum chemistry and oxidative stress parameters were analyzed as well as immunohistochemistry of TNF-α. The results revealed that CP nephrotoxicity significantly increased serum creatinine levels and urea levels. Also CP significantly decreased antioxidant defense mechanism (superoxide dismutase SOD, glutathione peroxidase GPx) and increased lipid peroxidation level (malondialdhyde MDA) with elevated TNF-α level. In contrast FXO increased antioxidant enzymes (SOD, GPx) and reduced lipid peroxidation MDA and serum creatinine , urea levels and TNF-α expresion .Dietary FXO supplementation ameliorated CP induced specific metabolic alterations and oxidative damage due to its intrinsic biochemical antioxidant properties.

INTRODUCTION

Cisplatin, cisplatinum, or cis-diamminedichloroplatinum (II) as well as other platinium compounds are well known for their effectiveness in treatment of various solid tumors **(The American Society of Health-System Pharmacists, 2016).** However, Cisplatin as well as platinum compounds are toxins so they caused severe side effects such as nausea and vomiting, decrease in blood cell count and platelet production in bone marrow (myelosuppresion) and decreased response to infection (immunosuppression). More specific side effects include damage to the kidney (nephrotoxicity), damage of neurons (neurotoxicity) and hearing loss (ototoxicity) **(Desoize, and Madoulet, 2002; Florea and Büsselberg, 2006; Shah and Dizon, 2009 and Tsang, et al., 2009)**, damage in liver (hepatotoxicity) **(Dos Santos, et al., 2007)** , damage in heart (Cardiotoxicity) **(Al-Majed, et al., 2006).**

Cisplatin nephrotoxicity is dose and duration dependent which cause restrictions in treatment with this compound **(Schrier, 2002).**

About 25-35 % of patients show symptoms of nephrotoxicity after single dose administration of CP **(Saad, et al., 2007).** The excessive CP accumulation, in tissue of kidney, factors in nephrotoxicity resulted from cisplatin also known as acute kidney injury (AKI) **(Arany and Safirstein, 2003)**. After treatment with CP the kidney shows various functional changes such as alteration in the volume of urine as well as elevation in both serum creatinine and blood urea nitrogen **(Dauggard, 1990).** The morphological changes in the kidney related to treatment with CP occur in in the external stripe of the medulla specifically on the pars recta of the proximal tubule **(Dobyan, et al., 1980)**. Histological alterations are constant with both apoptosis and necrosis **(Schumer, et al., 1992)**.

The pro-inflammatory nature of AKI resulted from cisplatin has been well documented **(Deng, et al., 2001)**. Both urine and serum levels of TNF-alpha is elevated by CP **(Ramesh and Reeves Brian, 2002; Liu, et al., 2006)**. CP causes the phosphorylation and consequent translocation of nuclear transcription factor-kappa B (NF-κB) to the nucleus, as CP cause inhibitory protein (IκBα) degradation **(Sung, et al., 2008)**.Within the nucleus, activated NF-κB results in inflammatory mediators transcription such as tumor necrosis factor (TNF-alpha) **(Ramesh, et al., 2007)**. In turn, TNF-alpha results in induction of other inflammatory cytokines expression and inflammatory cells recruitment into renal tissue **(Sanchez-Gonzalez, et al., 2011)**. The role of NF-κB in AKI resulted from cisplatin remains to be studied yet.

Moreover, the oxidative stress resulted from CP administration has been strongly suggested to involve in AKI **(Gonzales et al., 2005).** As production of reactive oxygen species (ROS) as a result of CP administration are directly linked to CP cytotoxicity. Injury induced by CP as a result of ROS production can be ameliorated with free radical scavengers **(Dickey, et al., 2005)**,and superoxide dismutase (SOD) **(Davis, et al., 2001)**. Moreover, CP induced cellular stress resulted in activation of MAPK (mitogen-activated protein kinases) pathways including c-Jun N-terminal kinases (JNK), extracellular signal-regulated kinases (ERK), and p38. Specific reduction of MAPK, p38, JNK or ERK inhibits the activation of caspase, the inflammatory response, apoptosis, as well as renal injury **(Clark, et al., 2010)**.

Hence the continuous search for different agents that helps in nephron-protection in adverse of CP and other platinum drugs **(Ali and Al Moundhri, 2006)**. These involve antioxidants, modulators of nitric oxide, diuretics, and cyto-protective and apoptotic agents **(Conklin, 2004; Ali and Al Moundhri, 2006; Cetin, et al., 2006)**. However, none of these were found to be safe and suitable for clinical use in protecting against CP nephrotoxicity. The technique of recognizing naturally occurring dietary sources and applying them as cyto-protectants provides a strategy that is of great interest for CP chemotherapy. Nutritional recommendations have recently promoted the elevated need to consume omega-3 (omega-3/n-3) polyunsaturated fatty acids (PUFAs) **(Simopoulos, 1999)**. Flaxseed *Linum usitatissimum* is the richest dietary source of omega-3 fatty acids among plant sources **(Chen, et al., 2002; Lin, et al., 2002; Newairy, & Abdou, 2009)**. The essential fatty acids specifically omega-3 fatty acids in flaxseed oil are regarded as the key healing components **(Larsson, et al., 2004)**. The

concentration of α-linolenic acid (omega-3 PUFA), a strong anti-carcinogen, in flaxseed oil, varies from approximately 40–60% **(Williams, et al., 2008).** Flaxseed has hypolipidemic and antioxidant effects **(Shahidi, 2000; Newairy, & Abdou, 2009)**.

Flaxseed oil prevents lead induced neurotoxicity and nephrotoxicity **(Abdel-Moniem, et al., 2010; 2011)**. FXO have recently reported to ameliorates CP induced hepatotoxic effects **(Naqshbandi, et al., 2012)**. Though, the reno-protective potential of FXO in CP nephropathy has not yet been explored.

Regarding the CP potential therapeutic uses and FXO numerous health benefits, researches was undertaken to study the effect of FXO on biochemical mechanisms of CP induced nephrotoxicity due to FXO antioxidant and intrinsic biochemical properties that would improve both metabolism and antioxidant defense mechanism of the kidney.

MATERIALS AND METHODS

1.1. Chemicals and drugs

Pure cold pressed fortified Flax seed oil, Cisplatin 10 mg (CP) and all other chemicals and kits used for achieving this study were purchased from Sigma Company, Cairo, Egypt.

1.2. Diet

The basal control diet was obtained from (MERC) Faculty of Medicine, Mansoura University. The diet produced In the form of pelleted rat diet composed of (Casein, L-Cystine, Soya bean, Lard, Corn starch, Mal todextrin, Sucrose, AIN-93 vitamin mix, AIN-93 G mineral mix, Choline chloride, Cellulose, and t-BHQ).

1.3. Experimental design

Forty two wistar albino male rats (weighting 185 ± 10 grams) were purchased from (MERC), center in Faculty of medicine, Mansoura University, Egypt for medical experimental researches. The animals were housed at room temperature (22±4 C), and humidity (5± 10 %) and at half day light –dark cycle and fed with commercial pellet diet and water ad libitum for 7 days as acclimatization period. All the experiments were conducted according to the ethical guidelines of international association for studying pain, **(Zimmermann, 1983)**.

The animals were divided into 6 groups of 7 rats each –control group (G1), FXO only group (G2), CP groups in different concentration (G3, G4) (3 mg/kg, 5 mg/kg respectively), FXO+CP 3 mg/kg (G5), FXO+CP 5 mg/kg (G6) – all groups were fed on normal control diet, FXO was given daily to rats in (G2, 5, 6) via stomach tube 500 mg/kg body weight **(Wahba and Ibrahim 2013).** After 10 days, rats in groups G3, G5 were injected with CP 3mg/kg intraperitoneally and rats in groups G4, G6 were given CP intraperitoneally with a dose 5 mg/kg body weight **(Robert, et al., 1987)**, G1, G2 received an equivalent amount of normal saline. 4 days after CP administration rats were sacrificed under thiopental sodium anesthesia 20 mg/kg **(Ebling, et al., 1991)** blood samples were collected and kidneys were removed for preparation of tissue homogenate in addition to immune histo- chemistry analysis.

1.4. Preparation of blood samples

Blood samples withdrawal through cardiac puncture from the heart, immediately the blood samples were placed in sterile, dry, capped tubes. The tube containing blood sample for serum separation was left in vertical position at room temperature then centrifugation at 3000 rpm for 15 minutes, a clear, straw colored serum samples were aspirated by automatic pipette and transferred into clean, dry, labeled tubes and kept in freezer -20 C.Serum samples used for determination of serum creatinine level and serum urea level.

1.5. Preparation of tissue samples

- **Tissue homogenate samples**

Left kidney from 7 animals of each of 6 groups were dissected and preserved in normal saline. The collected tissue samples were immediately stored in refrigerator for the first 24 hours then stored at (-20 C) for subsequent analyses. Then it used after preparation for determination of SOD, GPx activities and MDA level.

Before dissection, tissue was perfused with a phosphate buffered saline (PBS) solution, pH 7.4 for removal of any RBCs and clots. Tissue was homogenized 5 – 10 ml cold buffer (i.e., 50mM potassium phosphate, pH 7.5) per gram tissue. Samples were centrifuged at 4000 r.p.m for 15 min. The supernatant was removed and freezed at – 80^{o} C.

- **Tissue for Immune histo-chemistry analysis**

Right kidney from 7 animals of each of 6 groups were dissected and preserved in formalin 10% for Immune histo-chemistry analysis of TNF-α.

For **Immunehistochemistry analysis** the isolated kidney tissue was fixed by 4% paraformaldehyde and blocked in paraffin. Sections of tissue were placed on glass slides and xylene was used to deparaffinize and graded ethanol to rehydrate. Endogenous peroxidase was blocked with 3% hydrogen peroxide for 20 min, and the samples on slides were then rinsed with PBS. To obtain an adequate signal, samples were treated with pepsin at 428C for 5 min. After treatment with the blocking buffer, the slides were incubated overnight at 48C with primary antibodies for TNF-α.

1.6. Assay of serum creatinine and urea

Serum creatinine levels were determined colorimetrically by using ready kits **(Bartels and Böhmer 1971).** Urea levels were determined colorimetrically by using ready kits provided by Diamond, **(Young, 2001).**

1.7. Assay of enzymes involved in free radical scavenging and lipid peroxidation

SOD enzyme activity was assayed **(Nishikimi, et al., 1972).** GPx activity was determined **(Paglia, and Valentine, 1967).** Lipid peroxidation was measured by MDA **(Satoh, 1978 ; Ohkawa, et al., 1979).**

Statistical Analysis

Statistical differences between mean of biochemical variables in control and diseased rats were assessed using Kruskal-Wallis test

with post hoc Dunn multiple comparison tests. Mann- Whitney test Data was used for assessment statistical differences between diseased and treated rats using statistical software program (Graph Pad Prism for Windows version 5.0; GraphPad Software, Inc., San Diego, CA, USA). $P < 0.05$ was considered as statistically significant in all tests (**Steel and Torrie, 1960).**

RESULTS

The effect of different doses of CP and CP+FXO -on parameters of nephrotoxicity in serum, and enzymes of oxidative stress in tissue homogenate of rat's kidney also in immunehistocheistry of TNF-α in kidney tissue- was studied the results revealed that:-

1.8. The effect of FXO on CP treated rats in different doses on kidney function test (urea and creatinine)

Results of this study showed that cisplatin groups (G3, G4) exhibited a significant increase in serum creatinine and urea levels when compared to control group (G1) with a significant decrease in both creatinine and urea levels in groups treated with FXO+CP (G5, G6) when compared to diseased groups (G3, G4) and serum creatinine level returned to normal in group treated with FXO+CP3mg/kg (G5). **Table (1).**

Table (1) showing the effect of FXO on CP treated rats in different doses on kidney function test (urea and creatinine)

Kidney function tests		
	Creatinine (mg/dl)	**Urea (mg/dl)**
Groups (n=7)	**Mean±SD**	**Mean±SD**
(G1)	0.59 ± 0.029^{c}	40.32 ± 5.17^{d}
(G2)	0.6 ± 0.076^{c}	38.5 ± 6.33^{d}
(G3)	0.93 ± 0.233^{bc}	118.1 ± 6.67^{b}
(G4)	2.83 ± 0.622^{a}	142.4 ± 19.18^{a}
(G5)	0.7 ± 0.335^{c}	66.05 ± 17.29^{c}
(G6)	1.4 ± 0.562^{b}	130.82 ± 11.60^{ab}

The mean with the same letter in column revealed a non- significant change ($P>0.05$) and the mean with different letter in column revealed a significant change ($P<0.05$).

1.9. The effect of FXO on CP treated rats in different doses on oxidative stress

In table (2), results show that there was a significant decrease in SOD and GPx activities as well as increase in lipid peroxidation (MDA) level in kidney tissue homogenate in CP groups (G3, G4) when compared to control group (G1) while there was a significant up regulation in both SOD and GPx activities as well as increase in lipid peroxidation (MDA) level in groups treated with FXO+CP (G5, G6) when compared to diseased groups (G3, G4) and MDA level returned to normal in group treated with FXO+CP3mg/kg (G5).

Table (2) showing the effect of FXO on CP treated rats in different doses on oxidative stress and lipid peroxidation (SOD, GPx and MDA)

Antioxidant enzymes activity in kidney tissue homogenate			
	SOD (U/ml)	**GPx (µmoles of NADPH oxidized/mg tissue/min)**	**MDA (nmoles of MDA/gm tissue)**
Groups (n=7)	**Mean±SD**	**Mean±SD**	**Mean±SD**
(G1)	348.2±19.77^{b}	27.05±2.11^{c}	0.055±0.0395^{c}
(G2)	381.6±12.06^{a}	36.85±3.17^{c}	0.122 ±0.059^{c}
(G3)	257.0±8.67^{d}	19.45±0.93^{bc}	0.192 ±0.089^{bc}
(G4)	198.5±11.50^{e}	13.95±0.67^{a}	0.34 ±0.035^{a}
(G5)	306.8±13.63^{c}	22.8±1.53^{c}	0.12 ±0.039^{c}
(G6)	245.5±16.25^{d}	21.45±1.08^{b}	0.276 ±0.212^{ab}

The mean with the same letter in the same column revealed a non- significant change (P>0.05) and the mean with different letter in the same column revealed a significant change (P<0.05).

1.10. The effect of FXO on CP treated rats in different doses on immune histo-chemistry of TNF-alpha in kidney tissue

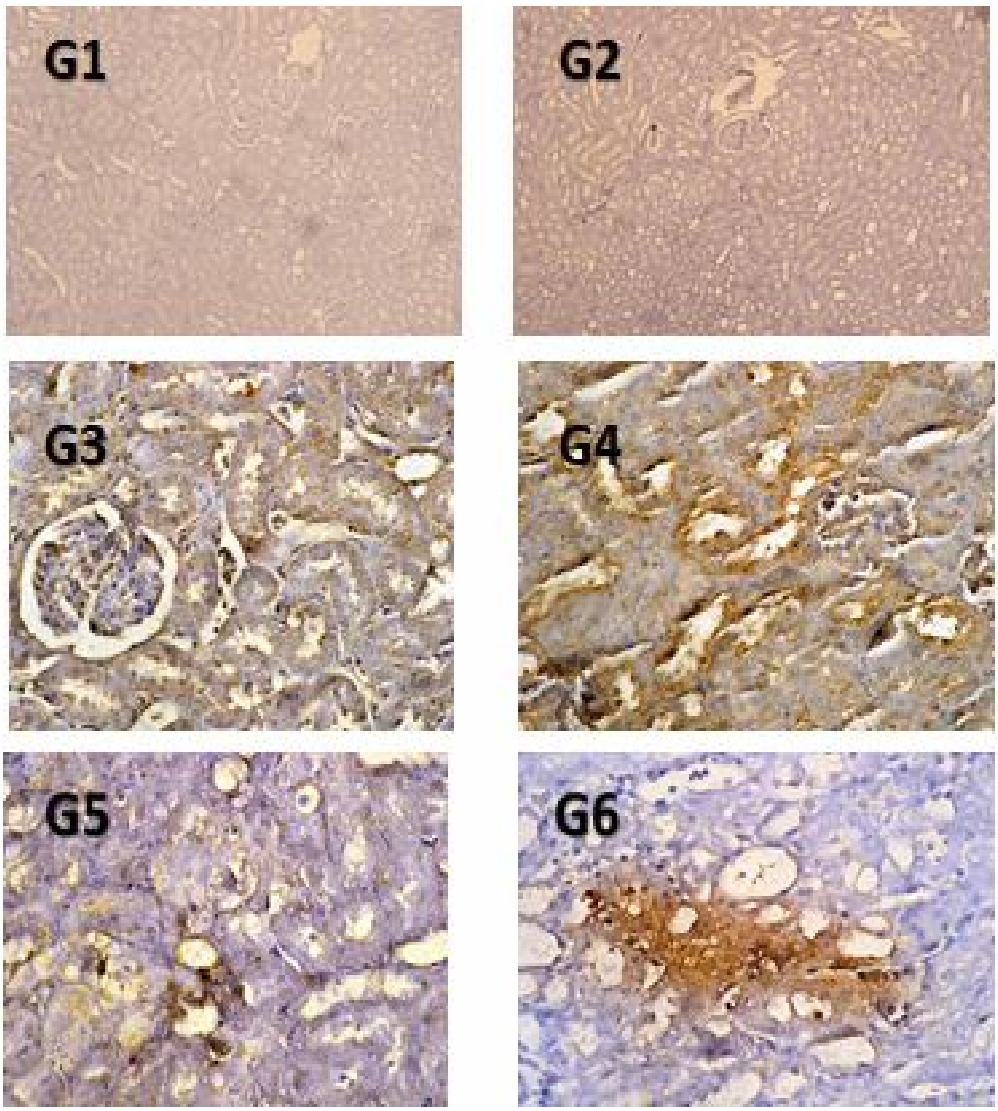

Figure (1): Immune histo-chemistry of NF-KB in rat kidney of rats in different groups.

The received results indicate that in both **(G1, G2)** Kidney is showing negative brown immunostain for NFKB. (IHC, DAB immunostain, Hematoxylline counter stain, 400x). While in **(G3)** kidney is showing moderate brown immunostain for NFKB. (IHC, DAB immunostain, Hematoxylline counter stain, 400x). Also **(G4)** shows that kidney is showing strong brown immunostain for NFKB. (IHC, DAB immunostain, Hematoxylline counter stain, 400x). However in **(G5)** Kidney is showing mild positive brown immunostain for NFKB. (IHC, DAB immunostain, Hematoxylline counter stain, 400x). Also **(G6)** shows Kidney is showing moderate brown immunostain for NFKB. (IHC, DAB immunostain, Hematoxylline counter stain, 400x). **(Figure, 1)**

DISCUSSION

Worldwide nephrotoxicity is considered a major health and economic problem. Nephrotoxicity resulted from drug usage for therapeutic needs are one of the main causes of cases suffering from acute kidney injury (AKI) **(Taber, and Mueller, 2006)**. As a result of that, seeking strategies for nephrotoxicity prevention has become an active field for investigation. In addition to drug targeting and medical chemistry for new and safer molecules, scientist developed a great interest in the identification of renal protective adjuvants for co-administration with suspected nephrotoxic drugs, such as cisplatin and other platinum compounds used for cancer treatment. Various protective agents such as antioxidants **(Pabla, and Dong, 2008)** have been studied for their useful effects on nephrotoxicity resulted from cisplatin administration **(Ali, and Al Moundhri, 2006; Chirino, and Pedraza-Chaverri, 2009)**.

Roughly during the last ten years a remarkable interest in omega-3 PUFAs has observed considerably due to their useful effects on health **(Simopoulos, 1999; Al-Nouri, et al., 2012; Komprda, 2012)**.

Flaxseed has been recognized as an important alternative source of omega-3 fatty acids **(Gebauer, et al., 2006; Harper, et al., 2006)**. In Africa, Europe and Asia, people have used Flaxseed (L. usitatissimum) meal and flaxseed oil as a source of food for centuries. Flaxseed is regarded beneficial for both human and animals due to its composition as it contains three main components; a high percentage of omega-3 PUFA which is named α-linolenic acid (18:3 n-3), a high content of dietary fiber, soluble as well as insoluble altogether; and flaxseed also contains the highest amount of phyto-estrogenic lignans **(Lay, and Dybing, 1989; Harris, and Haggerty, 1993)**. Dietary supplementation of flaxseed/ FXO proved to benefit the health in both normal and pathological cases **(Abdel-Moniem et al., 2011; Chen et al., 2002; Lin et al., 2002)**.

In this study we investigate the effect of flaxseed oil in rats with nephrotoxicity resulted from cisplatin treatment and effect of flaxseed oil on levels of (creatinine, urea); antioxidant enzymes activity (SOD, GPx and lipid peroxidase MDA) and tumor necrosis factor-alpha (TNF-α).

In **tables 1, 2** results indicate that single CP injection in different doses (3mg/kg, 5mg/kg) produced a typical pattern of nephrotoxicity which is showed by elevation in both serum creatinine and urea levels. Also results prove that FXO when given for 14 days before and after CP administration provided protection against CP induced harmful effects on kidney as it significantly reduced CP-induced elevation in S.creatinine and BUN levels **(Khan et al., 2009).**

One of major suspected causes of Cisplatin toxicity is elevated production of reactive oxygen species ROS and free radicals **(Xiao, et al., 2003; Atessahin, et al., 2005)**. Nevertheless, the cause of oxidative stress could be either the elevated ROS production and/or reduced antioxidant enzyme system such as SOD and GPx which known to provide protection to cells against cytotoxic ROS. SOD and catalase together initiate conversion of superoxide radicals primarily to hydrogen peroxide (H_2O_2) and then to O_2 and H_2O. Also enzymes like GPx use thiol-reducing power of glutathione to reduce oxidized lipids and protein targets of ROS. Due to compromised antioxidant enzyme status, lipid peroxidation in the cellular and subcellular membranes is the expected result of ROS injury **(Yu &**

Anderson, 1997; Irmak, et al., 2001; Fadillioglu, et al., 2004).

The results of this current study showed that CP causes down regulation in both SOD and GPx activity and increase lipid peroxidation MDA when compared to normal control group while CP+FXO elevated both SOD and GPx activities and reduced MDA when compared with CP groups **(Tables 3, 4, 5)**. As a reference to this results **(Mistry, et al., 1991)** found that CP inhibit antioxidant enzymes in renal tissues, also causes lipid peroxidation (MDA) up regulation which indicates renal tissue damage and deplete Glutathione (GSH) and protein thiols. **Fatima et al., 2004; Gonzales et al., 2005; Khan et al., 2009**, stated that CP causes a defined decrease in SOD, catalase and GPx activities and leads to LPO enhancement in the kidney indicating oxidative stress resulted by CP treatment. In the opposite CP caused effect, FXO resulted in a marked elevation in the activity of the antioxidant enzymes when used alone without CP and also FXO reduced CP oxidative stress when administrated with CP. The addition of FXO to diet during treatment with CP reduced lipid peroxidation (MDA) and elevated the activity of antioxidant enzymes.

The two suggested possible mechanisms of ameliorating effect of FXO regarding the oxidative damage caused by CP administration are: First, FXO addition to diet elevates the of SOD, catalase and GPx levels in the proximal tubular epithelial cells leading to enhancement in defense against ROS. Second, the replacement of the polyunsaturated fatty acid constituent of the BBM, that had been attacked by oxygen free radicals, by the component omega-3 PUFA of dietary FXO **(Ozgocmen, et al., 2000)**, thereby improving membrane integrity.

Moreover, Researches suggest that one of the important mechanisms in pathogenesis of AKI induced by CP is inflammatory mechanism **(Ramesh, and Reeves, 2002; Arany, and Safirstein, 2003)**. Cytokines, such as TNF-α, appear to have a role in renal damage resulted from CP administration, in addition to large scale activation of cytokines and chemokines after CP injection in kidney **(Ramesh, and Reeves, 2002).** Researches showed that both IL-1β and TNF-α is pro-inflammatory cytokines that often increase in parallel. Also, both of them stimulate the other's production **(Aggarwal, et al., 2001)** and both up regulate the other cytokines and chemokines production **(Banas, et al., 1999)**. TNF-α mRNA levels is elevated in both ischemic and CP induced kidney damage **(Deng, et al., 2001)** which agrees with results of the present study that showed positive immunostain for TNF-α in kidney tissue in CP only groups and decrease in positivity of immunostain for TNF-α in kidney tissue in CP+FXO groups when compared to CP only ones

Previous studies showed the anti-inflammatory and antioxidant potential of FXO **(Calder, 2001)** and that the important anti-atherogenic role of ALA may involve a potent anti-inflammatory role **(Rodriguez-Leyva, et al., 2010)**. FXO results in inhibition of TNF-α though the specific mechanism for both IL-1β and TNF-α synthesis inhibition via dietary n-3 FAs is not fully known, but may be attributed to the fact that n-3 fats inhibit the synthesis of n-6 eicosanoid, which in turn affect the synthesis of cytokines. Also, leukotriene B4 is studied as a possible reason for FXO ability to inhibit TNF-α and IL-1β production as it is known for simulating the production of both TNF-α and IL-1β and can be inhibited by n-3 fatty acids as well **(Pedersen, 2000).**

In conclusion supplementation of flaxseed oil to rats with CP treatment result in a significant elevation in SOD and GPx activity with a significant decrease in the mean value of serum creatinine, urea, and MDA when

compared to non-supplemented rats with CP treatment. Also, supplementation of flaxseed oil to rats with CP treatment causes down regulation of TNF-α when referred to non-supplemented rats with CP treatment. These results indicate that dietary FXO supplementation ameliorates CP induced nephrotoxicity in rats.

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الملخص العربي
تأثير زيت الكتان علي اصابات الكلي المستحثة بالسيسبلاتين في الفئران

هند سامي خيرة، شيماء عبد السلام السيد، جهاد رمضان السيد

قسم الكيمياء الحيوية و كيمياء التغذية، كلية الطب البيطري، جامعة المنصورة

يعد السيسبلاتين احد الادوية الفعالة المستخدمة في علاج الاورام الخبيثة، و لكن نتيجة للاعراض الجانبية السمية الناتجة عن استخدام السيسبلاتين التي تتسبب في الحد من جرعة و مدة العلاج، و تعد السمية الكلوية احد الاعراض السمية للعلاج بالسيسبلاتين، و قد اجريت العديد من الابحاث و الدراسات للتغلب علي الاثار الجانبية للعلاج بالسيسبلاتين علي الكلي و لكن لم يتم التوصل الي عوامل او استراتجيات امنة و فعالة حتي الان، يعتبر زيت بذرة الكتان من اغني الزيوت النباتية المحتوية علي الدهون الغير مشبعة (الاوميجا ٣) و المعروفة بخصائصها المضادة للاكسدة و الالتهابات، و يتم دراسة الاثر الوقائي لزيت بذرة الكتان ضد التسمم الكلوي و الاثار الضارة بالصحة الناتجة عن العلاج بالسيسبلاتين، تم تجريع الفئران بزيت بذرة الكتان الفئران طوال فترة التجربة و حقنها بالسيسبلاتين بعد ١٠ ايام من بداية التجربة بجرعة واحدة من السيسبلاتين بتركيزين مختلفين (٣ مجم/كجم، ٥ مجم/كجم من وزن الجسم) مع استمرار التجريع بالسيسبلاتين، تم قياس العديد من العوامل كالكرياتنين و اليوريا في الدم و قياس ال SOD و GPx و MDA في خلايا الكلي و تحليل الكيمياء الهيستولوجية المناعية TNF-α في انسجة الكلي.

الخلاصة:

لوحظ تسبب السيسبلاتين في ارتفاع نسبة الكيرياتنين و اليوريا و نسبة ال MDA مع انخفاض نسبة ال SOD و GPx في حين تسبب زيت بذرة الكتان في ارتفاع ملحوظ في نسبة الانزيمات المضادة للاكسدة و انخفاض نسبة اليوريا و الكيرياتنين و ال MDA وال TNF-α ، و قد تسبب زيت بذرة الكتان في تحسين اصابات الكلي الناتجة عن العلاج بالسيسبلاتين في الفئران. و من هنا نستخلص ان تناول زيت بذرة الكتان يقلل من التاثير السمي للسيسبلاتين علي الكلي في الفئران و ذلك عن طريق رفع نشاط انزيمات الاكسدة و تقليل المالون الدهيد في الفئران.