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Protective role of selenium against diclofenac sodium induce nephrotoxicity in female rats

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Abstract

Selenium is an essential trace mineral found in soil, water, and some foods. It increases antioxidant effects in the body. Diclofenac sodium is one of the world's most commonly prescribed nonsteroidal anti-inflammatory drugs (NSAIDs). This study aimed to evaluate the histological changes induced by diclofenac sodium on the kidney of adult female rat and evaluating the possible ameliorative role of selenium. Healthy female rat 4 month old weighting 140-235g were divided equally into four groups. The first group was received only distilled water and considered as control group. The second group animals were received selenium at a dose of 0.25 mg/kg b/w by oral gavage daily for 21 days. Third group were receive diclofenac sodium at dose of 10mg/kg b/w by oral gavage daily for 14 days. And the fourth group the animals were given same dose of selenium for 7 days, during the next two weeks, animals given same dose of diclofenac sodium. Histological examinations revealed that administration of diclofenac sodium only exhibited marked glomerular and tubular lesion included vacuolization and swelling the lining epithelium of tubules, inflammatory cells infiltration and hemorrhage in the interstitial tissue, necrotic or desquamation lining epithelium in the luminal tubules, intratubular esinophilic casts in some renal tubules, completely obscured or stenosis urinary space of renal corpuscles, shrinkage glomeruli of some renal corpuscles with widening urinary space, many glomeruli appeared with less cellularily, dilation and congestion blood vessels. In contrast administration of selenium along with diclofenac sodium induced ameliorating change in renal tissue of diclofenac sodium intoxicated rat.

Key words: selenium, diclofenac sodium, Histopathological, kidney, female rat

Introduction

The kidney is one of the body's vital organs. Blood pressure, acid-base balance, and bodily electrolytes are all controlled by the kidney. Nephrotoxicity is actually the lethal effect of some materials including both noxious substances and medications on the kidney. These materials are known as nephrotoxins. Among nephrotoxins, drugs are the leading cause of nephrotoxicity. These drugs produce their poisonous effects by one or more common pathogenic mechanisms, which may include altered intraglomerular haemodynamics, tubular cell toxicity, inflammation, crystal nephropathy and rhabdomyolysis (*1*). Non-Steroidal anti-inflammatory drugs (NSAIDs) are the most frequently prescribed therapeutic agents, as they have analgesic, antipyretic and anti-inflammatory actions. They are commonly used by more than 30% in developed countries (*2, 3*). Diclofenac Sodium (Voltaren) is one of the most widely prescribed nonsteroidal anti-inflammatory drugs (NSAIDs) in the world. It is used mainly to relieve symptoms across multiple clinical indications, including inflammation, pain, osteoarthritis, rheumatoid arthritis and ankylosing spondylitis (*4*). They exert their anti-inflammatory, antipyretic and analgesic effects via the suppression of prostaglandins (PGs) synthesis, by inhibiting the enzyme, cyclooxygenase (COX), which has two isoforms, COX-1 and COX-2 (*5*). They inhibit both COX-1 and COX-2 the rate-limiting enzymes for the production of prostaglandins and thromboxane (*6*). Normal therapeutic dose of diclofenac is safe, effective and widely used but extensive use of this agent, leading to toxicity and untoward effects many times especially when therapy of pain, inflammation and fever involves use of higher dose for longer period (*7*) .

The use of these agents therefore may lead to adverse effects on body tissues or cause cellular injuries, ranging from acute to long-term chronic disorders and may include conditions considered to be degenerative. These include alterations in renal function, effects on blood pressure, hepatic injury, platelet inhibition, gastrointestinal and cardiovascular disorders (*8*)**.** In histological examination of the kidneys from diclofenac-treated rats received 10mg/kg intramuscularly injection of diclofenac daily for 7days revealed distorted renal corpuscles, hyper-infiltration of the glomerulus, increased mesangial matrix, severe and widespread renal tubule necrosis (particularly in the proximal tubules), dilatation of renal vessels, tubular cell desquamation, intraluminal cast formation, and infiltration of inflammatory cell (*9*).

Humans have always relied on nature for their basic needs. For thousands of years, plants and their derivatives have formed the basis of sophisticated traditional medicine and have been an invaluable source of bioactive compounds with therapeutic potential. They play an important role all over the world in the treatment and prevention of human diseases (*10*). Selenium is an essential trace mineral found in soil, water, and some foods. It is an important factor in many body processes. Selenium increases antioxidant effects in the body. The amount of selenium in soil varies, and foods grown in different soils have different selenium levels. **It** is present at extremely low levels in nature compared with other microelements (*11*) .

Selenium occurs naturally in two chemical forms, organic selenium (Incorporated within the amino acids methionine and cysteine) , Inorganic selenium (Depend on the oxidation state as selenite, selenate or selenide) (*12*). *In vivo* studies were conducted to examine the effects of Selenium in various tissues such as Brain (*13*). kidneys and muscles (*14*). This element contributes in various biological processes such as antioxidant defense ,thyroid hormone production, and immune responses (*11, 15*).

Selenium supplementation increases enzymatic anti-oxidant activity and reduces lipid peroxidation (*16*) . A previous study showed that Selenium protects against inflammatory indices in rat. It can reduce oxidative stress- and inflammation-related consequences of renal ischemia-reperfusion (*17*)**.** Therefore, the histopathological effect of the Selenium in kidney was studied in female albino rats against nephrotoxicity induced by diclofenac sodium.

Material and Methods

Diclofenac sodium 50mg tablets (votrex company) and selenium 200 mcg capsules will be used in this study.

Experimental animals and treatment

Healthy female adult albino rats (4 month old and weighing 140-235g) were obtained from the Animal Breeding House of faculty of veterinary medicine, Omar Al-Mukhtar University, Al Bayda-Libya. They were housed in the laboratory animal room in clean plastic cages (7 rats/ cage).The animals were maintained on standard commercial pellet diet and clear drinking water ad libitum. The rats were acclimatized for a 2 week prior to the start of experiments. The rats were divided equally into 4 equal groups of seven rats each and subjected to the following treatments:

- The first group were received only standard diet and clear drinking water and considered as control group.
- The second group (selenium group), the animals were received selenium at a dose of 0.25 mg/kg body weight by oral gavage (*18*) daily for 21 days at early morning.
- Third group were received diclofenac sodium at a dose of 10mg/kg body weight by oral gavage (*19*) daily for 14 days.
- The fourth group the protective group (selenium and diclofenac sodium). The animals were given selenium at a dose of 0.25 mg\kg body weight by oral gavage daily for 7 days. Then at next two weeks, animals were given selenium at a dose of 0.25 mg/ kg orally in the early morning after two hours. The animals were given diclofenac sodium at a dose of 10 mg/ kg orally.

Histopathological study:

At the end of the experimental period the animals from both control and experimental groups were dissected after sacrificed by cervical dislocation. For the light microscopic examination the kidney samples were collected from both control and experimental animals and immediately fixed in aqueous Formaldehyde 10% fixative, dehydrated in ascending grades of ethyl alcohol, cleared in xylol, impregnated in paraffin wax (melting point between 56°C and 58°C), sectioned with rotary microtome (Leica RM 2125) at 5 µm thicknesses and stained with Harri's Hematoxylin and Eosin according to (*20*). Stained sections were examined under light microscope and histopathological changes were recognized.

Results

The renal tubules and glomeruli in kidney slices from the control group had their normal histological structure. Additionally, the medulla region's tubules showed their usual characteristics (Figs.1 and 2). Sections from second group were no detectable histopathological lesions in kidney sections of rat treated only with selenium for 21 days. histological sections of kidney were showed regular structure with well distributed glomeruli and related tubules (Fig.3). In comparison, the opposite situation was found in the kidney sections of rat treated with diclofenac sodium were detected in glomeruli and in convoluted tubules (Fig.4-8) compared to those of control group. The main characteristic abnormalities were the appearance of vacuolization and swelling the lining epithelium of tubules, inflammatory cells infiltration and hemorrhage in the interstitial tissue, necrotic or desquamation lining

epithelium in the luminal tubules, intratubular esinophilic casts in some renal tubules and such alteration were more prominent in proximal convoluted tubules. Beside that renal corpuscles with completely obscured or stenosis urinary space were frequently observed and shrinkage glomeruli of some renal corpuscles with widening urinary space, many glomeruli appeared with less cellularily , dilation and congestion blood vessels. While, administrated of selenium with diclofenac sodium induced marked improvement in the histological structure of kidney in comparison to diclofenac sodium only treated group, Increased inflammatory cell infiltration (Figs.9 and 10).

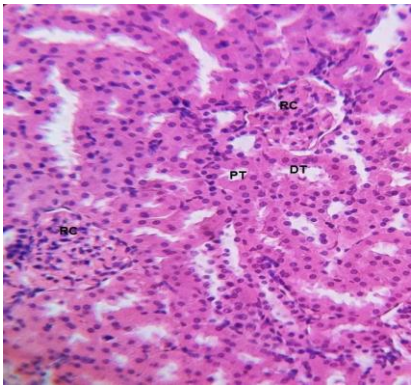

Fig. (1): A section of kidney from control group showing renal cortex normal of proximal tubules (PT) and distal tupules (DT) and renal corpuscles (RC) (H&E stain,X400).

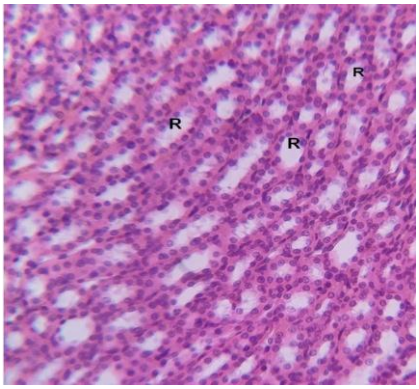

Fig. (2): A section of kidney from control group showing normal renal tubules (R) in the medulla region (H&E stain,X400).

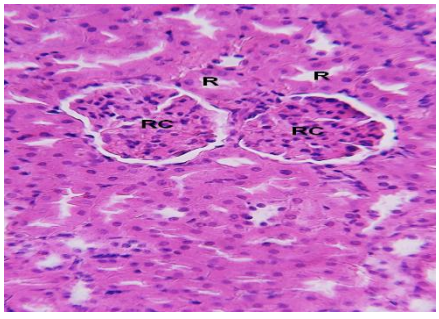

Fig. (3): A section of renal cortex of rat treat with selenium for 3 weeks showing normal of renal tubules (R)

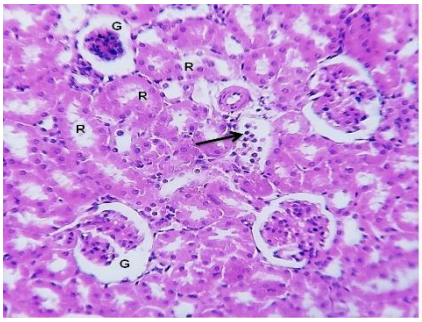

Fig. (4): A section of renal cortex of rat treat with diclofenac sodium for 3 weeks showing Glomerular shrinkage

and renal corpuscles (RC) (H&E stain,X400).

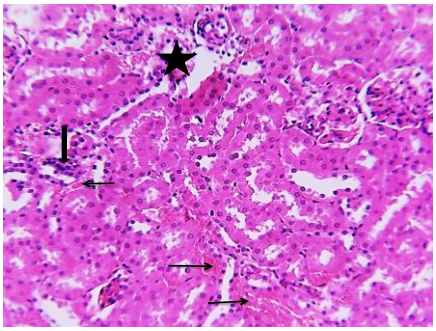

Fig. (5): A section of renal cortex of rat treat with diclofenac sodium for 3 weeks showing hemorrhage in interstitial tissue (Arrows), necrotic changes in tubules (Star), inflammatory cells infiltration (Thick Arrow) (H&E stain,X400).

(G) with widening urinary space , Inflammatory cells infiltration (Arrow), degeneration and necrotic renal tubules (R) (H&E stain,X400)

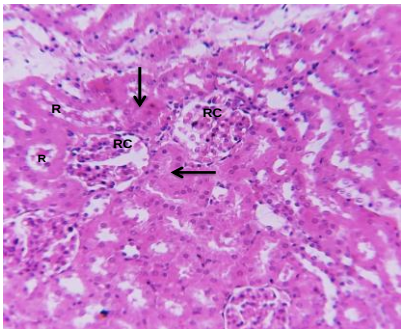

Fig. (6): A section of renal cortex of rat treat with diclofenac sodium for 3 weeks showing degeneration of renal corpuscles (RC) with less cellularily, cloudy swelling of renal tubules (Arrows), note also the intratubulur casts (R) (H&E stain,X400).

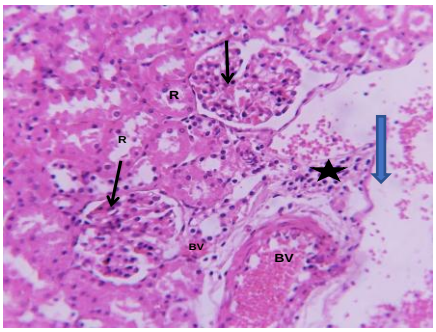

Fig. (7): A section of renal cortex of rat treat with diclofenac sodium for 3 weeks showing degeneration and necrotic renal tubules and debris in lemun (R), congestion of glomerular tuft (Arrows), necrotic areas (Blue Arrow), congestion blood vessel (BV), inflammatory cells infiltration (Star) (H&E stain,X400).

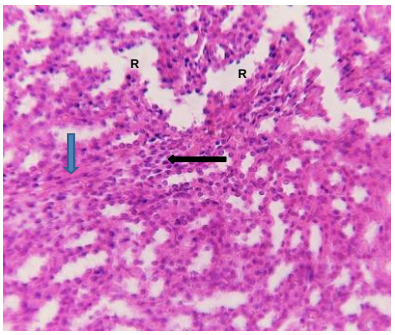

Fig. (8): A section of renal medulla of rat treat with diclofenac sodium for 3 weeks showing inflammatory cells infiltration in the interstitial tissue (Black Arrow), hemorrhage in interstitial tissue (Blue Arrow),dilation of renal tubule(R) (H&E stain,X400)

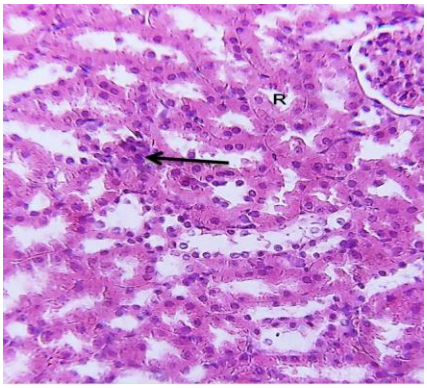

Fig. (9): A section of renal cortex of rat treat with diclofenac sodium and selenium showing an improvement in the renal tubules (R) and renal corpuscles (RC), inflammatory cells infiltration (Arrow) (H&E stain,X400).

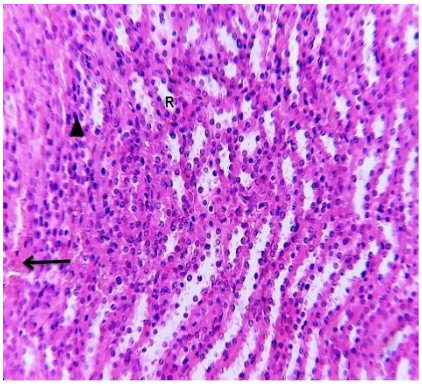

Fig. (10): A section of renal medulla of rat treat with diclofenac sodium and selenium showing normal renal tubule (R), inflammatory cells infiltration (Head Arrow), hemorrhage in interstitial tissue (Black Arrow) (H&E stain,X400).

Discussion

The kidney is the target of many xenobiotics toxicants. There are many factors that contribute to the sensitivity of the kidney via, presence of variety of metabolizing enzymes and xenobiotic transporters, large blood flow and concentration of solutes during urine production. Further physiological, anatomical, and biochemical features of the kidney make it particularly sensitive to many toxins and drugs (*21*). Present investigation was carried out with the aim of evaluating the possible role of selenium in modulating the *in vivo* toxicity and oxidative renal injury of diclofenac sodium consumption. In the present research no detectable histopathological lesions in kidney sections of rat treated only with selenium. While, many histopathological lesions in glomeruli and in convoluted tubules were detected in diclofenac sodium only treated group.

The glomerulus is the initial site of exposure to chemicals in the nephron, and various nephrotoxic substances produce lesions on this location. In some cases, the chemical change glomerular permeability to proteins by altering the size and charge-selective functions. Circulating immune complexes can be trapped within the glomerulus which may result in complement activation, attraction of neutrophils and phagocytosis. Neutrophils and macrophages are commonly seen in the glomerulus in membranous glomerulonephritis, and the local release of cytokines and

reactive oxygen species may contribute to glomerular injury (*22*). A previous study found that massive areas offibrosis and ongoing cell apoptosis. Glomerular affection in the form of thickening of glomerular membrane (*23, 24*).

Current findings were in parallel with resent study found that diclofenac sodium altered the regular morphology of the cortical layer of renal tissue, observed astubular cell dilatation together with flattening of the renal epithelium and disruption of thebrush borders in the proximal convoluted tubes, as well as deformation in glomeruli shape and Bowman's capsule injury(*25*). Alterations in histology of kidney may refer that diclofenac sodium caused significant increase in the plasma levels of creatinine and urea and activities of liver enzymes, including bilirubin level, pro-inflammatory markers, and plasma prostaglandin E2. It also caused significant alteration in renal and hepatic prostaglandin E2, antioxidants, lipid per-oxidation (malondialdehyde), andhematological indices. These toxic effects were confirmed by histological studies and levels of inflammatory infiltration (myelo-peroxidase) (*9*). A previous study showed that selenium protects against inflammatory indices in rat and It can reduce oxidative stress- and inflammation-related consequences of renal IR (*17*). The potential reno-protective properties of green biosynthesized lycopene-coated selenium on renal damage relative to the sole treatment with either sodium selenite or lycopene-coated selenium via assessment of oxidative stress, inflammation, apoptotic, and necroptotic signaling pathways in rats (*26*). Seleno-protein protects cells against oxidative stress–induced damages (*27*) . More than 30 seleno-proteins have been identified (*28*). One of the predominant selenoprotein families critical for human health are the glutathione peroxidase (GPx) enzymes. Of the 7 characterized GPx enzymes, 5 are selenium dependent (*12*) .

Conclusion

According to the findings of this study, it can be concluded that administration of diclofenac sodium for 21 days can lead to renal disorder in rat. The Co-administration of selenium with diclofenac sodium partially resulted in decreased of renal injuries caused by diclofenac sodium exposure.

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الدور الوقائي للسيلينيوم ضد ديكلوفيناك الصوديوم المسبب للسمية الكلوية في إناث الجرذان

انصاف حسن عبدالواحد ، سماح عبدالسلام خليفة ، مرفوعة صالح علي و نورا إبراهيم الزاعل

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المستخلص العربي

السيلينيوم هو معدن أساسي موجود في التربة والماء وبعض الأطعمة. يزيد من التأثيرات المضادة للأكسدة في الجسم. يعتبر ديكلوفيناك الصوديوم أحد أكثر العقاقير غير الستيرويدية المضادة للالتهابات شيوعًا في العالم. هدفت هذه الدراسة إلى تقييم التغيرات النسيجية التي يسببها ديكلوفيناك الصوديوم على التغيرات النسيجية في كلية أنثى الجرذ البالغة وتقييم الدور التحسيني المحتمل للسيلينيوم. تم تقسيم اناث الجرذان البالغة من العمر 4 أشهر والتي تزن 140-235 جم بالتساوي إلى أربع مجموعات. تلقت المجموعة الأولى الماء المقطر فقط واعتبرت كمجموعة ضابطة. تلقت المجموعة الثانية من الحيوانات السيلينيوم بجرعة 0.25 مجم / كجم من وزن الجسم عن طريق الفم يوميًا لمدة 21 يومًا. تلقت المجموعة الثالثة ديكلوفيناك الصوديوم بجرعة 10 ملجم / كجم من وزن الجسم عن طريق الفم يوميًا لمدة 14 يومًا. وأعطيت المجموعة الرابعة من الحيوانات نفس جرعة السيلينيوم لمدة 7 أيام، خلال الأسبوعين التاليين، وأعطيت الحيوانات نفس جرعة ديكلوفيناك الصوديوم. اظهرت الفحوصات النسيجية أن إعطاء ديكلوفيناك الصوديوم سبب تلف للكبيبة والأنابيب الكلوية مع وجود تجويف وتورم للنسيج الطلائي المبطن للأنابيب، وتسلل الخلايا الالتهابية ونزيف في النسيج الخلالي ، ونخر أو تقشر بطانة النسيج الطلائي للأنابيب الكلوية، انكماش الكبيبات لوحظ في بعض الكبيبات الكلوية مع اتساع المساحة البولية، ظهرت أيضا العديد من الكبيبات مع تراكيب خلوية أقل، كما لوحظ تمددًا واحتقان للأوعية الدموية. على النقيض من ذلك فتناول السيلينيوم مع ديكلوفيناك الصوديوم أظهر دورا محسن في الأنسجة الكلوية لمجموعة الجرذان التي تلقت ديكلوفيناك الصوديوم.

الكلمات المفتاحية: السيلينيوم ، ديكلوفيناك الصوديوم ، أمراض أنسجة ،الكلى ، أنثى الجرذ