JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2015. Vol. **9**(3), p. 1805-1816

Selenium – Induced Protection against Citrinin Nephrotoxicity Associated with the Use of Contaminated Dry Black Lemon Extracts in Male Rats

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(Received: 22 April 2015; accepted: 09 June 2015)

Citrinin is a nephrotoxic mycotoxin produced by several fungal strains belonging to the genera *Penicillium*, *Aspergillus*, and *Monascus.* The aim of this study was to determine the protective effect of Se in citrinin-induced nephrotoxicity in male rats. Fifty white male Wistar Lewis rats were divided randomly into 5 groups. Group G1 was daily gavaged with distilled water. Group G2 daily gavaged with a citrinin (10mg/Kg) for 2 weeks. Group G3 daily gavaged with a soup dried black lemon extract 2ml/kg (3.4mg/Kg) B.W for 2 weeks. Group G4 was gavaged 2ml/kg (3.4mg/Kg) B.W of soup dried black lemon extract and fed diets containing (0.04 mg/Kg) selenium for 2 weeks. Group G5 was fed diets containing (0.04 mg/Kg) selenium for 2 weeks. Serum from all groups were collected to measure several biochemical indicators to assess kidney function, such as urea (BUN) and creatinine (SCr). The results of this study revealed that G2 and G3 induced renal dysfunction as reflected by a significant elevation in serum levels of urea, creatinine , total antioxidant status and tumor markers (AFP,CEA and PKM2). Histological changes of G2 group revealed damaged cell. Whereas the histological changes of G3 group revealed the kidney tubules markedly affected .Treating male Wistar Lewis rats, with selenium antagonized the effects induced by Citrinin, and increase in total antioxidant status and decrease in tumor markers (AFP,CEA and PKM2). In addition to the positive impact on the shape of the cell. This study revealed the use of selenium has the ability of protecting kidney dysfunction induced by citrinin.

Key words: Citrinin, *P. citrinum,* Selenium, creatinin, urea, nephrotoxicity, rats.

Inadequately stored products and agricultural by-products exposed to high humidity and high temperatures simplify the development of fungi. The presence of these microorganisms, in addition to damaging the products, reduces their quality and favors the development of mycotoxins (Pitt, 2000).

Maycotoxins are fungal toxic agents, which are metabolites able to affect the functions of numerous tissues and organs such as kidneys, intestines and the immune system (Maresca and Fantini, 2010). Consumption of a mycotoxin-contaminated diet may induce acute and long-term chronic effects in animals and humans, resulting in teratogenic, carcinogenic and oestrogenic or immune suppressive effects (Pestka, 2007). Direct consequences of consumption of mycotoxin-contaminated animal feed include: reduced feed intake, feed refusal, poor feed conversion, diminished body weight gain, increased incidence of disease (due to immune-suppression) and reduced reproductive capacity which lead to economic losses (Binder *et al*., 2007). The most prevalent toxigenic fungi belong to the genera *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria*. These fungi pose serious

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phytopathological and mycotoxicological risks at preharvest and postharvest stages, as well as in processed food products (Castori a*et al.*, 2008). The toxins produced by *Penicillium* species can be placed in two general groups: those that affect the liver and kidney function, and those that are neurotoxic. The *Penicillium* toxins that affect liver or kidney function are asymptomatic or cause generalized debility in humans or animals while the neurotoxins are characterized by sustained trembling (USDA, 2006).

The mycotoxincitrinin(4, 6-dihydro-8-hydroxy-3, 4, 5-trimethyl-6-oxo-3H-2-benzopyran-7-crboxylic acid) is a toxic secondary metabolite, first isolated from filamentous fungus *Penicillium citrinum* (Hetherington and Raistrick, 1931). Citrinin was investigated as an antibiotic (Wong and Koehler, 1981), but relative toxicity studies showed that this secondary metabolite acted in animals as a nephrotoxin(Betina, 1989), damaged the proximal tubules of the kidney (Phillips *et al*., 1980), and was implicated as a potential causative agent in human endemica Balkan nephropathy (IARC, 1986). Citrinin contamination by *P. citrinum* has been reported in agricultural commodities, food, feedstuffs and it has been implicated in mycotoxic nephropaty (Xu *et al*., 2006). Citrinin found as a natural contaminant of maize (Nelson *et al*., 1985), wheat, rye, barley, oats (Scott *et al*., 1972), rice (Tanaka *et al*., 2007), and dry lemon (Elsawi *et al*., 2012). Thus, citrinin is a potentially important mycotoxin that may be ingested by humans and other animals and could cause chronic disease (Hald and Krogh 1973).

The mycotoxin citrinin is produced by a variety species of *Penicillium* and *Aspergillus*. The most important fungus is *P. citrinum*, which was reported as one of the causative fungi of " yellow rice toxicosis " in Japan (Saito *et al*., 1971). Other *Penicillium* species that produce citrinin include P. *fellutanum, P. lividum, P. implicatum, P. jeneni, P. citreoviride, P. steckni, P. expansum, P. velutinum, P. canescens, P. notatum, P. viridicatum, P. palitans* and *P. claviforme*. The aspergilli that produce citrinin include *A. terreus, A. niveus, A. candidus, Crotolaria crispate* and *Blennoria* spp (Deshpande, 2002).

Citrinin is not mutagenic in conventional bacterial assays, either with or without metabolic activation by the S9 fraction from rat or human liver or rat kidney It is not carcinogenic according to recent knowledge. The International Agency for Research on Cancer (IARC, 1986) concluded that there was limited evidence for the carcinogenicity of citrinin to experimental animals and that no evaluation could be made of the carcinogenicity of citrinin to humans. Citrinin is classified in group 3 (not classifiable as to its carcinogenicity to humans) (Knasmüller *et al*., 2004).

The mean oral lethal dose (LD50) of citrinin for rats is 50 mg/ kg B.W. (Sakai, 1955), while subcutaneous LD50 is 67 mg/ kg B.W. (Ambrose and DeEds, 1945). The subcutaneous treatment of pregnant rats with 35 mg/ kg on days 6, 9, and 10 of pregnancy resulted in 50 % or higher maternal mortality (Reddy *et al*., 1982). Oral LD50 for Dutch Belted rabbit is 134 mg/ kg, and in the New Zealand White rabbit it is about 120 mg kg^{-1} (Hanika*et al*., 1984). Acute lethal doses administered to rabbits, guinea pigs, rats, and swine caused swelling of the kidneys and acute tubular necrosis (Krogh *et al*., 1973).

Citrinin induces mitochondrial permeability pore opening (Da Lozzo *et al*., 1998) and inhibits respiration by interfering with complex I of the respiratory chain (Chagas *et al*., 1995). Antioxidants are of great importance in the protection against mycotoxins (Galvano *et al*., 2001). Selenium and vitamin E are key ingredients of food and they play the role in antioxidative protection. Vitamin E, as an integral component of lipid membrane, neutralizes free radicals. It represents the first line of cell's defence against free radicals and it is the keeper of cell's integrity. Selenium plays its role through the enzyme GSH-Px. The level of this enzyme increases in plasma along with the increase of the concentration of selenium in food or water, what is a good indicator of biological adoption of selenium (Mihailovie *et al*., 1991).

The effects of citrinin contaminated dry lemon extract on some biochemical parameters and histological changes on kidneys of male Wister Lewis rats has been examined *in vivo* study. Elsawi *et al*. (2012) concluded the use of dry lemon as a traditional food supplement in the Gulf region may pose some risk of food poisoning due to the presence of citrinin. The present study was designed to determine the protective effect of

selenium (Se) in citrinin contaminated dry lemon extract induced oxidative stress and nephrotoxicity in rats.

MATERIALS AND METHODS

Fifty white male Wistar Lewis rats weighs (220 - 250 g) were obtained from the animal facility of King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Saudi Arabia. Dried black lemon purchased from local market that made traditionally. Dry black lemon which use as an appetite enhancer in Saudi Arabia have been found residues of citrininmycotoxins (Elsawi *et al*., 2012).

Extraction of cirinin for thin layer chromatography (TLC)

Citrinin extracted according to method of Molinie *et al*. 2005. It was extracted from crashed dry black lemon (50 g) with 100 ml chloroform and 50 ml water. To the chloroform extract, 150 ml 0.1M Na HCO_3 were added. The Na HCO_3 solution was acidified to PH 1.5 with concentrated HCLand extracted three times with chloroform. The chloroform phases were evaporated to near dryness in a 40 °C water bath at low speed, protecting the flask from light. Two ml of methanol were added and finally evaporated to dryness in an air. The Citrinin readily crystallized from hot absolute methanol, and yields were determined by weight.

Citrinin was monitored by comparison with an authentic sample by using TLC plates. The TLC plates were impregnated with oxalic acid by dipping them in an oxalic acid – methanol solution before spotting the extract concentrate. The plate was developed in mobile phase condition chloroform / methanol (9:1 v/v). After development, the plate was observed under UV light.

Preparation of soup extract

Whole dried black lemons (local market grade) weighed about 150 g were homogenize and soak in 450 ml warm water, under agitation at 100 rpm for 5 min and kept in refrigerator for later use (Elsawi *et al*., 2012).

All rats were subjected to the following schedule of treatments: Group G1 was daily gavaged with distilled water. Group G2 daily gavaged with a citrinin (10mg/Kg) for 2 weeks. Group G3 daily gavaged with a soup dried black lemon extract 2ml/kg (3.4mg/Kg) B.W for 2 weeks. Group G4 was gavaged 2ml/kg (3.4mg/Kg) B.W of soup dried black lemon extract and fed diets containing (0.04 mg/Kg) selenium for 2 weeks. Group G5 was fed diets containing (0.04 mg/Kg) selenium for 2 weeks.

Biochemical Studies

Urea has been determined according to the modified procedure of Talk and Schubert (1965). The urea ($H_2NCONH_{2)}$ reacted with water (H_2O) in the presence of urease enzyme to produce ammonia (NH3), then ammonia react with specific indicator to produce dye. The reflection density of the dye is measured and is proportional to the concentration of urea in the sample. The results calculated automatically by the analyzer. Determination of Serum Creatinine Concentration was by using The VITROS CREA Slide method. Serum Total Antioxidant Status (TAS) Concentration has been determined by kits which was purchased from *Randox laboratories Ltd.*, Serum, Alpha Fetoprotein (AFP) has been determined by kits which was purchased from *Uscn life Sience Inc. Wuhan-China*. Serum Carcinoembryonic Antigen (CEA) has been determined according to the method reported by Munjal *et al*. 1984. This kit was purchased from *Uscn life Sience Inc*. Serum Pyruvate Kinase isoenzyme type M2 (M2PK) has been determined by kits which was purchased from*Uscn life Sience Inc. Wuhan-China*.

Histological Studies

For histological studies, the animals were killed by cervical decapitation and dissected. The abdomen and thorax were opened. The two kidneys were removed, cut sagittally and re-fixed in the same fixation for 24 hours until processing for 5µm paraffin section. The slides were stained with haematoxylin and eosin. The procedures of preparation and staining were done according to (Drury and Wallington, 1980). Stained sections were examined and photographed using digital camera, attached to Olympus CX51 light microscope and connected to computer.

Statistical Analysis

Statistical analysis were performed using Microsoft office excel (2007) and origin professional 8 program. The variability degree of the results is expressed as mean ± standard error of means (Mean ± SE). The significance of the difference between samples was determined using

one way ANOVA (SPSS V16). The difference was regarded as significant when $P \leq 0.05$, and non-significant when $P > 0.05$, where P is a value for comparing between groups.

RESULTS

Thin Layer Chromatography (TLC)

Citrinin was detected by Thin Layer Chromatography (TLC) in dried black lemon (Fig. 1) and its extract was found to contain citrinin which was detected using TLC (R_f = 0.546).The amount of citrinin was extracted from 50 grams of dry black lemon = 80 mg

Effect of lemon extract on biochemical characters of treated Rats

Blood Urea Nitrogen (BUN) and Serum creatinine levels (SCr)

Table (1) show the effect of selenium supplemented on kidney function in rats treatment with citrinin. The mean values of BUN and SCrin the G2 and G3 groups were higher than that of the G1 group. The differences were significant at (P<0.005), when compared to the G1.

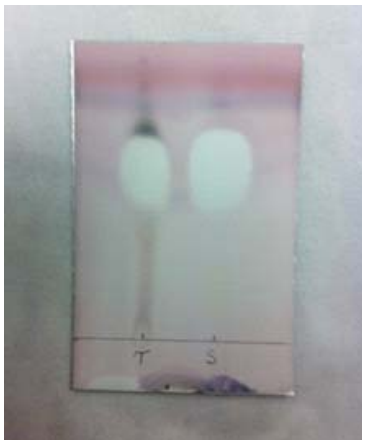
Total antioxidants stress (TAS)

The data in Table (1) indicate that the mean values of TAS in the G2 group were lower than that of the G1 group). In G4, the mean values of TAS were higher than that of the G2 and G3 groups. The differences were non significant at (P<0.005) when compared to the G1. While the mean values of TAS in G5 were higher than that of the G1 group and the G2 and G3. However, differences werenon significant at (P<0.005) when compared to the G1.

Tumor markers

Serum α fetoprotein (AFP)

Table (1) represented the mean values of AFP in the G2 and G3 groups were higher than that of the G1 group. The differences weresignificant at (P<0.005) when compared to the G1. In G4, the mean values of AFP were lower than that of the G2 and G3 groups. The differences weresignificant at (P<0.005) when compared to the G1.



T: test sample (dried lemon), S : standard citrinin

Fig. 1. Thin Layer Chromatography (TLC).

Table 1. Effects of selenium (0.04 mg/kg/day) on the levels of blood urea nitrogen, serum creatinine and some tumor markers analysis inrats treated with citrinin.

GroupsParameters	G1	G2	G3	G4	G5
BUNmg/dl	9.26±0.42	22.37[a]±0.37	12.91[a,b]±0.70	11.90[b]±0.46	11.62[b]±0.13
SCrmg/dl	77.30± 5.45	104.12±1.68	112.40[a]±5.51	103.62[a]±2.58	96.20±1.05
TASmmol/l	1.55±0.24	1.53[c]±0.08	2.10[b,c]±0.14	3.59[b]±0.14	4.40[a]±0.25
AFPng/ml	0.015±0.001	0.032±0.001	0. 037±0.006	0.024±0.006	0.022±0.003
CEAng/ml	2.32±0.14	3.17[d]±0.14	3.13±0.26	1.82±0.16	1.96±0.22
M2PKu/ml	1.46±0.15	2.84±0.25	3.13±0.78	0.87±0.02	0.75±0.06

G1= Control group
G2= Citrinin Group
G3= Soup extract group
G4= Soup extract + selenium food group
G5= Selenium food group

BUN= blood urea nitrogen
SCr= creatinine
TAS= Total antioxidants stress
AFP= α fetoprotein
CEA= Carcinoembryonic Antigen
M2PK= Pyruvate kinase isoenzyme type M2

Values are expressed as mean value of ± S.E (one – away ANOVA)
a: very highly significant at 0.1% (P< 0.001) from G1; b:very highly significant at 0.1% (P< 0.001) from G2; c:very highly significant at 0.1% (P< 0.001) from G3.

Serum Carcinoembryonic Antigen (CEA)

The mean values of CEA in the G2 and G3 groups were higher than that of the G1 group, Table (1). The differences werenon significant at (P<0.005) when compared to the G1. In G4 and, the mean values of CEA were lower than that of the G2 and G3 groups. The differences werenon significant at (P<0.005) when compared to the G1. While the mean values of CEA in G5 were lower than that of the G1, G2 and G3. However, differences werenon significant at (P<0.005) when compared to the G1.

Pyruvate kinase isoenzyme type M2 (M2PK)

The mean values of M2PK in the G2 and G3 groups were higher than that of the G1 group. The differences weresignificant at (P<0.005) when compared to the G1. In G4, the mean values of M2PK were lower than that of the G2 and G3 groups. The differences weresignificant at (P<0.005) when compared to the G1. While the mean values of M2PK in G5 were lower than that of the G1, G2 and G3 groups. However, differences weresignificant at (P<0.005) when compared to the G1.

Histopathological investigations

The histological examination of kidney sections of the control animals (Fig. 2 A-B) shows Bowman's capsule surrounded renal Glomerulus (G), and separated by a distance of Urinary space (US), also showing Proximal tubule (PT) and Distal tubule (DT). Kidney of the G2 group which was

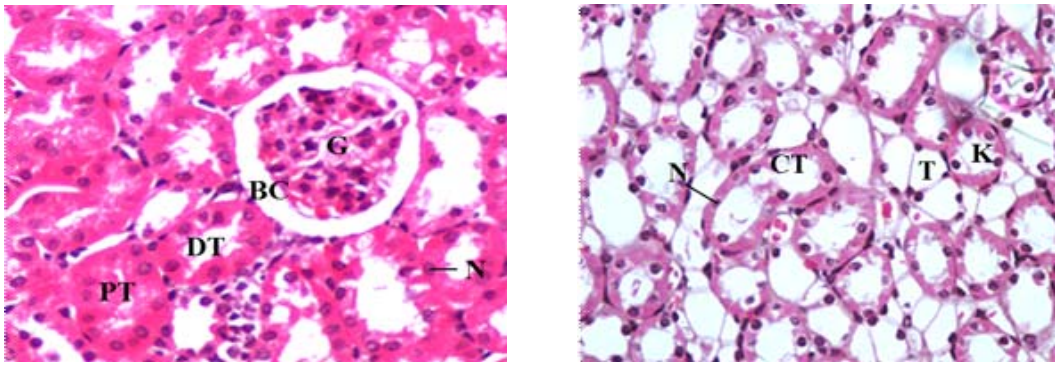


Fig. 2. Section of Control Rat Kidney A: the slide showing Bowman's capsule surrounded renal (G) Glomerulus, and separated by a distance of Urinary space (US), also showing (PT) Proximal tubule and (DT) Distal tubule. N: the nuclei of cells lining the proximal convoluted tubules. B: the slide showing Collecting tubule (CT), and Thin limb of Henle Loop (T), and Thick limb of Henle Loop (K) . N: the nuclei of cells lining the proximal convoluted tubules. (H &E X100)

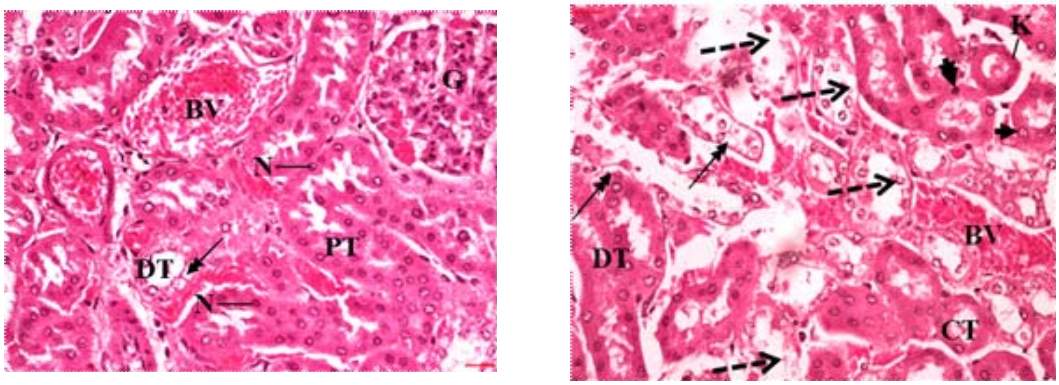


Fig. 3. Section of Citrinine group in Rat Kidney: A: the slide showing the cytoplasm free of dye Empty looking cytoplasm (double arrow), and show a renal (G) Glomerulus, and nuclei (N) Nucleus and (PT) Proximal tubule, and coiled distal (DT) Distal tubule, and a blood vessel (BV). B: the slide showing crash cells Damaged cell (shares), combining nuclear chromatin behind the nuclear Nuclear Margination (arrowhead), and Nuclear pyknosis (the top of a double arrow), and the cytoplasm free of dye Empty looking cytoplasm (double arrow) , showing (DT) Distal tubule, thick limb of Henle Loop (K), and Collecting tubule (CT), and a blood vessel (BV). (H &E X100)

regularly supplied by citrinin shows damaged cell, combining nuclear chromatin behind the nuclear (Nuclear Margination), and Nuclear pyknosis , and the cytoplasm free of dye (Fig. 3 A-B).

The rat kidneys of the G3 group which was regularly supplied by 2ml/Kg (3.4mg/Kg) of body weight of soup extract shows Hyaline cast, and cells Inflammatory cell, and Nuclear pyknosis, collect nuclear material behind the nuclear envelope: Nuclear Margination (Fig. 4 A-B).

On the other hand, the histological examination of kidney sections which represents the renal of G4 treated group shows part damaged cell, also show some Collecting tubule (CT) padded cell with cytoplasm free of dye (Fig. 5 A-B). The rat kidneys of the G5 group represents the renal of selenium (0.04 mg/Kg) treated group showing histological natural part of the cortex by Bowman's capsule (BC) surrounded renal Glomerulus (G), also Proximal tubule (PT) and Distal tubule (DT) (Fig. 6 A-B).

DISCUSSION

Citrinin is one of the first isolated mycotoxins; however, the data on the mechanism of its toxicity are still controversial and most have been obtained *in vitro*. The major target organ of citrinin toxicity is the kidney (Gupta *et al*., 1983). Nephrotoxic effects have been reported for citrinin include reduced glomerular filtration rate and renal blood flow resulting in increased blood urea

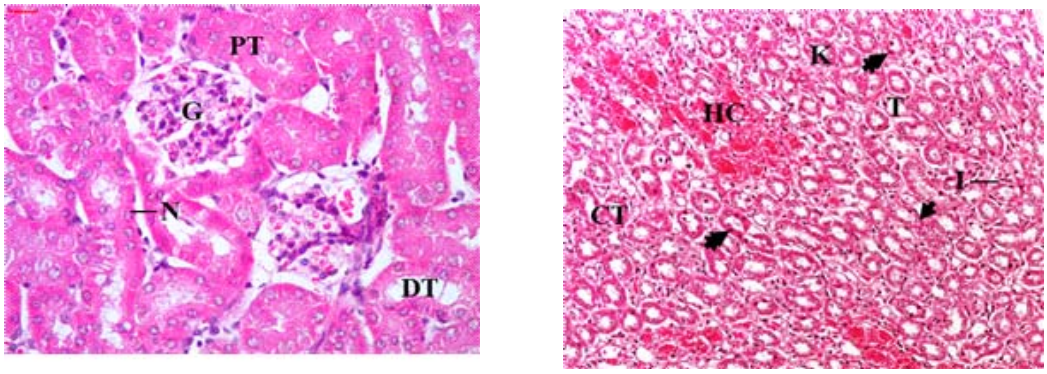


Fig. 4. Section of soup extract group in Rat Kidney. A: the slide showing a renal Glomerulus (G), and (PT) Proximal tubule, and (DT) Distal tubule and nuclei (N) Nucleus. B: the slide showing Hyaline cast (HC), and cells Inflammatory cell (I), and Nuclear pyknosis (arrowhead double), collect nuclear material behind the nuclear envelope: Nuclear Margination (arrowhead), as they appear (DT) Distal tubule, and thick limb of Henle Loop (K) and Thin limb of Henle Loop (T), and Collecting tubule (CT).(H &E X100)

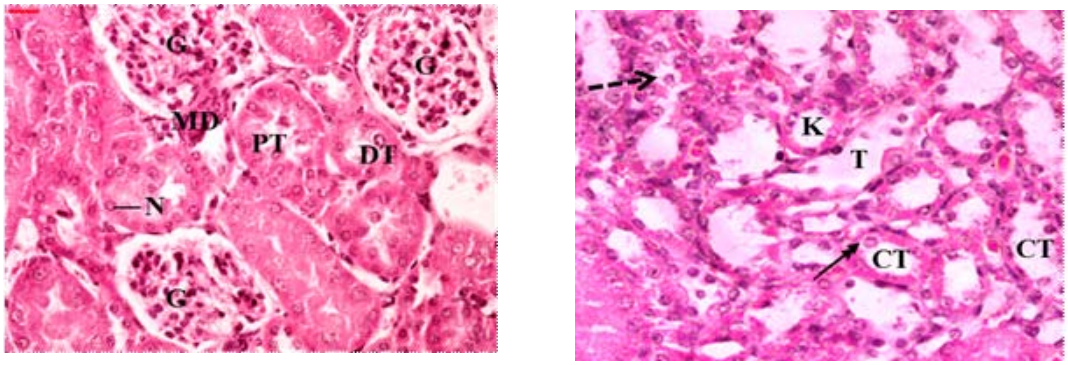


Fig. 5. Section of soup extract + Selenium in Rat Kidney. A: the slide showing a radar installation for the crust closer to the natural location. Note the presence of renal kippah (G) Glomerulus, and wrapped (PT) Proximal tubule and (DT) Distal tubule, as they appear dense spot Macula densa (MD). B:the slide showing part Damaged cell (shares), also show some Alonbebat Collecting tubule (CT) padded cell with cytoplasm free of Empty looking cytoplasm (double arrow). And Thin limb of Henle Loop and , K: Thick limb of Henle Loop.(H &E X100)

nitrogen concentration, urinary lactic acid dehydrogenase, aspartate amino transferase, and isocitric dehydrogenase activities, as well as proteinuria, glucosuria, lowered urinary specific gravity, and the presence of necrotic cells in the urinary sediment (Kogika *et al*., 1996).

Previous studies reported a protective effect of Se on mycotoxins. Se is shown to have protective effects against T-2 toxicity (Shokri *et al.*, 2000), to decrease damaging effects of Alphatoxin B1(AFB1) (Choi *et al.*, 1995) and to prevent Deoxynivalenol (DON) toxicosis (Atroshi *et al.*, 1997).

The first acute renal marker is urea, which increase when the kidney undergo any kind of injury. Further the very high significant increase in the levels of urea and creatinine , which are indicators of impaired renal function (Dobson *et al*., 2008).

In the present study, the serum BUN and SCr levels were significantly increased 2 weeks after administration of G2 and G3 groups compared to the control group (G1). Elevated BUN usually indicates glomerular damage. Whereas creatinine is a metabolite of creatine and is excreted completely in the urine *via* glomerular filtration, an elevation of its level in the blood is an reference to impaired kidney function (El-Shenawy *et al*., 2008).

Previous studies reported a change of kidney function tests upon exposure to mycotoxins. El-Sawi *et al*. study (2001) reported an alteration of creatinine in mice exposed to Verrucarian J mycotoxins. An increase in SCr and BUN were observed by Edrington *et al.* (1995) in lambs and by Bucci *et al.* (1998) in rats exposed to fumonisins mycotoxins.

In the present study, citrinin-induced increase in SCr and BUN levels was significantly blocked by Se administration in (G4) compared to G3. The protective effect of Se on creatinine and urea concentrations can be predicated to its antioxidant properties, as it has been found that ROS may be involved in the impairment of GFR (Pedraza-Chaverri *et al*., 2000). This result is in agreement with Randjelovic *et al*. (2012) that showed the Gentamicin -induced increase in serum creatinine and urea levels was significantly blocked by Se administration.

In the current study, serum concentration of the TAS was decreased in G2 compared to the control(G1). This reduce was most probably point to existence of an oxidative stress due to mycotoxicty (Johannessen *et al.*, 2007). This result is in agreement with Wojtowicz-Chomicz *et al*. (2011) that showed a significant decrease in TAS in rat blood is related to oxidative stress induced by AFB1 mycotoxin. However, serum concentration of the TAS was increased in G3 compared to the control(G1). The results could indicate the presence of oxidant/antioxidant imbalance due to citrinin which causes increased production of ROS and activation of antioxidant systems of cells. Similar finding was observed by Capcarova *et al*. (2014) who found that total antioxidant stress of porcine

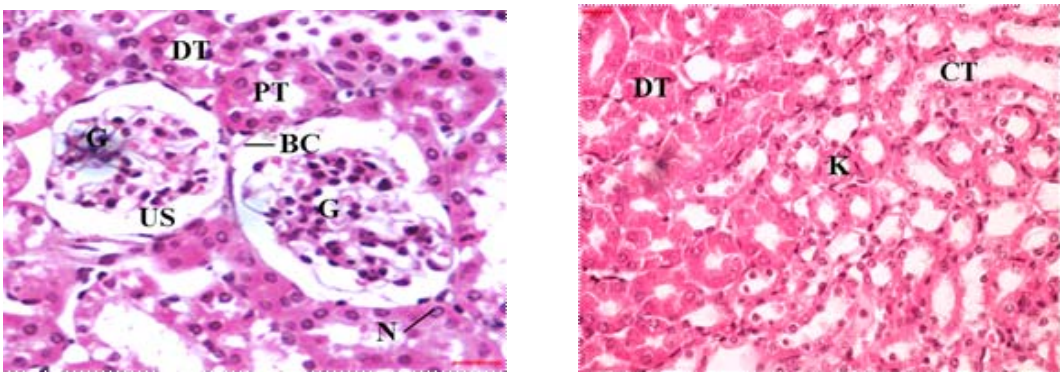


Fig. 6. Section of Selenium group in Rat Kidney. A: the slide showing Histological natural part of the cortex by Bowman's capsule (BC) Bowman's capsule surrounded renal (G) Glomerulus, also (PT) Proximal tubule and (DT) Distal tubule. N: the nuclei of cells lining the proximal convoluted tubules. B: the slide showing a portion of the end zone the crust and (DT) Distal tubule, and another part of the area of the bone and Thick limb of Henle Loop (K) look like a normal synthesis and Collecting tubule (CT).(H &E X100)

granulosa cells exposed to mycotoxins *in vitro* was increased against the control. This results was disagree with another study of Elsawi *et al*., 2012 that TAS concentration has decreased significantly after 2 weeks after administration of black lemon soup extract. However, Se supplementation for 2 weeks to rats pretreated with soup extract showed an increase TAS compared to those fed only soup extract. The pronounced increase in serum concentration of the TAS was detected after consumption of Se in diet in G5. These results may be due to ability of Se to enhance antioxidant ability by enhancing the activities of antioxidant enzymes and by increasing contents of the antioxidants. Xia *et al*. (2003) reported that Se is critical in several enzymes with physiological antioxidant properties, including GSHPx and thioredoxin reductase. GSHPx scavenges H_2O_2 and lipid hydroperoxides, using reducing equivalents from glutathione and protecting membrane lipids and macromolecules from oxidative damage (Watanabe *et al*., 1997). So, Se could be useful as a free radical scavenger compound against stress conditions in several tissues, including the kidney.

Serum α fetoprotein (AFP) and Serum Carcinoembryonic Antigen (CEA)are considered specific biomarkers for liver cancer and they are synthesized mainly in the fetal stage; practically no production of these markers occurs in the normal adult. However, when some adult cells are transformed to cancer cells, the synthesis of AFP and CEA commences again (Ersoy, 2005). The level of AFP and CEA in the current study was increased after 2 weeks in G2 and G3 compared to the control group (G1). It is well documented that citrinin is a potent hepatonephrotoxic (Berndt, 1990). Serum elevation of AFP has been reported in renal cell carcinoma (Aoki *et al*., 2001). However, in the present study, no signs of precancerous lesion or cancer can be detected by light microscopic studies in kidneys of G2 and G3 treated groups. May if the duration increase than 2 weeks may will lead to renal cell carcinoma. Identical result was showned by Sell *et al.* (1998), Abdel-Wahhab*et al.* (2006) who found that aflatoxins feeding to ducks for longer term and rats for 6 days produced an elevation in the level of serum AFP. In Al-Seeni *et al*. study (2011) that observed Zearalenone feeding to mice for two weeks produced an increased in the level of serum AFP. However, Se supplementation for 2 weeks to rats pretreated in G4 and G5 showed an decreased AFP and CEA compared to G3. These results may be due to anticancer properties of Se. These protective effects of Se seem to be primarily associated with its presence in the glutathione peroxidases, which are known to protect DNA and other cellular components from damage by oxygen radicals (Schrauzer, 2000).

In the present study, serum concentration of PKM2 increased 2 weeks in G2 and G3 compared to the control group (G1). These results were in agreement with the results obtained by Mazurek *et al*., 2005) who reported that increased aerobic glycolysis is one of the most common metabolic abnormalities occurring in tumour cells. Proliferating cells and particularly tumour cells express the PKM2, which acts as an enzymatically highly active tetramere in proliferating non-tumour cells. High plasma Tumor M2-PK level was recently shown to correlate with various malignant diseases, e.g., renal cell carcinoma, gastrointestinal cancer, and colon, breast, and lung cancers (Ugurel *et al*., 2005). The decrease in PKM2 was detected in G4. The improvement of the citrinin-induced isoenzyme M2- PK elevation could be attributed to the anticancer efficacy of Se.

Histological study of kidney may explain the above biochemical changes compared to control. The kidneys of the G1 group showed normal histological features but the G2 group marked crash cells (damaged cell), combining nuclear chromatin behind the nuclear margination, and nuclear pyknosis, and the cytoplasm free of dye Empty looking cytoplasm. The kidneys of the G3 group showed hyaline cast, and inflammatory cells, and nuclear pyknosis, collect nuclear material behind the nuclear envelope: Nuclear Margination, as they appear distal tubule, and thick limb of henle loop and thin limb of henle loop, and collecting tubule. Renal tubules especially distal were more affected than filtration apparatus (glomerular capillaries). The toxic chemical substances including mycotoxins (Bokhari and Ali, 2008) were excreted via distal tubules resulting in degenerative necrotic changes and this what was observed here where marked tubular necrosis was found to be associated with presence of hyaline casts cells inflammatory cell, and nuclear pyknosis. Citrinin has been suggested as a causative factor in renal

disease and death among livestock, poultry and perhaps humans. It is commonly found in field Samples along with ochratoxin A. these two mycotoxins have been suggested as the cause of porcine nephropathy in Denmark (Elling and Moller, 1973).

Tubules filled with eosinophilic (hyaline) proteinaceous casts generally indicate that there is increased glomerular permeability in that nephron and are often associated with glomerular damage. However, large amounts of protein within tubules can induce damage to the surrounding tubular epithelium as well. Casts are a common feature accompanying chronic nephropathies in rats and mice and their number increases with advancing age (Alden, 1986).

The functional changes of citrinin are associated with acute tubular necrosis. The location of the most severe tubular damage varies from species to species. In the mouse (Jordan and Carlton, 1977) and in hamsters (Jordan *et al*., 1978b) the distal portion of the nephron is mostly affected. Despite this, the specific pathologies resulting from acute citrinin exposure are remarkably similar in all species. Initial pathological observations report the kidneys of acutely exposed mice to be swollen and pale, with stippling of the capsule cortex and outer medulla (Hanika and Carlton, 1983).

Mehdi *et al*. (1981) observed degeneration and necrosis of tubular epithelial cells of both proximal and distal convoluted tubules in broiler chicks administered with 95 mg citrinin/kg body weight by crop gavages or mixed with diet. Degenerative and necrotic changes were reported in the distal convoluted tubules, loop of Henle and in a few proximal convoluted tubules. Acidophilic granules in the cytoplasm and pyknotic nuclei in some of the cells were also noticed. Maryamma*et al*. (1990) could notice disruption of the cytoplasmic membrane and desquamation of cells into the lumen in several foci after 4, 8 and 12 h of feeding citrinin as crop intubation at the rate of 125 mg/kg body weight. Uma and Reddy (1995) also observed similar changes in broiler chicken fed with 125 and 250 ppm citrinin for six weeks.

The kidney tubules changes were considerably mild in the G4 group that showed part damaged cell, also show some Alonbebat collecting tubule padded cell with cytoplasm free of Empty looking cytoplasm. Thus, the improvement of the citrinin-induced histological alterations could be attributed to the antioxidant efficacy of Se.

The nephropathy produced in swine by citrinin and ochratoxin has structural and functional similarities to the fatal human renal disorder, endemic Balkan nephropathy, prevalent in the Danube River basin in Yugoslavia, Bulgaria and Rumania. The kidneys are unquestionably a primary site of action of citrinin (Phillips *et al.*, 1980). No signs of malignant changes were observed in kidney tissues with all doses and this explains the biochemical data regarding tumor biomarkers. On the other hand, it rather caused necrotic tubular changes which points that the present dose was of toxic potential rather than carcinogenic. Histological studies made by Shinohara *et al.* 1976 on the nephrotoxic effect of citrinin on the kidneys of rats showed that administration of 0.02% or 0.05% citrinin alone caused signs of kidney injury but did not induce kidney tumors. However, they added that citrinin in combination with NDPS N-(3,5-dichlorophenyl) succinimide (NDPS) and N-nitrosodimethylamine (DMN) can induce kidney tumor in rats, which was renal cell tumor (adenoma). Their results pointed that citinin can potentiate the carcinogenic effect of some chemicals.

In concolusion, based on previous studies, citrinin considered as carcinogenic. The majority of biochemical as well as histological results observed in the present study indicated that citrinin could have potential nephrotoxic rather than carcinogenic effect in rats.A dose of 3.4mg/Kg of citrinincaused nephrotoxicity as revealed by the elevation in kidney function parameters and the histopathogical signs. While, daily consumption of 0.04mg/Kg Se had protective effects on the kidney structure and function against citrinin toxicity. These protective effects of the Se may be attributed to antioxidant properties. So, the current study recommends the avoidance of food contamination with citrinin because it has many adverse effects on health. Therefore, Se can be used to protect animals and humans against the adverse health effect of citrinin toxicity.

ACKNOWLEDGMENTS

This work was supported by King abdulaziz City for Science and Technology (KACST) [grant number 89-35 AS].

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