Nephrotoxicity Associated with the Use of Contaminated Dry Lemon Extract in Male Rats

Nagwa M. Elsawi[1], Eman A. Al-Muhaini[2], Safaa Y. Qusti[2], Ahmed N. Abo-Khatwa[2], Magda M. Aly [3] and Sabry H. H. Younes[1]

[1]Chemistry Department, Faculty of Science, Sohag University, Sohag 82524, Egypt.
[2]Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
[3]Biology Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
elsawinagwa@yahoo.com

Abstract: The contamination of food and feedstuff with mycotoxins represents worldwide problem for both humans and animals. Citrinin, one of the well known mycotoxins which cause renal disease and death among livestock, poultry and perhaps humans as well since it is commonly found in food samples. Nephrotoxicity is produced in swine by feeding grain contaminated with *Penicillium. citrinum*. This study deals with the effect of dry lemon extract on some biochemical parameters and histological changes on kidneys of male "Wister Lewis rats" after being found to be moldy and having residues of citrinin mycotoxin. Fifty inbred weaned white male "Wistar Lewis rats" were divided randomly into 5 groups (10 rats each). One control group was daily gavaged with distilled water and four treated groups were daily gavaged with a soup lemon extract (2 ml/kg B.W) for 2 weeks (T1), double the dose (T2), triple the dose (T3). Group T4 was gavaged (2 ml/kg B.W) of yellow lemon soup for 2 weeks. Sera from all groups were collected to measure several biochemical indicators to assess kidney function, such as urea (BUN) and creatinine (SCr). Serum BUN increased significantly in all treated groups as compared to control. In addition, serum SCr increased significantly in all treated groups as compared to the control. On the other hand, total antioxidant concentration was significantly lower in all treated groups as compared to the positive control. However, alpha fetoprotein and carcinoembrionic antigen remained unchanged while pyruvate kinase isoenzyme M2 was decreased significantly. Histopathological changes of rat kidney revealed tubular degenerative changes in (T3) which explain biochemical changes. In conclusion, 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.
[Nagwa M. Elsawi, Eman A. Al-Muhaini, Safaa Y. Qusti, Ahmed N. Abo-Khatwa, Magda M. Aly and Sabry H. H. Younes. **Nephrotoxicity Associated With the Use of Contaminated Dry Lemon Extract in Male Rats.** *J Am Sci* 2012;8(10):480-489]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 70

Key words: Citrinin**,** *P. citrinum*, creatinine**,** rat**,** urea**,** Nephrotoxicity,

1. Introduction

Dried lemons are commonly used as food flavoring supplements in the making of many Arabian dishes, such as rice and soups. It gives food a spicy and lemony flavor therefore; it is used as an appetite enhancer. Petterson (2004) reported that moulds can grow and produce mycotoxins in plant material during the whole chain from field to table. More often, under certain drying and storing conditions, some molds can grow on the surface of these fruits. Some of these molds can produce mycotoxins during growth, which may pose a potential health hazards to consumers. Diets in many developing countries are based on crops susceptible to mycotoxins, leading to high levels of chronic health problems in tropical and developing countries (Visconti and Perrone, 2008). Mycotoxins are secondary metabolites produced by certain filamentous fungi, which can be produced in foods because of fungal growth (Sweeney and Dobson, 1998). Scientific and medical literature has increased the awareness of mycotoxin producing fungi as causing mycotoxicosis and possibly other human diseases (Mazur and Kim, 2006).

The mycotoxigenic fungi involved with the human food chain belong mainly to three genera: *Aspergillus, Fusarium and Penicillium*. While *Fusarium* species are destructive plant pathogens producing mycotoxins before, or immediately post harvesting, *Penicillium and Aspergillus* species are more commonly found as contaminants of commodities and foods during drying and subsequent storage (Sweeney and Dobson, 1998). *Penicillium* is a large genus with over 150 recognized species and at least 50 species of common occurrence. The discovery of penicillin in 1929 gave impetus to a search for other Penicillium metabolites with antibiotic properties. Nearly 100 Penicillium species have been reported as toxin producers. The mycotoxins produced by *Penicillium* species are potentially significant to human health: citreoviridin, citrinin, cyclopiazonic acid, ochratoxin A, patulin, penitrem A, Roquefortine C and Secalonic acid D. (USDA, 2006). While the most important Penicillium mycotoxins are: Ochratoxin A, Patulin and Citrinin.

Citrinin is a fungal metabolite that has been known since 1931, when it was isolated from

Penicillium citrinun (Hetherington and Raistrick 1931) and later from the leaves of the Australian plant Crotolaria crisbata (Ewart, 1933). Eight years later it was characterized as an anti-bacterial antibiotic (Raistrick and Smith, 1941) and later tested for activity against bacteriophages, sarcoma tissues, protozoa, animal cells and higher plant cells (Robinson and Park, 1966). The antifungal properties of citrinin have been described (Robinson and Park, 1966; Betina and Ruckova, 1972). Citrinin was also implicated in porcine nephropathy (Krogh et al., 1970) and it has been found as a natural contaminant of corn, rice, wheat, rye, barley, oats and decaying tomato fruit (Mislivec and Ruite, 1970; Harwig et al., 1979). Thus, citrinin is a potentially important mycotoxin that may be ingested by humans and animals and could cause chronic disease (Hald and Krogh, 1973).

Citrinin (4,6-dihydro-8-hydroxy-3, 4, 5-trimethyl-6-oxo-3H-2-benzopyran-7-crboxylic acid) (Fig. 1) is produced by several fungi e.g. Aspergillus laceae and Penicillium citrinum (Hetherington and Raistrick, 1931).

Citrinin is commonly found in field samples along with Ochratoxin A. These two mycotoxins have been suggested as the cause of porcine nephropathy in Denmark and nephrotoxicity was produced in swine by feeding grain contaminated with P. citrinum. Swine fed on moldy batches of barley, Ochratoxin–contaminated feed or crystalline citrinin developed nephrotoxicity characteristic of porcine nephropathy. Acutely lethal doses of citrinin administered to rabbits, guinea pigs, rats and swine caused swelling of the kidneys and acute tubular necrosis. 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 is unquestionably a primary site of action of Citrinin (Phillips et al.,1979) and in addition, it impairs liver metabolism (Ramadoss and Shanmugasundaram, 1973), binds to serum protein in vitro (Damodaran, 1977) and has mutagenic properties (Stark et al.,1980). As already been mentioned, citrinin has phytotoxic properties (Mirchink et al., 1967; Damodaran et al., 1975).

Citrinin is produced in liquid media under static or submerged conditions and on cereals or fruits. Curtis et al. (1968) found that, cultivated Penicillium citrinum in a medium containing sucrose, potassium hydrogen phosphate and corn steep liquor (50% dry weight - 20 g /L) was produced the highest citrinin level. Moreover, Betina et al. (1973) observed that the highest citrinin level was achieved by P. janthinellum in submerged culture.

The present study was designed to investigate the effects of contaminated dry lemon extract on some biochemical parameters and histological changes on kidneys of male Wister Lewis rats. The wide distribution of the frequency of the damaged level resulting from cytotoxicity in host response to citrinin and whether this citrinin can cause nephrotoxicity in the kidneys of male rats was also determined.

2. Materials and Methods

Fifty white male Wistar Lewis rats weighs (180-200 g) were obtained from the animal facility of King Fahd Medical Research Center, King Abdul-Aziz University, and Jeddah, Saudi Arabia. The dried lemon that used in this study were collected from two sources: Dried lemon purchased from local market in the Saudi Arabia that made traditionally (Fig. 1) and homemade dried lemon, prepared by leaving them at room temperature opposite to an open window, naturally without any additives or any special precautions for 3-4 months. The two types were tested to investigate any fungal contamination that could produce mycotoxin products.

Fungi isolation from dried lemon

The two types of lemon fruits were crashed and 0.25g of the each powder was soaked in 5 ml sterile water for 15 minutes, then 1 ml of each suspension were spread on Potato Dextrose Agar (PDA) Petri dish which were incubated at 37°C for 24-48 hours. The obtained fungal colonies were purified on the same medium and the mycotoxin production by each colony was detected.

Preparation of oral feeding material (lemon extract)

The lemon extracts of the two tested lemon (Rationally prepared and homemade) were prepared and sterilized using 0.45 µm blue bacterial filters. Three doses of traditional made dried lemon extract (100%, 75% and 50 %) were prepared and compared with homemade lemon extract. All extracts were kept in refrigerator for later use. Animals were divided randomly into five groups (10 rats each group). For rats feeding, 2ml/kg body weight was given for each rat; this volume was calculated by using a conversion factor that equivalent to man (Goodman et al.,1992). All rats were subjected to the following schedule of treatments: Control group (C): Rats were fed daily by oral gavage with 2 ml/kg B.W. of water for 2 weeks before dissection. Treated group 1 (T1): Rats were fed daily with 2 ml/kg of 100% lemon extract. Treated group 2 (T2): Rats were fed daily with 2 ml/kg of 75% lemon extract. Treated group 3 (T3): Rats were fed daily with 2 ml/kg of 50% lemon extract. Treated group 4 (T4): Rats were fed daily with 2ml/kg of 100% homemade lemon extract .

Biochemical studies

Urea has been determined according to the modified procedure of Talk and Schubert (1965). This assay was carried on VITROS BUN/UREA Slide method using the VITROS BUN/UREA Slides. Urea (H_2NCONH_2) reacted with water (H_2O) in the presence of urease enzyme to produce ammonia (NH_3), 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. Creatnine has been determined using VITROS CREA Slide method. Total antioxidant status has been determined by kits which was purchased from Randox laboratories Ltd. Alpha Fetoprotein (AFP) has been determined by kit was purchased from Uscn life Science 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 Science Inc. Wuhan-China. Serum Pyruvate Kinase isoenzyme type M2 (M2PK) has been determined by kit purchased from Uscn life Science Inc., Wuhan-China.

Determination of citrinin by Thin Layer Chromatography (TLC)

This method was used for determination of citrinin in dried lemon (before cooking), Dried-boiled lemon (cooked) and lemon extract.

The lemon was initially crashed, and the powder then extracted with aqueous acidified acetonitrile solution, the resulting solution was partially purified by several partition steps, and then concentrated. The thin layer chromatography (TLC) plates were impregnated with oxalic acid by dipping them in an oxalic acid-methanol solution before spotting the extract concentrate. Each type of lemon extract has been concentrated by freeze drying and then the concentrate was loaded on the TLC plates.

Histological studies

For histological studies, the treated and control 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 connected to computer.

Statistical Analysis

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.

3- Results

Using Thin Layer Chromatography (TLC) dried black lemon collected from Jeddah market and its extract was found to contain citrinin which was extracted and detected using TLC (RF= 0.42) as shown in table (1). On the other hand, dried yellow lemon kept in a controlled clean area was proved to be free of fungal growth using Potato Dextrose Agar media and mycotoxin citrinin

Effect of lemon extract on biochemical characters of treated rats

Blood urea nitrogen (BUN)

The data in Table 2 indicate that serum blood nitrogen levels increased significantly ($P \leq 0.05$) for all four treated groups (T1, T2, T3 and T4) after 2 weeks as compared to control group.

Serum creatinine levels (SCr)

Table 2 represented serum creatinine levels (mg/dL) which indicating a significant increase ($P \leq 0.05$) in the mean value of treated groups (T1, T2, T3 and T4) after 2 weeks compared with control group.

Total antioxidants stress (TAS)

Analysis of total antioxidant concentration in sera of both treated and control groups was shown in Table 2 which revealed that TAS concentration has decreased significantly ($P \leq 0.05$) after 2 weeks of the four treated groups (T1, T2, T3 and T4) as compared with control group.

Serum α fetoprotein (AFP)

Table 2 indicated that α fetoprotein levels (ng/ml) in serum have no significant changes in the mean value ($P > 0.05$) of treated groups after 2 weeks (T1, T2, T3 and T4) as compared to control group.

Serum Carcinoembryonic Antigen (CEA)

No significant change in the level of serum carcinoembryonic antigen of all treated groups was observed as shown in Table 2 ($P > 0.05$) when compared to control group.

Pyruvate kinase isoenzyme type M2 (M2PK)

Pyruvate kinase isoenzyme type M2 concentration (U/ml) in serum is shown in Table 2, which indicate a significant decrease ($P \leq 0.05$) in the mean value of all treated groups after 2 weeks as compared to control group.

2- Histological result

Control animals

Examination of paraffin sections stained with haematoxylin, eosin from the kidney of control animals revealed a normal structure of rat kidney. It was formed of well known outer cortex containing

the renal corpuscles and renal tubules (mainly proximal and distal) and an inner medulla with the rest of renal tubules (Loop of Henele and collecting ducts) as shown in Fig.4.

Group 1 (T1)

In rat treated with 2ml/kg B.W. of soup extract and sacrificed after 2 weeks, slight changes in kidney tubules were observed .The cells of some tubules showed accumulation of acidophilic intracellular substances. Some tubular lumina contain desquamated nuclei. Focal atrophy of few renal corpuscles was observed in Fig. 5.

Group 2 (T2)

After 2 weeks of 2 ml/kg B.W. of soup extract (double dose) similar focal atrophy of glomerular capillaries, accumulation of acidophilic material within tubular cells and desquamation of degenerated cells into tubular lumen were also observed in (Fig. 6).

Group 3 (T3)

After 2 weeks of 2ml/kg B.W. of soup extract (triple dose), marked degeneration (decrease in height and small dark nuclei) of renal tubular epithelium with the appearance of cellular or protein casts within the lumina were observed in Fig. 7.

Group 4 (T4)

The light microscopic examination of paraffin section of rat treated with 2ml/kg B.W yellow lemon for 2 weeks, showed slight disruption of renal tubules in the form of focal cellular degeneration and presence of nuclei within the lumina of affected tubules (Fig. 8).

4. Discussion

Food poisoning is the result of eating food stuffs contaminated with organisms or their toxins which caused by a wide range of toxigenic substances or toxins of pathogenic microorganisms, such as bacteria, protozoa, viruses, algae and fungi that may be present in food (McLaughlin J and Little, 2007). Mycotoxins are known to be secondary metabolites produced by many fungal species, such as poisonous mushrooms and certain filamentous fungi, which can grow and proliferate in stored food items (Michael et al., 1998). In present investigation citrinin was significantly high in dried black lemon compared with homemade yellow lemon extract. Prepared No previous literature was available regarding the presence of citrinin in citrus fruit including dried lemon. Thus, this study could be considered the first to investigate the presence of citinin in this variety of feed stuff commonly used as food flavor in gulf area. However, it was known that mycotoxins produced by many species of fungi were found to contaminate wide variety of feedstuff, subjected to bad storage or handlings (Bhatnagar et al., 2004).

In the presence study the effect of lemon extract containing citrinin on serum urea, creatinin, cancer marker (AFP, CEA, and M2PK) and Total antioxidants stress was studied in Wister Lewis male rats. Histological study of kidney parenchyma was also checked to evaluate citrinin nephrotoxicity.

Kidney plays an important role in the total body homeostasis. The importance of kidney functions include excretion of metabolic waste products and foreign chemicals, regulation of water and electrolyte balances, regulation of body fluid osmolarity and electrolyte concentrations, regulation of arterial pressure regulation of acid-base balance, secretion, metabolism and excretion of hormones and gluconeogenesis. A toxic insult to the kidney can disrupt any or all of these functions and result in acute or chronic toxic effect on body (Sebastian, 2007).

Concentration of toxic chemicals within the cells of renal epithelium, npredominantly the proximal tubules (PT), may result in selective renal toxicity. This based on presence of certain enzymatic pathways within the kidneys, involved in enzyme metabolism (Cai et al., 2009).

The kidney excretes urea, a product of protein metabolism. Urea concentration in the glomerular filtration is the same as plasma. Tubular reabsorption of urea varies inversely with the rate of urine flow. Thus, urea is less useful measure of glomerular filtration than is creatinine, which is not reabsorbed. Blood urea nitrogen (BUN) varies directly with protein and inversely with the rate of excretion of urea (El-Sawi et al., 2001).

In the present study, the urea serum level was significantly increased 2 weeks after administration of black lemon soup extract in group T1, T2, T3 and T4 compared to control. However, the mean value of urea serum level of T4 (yellow lemon) was decreased compared to treated T1 (black lemon) for the same dose.

The possible reasons could be referred to possible increase in nitrogen metabolism because of diminished renal blood flow or decreased glomerular filtration rate in kidney (El-Sawi et al., 2001). On the other hand, the mean value of serum urea level in T4 showed a significant decrease when compared to the T1 group. Yellow lemon used in this study was evaluated for fungal contamination and was found to be free from any fungal growth, and this explained the biochemical results observed herein.

Rats treated with black lemon extract showed a significant increase in creatinine (SCr) after 2 weeks in group T1, T2, T3 and T4 compared to control. The increased level of SCr may be due to acute renal insufficiency (Murray, 1991). No significant changes in the mean value of SCr were observed between T4

and T1 groups. An increase in creatinine (SCr) level was reported by many authors in most cases of mycotoxin nephrotoxicity (Bokhari and Ali, 2008).

Antioxidants neutralize free radicals and other reactive chemicals. They can act at any different stages on an oxidative sequence, such removing oxygen or decreasing local oxygen concentrations, removing catalytic metal ions, removing key reactive oxygen species (ROS) such as O_2 and H_2O_2. Antioxidants protection can operate at several different levels within cells (Gutteridge, 1995; Sies, 1995). The intracellular redox state is characterized by the balance of oxidant production and the antioxidant capacity of the cell based on a variety of antioxidants enzymes such as total antioxidants include superoxide dismutase (which reduce O_2 to H_2O_2), catalase and glutathione peroxidase (which reduce H_2O_2 to H_2O). Also, a part from antioxidant enzymes, all cells contain a variety of reducing substances, e.g. the vitamin C and E, lipoate, thiols, urate, ubiquinone, glutathione, thioredoxin and glutaredoxin which efficiently scavange ROS and the antioxidant enzymes balance the ratio of the concentration of oxidizing equivalents to the concentration of reducing equivalents (Gamaley and Klyubin, 1999).

In the current study, the concentration of the total antioxidant stress was decreased in T1, T2, T3 and T4 compared to the control. This decrease was most probably point to presence of an oxidative stress due to mycotoxicty (Johannessen et al., 2007). However, the mean value of total antioxidant was increased in T3 compared to T2 and T1. Possible increase in the activity of organs producing anti oxidant such as liver could be of concern as a reflection a body defense against oxidative toxicants (Harfenist and Murray, 1991).

Production of alpha fetoprotein (AFP) is common in hepatocellular carcinoma, hepatoma and liver metastasis. Some extrahepatic carcinoma including stomach (Libman et al., 1979; Unno et al., 2000), colon (Arnaud et al., 1978; Kurihara et al., 1997) and gallbladder (Laurent et al., 1999) have elevated AFP levels associated with poor prognosis or with undifferentiated foci of the carcinoma. Serum elevation of AFP has also been reported in renal cell carcinoma (Aoki et al., 2001).

In the present study the mean value of AFP concentration showed no significant differences in all groups compared to control group. This means that the soup extraction of black lemon which contains citrinin by analysis does not affect on AFP. The present results means that citrinin in dry lemon soup was not present in such a dose that induce any cancerous or precancerous changes and this was confirmed by histological studies discussed latter on.

Carcinoembryonic antigen (CEA) is a member of a family of cell surface glycoproteins that are produced in excess in essentially all human colon carcinomas and in a high proportion of carcinomas at many other sites. The function of this widely used tumor marker and its relevance to malignant transformation is therefore of considerable interest (Benchimol, 1989).

In the present results, serum CEA showed no significance difference in T1, T2, T3 and T4 compared to control group. Similar explanation to what was given regarding AFP could be reported here.

Pyruvate kinase isoenzymes normally exist as enzymatically highly active tetramers .Several isoforms of pyruvate kinase are tissue-specific: type L in liver and kidney; type R in erythrocytes; type

M1 in muscle, heart, and brain; and type M2 in lungs and kidneys as well as in undifferentiated and proliferating tissues. (mazurek et al., 2005).

In the present study, the concentration of M2PK was decreased in T1, T2 and T4 compared to control group this means that lemon soup extract has no effect on serum M2PK which go in hand with the previous two parameters AFP and CEA as tumor markers. It is well documented in literature that the first step during multi-step carcinogenesis is the loss of the tissue-specific isoenzymes, e.g. L-PK in liver and kidney and M1-PK in brain and muscle, followed by the subsequent expression of the M2-PK isoenzyme as was demonstrated for renal cell carcinoma (Brinck et al., 1994 ؛Wechsel et al., 1999). However, to confirm such relationship in the present study was difficult and needs further investigation.

The increasing in the mean value of M2PK in group T3 (triple dose) compared to group T2 and T1 could not be explained. The previous authors reported that this isoenzyme was overexpressed in later stages of carcinogenesis as demonstrated for renal cell carcinoma. However, to confirm such relationship an immunohistochemistry for renal cell carcinoma was needed.

Histological examination of kidney tissue from animals receiving black lemon extract revealed that the effect was dose dependant. Renal tubules especially distal were more affected than filtration apparatus (glomerular capillaries). It was well known that 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 degeneration was observed, tubular necrosis was found to be associated with presence of hyaline and cellular debris casts filling their the lumina. 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, 1973). Swine fed mouldy batches of barly ochratoxin–cotaminated feed or crystalline citrinin developed nephrotoxicity characteristic of porcine nephropathy (Phillips et al., 1979).

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., 1979. Chung et al., 2011). 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.

Both biochemical and histological studies confirmed the potential nephrotoxic effect of soup prepared by boiling dried lemon although the amount of mycotoxin content estimated in the present study was less compared to dried lemon. This could be explained in view of what was reported by LEE, et al., 2007 who reported that the citrinin level started to increase as the wet heat condition was increased to 90C°.and cytotoxicity was a result in its degradation into more toxic compounds. Trivedi et al., reported that the toxicity of wet heated citrinin evaluated by cytotoxicity assay was 10 fold higher on a weight basis than that of citrinin.

In conclusion, the majority of biochemical as well as histological results observed in the present study indicated that soup prepared from boiled dry lemon either black and yellow could have potential nephrotoxic rather than carcinogenic effect in rats. On another hand, it must put in concern, the route of administration as well as the dose could determine the potential effect of citrinin or citrinin contaminated feedstuff regarding the bioavialbilty of the mycotoxin.

Table 1. Toxicity and mycotoxin of dried lemon in addition to lemon extract

Sample	Toxicity (brine shrimp larvae)	Mycotoxin identified
Dried black lemon	100 %	Citrinin (127 µg/g)
Homemade lemon	90%	Citrinin (0.0 µ g/g)
Extract of black lemon	15%	Citrinin 5µg / 100 ml

Table 2. Effect of lemon extract (2 ml/Kg B.W) on blood urea nitrogen, serum creatinine and some tumor markers analysis.

Groups Parameters	Control	T1 group	T2 group	T3 group	T4 group
BUN mg/dl	25.37±0.71	32.35±0.41*	28.9±0.41*	33.65±0.81*	30.23±0.51*
SCr mg/dl	0.4± 0.021	0.5±0.021*	0.73±0.026*	0.58±0.025*	0.54±0.027*
TAS mmol/l	93.1±0.189	53.95±0.7*	28.738±0.81*	84.5±0.191*	47.49±0.39*
AFP ng/ml	0.722±0.025	0.663±0.026 N.S	0.695±0.019 N.S	0.652±0.007 N.S	0.683±0.02 N.S
CEA ng/ml	0.272±0.005	0.282±0.001 N.S	0.28±0.001 N.S	0.28±0 N.S	0.284±0.001 N.S
M2PK u/ml	25.996±1.04	21.08±0.41*	22.077±1.3*	28.203±0.6*	22.55±0.61*

Values are expressed as mean value of ± S.E, No. of samples of each group equal 10

* Significant ($P \leq 0.05$), N.S = Non significant ($P > 0.05$)

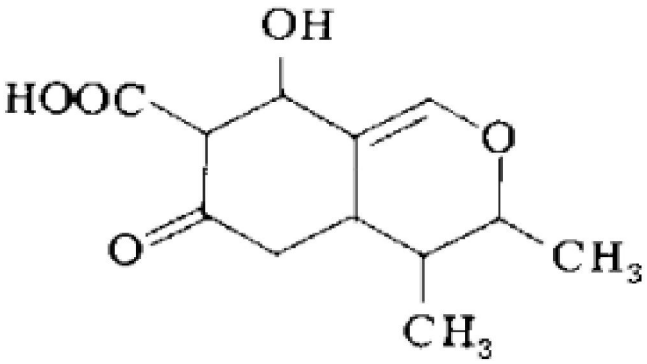


Fig. 1: Structure of Citrinin, 4, 6-dihydro-8-hydroxy-3, 4, 5-trimethyl-6-oxo-3H-2-benzopyran-7-crboxylic acid (Iwahashi et al., 2007)

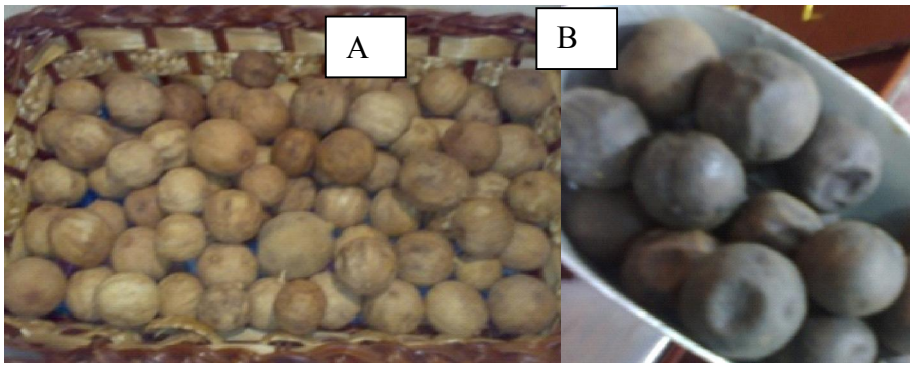


Fig.2. Homemade dried lemon (A) and black lemon obtained from Jeddah market (B)

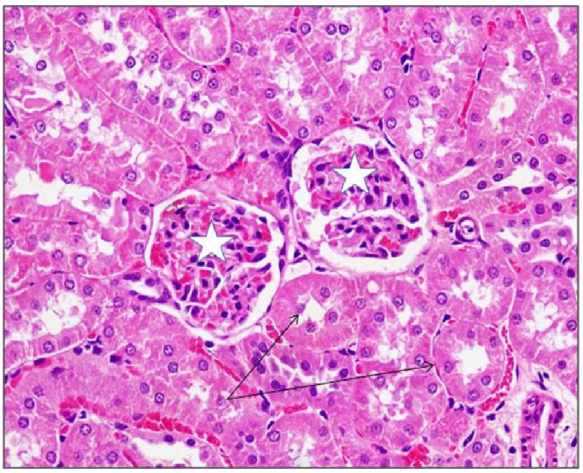


Fig. 4: Section of control rat kidney showing normal renal corpuscles (stars), and tubules (black arrows). (H&E, slide observed at 40X).

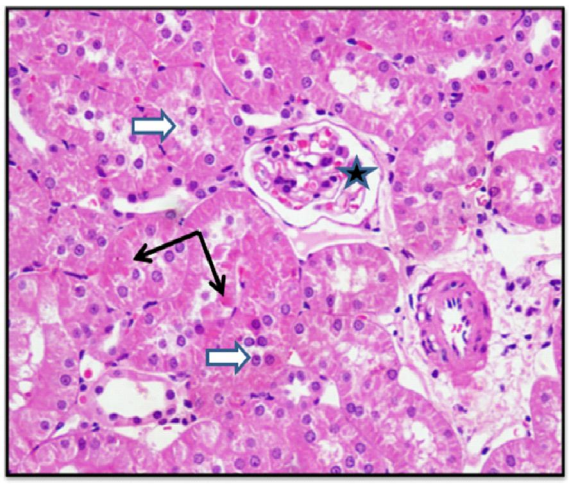
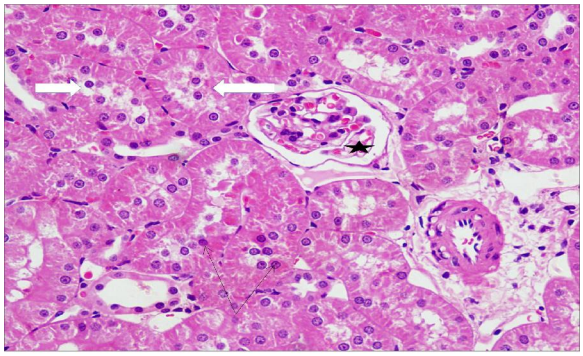


Fig. 5: Section in rat kidney of T1 showing slight changes in kidney tubules. The cell of some tubules showed acidophilic intracellular substances (arrows). Some tubular lumina contain desquamated nuclei (white arrows). Notice the focal atrophy of renal corpuscle (star). (H&E, slide observed at 40X)



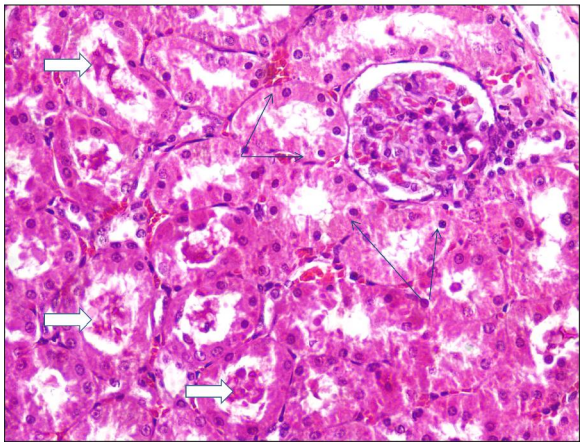


Fig. 7: section from rat kidney of T3 showing marked degeneration (decrease in height and small dark nuclei) of renal tubules with appearance of cellular protein casts or within the lumina (arrows). (H&E, slide observed at 40X).

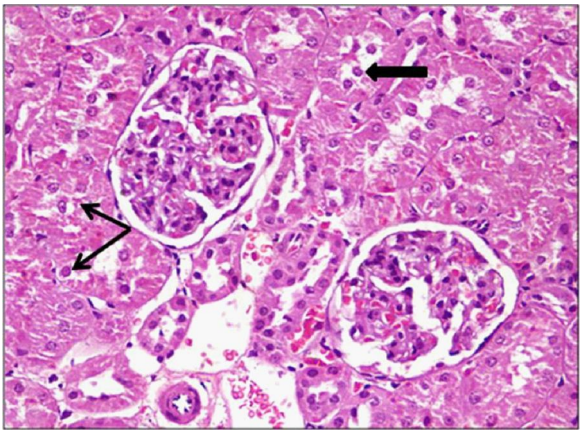


Fig. 8: Section from rat kidney of T4 showing slight disruption of renal tubules in the form of focal cellular degeneration (arrows) and presence of nuclei within nuclei (white arrows). (H&E, slide observed at 40X).

Corresponding authors
Nagwa M. Elsawi
Chemistry Department, Faculty of Science, Sohag University, Sohag 82524, Egypt.
Email: elsawinagwa@yahoo.com

References

Aoki, K., Nakanou, I., Takashima, K., Hirao, K., Hiramatsu, T., Fujimoto, K., Kagebayashi, Y., Ozono, S. and Hirao, Y. (2001). A case of renal cell carcinoma ptoducing alpha-fetoprotein, Hinyokika kiyo 47:477-480.

Arnaud, J. P., Isaac, J. P., Wanger, J. D. and Adolff, M. (1978). serum alpha fetoprotein ans extrahepatic digestive cancers. Apropos of 4 futher cases, J Chir (Paris) 115:591-594.

Benchimol, S., Fuks, A., Jothy, S., Beauchemin, N., Shirota, K. and Stanners, C. (1989). Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule Cell 57:327-334.

Betina, V. and Ruckova, E. (1972). Effects of Citrinin Desertomycin and of Polyene Antibiotics on Plant and Animal Cytoplasmic. Membranes Biologia (Bratislava). 27:463-468.

Betina, V., Balint, S., Hajnicka, V. and Nadova, A. (1973). Diphasic Production of Secondary. Metabolites hy Penicillum notatum WESTLING S-52 Folia Microbiol. 18:40-48.

Bhatnagar, D., Payne, G. A., Cleveland, T. E. and Robens, J. F. (2004). Mycotoxins: current issues in USA. In: Meeting the mycotoxin menace. Barug, D., Egmond, H. V., Lopez-Garcia, R., Osenbruggen, T. V. and Visconti, A., eds., Wageningen Academic Publishers, ISBN 9076998280: 17-47.

Bokhari, F and Ali, S. S. (2008). Ochratoxin A (OTA) contaminated Arabian Coffee seeds: Effects on liver and kidney functions and possible protective role of Vitamin C. Advances in Biolog. Rese. 2 (1-2): 17-25.

Brinck, U., Eigenbrodt, E., Oehmke, M., Mazurek, S. and Fischer, G. (1994). L- and M2-pyruvate kinase expression in renal cell carcinomas and their metastases. Virchows Arch. 424:177–185.

Cai, H., Agrawal, A. K., Putt, D. A. Hashim, M., Reddy, A., Brodfuehrer, J., Surendran, N., Lawrence, H. and Lash, L. H. (2009). Assessment of the renal toxicity of novel anti-inflammatory compounds using cynomolgus monkey and human kidney cells. Toxicology 258:56-63.

Curtis, R.F., Hassall, C.H. and Nazar, M. (1968). The biosynthesis of phenols. XV. Some metabolites of

Penicillium citrinum related to citrinin J. Chem. Soc, C. 85-93.

Damodaran, C. (1977). In vitro binding of citrinin to serum protein. Experientia. 33:598-599.

Drury N. and Wallington L (1980). Carleton's Histological Technique (seventh ed.), Drury R.A.B. and Wallington E.A. (eds) Oxford University Press, New York, Toronto.

Elling, F. & Moller, T. (1973) Mycotoxic nephropathy in pigs. Bull. Org. nond Sante, Bull. Wld Hlth Org. 49: 411-418.

El-Sawi N., Mohamed M., El-wessimy, M. M. and Youssef, O. A. (2001). Effect of Verrucarin J Toxin On Liver And Kidney Tissue of Male Mice, Texas J. SCI. 53(4):375-384.

Ewart, A.J. (1933). The presence of citrinin in Crotalaria crispate. Ann Bot. 47:913-915.

Gamaley, I. A. and Klyubin, I. V. (1999). Roles of reactive oxygen species: signaling and regulation of cellular functions. Int. Rev. Cytol., 188: 203-255.

Goodman, L. S. and Gilman, A. (1992). The pharmacological basis of therapeutics. 8th.ed.645,Macmillam publishing Co. Inc. New York.

Gutteridge, J. M. C. (1995). Signal messenger and trigger molecules from free radical reactions and other control by antioxidants. In: "Signaling mechanism-from transcription factors to oxidative stress". Packer, L. and Witz, K., eds., NATO ASI Series,Vol. H92, Springer-Verlag Berlin Heidelberg.

Hald, B. and Krogh, P. (1973). Analysis and chemical confirmation of citrinin in barley J. Ass. Offic. Anal. Chem. 56:1440-1443.

Harfenist, E. J. and Murray, R. K. (1991). Plasma proteins, immunoglobulins, and clotting factors. In: Harper's biochemistry, 22nd Edition, Appleton and Lange, Norwalk, Connecticut; Los Altos, California. pp., 611.

Harwig, J., Scott, P.M., Stolz, D.R. and Blanchfield, B.J. (1979). Toxins of molds from decaying tomato fruitAppl. Environ. Microbiol. 38:267-274.

Hetherington, A.C. and Raistrick, H. (1931). Studies in the Biochemistry of Micro-organisms. On the production and chemical constitution of a new yellow colouring matter, citrinin, produced from glucose by Penicillium citrinum. Trans. Roy. Soc., (London), B., 220, 269-295

Krogh, P., Hasselager, E. and Friis, P. (1970). Studies on fungal nephrotoxicity. Isolation of two nephrotoxic compounds from penicillium viridicatum Acta Pathol. Microbiol. Scand. Section B: 78:401-413.

Kurihara, K., Konishi, F., Kanazawa, K., Fujii, T. and Saito, K. (1997). Alpha fetoprotein producing carcinoma of the colon : report of acase, Surg Today 27:453-456.

Laurent, M. St., Esterl, Jr, R. M., Halff, A. G. and Speeg, K. V. (1999). Gallbladder carcinoma producing alpha fetoprotein, J Clin Gastroentrol., 28:155-158.

Libman, E., Lemberger, J. and Kollin, J. (1979). Alpha fetoprotein in the serum of patients with primary gastric cancer and liver metastases, Acta Hepatogastroenterol (Stuttg) 26:198-202.

Mazur, L.J. and Kim, J. (2006). Spectrum of noninfectous health effects from molds. Pediatrics. 18: 1909-1926.

Mazurek, S., Boschek, C.B., Hugo F. and Eigenbrodt, E. (2005). Pyruvate kinase type M2 and its role in tumor growth and spreading, Sem Cancer Biol. 15:300–308.

McLaughlin, J. and Little, C. (2007). Food poisoning and food hygiene. 7th edition. Hodder Arnold , animprint of Hodder education and a member of the Hodder Headline Group, an Hachette live UK company. London NW13BH. 59-153.

Michael, J., Alan, D. and Dobson, W. (1998). Mycotoxin production by Aspergillus, fusarium and Penicillium species. International Journal of Food Microbiology.(43) 3: 141-158.

Mirchink, T.G., Blagoveshchenskii, V.S. and Fedorov, V.A. (1967). Identification of the toxin formed by. Penicillium citrinum Microbiologiya. 36:1036-1040.

Mislivec, P.B. and Ruite, J. (1970). Species of Penicillium occurring in freshly-harvested and in stored dent corn kernels Mycologia. 62:67-74.

Munjal, D.D., Picken J. and Pritchard, J. (1984). Combined measurement and significance of lipid-bound sialic acid and carcinoembryonic antigen in detection of human cancer, Diagn. Immunol. 2:36–43.

Petterson, H. (2004). controlling mycotoxin in animal feed. In: Mycotoxins in food edited by: Magan, N. and Olsen, M. CRC Press. Woodhead publishing limited, Cambridge, England. 262.

Phillips, R.D.,Berndt, W.O. and Hayes, .W. (1979). Distribution and excretion of (14C) citrinin in rats. Toxicology. 12:285-298.

Raistrick, H. and Smith, G. (1941). Anti-bacterial substances from moulds Chem. Ind. London. 6:828-830.

Ramadoss, C.S. and Shanmugasundaram, E.R.B. (1973). Effect of citrinin on liver metabolism in rabbits Indian J. Biochem. Biophys. 10:296-297.

Robinson, P.M. and Park, D. (1966). Citrinin-a fungistatic antibiotic and narrowing factor. Nature. London. 211:883-884.

Sebastian, M. M., Baskin, S. and Czerwinski, S. E. (2007). In: Renal toxicity, Gupta, R. C. (ed.) Veterinary Toxicology. 161:176.

Sies, H. (1995). Signalling mechanism-from transcription factors to oxidative stress. In "Strategies of antioxidant defense:Relations to ixudative stress". Packer, L. and Wirtz K, eds., NATO ASI Series, Vol. H92, Springer-Verlag Berlin Heidelberg. pp., 165-186.

Stark, A.A. (1980). Mutagenicity and carcinogenicity of mycotoxins: DNA binding as a possible mode of action .Annu. Rev. Microbiol. 34:235-262.

Sweeney, M. j. and Dobson A. D.W. (1998). Review: Mycotoxin production by Aspergillus fusarium and Penicillium species. International Journal of Food Microbiology. 43:141–158.

United States Department of Agriculture. (2006). Fungi and Fungal Diseases of Plants. In: Grain Fungal Diseases and Mycotoxin Reference. Grain Inspection, Packers and Stockyards Administration.

Unno, J., Shirozaki, T., Inoue, K., Watanabe, Y., Arai, S. and Okuyama, H. (2000). A Case study of alpha fetoprotein (AFP) producing gastric carcinoma with multiple liver metastases, in which chemotherapy was effective enough to once save a life in rapid decline, Gan To Kagaku Ryoho 27:739-743.

Visconti, A. and Perrone, G. (2008). The EU mycoglobe project: global integration of mycotoxin and toxigenic fungal research for enhanced food safety. In: Mycotoxin: detection methods, management, public health and agriculture trade. Edited by: Leslie, J. F., Bandyopadhyayand, R. and Viscont, A. Cromwell press, UK. 3-4. Access date: January, 25, 2010.

Wechsel, H.W., Petri, E., Bichler, K.H. and Feil, G. (1999). Marker for renal cell carcinoma (RCC): the dimeric form of pyruvate kinase type M_2 (Tumor M2-PK). Anticancer Res. 19:2583–2590.

8/25/2012