Biochemical and Histological Studies on the effect of Zearalenone Mycotoxin and Thymoquinone on Male Mice Kidney

Nagwa Mohammed EL-Sawi[1], Madeha Nooh Al-Seeni [2], Sabry H. H. Younes[1], Salma Mohammed AL-Jahdali[2], and Soad Shaker Ali[3]

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

Abstract: ZEN was evaluated in male adult mice; given single and repeated doses of ZEN (2.7 mg/kg B.W). Also effect of Thymoquinone extracted from *Nigella Sativa* was assessed. Mice were divided into 4 groups. G1: receiving toxin once and dissected 48 hrs later, G2: given toxin twice a week for one week, G3: given toxin twice a week for 2 weeks, G4: pretreated o rally by Thymoquinone (10 mg/Kg B.W) for 7 days prior to administration of ZEN twice a week for two weeks. Blood serum urea nitrogen creatinine, alpha-fetoprotein, pyruvate kinase isoenzyme tumor M2 and total antioxidant status was assessed. All groups compared to control. The recovery occurred in TQ group based on changes in level of all groups when comparing with G3. Histological changes of mice kidney coincided with biochemical changes.

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1. Introduction

Alpha Zearalenol (α-ZOL) and β-Zearalenol (β-ZOL) (Figure 1) are considered the major metabolites of ZEN in various species, especially α-ZOL which is described as the major ZEN metabolite in rats and ruminants (Mirocha *et al*., 1981; Fitzpatrick *et al*., 1988). It has been suggested that the reduction of ZEN to α- and β-ZOLs occurs most actively in liver. This was demonstrated in many species such as rats (Ueno *et al*., 1977; Tashiro *et al*., 1983), swine (James and Smith, 1982), turkeys (Olsen *et al*., 1986) and hens (Danicke *et al*., 2002).

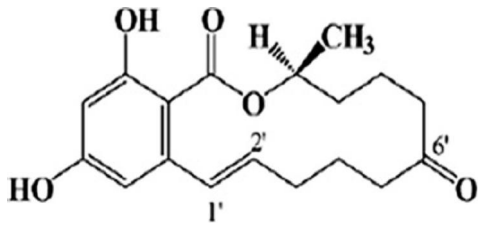

Figure 1: Chemical structure of Zearalenone (Zourgui, 2008)

Erythrocytes are also able to metabolize ZEN to α-ZOL and β-ZOL (Chang and Lin, 1984), as well as, intestinal mucosa and gut microflora (Biehl *et al*., 1993; Kollarczik *et al*., 1994).

Studies of elimination in various animal species indicated that α-and β-ZOL metabolites are excreted both as free compounds and as glucuronide conjugates *via* urine, but mainly *via* faeces (Olsen *et al*., 1985; Fitzpatrick *et al*., 1988; Danicke *et al*., 2001) . Likewise, extensive biliary excretion was observed in pigs (Biehl *et al*., 1993), cattle (Kennedy *et al*., 1998) and mice (Appelgren *et al*., 1982).

Nigella sativa (*N.S.*) L. seeds (Ranunculaceae) also known as black caraway seed and "the blessed seed". It is an annual herb, which grows in countries bordering the Mediterranean Sea, Pakistan and India (Figure 2A) (Ali and Blunden, 2003). It has been used for thousands of years as a spice and food preservative, as well as a protective and curative remedy for numerous disorders (Chopra *et al*., 1956; Nadkarni, 1976).

Thymoquinone (TQ) (Figure 2B) has been shown to attenuate a variety of renal toxicities that are the consequence of oxygen free radical damage, such as cisplatin-induced nephrotoxicity in rats and mice (Badary *et al*., 1997).

The aim of this study is to answer the following questions:

1. Does Zearalenone induce biochemical changes of kidney functions at the used dose level?
2. To what extent certain tumor markers are affected?
3. Is it possible to protect against the toxic action of Zearalenone by a prophylactic administration of a potential natural antidote, such as thymoquinone?

Figure 2: (A) The black seed herb and (B) its bioactive component thymoquinone (Gali-Muhtasibi *et al.*, 2006).

2. Materials and Methods

2.1. Materials

2.1.1 Zearalenone mycotoxin:

Zearalenone, from a *Fusarium* species (5 mg) was obtained from SIGMA-ALDRICH. It is a white powder, which was dissolved in DMSO (dimethyl sulfoxide) and diluted to 1:100 in a sterile saline solution (0.9 % NaCl) to produce a working stock stored at -20 °C (Hughes *et al.,* 1989).

2.1.2 Thymoquinone:

Thymoquinone from *Nigella Sativa* (28 mg) was obtained from FRINTON LABORATORIES, INC. It is a yellow powder, which was dissolved in DMSO and diluted to 1:100 in a sterile saline solution (0.9 % NaCl) to produce a working stock (Badary *et al.,* 2000).

2.1.3 Animals:

Inbreeding (8) weeks white male mice, Balb/C (70 animals); each weighs 20-30 g were obtained from King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. The animals were conditioned for two weeks at room temperature. A commercial balanced diet and tap water, *and labium* was provided throughout the experiment. The timing of blood sampling was selected after a preliminary study with Zearalenone showing that the maximum effect on kidney functions was two weeks after treatment**.**

Animals were divided into (4) groups (10 mice each), each group has its corresponding control. They were subjected to the following schedule of treatments:

Control of T1: which was gavage fed with single dose of 2.7 mg/kg B.W of DMSO saline solution and left for 48 h before dissection?

T1: was gavage fed with single dose of 2.7mg/kg Zearalenone (dissolved in 1% DMS saline)[21] and kept on normal feed for 48 h.

Control of T2: which was gavage fed with two doses of 2.7 mg/kg B.W of DMSO saline solution and left for one week before dissection?

T2: was gavage fed with two doses of2.7 mg/kg of Zearalenone and left for one week.

Control of T3: which was gavage fed with two doses (twice a week) of 2.7 mg/kg B.W of DMSO saline solution mg/kg B.W of DMSO saline solution and left for two weeks before dissection?

T3: was gavage fed with two doses (twice a week) of 2.7 mg/kg of Zearalenone and left for two weeks.

T4: was gavage fed with TQ (10 mg/kg B.W)[22] for 7 days prior to giving Zearalenone. Thereafter, they were gavages fed with two doses (twice a week) of 2.7 mg/kg B.W of Zearalenone and left for two weeks.

2.2. Methods

At the end of each specified period, mice were anesthetized using diethyl ether and blood samples were collected for biochemical assay (blood urea nitrogen, creatinine, Alpha-fetoprotein, Pyruvate kinase isoenzyme tumor M2 and total antioxidant status). Tissue samples from kidney was taken from all groups after saline and 10% neutral buffered formalin (NBF) intra-cardiac perfusion to ensure good tissue fixation. Small pieces of each organ (2×2 m) were refixed in 10% NBF processing for 5 um thick paraffin sections was preformed at King Abdul Aziz University Hospital (KAUH) pathology lab. and stained by Haematoxylin and eosin, PAS (periodic acid Schiff). Then the samples were examined by light microscope and photographed using digital camera.

3. Results

3.1. Effect of zearalenone (2.7 mg/kg B.W) on kidney function BUN concentration

The data in table **1** indicated that the mean value of serum BUN concentration (mmol/L) showed no significant difference ($P > 0.05$) in the mean value compared with control after 48 h. Highly significant increase ($P < 0.001$) in T2 (one week) compared to control was observed. Also, there was significant increase ($P < 0.05$) in T3 (two doses/ week /two weeks) compared to control. Highly significant

decrease was observed in T4 (TQ treated) compared to non treated group T3 (two weeks). In T4 (TQ) there was significant decrease in the mean value of BUN concentration compared with control.

3.2. CRE concentration

Table **1** showed that there was no significant difference (P> 0.05) after 48 h group in the mean value compared with control. Highly significant decrease (P<0.001) in the mean values of serum CRE (µmol/L) in T2 (one week) and T3 (two weeks) when compared with control were observed. In T4 (TQ), there was no significant difference compared to control. Highly significant increase (P<0.001) in the mean values of serum CRE (µmol/L) in T4 (TQ treated) compared to non treated T3 (two weeks).

3.3. AFP concentration

Table **1** showed that the mean value of AFP concentration (ng/L) in serum was significant increased (P<0.05) in T3 (two weeks) compared with controls. On the other hand, there was no significant difference (P > 0.05) after either 48 h for (T1) or one week for (T2) groups compared with control. In T4 (TQ) group significant decreased was observed compared to T3 group after (two weeks)

3.4. Tu M2_PK concentration

The mean value of Tu M2_PK concentration (U/ml) in serum was shown in table **1**. There was highly significant increase (P < 0.001) in T1 (48 h), T2 (one week) and T3 (two weeks) when compared with controls. Highly significant decrease (P <0.001) of Tu M2_PK concentration in T4 (TQ) group compared to T3 (two weeks).

3.5. TAS concentration

Analysis of TAS concentration (mM) in serum of both treated and control groups were shown in table **1**. It revealed significance increase (P <0.05) in the mean values in group T1 (48 h) and T3 (two weeks) when compared with controls. On the other hand, there was highly significant increase (P <0.001) in group T2 (one week) when compared with control. In T4 (TQ) there were no significant differences in TAS concentration compared to control. While, there was highly significant decrease in T4 (TQ) compared to T3 (two weeks).

4. Histological results

4.1. Control animals

Figure **3** revealed the normal structure of mouse kidney described in previous literatures. It was formed of an outer cortex and inner medulla. The former contains the renal corpuscles (Bowman capsule and glomerular capillaries) and the tubular elements. Proximal and distal tubules could be distinguished among the cortex by their characteristic features. Proximal tubules have narrow lumina. The cytoplasm of the lining cells (3-5 cells) were stained darkly acidophilic. The nuclei are rounded and vesicular. Distal tubules have wide lumina. The cells were shorter with rounded nuclei with highly acidophilic cytoplasm. In between the tubules thin walled blood capillaries could be seen. The inner kidney medulla was formed mainly of collecting ducts, besides the thin parts of loop of Henle together with their associated blood vessels giving the medulla a striated appearance.

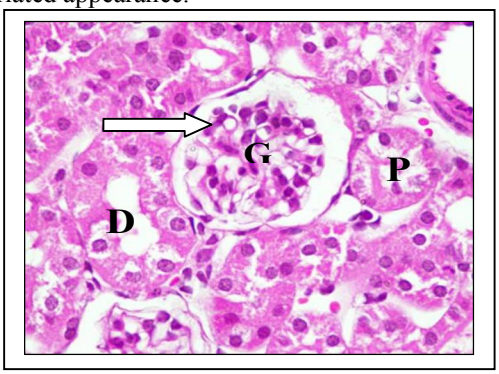

Figure 3: Photomicrograph of magnified part of control mice kidney showing: Normal architecture, renal corpuscle (arrow) with normal glomerular capillaries (GC), proximal tubules (PT) and Distal tubules (DT). (Hx & E X 600).

Table 1: Effect of zearalenone (2.7 mg/Kg B.W) on some biochemical parameters of male mice.

Parameter / Groups	BUN (mmol/L)	CRE (µmol/L)	AFP (ng/ml)	Tu M2_PK (U/ml)	TAS (mM)
C1 (48 hours)	9.543± .328	33.545± .813	.182±.0157	1.698±.126	.376±.003
T1 (48 hours)	10.785±.813 N.S	32.1±.589 N.S	.143±.021 N.S	20.376±.653 **	.462±.034 *
C2 (one week)	6.360±.068	19.575±.4535	.339±.036	.992±.048	.430±.009
T2 (one week)	6.852±.099 **	14.250±.258**	.490±.141 N.S	12.611±.636**	.507±.014**
C3 (two weeks)	8.760±.157	33.239±.462	.100±.000	1.795±.086	.519±.008
T3 (two weeks)	9.308±.191*	28.725±.372**	.640±.143*	6.284±.169**	.577±.022*
T4 (TQ+ two weeks)	8.318±.143 *, a**	32.325±.325 N.S, a**	.506±.133 , a**	3.938±.182 , a***	.481±.008 N.S, a**

Values are expressed as mean of 10 mice ± S.E C1, C2, C3 The control group with DMSO saline solution
T1, T2, T3 The treated group with zearalenone T4 The treated group with zearalenone + thymoquinone
** The highly significant (P<0.001) * The significant (P<0.05)
N.S The non significant (P > 0.05) a = T4 (TQ+two weeks) compared to T3 (two weeks)

Group T1 (2.7 mg/Kg B.W ZEN/48 hours)

Marked disruption of kidney parenchyma was observed. There was atrophy of glomerular capillaries, deformity, degeneration of tubular epithelial lining and intertubular haemorageic areas (Figure **4A**). Both tubular necroses with scattered apoptotic changes were observed (Figure **4B**). Degeneration of proximal tubular cells and dilation and cast deposition in distal tubules were the most evident changes (Figure **4C**).

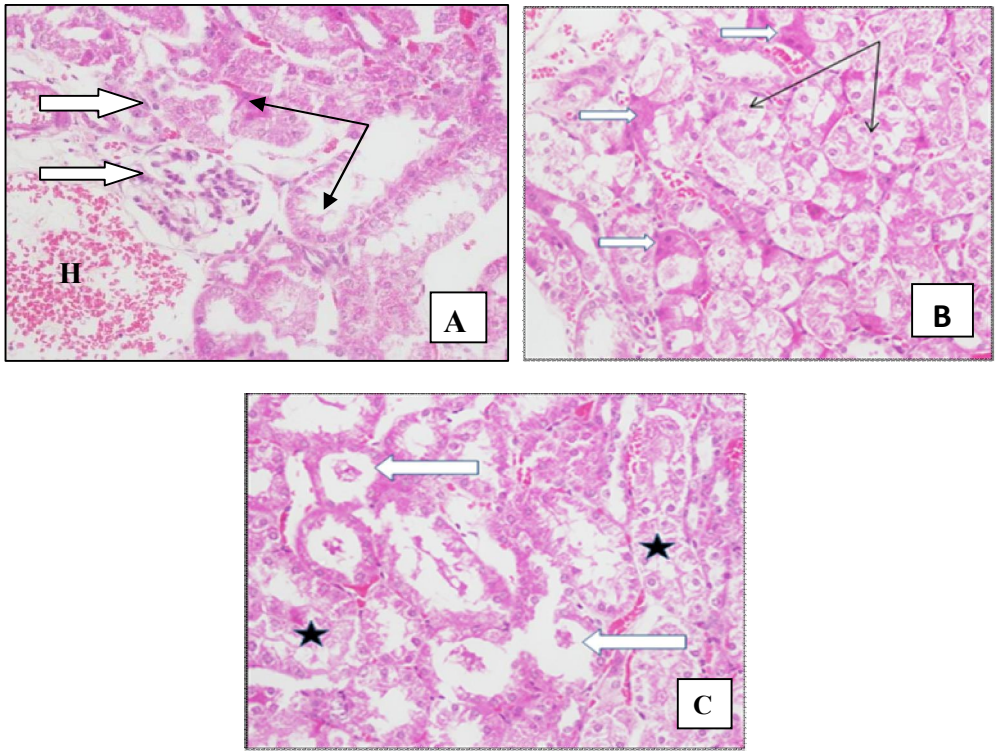

Figure 4: Photographs of magnified part of mice kidney in T1 (48 h after oral administration of ZEN) (2.7 mg/kg B.W)
Showing: Marked disruption of kidney parenchyma Notice

Fig. 4A Atrophy of glomerular capillaries (white arrow), deformity, degeneration of tubular epithelial lining (thin arrows) and intertubular haemorageic areas (H).

Fig. 4B Marked tubular necrosis (black arrows) and scattered apoptotic cells (white arrows) are seen following 48 hours of ZEN (2.7 mg/kg B.W) oral administration.

Fig. 4C Degeneration of proximal tubule cells (stars) and dilation of distal tubules > notice the cast deposition within their lumina (white arrows). (Hx & E X 400).

Group T2 (2.7 mg/Kg B.W ZEN / one week / twice a week)

After one week of oral administration of 2.7 mg/Kg B.W ZEN, (twice a week) congestion of glomerular and peri-tubular capillaries was observed. Dilatation of tubular lumina was observed in some animals (Figure **5A**). In other animals marked shrinkage and degeneration of tubules were observed. The lining cells of the shrunken tubules showed dark stained cytoplasm and small dark nuclei which could be considered signs of apoptosis described in literature. The interstitial connective tissue between the tubules of those samples showed extravasted blood cells (Figure **5B**).

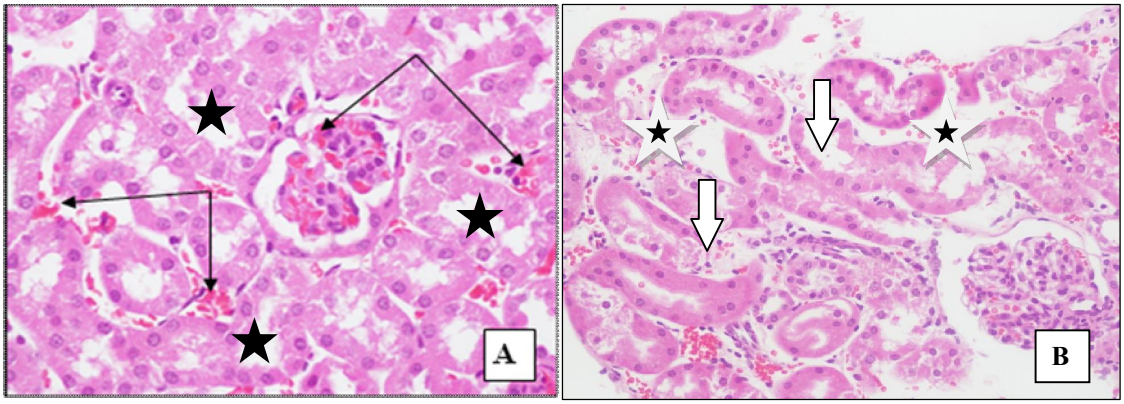

Figure 5: Photomicrograph of magnified part of mice kidney in T2 (one week after administration of ZEN 2.7 mg/kg B.W) (twice a week) Showing:
Fig. 5A Congestion of glomerular (GC) and peritubular capillaries (arrows) and slight dilation of tubular lumina (star).
Fig. 5B Shrinkage and degeneration (white arrow) of tubules with intertubular haemorrage (star). Cells lining the tubules show dark cytoplasm and small deeply stained nuclei which could be considered signs of apoptosis. (Hx & E X 400).

Group T3 (2.7 mg/Kg B.W ZEN/two weeks/twice a week for two weeks)

After two weeks of 2.7 mg/Kg B.W ZEN, marked dilation of renal tubular lumina and focal atrophy of glomerular capillaries were observed (Figure **6A**). Either apoptotic or necrotic changes in the lining epithelium of renal tubules were the most characteristic (Figure **6B**).

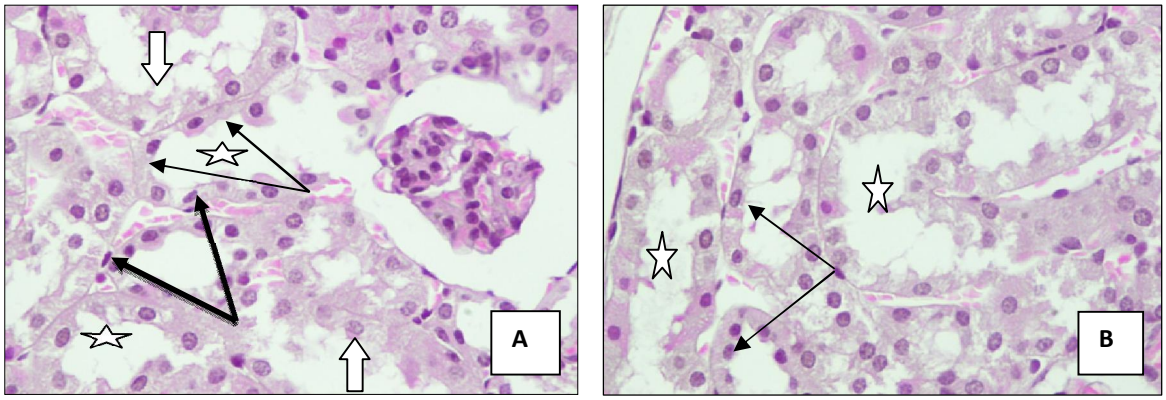

Figure 6: Photomicrograph of magnified part of mice kidney in T3 (two weeks after administration of ZEN2.7 mg/kg B.W)(twice a week for two weeks) Showing:
Fig. 6A Marked atrophy of tubular epithelium (black arrows) with dilation of the lumina (star). Glomerular capillaries looked shrunken and clumped. The cells lining the tubules are degenerated (white arrows). Other tubules are lined by small dark stained cells with small nuclei (apoptosis) marked by (thin black arrows).
Fig. 6B The renal tubules in the medulla shoed similar finding tubular dilation (star) and cellular degeneration. (Hx & E X 600)

Group T4 (daily oral 10 mg/kg B.W, TQ for 7 days before toxin (2.7 mg/Kg B.W two weeks / twice a week for two weeks).

Protective effect against nephrotoxic changes induced by ZEN was evident. The kidney parenchyma looked more or less similar to control. No necrotic or apoptotic changes or any signs of inflammation were observed (Figure **7 A, B**).

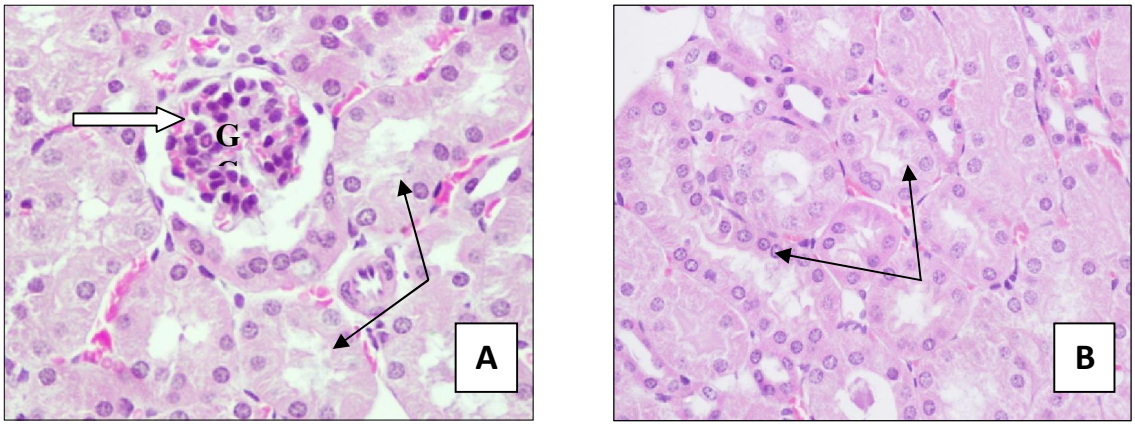

Figure7: Photomicrograph of magnified part of mice kidney in T4 mice treated with (10 mg/kg B.W) TQ for 7 days daily before toxin receiving for two weeks (twice a week for two weeks)
Fig. A, B: Showing marked improvement of kidney parenchymal architecture. Glomerular capillaries (GC) within Bowman capsule (white arrow) appeared normal with no atrophy. The tubules are well organized having cells with large vesicular active nuclei and striated acidophilic cytoplasm (black arrows). (Hx & E X 600)

5. Discussion

The present investigation has been focused on the effect of ZEN on kidney function, lipid peroxidation, cancer marker and histological changes on male mice kidney. The protective effect of Thymoquinone (TQ) extract and its possible ameliorative role of on ZEN-induced nephrotoxicity in Balb/C mice were also studied. Urea, an end product of protein metabolism is excreted by the kidney. The urea concentration in the glomerular filtration is the same as in the plasma. Tubular reabsorption of urea varies inversely with the rate of urine flow. Thus, urea is a less useful measure of glomerular filtration than is creatinine, which is not reabsorbed. Blood urea nitrogen (BUN) varies directly with protein uptake and inversely with the rate of excretion of urea (El-Sawi *et al*., 2001).

Blood urea nitrogen (BUN) was chosen because of its high predictive value because it is an integral marker of tissue necrosis, protein catabolism, and renal function. In the present study, the BUN level in serum was increased significantly after administration of ZEN for one week and two weeks. In the absence of conditions that enhance urea production, such as gastrointestinal bleeding, corticosteroid therapy, or a high-protein diet, elevations in BUN levels may represent a fall in glomerular filtration rate (GFR), increased of nitrogenous metabolism associated with diminished renal blood flow (El-Sawi *et al*., 2001).

Alteration in BUN was described in most cases of nephrotoxicity including those induced by mycotoxin exposure (Single, 1989; An *et al*., 2009). However, because urea reabsorption is a passive process that is linked to sodium and water reabsorption, conditions associated with increased water and sodium reabsorption lead to a rise in urea reabsorption. The resulting elevation of BUN that is not solely due to the fall in GFR (Aronson *et al*., 2008) resulting an acute renal failure (An *et al*., 2009). Creatinine (CRE) is an important analyte of clinical significance that is used for the determination of renal glomerular filtration rate and kidney dys-functioning and muscle disorder. CRE is a byproduct of amino-acid metabolism and is the energy source for muscle tissue (Pandey and Mishra, 2004).

Mice treated with ZEN showed a significant decrease in CRE after one week and two weeks compared to control. The decreased level of CRE may be due to the inhibition of t-RNA-synthetase accompanied by a reduced protein synthesis (Dänicke *et al*., 2006) and/or protein catabolism accompanied by kidney dysfunction (Abbès *et al*., 2006). Also, (Perrone *et al*. 1992) reported that low levels of CRE in blood may also indicate a decrease in muscle mass, some types of severe liver disease, low protein diets and by aging.

TQ treated mice offered some protection against ZEN induced toxicity after two weeks of treatment as compared to the control group. These results were confirmed with previous study made by Mutabagani and El-Mahdy in 1997 who found that TQ has an anti-inflammatory activity. Also, TQ has been shown to attenuate a variety of renal toxicities that are the consequence of oxygen free radical damage, such as cisplatin-induced nephrotoxicity in rats and mice (Badary *et al*., 1997) These results were disagree with other studies that showed significant increase in serum CRE concentration in animals exposed to mycotoxins (Bokhari and Ali, 2008; An *et al*., 2009). It is a well known fact that CRE production and excretion are reasonably constant in the absence of muscle disease,

however increased level of CRE resulting an acute renal dysfunction (An *et al*., 2009).

Alpha-fetoprotein (AFP) is a major serum protein synthesized by fetal liver and yolk sac cells and in trace amounts by the fetal gastrointestinal tract (Deutsch, 1991). AFP was first described in 1963 in the serum of mice with hepatoma (Zheng *et al*., 2003).

In the present study, the mean value of AFP concentration was increased in animal group receiving ZEN for two weeks (T3) compared to the control. It is well documented that ZEN is a potent hepatonephrotoxic (Abbès *et al*., 2007) and plays a role in its carcinogenicity resulting from oxidative stress and cytotoxicity (Abid-Essefi *et al*., 2004). Some extra hepatic 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). However, in the present study, no signs of precancerous lesion or cancer can be detected by light microscopic studies in kidneys of all ZEN treated groups. Yet apoptotic changes seen in some kidney tubules could point to some sort of cellular transformation prior to malignant changes. Apoptosis was activated to eliminate such cells (D'agostini *et al.,* 2001).

Pyruvate kinase (PK) catalyzes the second ATP generating step in glycolysis. PK facilitates the reversible transfer of a phosphate group from phosphoenolpyruvate (PEP) to ADP, giving ATP and pyruvate as products. There are several isozymes of pyruvate kinase. Tumor cells express the M2-PK form of this enzyme. Fluorophosphates, pyridoxal 5′-phosphate, creatine phosphate, oxalate, and L-phospholactate inhibit PK (Scatena *et al*., 2008). There are also PEP analogs with modified phosphate and carboxylate groups being designed to inhibit PK (García-Alles and Erni, 2002). Different tissue-specific isoforms of this enzyme exist and are homotetramers in their activate state. In tumor cells, the isoenzyme M2-PK is shifted to a dimeric form which is over expressed during multi-step carcinogenesis and present both in blood and other body fluids, presumably released from tumor cells by necrosis and cell turnover (Mazurek *et al*., 2005).

In the present study, the concentration of Tu M2_PK was increased in ZEN administration after 48 hours, one week and two weeks. These results were in agreement with the results obtained by Eigenbrodt *et al*. (1998) and Mazurek *et al*. (2000; 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 pyruvate kinase isoenzyme type M2, which acts as an enzymatically highly active tetramere in proliferating non-tumour cells. High plasma Tu 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).

However, no evidence of cell proliferation was observed in kidney parenchyma treated by ZEN, and only necrosis or apoptosis were observed. The only explanation was based on considering ZEN an analogue to estrogen (Glenn, 2007) with the possibility of proliferative changes anywhere that could be the source of increasing PK in the present study. More future precise work is needed to explain such increase. In the present study, the concentration of the total antioxidants was increased after 48 hours, one week and two weeks compared to control. In T4 (TQ treated) group the mean values was near to the control group. In previous literatures dealing with the effect of mycotoxin exposure, total antioxidants (superoxide dismutase; catalase; glutathione; glutathione peroxidase and glutathione-*S*-transferase) were reported to be decreased (Meki *et al*., 2004). This decrease was explained by their involvement in defense process against organ toxicity induced by mycotoxin exposure.

Antioxidants were known to 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 together with the antioxidant enzymes balance the ratio of the concentration of oxidizing equivalents to the concentration of reducing equivalents (Gamaley and Klyubin, 1999).

It was hypothesized that anti inflammatory processes were activated to counterbalance excessive levels of proinflammatory cytokines (Van der Poll and Van Deventer, 1999) or oxidative stress (Bovril *et al*., 2002). It was also possible that altered antioxidant defense (i.e. a significant change in serum total

antioxidant level) eventually leads to immune dysfunction and a poor outcome (Teng *et al*., 2002). In fact the present study was supported by a recent study reported that demonstrated a significant increase in glutathione and total antioxidant Abraham and sugumar (2008), TQ administration 7 days prior to ZEN administration for 2 weeks was found to protect mice kidney from nephrotoxic changes. TQ result in return of creatinine level to normal. Also, BUN serum level and total antioxidant returned to normal levels. Regarding tumor markers, both AFP and Tu M2_PK were significantly decrease compared to Group T3 (untreated / two weeks). The kidneys are known to be liable to exposed to numerous xenobiotics and their metabolites as the renal blood flow accounts for 22–25% of the cardiac output. The different mechanisms by which toxicants are excreted by the kidneys include glomerular filtration, tubular excretion by passive diffusion and active transport (Sebastian, 2009). Accordingly the structures functioning in those processes are markedly damaged by nephrotoxic agents. In the present study, it was observed that the administration of ZEN (2.7 mg/kg B.W) to laboratory mice, affect the histological structure of kidney parenchyma. The changes were time dependant showing that cumulative effects properties result in more parenchymatous toxicity in mice kidney. This could explain the alteration in kidney function tests mentioned before especially the increased in BUN detected after one, two weeks of ZEN administration. In the present study, ZEN giving orally as in a small dose (2.7 mg / Kg B.W) result in marked damage of renal tubules involving both proximal and distal tubules especially in group T2 (one week) and T3 (two weeks). Focal atrophy of glomerular capillaries, increasing Bowman space and interstitial blood extravasate were also among the changes observed. The changes showed individual variation in the extent and degree of histological changes. The changes could be considered focal and moderate sparing wide regions of normal kidney parenchyma.

Proximal convoluted tubule is the main component of nephron affected in nephrotoxicity. Cytochrome P450, involved in xenobiotics metabolism and bioactivation is present in the highest concentration in the renal cortex, mostly in proximal tubules (Sebastian, 2009) and this could explain the focal degenerative or necrotic changes of proximal tubules induced by exposure to ZEN in this study. Mycotoxin contamination is one of the most relevant and worrisome problems concerning food and feed safety. Nephrotoxicity was a common feature in mycotoxin exposure. (Bokhari and Ali 2008) described focal atrophy of glomeruli and loss of tubular cell brush borders in mice 8 weeks after administrated of Ochratoxin A contaminated coffee. Similar histological changes due to mycotoxin exposure were also described (Abbès *et al*. 2006). The primary effect of zearalenone is estrogenic. The basis for the estrogenic effect is well established and is due to a close structural similarity between ZEN (and many of its metabolites) and estradiol (Osweiler, 2000).

Studies in various species (rodents, pigs and monkeys) have shown that ZEN and its metabolites display estrogenic and anabolic activities (Etienne and Doumand, 1994) and is regarded as carcinogenic, causing likely hepatocellular and pituitary gland adenomas (Maaroufi *et al*., 1996; IARC, 1993). Literature regarding the effect of ZEN on kidney parenchyma was lacking. Zourguri *et al*., (2008) reported that ZEN lead to elevation of Hsp70 and Hsp27 in Balb/C mice kidney extract. Ferns *et al*. (2006) reported that this protein could protect cells from oxidation damage by decreasing ROS production and this may explain the minimal histological change observed here in. Blandamura *et al*. (2005) reported a rare case of collecting duct carcinoma (CDC) of kidney with an increase in AFP.

In the present study, no signs of cellalular hyperplasia or preneoplastic changes such as nuclear pleomorphism were observed. This coincides with the biochemical changes where AFP was insignificantly altered in most groups. The changes in renal tubules were mostly degenerative or cellular necrosis (swollen cells with faintly stained granular cytoplasm and nuclear karyolysis). However, scattered apoptotic foci dark stained and shrunken tubular cells were observed in distal tubules of some animals of group T2 (one week) and T3 (two week). The tubules had dilated lumina containing hyaline or protein casts. Studies of elimination in various animal species indicated that α and β ZOL metabolites are excreted both as free compounds and as glucuronide conjugates via urine (Olsen *et al*., 1985; Fitzpatrick *et al*., 1988; Danicke *et al*., 2001).

Cell death takes two distinct forms, necrosis and apoptosis. These are two fundamental types of cell death, which have been defined morphologically and biochemically (Kiechle and Zhang, 1998). Although necrosis is considered to be a degenerative phenomenon commonly associated with chemical injury, apoptosis, in contrast, appears to be an active endogenous process. Apoptosis (programmed cell death) is derived from Greek and refers to the dropping or falling of leaves from a tree. The term was introduced by Kerr *et al*. (1972) to define the morphologic features including cytoplasmic blebbing, chromatin condensation, cell shrinkage, nuclear fragmentation and cell rounding (loss of adhesion). The process of apoptosis is said to be implicated in the regulation of normal as well as preneoplastic and neoplastic tissues (Kroemer *et al*., 1995).

A homoeostatic role for apoptosis is often evident also in association with exposure to chemical. In fact, the occurrence of apoptosis in hamster kidney cells and HeLa cells (Seegers *et al*., 1994a), human lymphocytes (Seegers *et al.,* 1994b), after exposure to Ochratoxin A has been considered to be part of a regulatory mechanism triggered to restore the original size of the organ after the initial hyperplasia caused by chemical agents. Co-existence between apoptosis and necrosis was found in the present study after exposure of experimental animals to ZEN. The finding that apoptosis occurs not only in physiologic circumstances but also under conditions of tissue injury is interesting and may lead to speculate on common pathways. The mechanism by which some chemicals may elicit the induction of a form of cell death that appears to be genetically programmed in several physiological conditions is not known.

TQ administrated here result in protection of mice kidney from degenerative changes described in ZEN treated groups. Kidney parenchyma looked more or less similar to control. No signs of necrosis or apoptosis could be seen except of small foci which could be considered normal turn over changes. On the other hand kidney treated with TQ alone, showed well organized parenchymatous elements (renal corpuscles and glomeruli-proximal and distal tubules) which seemed to be more healthy and organized compared to control (Figure **4.1**).

This point that TQ may has stabilizing effect on cellular membrane acting as antioxidant preventing lipid peroxidation (Houghton *et al*., 1995) upon exposure to toxic agents. Thus a prophylactic mediated action can be suggested and explain why prior administration of TQ for 7 days ameliorate or even protect against ZEN nephrotoxicity described in this study.

In conclusion, the most common *Fusarium* toxins in feedstuffs are deoxynivalenol, fumonisin and zearalenone. In the present study, biochemical and histological effect of ZEN was time dependent. Variation in degree of response was observed in different animals in the same group. Also, the results were pointed to the potential protective effect of TQ extracted from *N.S.* which was proved by both biochemical and histological studies. TQ can be used to protect humans or animals against the adverse health effects of this mycotoxin.

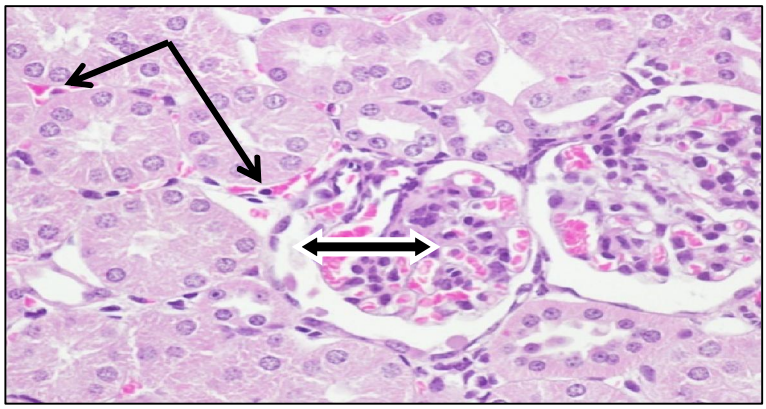

Figure 4.1: Photomicrograph of magnified part of control mice kidney (administration of TQ)(10 mg / Kg B.W) showing: Normal renal corpuscles (double head arrow). The proximal (PT) and distal (DT) tubules looked more organized than those of control. The nuclei of cells are rounded with uniform distribution of chromatin. Thin wall Blood capillaries can be seen around the renal tubules (thin arrows). (Hx & E X 600)

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