Mycological, Biochemical and Histopathological Studies on Acute Fusariotoxicosis In Sheep.

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ABSTRACT: One hundred cases of diseased sheep at desert districts in governorates of (Giza; 6th. October and El-Wadi-El-Gadid), were investigated. Sixty percent of these sheep sera had a mean levels of T-2, zearalenone and fumonisins (2.5±0.2, 4.3±0.5 and 25.0±2.0) respectively. The used feeds and underground water in breeding of this sheep were examined mycologically which revealed that all examined samples gave a variable rates of pollution. Seven genera and 15 species of fungi were recovered from feeds and water. The most predominant isolates belong to members of genus Aspergillus with a range of (5-100%), followed by Fusarium spp. with a range of (40-90%), Penicillium spp. with a range of (10-55%) and Mucor spp. with a range of (10-50). The Fusarium toxins were detected in same feed samples, the largest amount estimated in crushed yellow corn (60%) namely FB1, T2 and zearalenone with the mean levels of (48.4±1.0; 3.0±0.1 and 0.84±0.03) respectively. The significant high levels of FB1 in the present feed samples and serum of diseased sheep gave a large possibility that FB1 was responsible for this disease outbreak in sheep. On the other hand, the biochemical examination of diseased sheep sera for estimation of toxic effects is based on the assumption that the elevated activities in levels of serum enzymes such as (AST, ALT, GGT, LDH and urea). While, slightly decreases in ceratinine, calcium and phosphorus levels compared with the apparently healthy group. The pattern of protein electrophoresis showed a significantly decreased values in serum total protein, alpha globulin, beta globulin and while slightly increase in gamma globulin. The internal organs of dead cases during this disease had various significant pathological changes in vital organs including hemorrhagic, alveolar pneumonia and calcification in lung. The liver showed hemorrhage, oedema, vacuolar degeneration and necrosis of hepatocytes with evidence of preneoplastic stage in liver cells. Whereas, the kidney showed vacuolar degenerating changes and necrosis of the tubular epithelium, in addition to glomurular oedema and calcium deposition. This study increased awareness of the significant dangerous effect of environmental pollutions particularly fusarium species and their toxins. This study increased awareness of the significant dangerous effect of environmental pollutions particularly fusarium species and their toxins. [Life Science Journal 2010;7(3):49-57]. (ISSN: 1097-8135).

Keywords: pollution; biochemical alterations; fusarium

INTRODUCTION

The increased importance of animal production due to progressive elevated requirement of human consumption gave an intensive attention of animal health status. The environmental pollution is considered the essential cause of animal diseases particularly pollution with fungi and their toxins for the used feed and water in animal breading and elsewhere, contamination of human food. Mycotoxins are a group of structurally diverse, mold elaborated compounds that induce diseases known as mycotoxicosis in humans and animals. As much as twenty-five percent of the world's food crops are estimated to be contaminated with mycotoxins. Ingestion of sufficient quantities of mycotoxin-contaminated material leads to acute, and more commonly, chronic intoxication ***(Hassan et al., 2003; and 2009).*** The mycotoxins of greatest agricultural and public health significance include aflatoxins, ochratoxins, trichothecenes, fumonisins, zearalenone, and ergot alkaloids ***(Hassan et al., 2004; 2008 and 2009).*** However, the fungi of *Fusarium species* and their toxins are widely distributed through the world where they occur in soil, on plants, plants debris and similar organic subtracts. They cause significant economic losses in agriculture, morbidity and mortality in animals and immunological compromised humans, where it is capable of killing cells by causing extensive damage to cellular membrane **(*Ajello and Hay, 1998 and Mogeda et al., 2002).*** On the other hand, epidemiological studies associated with fusarium toxins had a wide range of biological effects, including pulmonary oedema in pigs and ruminants **(Harrison et al., 1990),** nephrotoxicity and liver cancer in rats **(Gelderblom et al., 1996).** Although, its effects on human are difficult to be determined. Fumonisin B9 had been statistically associated with a high incidence of oesophageal cancer in certain areas of Transkei, South Africa and also in China ***(Chu and Li, 1994).*** The International Agency for Research on Cancer has declared *F. moniliforum* form toxins as potentially carcinogenic to human. ***Gelderblom et al. (1994)*** proposed that FB1 was a tumour promoter at doses not causing significant liver pathology but when given at overtly hepatotoxic dose, it was also a weak initiator. Also, the lymphocytes decreased in response to Zeraralonone especially for LD50 dose. Many data showed that this mycotoxin induced immunosupression in depressing T or B lymphocyte activity ***(Berek et al., 2001).*** All the previous literatures recorded that the pollution affect upon the growth rate and health of human being and animals including aneamia , stunted growth , carcinogenic, tremorgenic, haemorrhagic, dermatitic, pulmonary edema, immunosuppressive and hormonal effects ***(Hassan, 1998 and 2003 ;and Hassan et al., 2003 ;2004;2008 and 2009)***. Whenever, sheep breeding and their production is the main source of food for human in the desert districts. So, the aim of the present

work was to investigate the problem of fungal and fusarium mycotoxins pollution of feed and underground water and its role in the health status of sheep at some deserts Governorates (Giza, El-Wadi El Gadid and 6 th October).

MATERIAL AND METHODS

Material:

Samples:

Serum, feed and water samples: One hundreds diseased cases of sheep at desert districts in governorates of Giza; 6th October and El-Wadi-El-Gadid were investigated. The cases of sheep suffered from loss of weight gain, low productivity, diarrhea, mastitis, disturbance in fertility and sudden mortality of some cases. From districts of diseased cases, 100 samples of sera, 150 feeds and 20 samples of underground water which used in breeding of diseased sheep were collected. The samples of feed and water were collected in sterile plastic container to prevent any contamination.

Internal organs: From the recently deed cases of animal from disease outbreak, the internal organs were collected and imbedded in bottles containing 10% formalin solution for further histopathological examination. These organs included liver, kidney, lung, bronchial lymph node and heart.

***Mycotoxins standards*:** Standers and immunoaffinity column of Zearalenon, T2 and FB1, were purchased from Sigma Chemical Company (USA).

Methods:

Mycological examination of samples:

The samples of feeds and underground water which used by symptomatically diseased sheep cases were subjected for isolation and identification of fungi as recommended by ***(Conner et al., 1992)*.**

Detection of mycotoxins in feed and sera of diseased sheep:

Detection of mycotoxins in serum of sheep and feed stuffs by fluerometric methods as described by ***Hansen (1993)*** using immune-affinity column method**.**

Biochemical investigations of sheep sera:

From each of investigated animal a blood samples were collected in small labeled dry and clean vials without anticoagulant in centrifuge tube, allowed to clot and then centrifuged at 3000 rpm for 90 minutes for separation of serum which used to assay the biochemical parameters The biochemical assays of serum gamma glutamyle transferase (GGT) and lactic dehydrogenase (LDH) activities were determined according to methods of **(Szase et al., 1976)** ,aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities according to ***Reitman and Frankel, (1957),*** serum urea according to **Wybenga *et al.* (1971**), serum creatinine level according to ***Henry (1974)***, Estimation of serum total protein and electrophoretic pattern were carried out after ***SonnenWirth and Jaret (1980) and Davis (1964),*** respectively.

However, measurement of calcium, ph. and Mg. were carried out as the technique described in the references ***(Brown et al., 1986 and Brown and Taylor, 1995)***.

Histopathological studies:

From the recent dead cases, tissue specimens were collected directly from lung, bronchial lymph node, heart, liver, spleen, kidneys and intestine for histopathological examination. They were kept in 10% neutral buffered formalin for at least 24 hours, routinely processed by the standard paraffin embedding technique and stained with Hematoxylin and Eosin. Prussian blue stain was used for hemosidrin pigments staining ***(Bancroft et al., 1994).***

STATISTICAL ANALYSIS: The obtained date were computerized and analyzed for significance, Calculation of standard error and variance according to ***(SPSS 14, 2006)***.

RESULTS AND DISCUSSION

The economical importance of sheep animals in desert districts Governorates were at the top to other part in Egypt, Where, peoples in these districts their life depend on its products such as meat, milk, wool and leather obtained from these animals ***(Agaoglu, 1991; Camas et al., 1994* and *Hassan et al., 2008***)

In this paper, the current data in table (1) showed that, sera of one hundred cases of diseased sheep outbreaks which suffered from loss of weight gain, low productivity, diarrhea, mastitis, disturbance in fertility and sudden mortality of some cases at desert districts in governorates of Giza; 6thOctober and El-Wadi-El-Gadid, contained significant levels of fusarium toxins. Meanwhile, sixty percent of these sheep had the mean levels of fusarium toxins as T-2, zearalenone and fumonisins (2.5±0.2, 4.3±0.5 and 25.0±2.0) respectively. .The results indicated that serum of diseased sheep contained higher mean significant level of FB1 than other types of fusarium toxins which suggested being the essential cause of disease. Mycotoxins in sera of sheep and cattle in Egypt in association with symptoms of toxicities were previously reported by ***Hassan (1994); Hassan et al. (2003; 2004 and 2009)***.

Table (1): Determination of fusarium toxins in serum of diseased sheep .

Animals	Prevalence of fusarium toxins			Mean levels of fusarium toxins (ppm)		
	No. of tested	No. of +ve	%	Fumonisins	T-2	Zearalenone
Sheep	100	60	60	25.0±2.0	2.5±0.2	4.3±0.5

The effects of fusarium toxins in human and animals ranged from carcinogenic and nephrotoxic and immunosuppressive health effects (**Morriss, 1997)**. Although the main route of human exposure to mycotoxins has been identified as the direct ingestion of contaminated cereals and grains **(Morriss, 1997)**, while, there are many studies about whether the ingestion of meat, milk, and eggs originating from mycotoxin-exposed food-production animals is a significant exposure pathway for mycotoxins among humans **(Hassan et al., 1997; Wafia and Hassan, 2000 and Hassan et al., 2004 and 2009).** The search focused to recovered the accurate causes and sources of this disease in sheep, therefore, the direct factors to the animal consumption were examined .The fungal examination of feeds , feedstuffs and underground water (which the only available source of water in these districts), the results revealed that all examined samples gave a variable rates of pollution. Seven genera and 15 species of fungi were isolated from feeds and water. The most predominant isolates belong to members of genus Aspergillus with a range of (5-100%), followed by Fusarium spp. with a range of (40-90%), Penicillium spp. with a range of (10-55%) and Mucor spp. with a range of (10-50%). Whereas, the frequency of isolation of other spp. as Rhizopus spp., C.albicanse and Rhodotorula spp. were relatively low. On the other hand, the fungal contamination of underground water was significantly high as compared with standard healthy water which must be free from any signs of pollution (Table, 2). However, F.moniliform, F.oxysporum and F. solani were the most frequent isolated members of Fusarium from feed samples (Table, 3). The fungus of F.moniliform was recovered from all examined feed samples at a rates ranged from (20-65%), while, F.oxysporum was isolated from lower examined samples (5-10%) with exception of wheat straw samples .Whereas, the species of F. nival and F. fusaroides were only isolated from (Soya bean meal and crushed yellow corn), respectively with the same rate (5%). It is clear from the result that crushed yellow corn and wheat straw were the most contaminated followed by hay, Soya bean and drawa. While, the underground water was the lowest contaminated samples. These differences in the level of contamination may be due to the exposure of the examined samples to different climatic condition either during preparation or transportation or storage. These findings were in agreement with the results of (**Hassan et al. 2003; 2004; 2008 and 2009)**, who recovered most of these fungi from the examined feed and water samples.

Table (2): Prevalence of fungi in feeds and underground water used for breeding of sheep

Fungal Species	Crushed yellow corn(30)		hay(35)		Wheat straw(20)		Soya bean meal(35)		Drawa (Leaves of yellow corn) (30)		Underground water (20)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Aspergillus sp.	20	100	19	95	20	100	15	75	10	50	1	5
A. flavus	18	90	17	85	18	90	7	35	40	20	1	5
A. niger	16	80	15	75	15	75	14	70	36	18	10	50
A. candidus	1	5	--	--	--	--	2	10	30	15	0	0
A. fumigatus	4	20	7	35	--	--	2	10	20	10	1	5
A. ochraceus	5	25	19	5	1	5	1	5	16	8	0	0
A. terrus	5	25	2	10	3	15	3	15	10	5	0	0
Fusarium sp.	10	50	18	90	15	75	8	40	8	40	0	0
Penicillim sp.	7	35	9	45	6	30	10	50	11	55	2	10
Mucor sp.	10	50	6	30	2	10	10	50	3	15	0	0
Rhizopus sp.	1	5	1	5	3	15	4	20	1	5	0	0
C.albicanse	2	10	0	0	0	0	1	5	2	10	1	5
Rhodotorula sp	1	5	0	0	1	5	0	0	2	10	2	10

When, the feed samples which contaminated with fusarium spp. were subjected for detection of Fusarium toxins, the results revealed that the largest amount was detected in crushed yellow corn (60%) namely FB1, T2 and zearalenone with the mean levels of (48.4±1.0; 3.0±0.1 ppm and 0.84±0.03 ppm)**,** respectively.

Table (3): Prevalence of fusarium species in feeds of sheep suffering from problems of animal diseases.collected from different districts at el Wadi El Gedid

Fusarium Species	**Crushed yellow corn**		**Hay**		**Wheat straw**		**Soya bean meal**		**Drawa (Leaves of yellow corn)**	
	No.	%	No.	%	No.	%	No.	%	No.	%
F.moniliforme	4	20	13	65	8	40	6	30	7	35
F.oxysporum	1	5	1	5	-	-	1	5	2	10
F.solani	1	5	1	5	4	20	-	-	-	-
F.sporotrichoides	1	5	-		1	5	-	-	-	-
F. aquaeductum	1	5	-	-	1	5	-	-	-	-
F. nival	-	-	-	-	-	-	1	5	-	-

F. fusaroides	1	5	-	-	-	-	-	-	-	-
F. equiseti	-	-	-	-	1	5	-	-	-	-
F. tricinctum	1	5	3	15	-	-	-	-	-	-

It was interesting to report here that the samples of wheat straw contained only FB1 at a rate of (70%) with a mean level of (20±0.9 ppm) (Table, 4). The significant levels of FB1 in the present feed samples and serum of diseased sheep gave a large possibility that FB1 was responsible for the disease outbreak in sheep**.**

Table (4): Detection of fusarium toxins in feeds .

Fusarium Species	**Prevalence of fusarium toxins**			**Mean levels of fusarium toxins (ppm)**		
	No. of tested	**No. of +ve**	**%**	**Fumonisins**	**T-2**	**Zearalenone**
Crushed yellow corn	10	6	60	48.4±1.0	3.0±0.1	0.84±0.03
Hay	10	5	50	17.0±1.3	-	0.71±0.0
Wheat straw	10	7	70	20±0.9	-	-
Soya bean meal	10	4	40	15.0±0.2	2.0	0.99±0.005
Drawa (Leaves of yellow corn)	10	4	40	27.0±3.22	1.0±0.01	1.50±0.0

The Food and drug administration has established recommended maximum levels for aflatoxins and fumonisins in animal feed. For swine, ruminants including sheep, and poultry, the recommended maximum levels of total fumonisins in complete feeds are 10, 30, and 50 μg/g, respectively **(FDA, 1994).** Therefore, the detected levels of FB1 were significantly over the permissible limits in feeds particularly FB1 toxin in examined sheep feed samples which ranged from (15.0±0.2-48.4±1.0 ppm). The same findings were detected by many authors as **(Hassan et al., 2002; 2003; 2004 ; 2008 and 2009) ; El-Hamaky, 2001 and El Ahle et al., 2006).**

On the other hand, the biochemical examination of diseased sheep sera for estimation of toxic effects is based on the assumption that the elevated activities in levels of serum enzymes such as (AST, ALT, GGT, LDH and urea) in Table, (5). While, a slightly decreases in ceratinine level compared with the apparently healthy group. These results reflect organs damage **(Cheng et al., 2001 and Asrani, et al., 2006).** The increased serum enzymes activity observed by feeding toxic diets in this study may be due to hepatic degeneration and subsequent leakage of enzymes into circulation. **(Chen** et **al., 2008 and Wang et al., 2008).** It is reported that the significant effect of fusarium toxins are the alteration in serum concentration of kidney and liver enzymes ,total protein, albumin, minerals and lipid profiles **(Kubena et al., 1997 and Mogeda et al., 2002).** The high concentrations of serum urea in sheep fed contaminated diet may be a result of increased ammonia absorption caused by altered protein turnover in the rumen micro-flora, or altered protein metabolism in sheep tissues. In ruminants, serum urea levels are affected by protein digestion and metabolism by the rumen biomass. A large portion of dietary protein is hydrolyzed and deaminated by rumen micro-flora, giving rise to peptides and free ammonia in the rumen **(Herdt, 2000).** A portion of the free ammonia is absorbed and is metabolized to urea in the liver. If microbial protein synthesis in the rumen is inhibited by mycotoxins, more free ammonia remains in the rumen, is absorbed into the blood, and is metabolized to urea, resulting in elevated blood urea concentrations. **Danicke et al. (2005)** observed that postprandial rumen fluid ammonia concentrations were consistently higher when *Fusarium* mycotoxin-contaminated wheat was fed to sheep**.** Inhibition of protein synthesis results in elevated concentrations of free Amino acid that are used for energy utilization, resulting in increased serum urea. The results of this study are in agreement with those of **Chowdhury and Smith (2004),** who observed that excessive serum concentrations of uric acid in laying hens were a result of feeding feedborne *Fusarium* mycotoxins. Moreover, in a subsequent study with laying hens, they found that feeding contaminated grains led to reduced hepatic fractional protein synthesis rates **(Chowdhury and Smith, 2005). Danicke et al. (2006)** also observed a reduction in fractional protein synthesis rates in the kidneys, spleen, and ileum of pigs exposed to DON.

At the same time concentrations of serum calcium and serum phosphorus were decreased due to feeding *Fusarium* mycotoxin-contaminated diets This resultes were agree with **Díaz and Smith (2006).**

Fusarium inducing significantly decreased values in serum total protein, alpha globulin, beta globulin and while slightly increase in gamma globulin, these results agree with **(Rotter et al., 1994**).

The globulin component (Table, 6) showed drop in α1, α2 and β2 globulin in all the experiment while decrease γ1 globulin. This may be attributed to that *Fusarium* fungi cause's hepatotoxic, nephrosis, hemorrhages (liver and kidneys) ***(Tietz, 1996)*** *Fusarium* mycotoxins might affect

the synthesis of globulins of hepatic origin as well as globulins of lymphoid origin. **Rotter et al. (1994)** suggested that *Fusarium* mycotoxins can directly affect α-globulin synthesis in the liver. In addition, *Fusarium* fungi has immunosuppressive effect inhibit nearly cellular and humeral immunologic reaction have been reported by **Rocha et al. (2005) i**ncluding disruption of normal cell function by inhibiting RNA, DNA, and protein synthesis; inhibition of cell division; stimulation of ribotoxic stress response; and activation of mitogen-activated protein kinases. It has been found that T-2 toxin is a potent member of the trichothecene group of mycotoxins produced by *Fusarium* fungi **(Bamburg et al.,1970).** It has been found that T-2 toxin is a mycotoxin with immunomodulatory activity, where it can stimulate (immune-stimulation) or inhibit (immune-suppression) the activity of the immune system **(Shinozuka et al., 1997and Pestka et al., 2004)].**

Table (5); Biochemical parameters in serum of diseases sheep cases at desert districts in comparison to healthy cases.

Parameter	Apparently healthy	Diseased group
AST u/l	53.67±4.91	124.9***±7.94
ALT u/l	40.66±2.18	93.6***±5.48
GGT u/l	97.57±1.38	111.56*±5.11
LDH u/l	718.4±22.36	811.0*±24.11
urea mg%	41.11±2.15	53.52**±3.81
Creatinin mg%	1.31±0.07	0.9±0.24
Uric acid mg%	3.17±0.37	5.1**±0.34
Calcium mg%	9.22±0.33	7.46**±0.41
Phosphorus mg%	6.31±0.32	5.77±0.17

Results are expressed as means ± SEM (n =15), student 't' test

To give complete idea about the effect of this disease in sheep, the internal organs of dead cases during disease outbreak in the same desert districts were subjected for histopathological studies. The results revealed that thickening of the pleural membrane was observed with infiltration of mononuclear inflammatory cells, hemorrhage and proliferation of the epithelial cells lining bronchioles. Moreover, in some cases the proliferation was severe and uncontrolled which lead to occluded the bronchial lumen and form nest of epithelial cells with clear eosinophilic cytoplasm giving the feature of preneoplastic stage (Fig. 1, a & b).Some alveoli were filled with red blood cells accompanied with mononuclear inflammatory cells (alveolar pneumonia). Destruction of the wall of some alveoli with infiltration of inflammatory cells (lymphocytes, macrophages and neutrophils) were noticed accompanied with hemorrhage, calcification was also detected (Fig. 2, a & b). Severe hemorrhages with infiltration of inflammatory cells with compensatory emphysema (Hemorrhagic pneumonia) were seen in some cases.

While, bronchial lymph node showed moderate to severe depletion of lymphoid follicles, where lymphocytes detected inside alveoli and interalveolar septa in pneumonia. The respiratory tract is the primary rout of entry for Fusarium spp. and their toxins based on the sinopulmonary involvement. It has been speculated that the fusarium toxins produced damage the tissues which allowing the fungus to spread more easily **(Ajello and Hay, 1998).** However**, Halloy et al. (2005) and (Haschek et al., 2001)** mentioned that the lung of experimentally fusariotoxicated piglets particularly with FB1 showed a minimal enlargement of the alveolar septa due to an increase in the macrophage, lymphocyte number and develop lethal pulmonary edema within 4-7 days. Whereas, muscles necrosis and oedema were evident in heart in our study. A various degrees of myocardial degeneration with foci or cellular infiltration and fibrosis were observed in rats with several doses of T-2 toxin, a trichothecene metabolite of Fusarium **(Schoental et al., 1979)**.

Table (6); Patterns of protein electrophoresis in serum of diseases sheep cases at desert districts in comparison to healthy cases (mg/dl).

Parameter	Apparently healthy	Diseased group
Alb	2.35±0.12	1.87**±0.07
T.alpha	0.96±0.1	0.87±0.09
Alpha1	0.41±0.03	0.4±0.02
Alpha1	0.55±0.02	0.47*±0.02
t. beta globulin	1.09±0.04	1.02±0.03
Beta1	0.5±0.02	0.55±0.04
Beta2	0.59±0.01	0.47*±0.04
Gamma1	1.59±0.11	1.53±0.05
Gamma2	0.34±0.03	0.520.03
Gamma globulin	1.93±0.15	2.05±0.1
T.globulin	3.98±0.33	3.940.29
A/G ratio	0.59±0.03	0.43**±0.03
T. protein	6.33±0.55	5.81±0.08

- Results are expressed as means ± SEM (n =15), student 't' test

Many researchers mentioned that fusarium toxins particularly FB1 produces a wide range of biological effects including nephrotoxicity and liver cancer in rats **(Gelderblom et al., 1996).** The present study revealed glissonian's cirrhosis in liver, vacuolar degeneration and necrobiotic changes of hepatocytes in addition to haemorrhages and oedema in between hepatocytes). Some liver cells arranged in irregular aceni (preneoplastic stage) (Fig. 3 a & b). Thickening of the wall of central vein was also noticed. Epithelial hyperplasia of bile duct was detected with the formation of newly formed bile ductules. There were aggregation of oval vesicular cells in the portal area with infiltration of mononuclear inflammatory cells and fibrous connective tissue formation. Similar lesions were illustrated caused by FB1 **(Abbes et al., 2006 and Voss et al., 2001)** and zearalenone **(James and Smith, 1982).** According to data of the **National Toxicology**

Program (USA) (1982), ZEN was found to produce hepatocellular adenoma. While**, Abbes et al. (2006**) mentioned that the histological examination of mice kidney that treated with two ZEN doses alone revealed a swelling in the epithelial cells of the proximal tubules, granular degeneration, shrunken glomeruli with the presence of eosinophilic cast in the lumen of tubules and blood vessels dilatation.

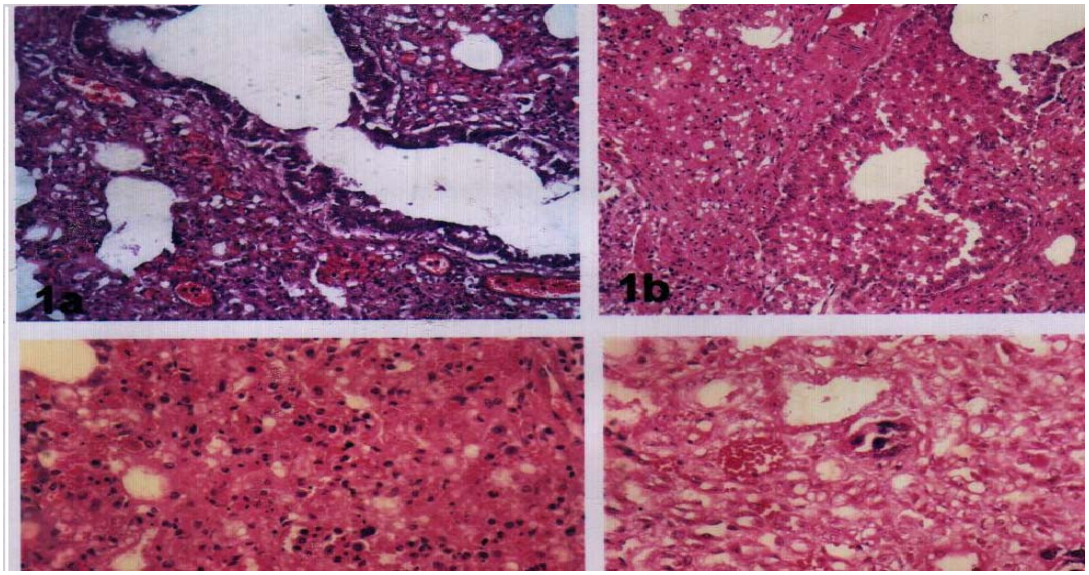

Fig. (1, a & b): Lung of sheep fed on mycotoxin (FB1, T2, ZNE) showing proliferation of the epitheliial cells lining bronchiols was severe, uncontrolled and form nest of epithelial cells giving the feature of preneoplastic stage (H & E X 100).

Fig. (2, a & b): Lung of sheep fed on mycotoxin (FB1, T2, ZNE) showing destruction of the wall of some alveoli with infiltration of inflammatory cells (lymphocytes, macrophages and neutrophils) accompanied with hemorrhage and calcification (H & E X a) 200, b) 400).

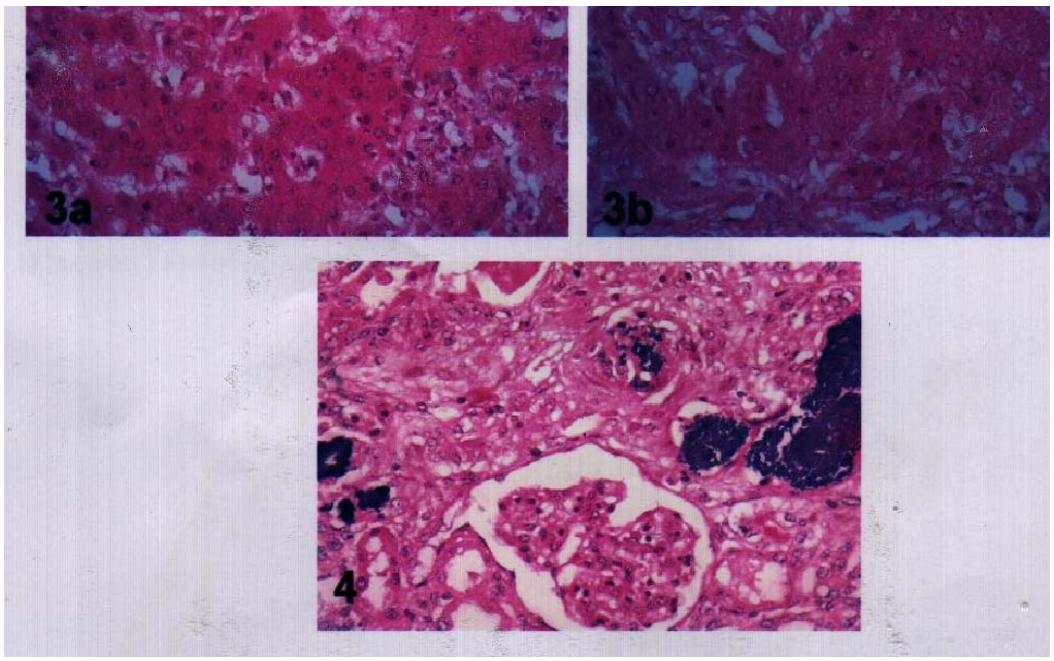

Fig.(3, a & b): Liver of sheep fed on mycotoxin (FB1, T2, ZNE) showing disorganization of hepatic cord (a) with tendency to formation of irregular aceni (preneoplastic stage) (b) (H & E X 400).

Fig. (4): Kideny of sheep feeding on mycotoxin (FB1, T2, ZNE) showing necrosis of renal tubular epithelium, gromerular oedema and calcium deposition. (H & E X 400).

These confirm our results showed in kidney in our study, where, the pathological examination of kidney revealed blood vessels dilatation. Vacuolar degeneration of epithelial cells lining the renal tubules were noticed, other were sloughed in the lumen forming renal casts. Meanwhile, some tubular epithelium revealed necrosis, glomerular oedema and calcium deposition were also detected (Fig.4). ***Voss et al. (2001),*** Mentioned that FB1 induces apoptosis of hepatocytes and proximal tubular epithelial cells. More advanced lesion in both organs is characterized by simultaneous cell loss (apoptosis and necrosis) and proliferation (mitosis). Microscopic and other findings suggest that an imbalance between cell loss and replacement develops a condition favorable for carcinogenesis. On the molecular level, fumonisins inhibit cermide synthase and disrupt sphingolipid metabolism and theoretically, sphingolipid-mediated regulatory processes that influence apoptosis and mitosis.

The previous literatures recorded that the pollution affect upon the growth rate and health of human being and animals including aneamia , stunted growth , carcinogenic, tremorgenic, haemorrhagic, dermatitic, pulmonary edema, immunosuppressive and hormonal effects ***(Hassan, 1998 and 2003 ;and Hassan et al., 2003 ;2004;2008 and 2009)***.These findings were confirmed in our study, where , the above results clearly observed the effects of fungal particularly fusarium species and their toxins in sheep at desert districts.

It can induce both toxicologic and immunotoxic effects in a variety of cell systems and animal species as cytotoxic effect to reticulocytes, fibroblasts and lymphocytes and the cellular toxicity appears to be mediated by the inhibition of protein synthesis as reported by ***(Ueno, 1983; Rotter et al., 1993; Mogeda et al., 2002 and Hassan et al., 2003 and 2009).*** Also, fusarium mycotoxin inhibits cell division, RNA/ DNA synthesis and apoptosis **(*Rotter et al., 1996).*** Growth retardation and immune suppression are the major toxic effects induced by *Fusarium* ingestion in farm animals and suppression of the normal immune function and super induction of pro-inflammatory cytokines have been also suggested as supplementary tools for making a diagnosis as mentioned by ***(Widestrand et al., 2004 ; Kinser et al., 2004 and Hassan et al., 2004).*** This study, focused the highlight of the dangerous effects of fusarium and their mycotoxins pollution of animal feeds and water which allows a certain generalization as to the solution of problems regarding sheep breeding, which is an important contributor to the country's economy (especially at desert districts) in the form of meat, milk, wool and leather, with respect to the effects of environmental factors.

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