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AN ATTEMPT TO ALLEVIATE THE HISTOLOGICAL ALTERATIONS OF SOME INTERNAL ORGANS OF RATS FED ON AFLATOXIN CONTAMINATED DIETS

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ABSTRACT

One hundred and twenty six albino male rats were divided into 14 dietary groups (3 replicates x 3 animals/group). They fed for 20 weeks on a control (C) diet, C + 0.5 or 1% tafla, C + 1 or 3% ammonia, C + 3 or 6% H_2O_2, C + 1000 ppb aflatoxins (A), C + A + tafla or ammonia or H_2O_2 (at the same previous levels). At the end of the feeding period, the relative weights of the internal organs (liver, kidneys, spleen, testes, heart, and lungs) increased by feeding the contaminated diets. The different dietary treatments did not improve the toxic effects of A. The contaminated diets (even those treated with tafla, ammonia or H_2O_2) led to histological alterations in liver [focal necrobiotic degeneration, apoptosis with karyomegalic nuclei, disfiguration and fibrosis, diffuse kupffer cells proliferation, and leucocytic inflammatory cells infiltration], kidneys [disfiguration of the epithelial cells lining the renal tubules, pus cells, fibrosis and focal hemorrhage, and inflammatory cells infiltration].

INTRODUCTION

Aflatoxins are potent liver toxins and their effects on animals vary with dose, duration of exposure, species, sex, age and nutritional status. These toxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Abdelhamid *et al.*, 1989). They are found often in most feed and food stuffs, particularly of plant origins (Abdelhamid and Saleh, 1996). These toxins may be lethal when consumed in large doses (acute exposure) and low levels (chronic exposure) can result in cancer (Wogan and Newberne, 1967; Sinnhuber *et al.*, 1977, Sekijima *et al.*, 1999 and Awney *et al.*, 2002). Rats are among animals resistant to the carcinogenicity of aflatoxin (Eaton and Ramsdell, 1992).

Aflatoxicosis is primarily a hepatic disease. Hepatic injury in aflatoxicosis can be demonstrated by changes in clinical chemistry values associated with liver function (Richard *et al.*, 1975; Thurston *et al.*, 1980 and Norred, 1986) and by histopathology, where lesions of bile duct proliferation, hepato cellular degeneration, necrosis, and fibrosis are seen in virtually all instances of clinical and experimental disease (Butter, 1974; Edds, 1979 and Hoerr *et al.*, 1986).

Scientific efforts were directed towards using physical, chemical and biological techniques for detoxification or inactivation of aflatoxins (Abdelhamid *et al.*, 1986 & 1992-a &b; Abdelhamid, 1993 and Abdelhamid & Mahmoud 1996). Many chemical have been tested for their ability to structurally degrade or inactivate aflatoxins, including oxidizing agents, and bases (Goldblatt & Doller, 1979 and Anderson, 1983). Ammoniation resulted in a significant reduction in the level of aflatoxins in contaminated peanut and

cottonseed meals (Gardner *et al.*, 1971; Park *et al.*, 1984 and Abdelhamid *et al.*, 2002-a). The safety of ammoniated corn was evaluated in rainbow trout (Brekke *et al.*, 1979), chickens (Hughes *et al.*, 1979) and rats (Southern and Clawson 1980). In a long term feeding study on rats, Norred and Morrissey (1983) reported that ammoniation of corn resulted in significant protection from toxicity and hepatic neoplasia in experimental animals.

Chokobarti (1981) reported that adding hydrogen peroxide at levels of 3 and 6% to the aflatoxic meal destroyed this toxin with negligible losses of proteins and lipids in meal. Adsorbent materials were used too for the detoxification, including clays (Nowar *et al.*, 1996 & 2000). However, the aim of the present study was to evaluate the effectiveness of ammonia, hydrogen peroxide and tafla in reducing hitopatological effects of aflatoxins in rats.

MATERIALS AND METHODS

Production of aflatoxins:

For producing aflatoxin, the strain of *Aspergillus flavus* NRRL 3357 (From Laboratory of Mycotoxins, National Research Center, Dokki, Cairo) was grown on synthetic media, namely yeast extract – sucrose broth (YES) containing 2% yeast extract and 20% sucrose. The substrate was dispensed in conical flasks. The flasks were then autoclaved for 15 minutes at 121°C then coold and inoculated with spores suspension and incubated for 9 days at 25 – 29°C. Aflatoxin concentration was determined using immunoaffinity column coupled with solution fluorometry or liquid chromatography postcolumn derivatization according to Truckess *et al.* (1991). The media contained a mixture of aflatoxins B_1, B_2, G_1 and G_2 at a total level of 18 ppm. The culture was added to feed to be contained 1000 ppb aflatoxins.

Animals:

One hundred and twenty six male albino white rats (average weight 105 gm) were bought from the focal market and randomly divided into 14 groups (9 animals for each group, i.e. treatment, at 3 replicates, i.e. cages). The animals were housed in wire cages (provided with feed and water troughs), three animals in each cage and fed *ad libitum* and water was available for 24 hours dialy, and cages were kept in a conditioned room.

Diets:

A basal diet was prepared from focally purchased ingredients according to Ahmed (1976). It contained 46% crushed wheat, 40% shredded barley, 9% fish meal, 3% dried milk, 1% yeast and 1% minerals and vitamins mixture. It was tested and proved that it was free of aflatoxins. The experimental dietary groups were control (basal) diet, control diet plus 0.5 or 1% tafla, control diet treated with 1 or 3% ammonia solution or with 3 or 6% hydrogen peroxide solution, control diet contaminated with 1000 ppb aflatoxins and aflatoxins contaminated diet plus 0.5 or 1% tafla, 1 or 3% ammonia solution and 3 or 6% hydrogen peroxide solution. The experimental diets were offered for rats for 20 weeks.

Criteria tested:

At the end of the experiment, three animals from each group were fasted for 14-hours, slaughtered and different organs were weighed and proportionated to live body weight (relative organs weight). Samples of liver and kidneys were fixed in formaline for histopathological investigation according to Bancroft *et al.* (1990).

Statistical analysis:

Numerical data obtained were statistically analyzed using MSTATC computer program package (Russell, 1986). When F-test was significant, least significant difference (LSD) was calculated according to Duncan 1955 for the comparison between means.

RESULTS AND DISCUSSION

1- Relative weights of rats' organs:

It is very clear from Table (1) that the aflatoxic diets increased obviously relative weights of all tested organs comparing with the aflatoxin – free diets. The additives (tafla, ammonia and hydrogen peroxide) at their both tested levels did not alter the organs weight; yet, they slightly diminished – to some extent – the negative effect of dietary aflatoxin inclusion on the relative weights of all tested organs.

Table (1): Effect of the dietary treatments on relative weights of different organs (% from the live body weights) of the experimental rats.

Dietary treatments	Liver	Kidneys	Spleen	Testes	Heart	Lungs
Control (C)	2.46^{e}	0.48^{c}	0.23^{c}	0.81^{d}	0.31^{b}	0.74^{b}
C + 0.5% tafla	2.37^{e}	0.48^{c}	0.23^{c}	0.79^{d}	0.31^{b}	0.73^{b}
C + 1% tafla	2.41^{e}	0.50^{c}	0.23^{c}	0.79^{d}	0.31^{b}	0.75^{b}
C + 1% ammonia	2.36^{e}	0.48^{c}	0.23^{c}	0.79^{d}	0.31^{b}	0.74^{b}
C + 3% ammonia	2.41^{e}	0.48^{c}	0.23^{c}	0.78^{d}	0.30^{b}	0.74^{b}
C + 3% H_2O_2	2.40^{e}	0.48^{c}	0.23^{c}	0.78^{d}	0.31^{b}	0.75^{b}
C + 6% H_2O_2	2.47^{e}	0.49^{c}	0.23^{c}	0.80^{d}	0.30^{b}	0.74^{b}
Aflatoxin (A)	3.86^{a}	0.73^{a}	0.33^{a}	1.15^{a}	0.44^{a}	1.06^{a}
A + 0.5% tafla	3.25^{cd}	0.64^{b}	0.31^{b}	1.07^{bc}	0.42^{a}	0.99^{a}
A + 1% tafla	3.22^{cd}	0.65^{b}	0.30^{b}	1.09^{bc}	0.42^{a}	0.99^{a}
A + 1% ammonia	3.16^{d}	0.66^{b}	0.31^{b}	1.07^{bc}	0.42^{a}	0.99^{a}
A + 3% ammonia	3.25^{cd}	0.67^{b}	0.30^{b}	1.07^{bc}	0.41^{a}	0.99^{a}
A + 3% H_2O_2	3.44^{b}	0.67^{b}	0.30^{b}	1.13^{ab}	0.44^{a}	1.03^{a}
A + 6% H_2O_2	3.35^{bc}	0.65^{b}	0.30^{b}	1.07^{bc}	0.42^{a}	1.00^{a}

a – e: Means in the same column followed by different letters differ significantly at $P \leq 0.05$.

In this respect, Singh *et al.* (1993 and 1998) found that dietary aflatoxin led to enlargement of liver and congestion of kidneys and lungs of rabbits. Moreover, Soliman *et al.* (2001) reported that aflatoxic rabbits reflected

increased relative liver weight but relative kidneys weight was decreased. However, Tamimi *et al.* (1997) noticed that there was significant decrease in relative weight of liver from aflatoxicated rats.

2- Histopathological examination:

2-1- Liver:

There was no histopathological alteration observed in the control liver as well as livers from all other groups fed on the basal diet plus tafla, ammonia and hydrogen peroxide (without aflatoxins). Their sections showed normal lobules of hepatocytes with normal central vein in the middle. The cells are arranged in thin plates with blood sinusoids in between. The portal tract is seen between the adjacent lobules shows the hepatic artery (Fig. 1).

In aflatoxic rats, focal necrobiotic degenerated areas were observed in the hepatic tissue parenchyma associated with focal mononuclear leucocytic inflammatory cells infiltration mainly surrounding the central veins as well as diffuse kupffer cells proliferation (Fig. 2).

Fig. (1): Liver of a control rat showing no histopathological alteration (H & E X 40).

Fig. (2): Liver of rat administrated aflatoxin showing focal necrobiotic areas with mononuclear leucocytic inflammatory cells infiltration mainly surrounding the central vein with diffused kupffer cells proliferation (H & E X 40).

The hepatocytes showed degeneration and apoptosis with karyomegalic nuclei (Fig. 3). There was disfiguration and alteration in the histological structure of the hepatocytes (Fig. 4).

The hepatocytes showed degenerative changes and karyomegalic nuclei associated with disfiguration in the normal histological arrangement as well as apoptosis beside diffuse proliferation of the kupffer cells (Figs. 5 & 6).

Rats administrated aflatoxin with 0.5% tafla showed diffused kupffer cells proliferation in between the degenerated hepatocytes (Figs. 7 and 8). Whereas in rats administrated aflatoxin with 1% tafla, the hepatic cells showed focal areas of necrobiosis and degeneration with karyomegalic nuclei associated with fibrosis in the portal area (Figs. 9 and 10), as well as in between the hepatocytes in focal manner beside diffused kupffer cells proliferation (Fig. 11).

Fig. (3): Liver of rat administrated aflatoxin showing karyomegalic nuclei in degenerated apoptotic hepatocytes (H & E X 160).

Fig. (4): Liver of rat administrated aflatoxin showing disfiguration and alteration in the normal histological structure of the hepatocytes (H & E X 160).

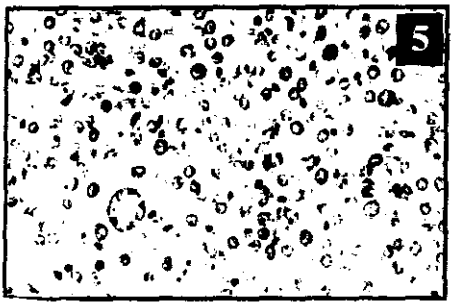

Fig. (5): Liver of rat administrated aflatoxin showing karyomegalic nuclei of degenerated hepatocytes with kupffer cells proliferation (H & E X 160).

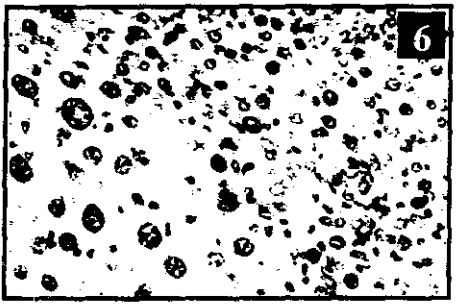

Fig. (6): Liver of rat administrated aflatoxin showing disfiguration of the histological structure in most of the hepatocytes, which had karyomegalic and apoptotic nuclei (H & E X 160).

Fig. (7): Liver of rat administrated aflatoxin with 0.5% tafla showing diffused kupffer cells proliferation with degeneration in some of hepatocytes (H & E X 40).

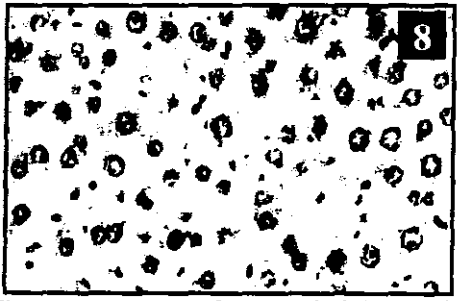

Fig. (8): Liver of rat administrated aflatoxin with 0.5% tafla showing the magnification of (Fig. 7) to identify and clarify the degenerated hepatocytes and kupffer cells proliferation (H & E X 160).

Fig. (9): Liver of rat administrated aflatoxin and 1% tafla showing focal necrobiotic degenerated areas with fibrosis in the portal area with karyomegalic nuclei of other hepatocytes (H & E X 40).

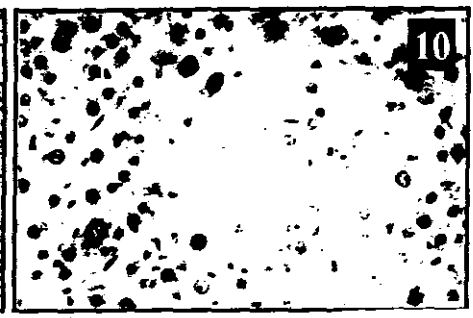

Fig. (10): Liver of rat administrated aflatoxin and 1% tafla showing the magnification of (Fig. 9) to identify the focal necrobiotic degenerated area and karyomegalic nuclei of hepatocytes (H & E X 160).

Fig. (11): Liver of rat administrated aflatoxin with 1% tafla showing diffused kupffer cells proliferation with focal fibrosis in between the hepatocytes (H & E X 160).

The examination of liver from rats fed on aflatoxic diet treated with the low level of ammonia revealed that the hepatic cells showed focal areas of necrobiosis and degeneration associated with fibrosis and leucocytic inflammatory cells infiltration in the portal area (Fig. 12). Whereas on aflatoxic diet treated with the high level of ammonia, the hepatic cells showed focal areas of necrobiosis and degeneration associated with fibrosis and leucocytic inflammatory cells infiltration in the portal area as well as diffused kupffer cells proliferation allover the hepatic parenchyma (Fig. 13).

However, rats fed the aflatoxic diet with low level of H_2O_2 presented hepatic cells showed focal areas of necrobiosis and degeneration associated with fibrosis and leucocytic inflammatory cells infiltration in the portal area (Fig. 14). Yet, high level of H_2O_2 with the toxic diet reflected hepatic cells showed focal necrotic areas (Fig. 15), karyomegalic nuclei and degeneration associated with leucocytic inflammatory cells infiltration in between (Fig. 16), as well as fibrosis with diffused kupffer cells proliferation (Fig. 17).

Fig. (12): Liver of rat administrated aflatoxin with the low dose of ammonia showing focal necrobiotic degenerated area with fibrosis and leucocytic inflammatory cells in the portel area (H & E X 40).

Fig. (13): Liver of rat administrated aflatoxin with the high dose of ammonia showing focal necrobiotic degenerated area of the hepatocytes with fibrosis and leucocytic inflammatory cells infiltration in the portal area in association with diffused kupffer cells proliferation (H & E X 40).

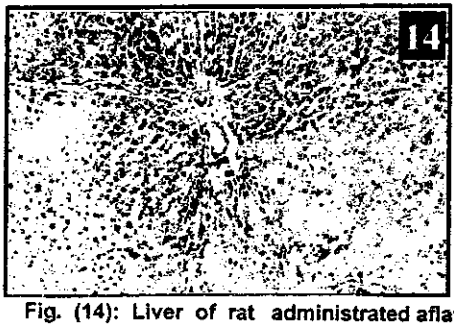

Fig. (14): Liver of rat administrated aflatoxin with the low dose of hydrogen peroxide showing focal necrobiotic and degenerated areas in the hepatocytes with fibrosis and leucocytic inflammatory cells infiltration in the portal area (H & E X 40).

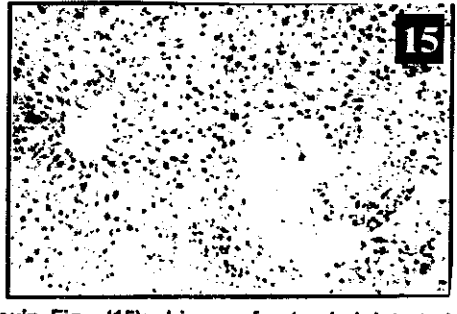

Fig. (15): Liver of rat administrated aflatoxin with the high dose of hydrogen peroxide showing focal necrotic area (H & E X 40).

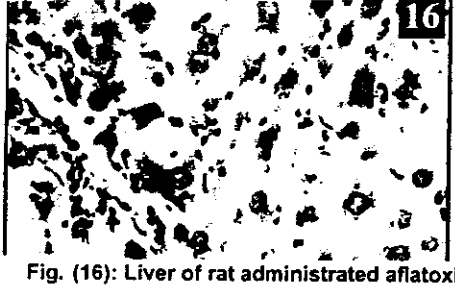

Fig. (16): Liver of rat administrated aflatoxin with the high dose of hydrogen peroxide showing karyomegalic nuclei in degenerated hepatocytes with inflammatory cells infiltration in between (H & E X 160).

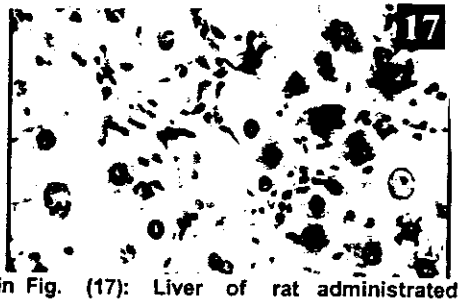

Fig. (17): Liver of rat administrated aflatoxin with the high dose of hydrogen peroxide showing fibrosis with kupffer cells proliferation in between the degenerated karyomegalic hepatocytes (H & E X 160).

From the previous show, it could be concluded that tafla, ammonia and hydrogen peroxide additions to the aflatoxic diet did not overcome or ameliorate the toxic effect of aflatoxin on the histological findings of rat's liver. Also, Soliman *et al.* (2001) reported that the use of H_2O_2 for the destruction of aflatoxins in contaminated diet induces adverse effects in the animals. Yet, Frayssinet and Frayssinet (1990) mentioned that ammonia treatment is a practical solution to the problem of the carcinogenic potency of contaminated oil cakes. The obtained results agree with those of other researcher (Abdelhamid *et al.*, 1995-a & b and 2002-b). However, Abd-El-Monem *et al.* (1996) reported that aflatoxic rat's liver showed severe congestion and vacuolar hydropic degeneration. Aflatoxin induced also degenerative changes in hepatic cells.

Liver is the target organ for aflatoxin, so it is extremely affected with main findings including bileduct cell proliferation (Llewellyn *et al.*, 1984 and Guerre *et al.*, 1996), large – degenerative cells and cells with distinct mitotic configurations (Ranjan, 1985). Also, many other manifestations were reported, e.g. marked degeneration, necrosis binucleated hepatic cells, karyomegally, hyperplasia of the bile duct epithelium, newly formed bile duct and ductules, infiltrated portal tracts by large numbers of round cells together with fibrous tissue proliferation (El-Mahdy *et al.*, 1988). Aflatoxins are causative for hepatocellular carcinomas (Morimura *et al.*, 1990) and hepatoma including nodular hyperplasia, hypertrophy, vacuolisation, degeneration, pseudolobulation, cellular infiltration and fibrosis (Rati *et al.*, 1991; Singh *et al.*, 1998 and Vinita *et al.*, 2003).

Aflatoxic livers showed also congestion of central and portal veins, and sinusoids. Hepatocytes in centrilobar and midzonal areas showed hydropic and fatty changes with focal areas of necrosis (Sahoo *et al.*, 1992). Singh *et al.* (1993) and Nowar *et al.* (1994) revealed presence of foci of coagulative necrosis with bile ductular hyperplasia, pericellular and periportal cirrhosis in the aflatoxic liver. Histopathological assessment was characterized by portal/central vein/artery congestion, sinusoid congestion, nuclear pyknosis and karyolysis, and hepatocyte vacuolation (Towner *et al.*, 2000). Aflatoxins are responsible for hepatocytes with dysplastic nucleic (Gelderblom *et al.*, 2002).

2-2- Kidney:

There was no histopathological alteration observed in the control section nor in sections of all kidneys from rats fed on the aflatoxin – free diets. So, intact glomeruli and normal renal convoluted tubules were shown (Fig. 18). Whereas, dietary aflatoxin inclusion presented the epithelial cells lining the renal tubules showed disfiguration in the shapes and normal arrangement in the basement membrane (Figs. 19 and 20). Focal area of aggregated dead neutrophils (pus cells) was observed in the both cortical and medullry portions (Fig. 21). Aflatoxin plus 0.5% tafla caused focal fibrosis in between the atrophied renal tubules (Fig. 22) but with 1% tafla, focal fibrosis with mononuclear leucocytic inflammatory cells infiltration were noticed in between the renal tubules as well as in the dilated perivascular areas (Fig. 23).

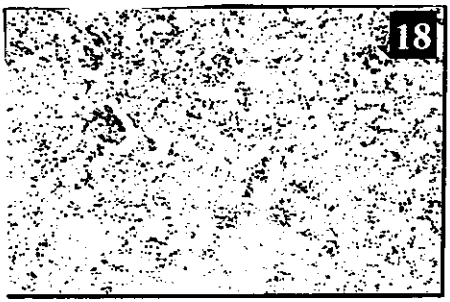

Fig. (18): Kidney of control rat showing no histopathological alteration (H & E X 40).

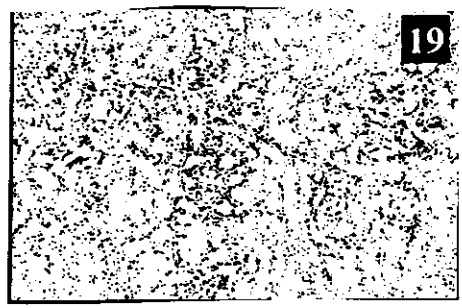

Fig. (19): Kidney of rat administrated aflatoxin showing disfiguration in the shape of epithelial cells lining some of renal tubules (H & E X 40).

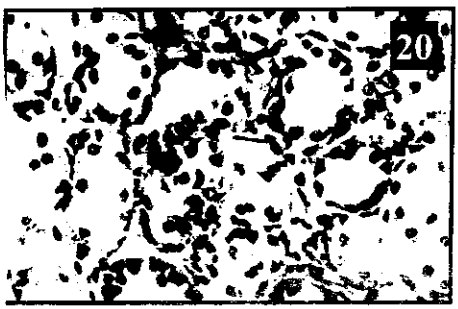

Fig. (20): Kidney of rat administrated aflatoxin showing the high magnification of (Fig. 19) to clarify and identify the disfiguration of epithelial cells lining the renal tubules (H & E X 160).

Fig. (21): Kidney of rat administrated aflatoxin showing focal area of aggregated dead neutrophils (pus cells) (H & E X 40).

Fig. (22): Kidney of rat administrated aflatoxin and low dose of tafla showing fibrosis in between the atrophied renal tubules in focal manner (H & E X 160).

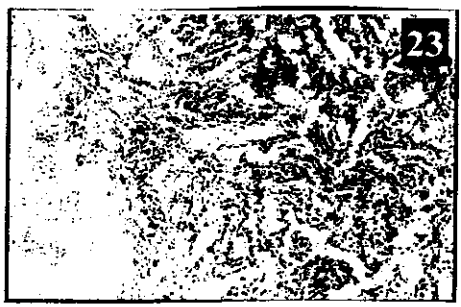

Fig. (23): Kidney of rat administrated aflatoxin and high dose of tafla showing focal fibrosis with mononuclear leucocytic inflammatory cells infiltration in between the renal tubules as well as in the perivascular area of dilated blood vessel (H & E X 40).

Low level of ammonia treated aflatoxic diet showed focal fibrosis in between the atrophied renal tubules (Fig. 24). Yet, high level of ammonia with toxic diet reflected focal leucocytic inflammatory cells infiltration with fibrosis and focal haemorrhagic areas noticed in between the renal tubules (Fig. 25). The corticomedullary portion had an areas of hemorrhages, hyperemic capillaries and inflammatory cells infiltration and fibrosis (Fig. 26). Kidneys of rats fed on the aflatoxin contaminated diet treated with low level of H_2O_2 show focal leucocytic inflammatory cells infiltration in between the degenerated renal tubules and glomeruli (Fig. 27). Whereas, those from animals fed on the aflatoxic diet with high H_2O_2 level reflect focal leucocytic inflammatory cells infiltration and fibrosis adjacent the glomerulus (Fig. 28).

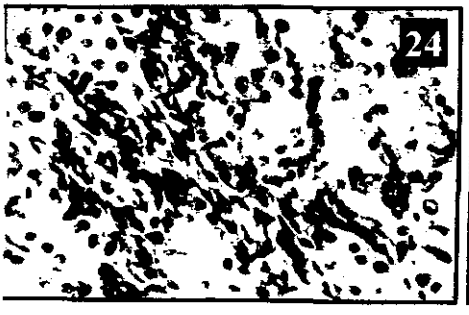

Fig. (24): Kidney of rat administrated aflatoxin with low dose of ammonia showing fibrosis in between the atrophied renal tubules in focal manner (H & E X 160).

Fig. (25): Kidney of rat administrated aflatoxin with high dose of ammonia showing focal mononuclear leucocytic inflammatory cells infiltration with fibrosis associated with focal haemorrhagic area (H & E X 40).

Fig. (26): Kidney of rat administrated aflatoxin with the high dose of ammonia showing focal area of hyperemic blood capillaries, haemorrhages and inflammatoy cells infiltration with fibrosis in the corticomedullary fraction (H & E X 40).

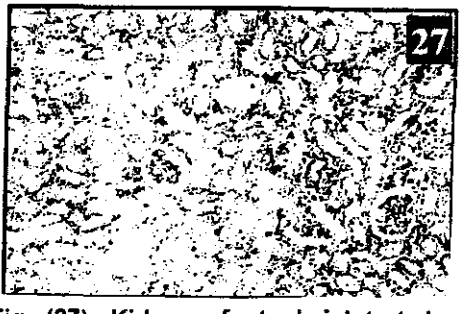

Fig. (27): Kidney of rat administrated aflatoxin with the low dose of hydrogen peroxide showing focal leucocytic inflammatory cells infiltration in between the glomeruli and degenerated renal tubules (H & E X 40).

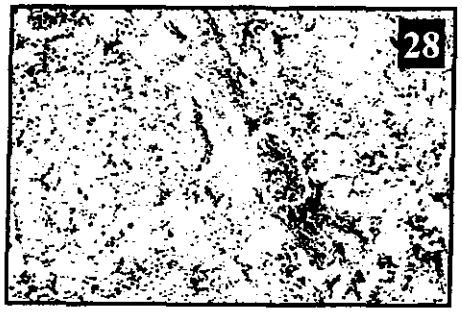

Fig. (28): Kidney of rat administrated aflatoxin with the high dose of hydrogen peroxide showing mononuclear leucocytic inflammatory cells and fibrosis in focal manner adjacent the glomerulus (H & E X 40).

Again, aflatoxins are harmful for kidneys as excretory organs for different toxic metabolites. So, Nowar *et al.* (1994 and 1996) and Abdelhamid *et al.* (2002-b) recorded that the histopathological examination of rabbits and rats, respectively treated with aflatoxin showed renal degenerative changes and focal coagulative necrosis. Although zeolite at 0.5 of the aflatoxic diet improved the histological picture (Abdel Wahhab, 1999), all the tested additives (tafla, ammonia and H_2O_2) did not succeed in lighten the histological alterations occurred in kidneys by aflatoxicosis.

It was reported very often that aflatoxin showed degenerative lesions of the kidneys (Trigo *et al.*, 1981; Maru *et al.*, 1987; El-Mahdy *et al.*, 1988; Bacawy, 1997 and Soliman *et al.*, 2001) and masses of non-differentiated cells with hyperchromatic nuclei in the medullary region of the kidneys (Ranjan, 1985). Cortical congestion and focal hemorrhages in the medulla were observed in the kidneys (Singh *et al.*, 1998). Vinita *et al.* (2003) reported congested glomeruli, hemorrhage and tubular epithelium necrosis in kidneys of aflatoxic rabbits.

CONCLUSION

Aflatoxins are very toxic natural substances negatively affected relative weight of different internal organs as well as their histological structures. Treating aflatoxic diet with tafla, ammonia or hydrogen peroxide did not ameliorate these negative effects of aflatoxins on rats. Therefore, it is a must to realize the wisdom said that "Prophylaxis is more better than treatment".

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محاولة تخفيف التأثيرات المرضية على أنسجة بعض الأعضاء الداخلية للجـــرذان المغذاة على عليقة ملوثة بالأفلاتوكسين٠

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٢) المعمل المركزى للأغذية والأعلاف – وزارة الزراعة – القـــاهـــرة٠

تم تغذية ١٢٦ من الجـــرذان البيضاء الذكـــور لمدة عشرين أسبوعا على ١٤ معاملـــة غذائية (٣ مكررات × ٣ حيوانات/معاملة) تتضمن عليقة خالية من الأفلاتوكسين بدون معاملـــة، أو مضافا إليهـــا ٥ر٠ أو ١% طفـــلة، أو معاملة بالأمونيـــا (١، ٣%)، أو معاملـــة بفـــوق أكســـيد الهيدروجين (٣، ٦%)، أو مضافا إليها ١٠٠٠ جـــزء/بليـــون أفلاتوكســـينات، أو مضافـــا إليـــها التوكســـــين والطفلة أو الأمونيا أو فوق أكسيد الهيدروجين (بنفس التركيزات السابقة)٠

فى نهاية التجربة وجد أن الأعضاء الداخلية (كبد – كلى – طحال – خصى – قلب – رئات) قـــد زاد وزنها النسبى بالتغذية الملوثة وأن المعاملات المختلفة لم تحسن الوضع٠ كمـــا أدت العلائـــق الملوثة (حتى المعاملة بالطفلة أو الأمونيا أو فوق أكسيد الهيدروجين) إلى تغييرات مرضيـــة فـــى أنسجة كل من الكبد [تدهور نكروزى بؤرى – انفصال الخلايا وهدمها – مسخ (تشوه) – تليـــف – انتشار تكاثر خلايا كوبفر – ارتشاح الخلايا الملهبة من كرات الدم البيضاء] والكلى [تشوه خلايـــا الطلائية المبطنة للأنابيب الكلوية – خلايا صديدية – تليف – أنزفة بؤرية – رشح الخلايا الملهبة]٠