

PATHOLOGICAL CHANGES IN LIVER, SPLEEN AND LYMPH NODES IN MICE TREATED WITH HYDATID CYST FLUID OF SHEEP ORIGIN AND ITS TOXIN FRACTIONS

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ABSTRACT

Swiss albino mice were injected, intraperitoneally, with hydatid cyst fluid (HCF) of sheep origin and its toxin fractions (TFs) at two concentrations (10 and 50 µg/ml). Changes in the weight and organ index of liver, spleen and lymph nodes, in addition to the histopathological changes occurred in these organs, and was investigated. Results demonstrate that the severity of the pathological lesions caused by TFs at both concentrations, is more than that caused by HCF.

التغيرات المرضية في الكبد ، الطحال والعقد اللمفية للفئران المعاملة بسائل الاكياس

العدرية من أصل أغنام واجزائه السامة

انتصار رحيم الكنانى* و نبيل عناد صالح** و اميمة عادل نجم عيو* فرغ الاراض ،
كلية الطب البيطري ، **قسم علوم الحياة ، كلية التربية ، جامعة الموصل

الخلاصة

حقنت الفئران السويسرية البيضاء ، داخل البريتون ، بسائل الاكياس العدرية والاجزاء السامة المعزولة منه من اصل اغنام بالتركيزين 10 و 50 مايكروغرام/مليتر. درست التغيرات الحاصلة في وزن ومعدل تضخم الكبد والطحال والعقد اللمفية ، فضلا عن التغيرات المرضية النسجية. اوضحت النتائج بأن شدة الافات المرضية التي احدثتها الاجزاء السامة ، عند كلا التركيزين ، كانت اكبر من تلك التي احدثها السائل العدري.

INTRODUCTION

Cystic hydatid disease (CHD), caused by the larval stage of *Echinococcus granulosus* , is one of the most important zoonotic diseases in man (1) and more than 70 species of ungulates (2). Pathogenesis tissue damage is caused by replacement of host tissues by growing cysts and, in some instances, by vascular compromise. The main result is the dysfunction of the organs in which cysts grow (3-4). Previous authors gave a clear picture of the histopathological changes which occur due to the presence of cysts in different organs (5-24). However, few studies have focused on the histopathology of tissues in hosts infected with hydatid disease and treated with immunomodulators of different sources (for references see 25).

Osuna *et al.* (26) suggested the presence of certain fractions in the cyst fluid of hydatid cysts, termed as toxin fractions which were found to be cytotoxic to the macrophages of the host *in vitro*. They turned to be lipid in nature (27). It has been demonstrated that these fractions, obtained from cysts of different host origin, differ in their cytotoxicity to the macrophages and suggested the possibility that they are strain-specific (28-30). Salih and Abbu (31) presented evidence that these fractions are strain-specific, and that they are cytotoxic not only to macrophages in the tissues, but to microphages in the blood as well (32).

Apart from the above mentioned studies on toxin fractions, no investigation seems to have been carried out on their cytotoxicity to host tissues, other than macrophages. The present study is, therefore, conducted to investigate the pathological effect of toxin fractions, obtained from hydatid cysts of sheep origin, on liver, spleen and lymph nodes of mice injected, intraperitoneally, with these fractions.

$$\text{Organ index} = \frac{\text{Organ weight}}{\text{Body weight} - \text{Organ weight}} \times 1000$$

MATERIALS & METHODS

Hydatid cysts of sheep origin were obtained from infected liver and lung of sheep slaughtered in Nineveh Slaughter House.

Protoscolices were removed from the cysts, aseptically, according to Smyth (33). After centrifugation at 7600 g (10000 rpm), using a cryocentrifuge 6-4 (Heracus) for 10 minutes at 4°C, the supernatant (HCF) was separated and kept in sterile containers in refrigerators at -20°C until used.

Cyst fluid fractions (CFFs) were separated according to Janssen *et al.* (30). Ammonium sulphate was added to the cyst fluid (49.35 gm/100ml) and the fluid was left in the refrigerator at 4 °C for 24 hrs to give enough time for precipitation of protein. The fluid was centrifuged at 37000g (22150 rpm) for 30 minutes at 4 °C using the above mentioned centrifuge. An equal volume of chloroform was added to the supernatant. Two layers were formed after centrifugation. The chloroform layer was separated and half volume of methanol (chloroform: methanol = 2:1 v/v) was added and centrifuged under the same conditions, mentioned above. The supernatant was dried by rotary evaporator. The chloroform-methanol soluble fractions (CMSFs), or TFs, were kept in refrigerator at -20°C until used. At use, they were dissolved in few drops of chloroform and completed by phosphate buffer saline (PBS).

Twenty one, parasite-free, laboratory-bred, 5-6 weeks old, Swiss albino male mice were used in the present study. 3 mice were used as control group (not injected), and 8 were injected, intraperitoneally, with HCF and its TFs as follows:

Six male mice were injected with crude hydatid cyst fluid (HCF) at a rate of 1ml/ mouse of sheep origin (from liver and lung). After 32 days mice were killed and their liver, spleen and lymph nodes were removed.

The same number of mice, were injected with TFs at a concentration of 10 µg/ml and also mice were killed after 32 days and their liver, spleen and lymph nodes were removed.

Also, the same number of mice, were injected with TFs at a concentration of 50 µg/ml and also mice were killed after 32 days and their liver, spleen and lymph nodes were removed.

Organ index was estimated according to Kroeze and Tanner (34) as follows:

For light microscopy, tissues (liver, spleen and lymph nodes) of mice treated with HCF and its toxin fractions were fixed in 10% neutral buffered formalin, processed routinely in alcohol and, finally, embedded in paraffin. and 4-6µm thick-sections were prepared. The sections were stained with hematoxylin-eosin (H & E) (35).

Student t-test was applied to determine the significance between the means at $P < 0.05$ (36).

RESULTS

It is obvious from table (1) that the weight and organ index of liver decreased significantly in mice treated with HCF and its TFs of liver origin, at both concentrations, compared to the control group. However, the decrease was higher in mice treated with HCF compared with those treated with TFs. A non significant difference in the weight of liver and its organ index appeared between mice treated with TFs, at 50µg/ml, and those treated with TFs, at 10µg/ml.

For spleen, a decrease in its weight and organ index in mice treated with HCF and its TFs, at both concentrations, was noticed compared with the control group. However the decrease was higher in mice treated with TFs at 10µg/ml than those treated with 50µg/ml. Non significant increase in the organ index was noticed in mice treated with HCF compared with the control group. In mice treated with TFs, at both concentrations, a non significant decrease in the organ index was noticed although it was significant in mice treated with TFs at 10µg/ml compared with those treated with HCF.

For lymph nodes, a significant increase in their weight and organ index was noticed in mice treated with HCF compared with the control group and those treated with TFs, at both concentrations.

Table (2) shows that a significant decrease in the weight of liver occurred in mice treated with HCF and its TFs of lung origin, at both concentrations, compared with the control group. The weight of liver decrease significantly in mice treated with HCF compared with those of TFs at 10µg/ml. However, this decrease was not significant in mice treated with TFs at 50µg/ml compared with those treated with TFs at 10µg/ml. The organ index decrease significantly in mice treated with HCF compared with the control group and with those treated with TFs, at both concentrations.

For spleen, a non significant increase in its weight and organ index was noticed in mice treated with HCF and a non significant decrease in mice treated with TFs, at both concentrations, compared with the control group. An increase in the weight of spleen was noticed in mice treated with HCF compared with those treated with TFs at both concentrations. However, the increase was significant when compared with mice treated with TFs at 50µg/ml but non significant when compared

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with those treated with TFs at 10µg/ml. A non significant increase in the organ index was noticed in mice treated with HCF and its TFs, at both concentrations, compared with the control group. However, the increase was higher in those treated with HCF.

An increase in the weight and organ index of lymph nodes was noticed in mice treated with HCF and its TFs, at both concentrations, when compared with the control group. However, the increase was higher but non significant, in mice treated with TFs at 50µg/ml compared with the other two groups.

Hepatomegally, splenomegally and swelling of lymph nodes, particularly medistinal and peripheral, were observed in mice treated with TFs at both concentrations (10 and 50 µg/ml).

Liver: in mice treated with TFs at 10 µg/ml, the histopathological changes are represented by focal coagulative necrosis around a central vein, congestion of central vein, fatty changes, and apoptosis was also noticed in some hepatocytes. Perivascular lymphocytic cuffing around the central vein was also observed with dilatation of sinusoids associated with kupffer cells hypertrophy . At the concentration 50 µg/ml the lesion is represented by severe fatty changes with coagulative necrosis at the periphery of lobules with severe hemorrhage and hemosiderosis. With the HCF the histopathological changes are characterized by presence of multifocal areas of coagulative necrosis, fatty change and focal lymphocytic aggregation (Figs 1-3).

Spleen: at the concentration 10 µg/ml the histopathological lesions are represented by focal area of necrosis in the red pulp, depletion of white pulp associated with proliferation of plasma cells and megakaryocytes, arteriosclerosis in the wall of splenic artery with hemosiderosis. At the concentration 50 µg/ml, the lesions appeared were more severe than the above mentioned ones, represented by focal area of necrosis associated with hemorrhage, proliferation of plasma cells replacing the lymphocytes .With HCF the histopathological changes revealed severe hemorrhage and hemosiderosis associated with depletion of white pulp and edema (Figs 4-6).

Lymph nodes; at concentration 10µg/ml, 50µg/ml the histopathological lesions was characterized by severe congestion with hemorrhage and thickness in wall of lymph nodes with proliferation of lymphocytes and plasma cells while in HCF this lesion is represented by congestion and hemosiderine pigments deposition (Figs 7 and 8).

Table 1: Changes in weight and organ index of liver, spleen and lymph nodes in mice treated with HCF and its TFs, obtained from liver cysts of sheep origin.

Organ	Con. µg/ml	Weight (gm)	Organ index
Liver	C.	1.8487 A	76.3363 A
	HCF	0.5093 C	40.2790 C
	TF 10	1.4203 B	60.6707 B
	TF 50	1.4157 B	57.5953 B
Spleen	C.	0.1387 A	5.5340 AB
	HCF	0.1087 A	7.2090 A
	TF 10	0.0910 A	3.7090 B
	TF 50	0.1340 A	5.1800 AB
Lymph node	C.	0.0030 B	0.1153 B
	HCF	0.0063 A	0.4720 A
	TF 10	0.0030 B	0.1210 B
	TF 50	0.0030 B	0.1147 B

C: Control group , HCF : Hydatid cyst fluid , TF : Toxin fraction

Table 2: Changes in weight and organ index of liver, spleen and lymph nodes in mice treated with HCF and its TFs, obtained from lung cysts of sheep origin.

Organ	Con. µg/ml	Weight (gm)	Organ index
Liver	C.	1.8487 A	76.3363 A
	HCF	0.6667 C	39.0567 B
	TF 10	1.1950 B	59.7217 A
	TF 50	0.9317 BC	63.8157 A
Spleen	C.	0.1387 AB	5.5340 A
	HCF	0.1897 A	11.3067 A
	TF 10	0.1273 AB	6.2237 A
	TF 50	0.1007 B	6.4913 A
Lymph node	C.	0.0030 A	0.1153 A
	HCF	0.0050 A	0.3007 A
	TF 10	0.0057 A	0.2627 A
	TF 50	0.0070 A	0.3697 A

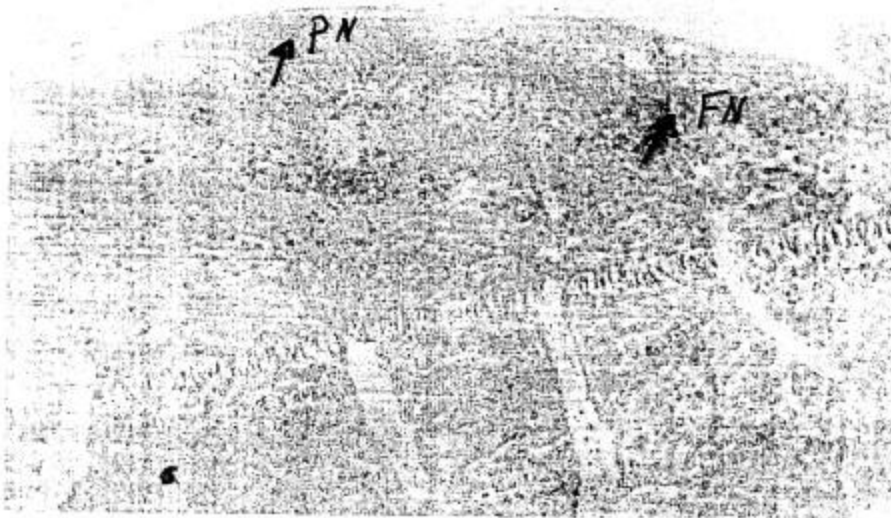


Figure 1. Histomicrograph of liver in mice treated with TFs (50 µg/ml) showing severe fatty changes associated with necrosis and dilatation of sinusoids H&E 400X.

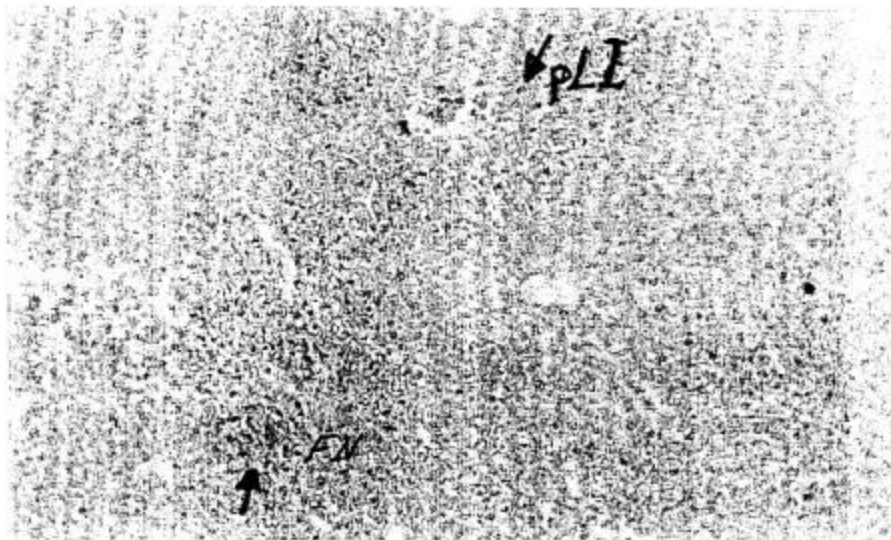


Figure 2. Histomicrograph of spleen in mice treated with TFs (10µg/ml) showing hemosiderin pigmentation associated with severe hemorrhage H & E 200X.

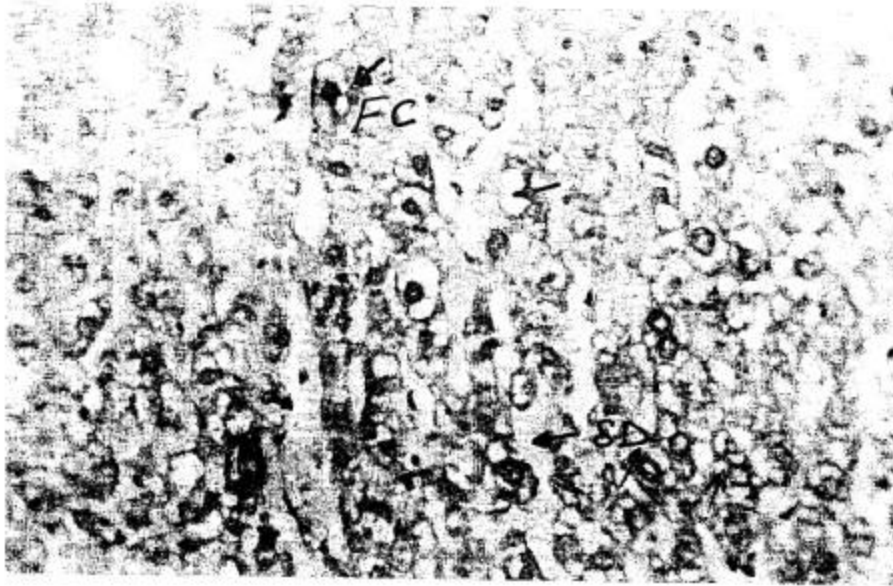


Figure 3: Histomicrograph of spleen in mice treated with TFs (50µg/ml) showing proliferation of megakaryocytes. H & E 400X

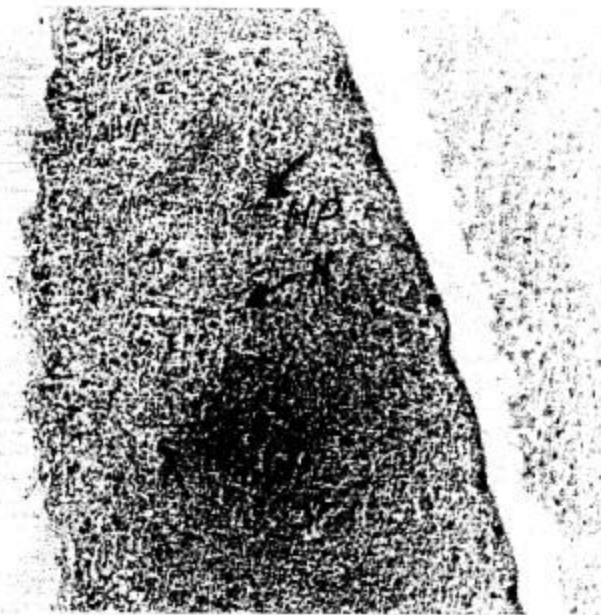


Figure 4: Histomicrograph of spleen in mice treated with HCF showing depletion of white pulp with edema. H & E 400X

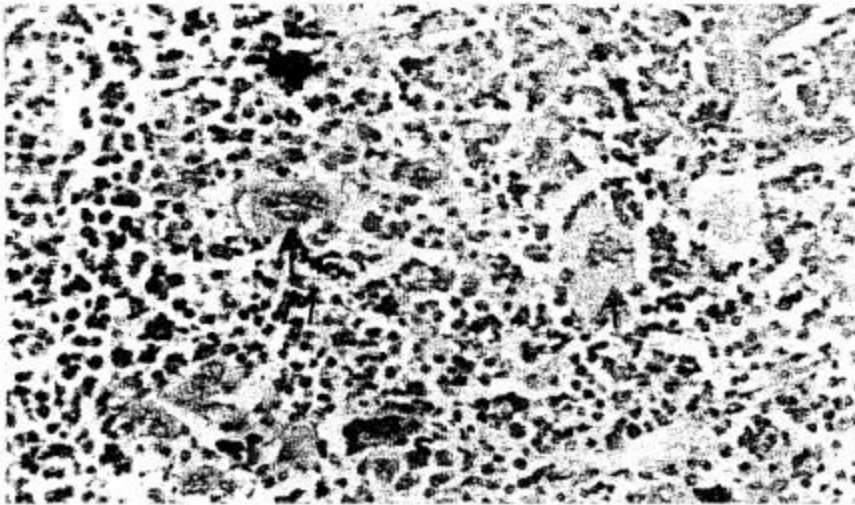


Figure 5 : Histomicrograph of lymph nodes in mice treated with TFs (10µg/ml) showing thickness in capsule congestion associated with plasma cell proliferation. H & E 400X

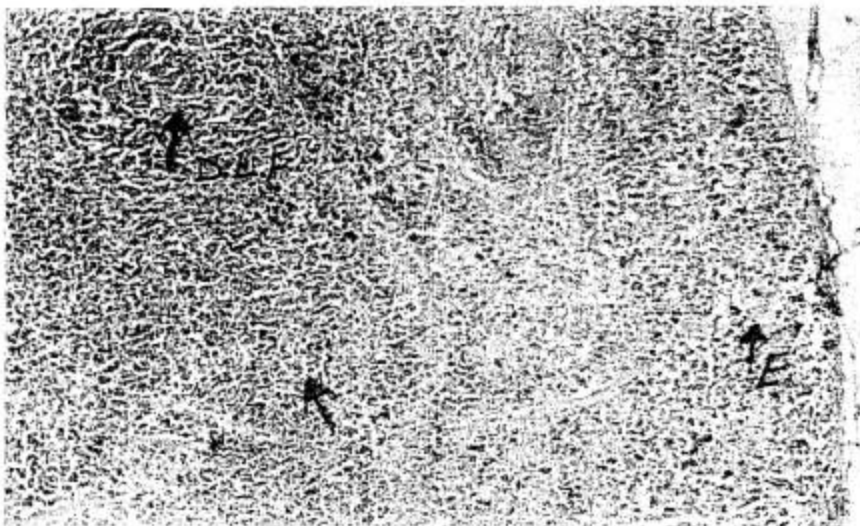


Figure 6 :Histomicrograph of lymph node in mice treated with TFs (50µg/ml) showing focal area of necrosis associated with lymphocyte infiltration and dilatation of sinusoid. H & E 400X

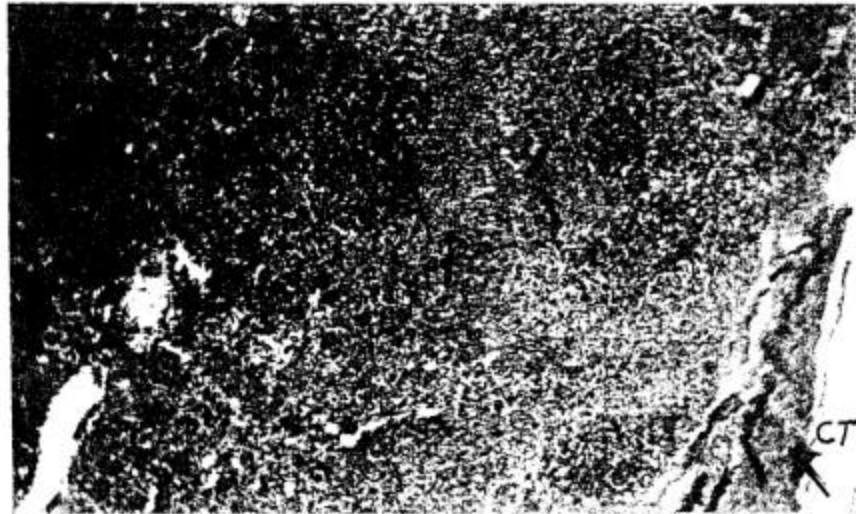


Figure 7: Histomicrograph of lymph nodes in mice treated with TFs (10 μ g/ml) showing thickness in capsule and congestion associated with plasma cell proliferation. H&E 400X.

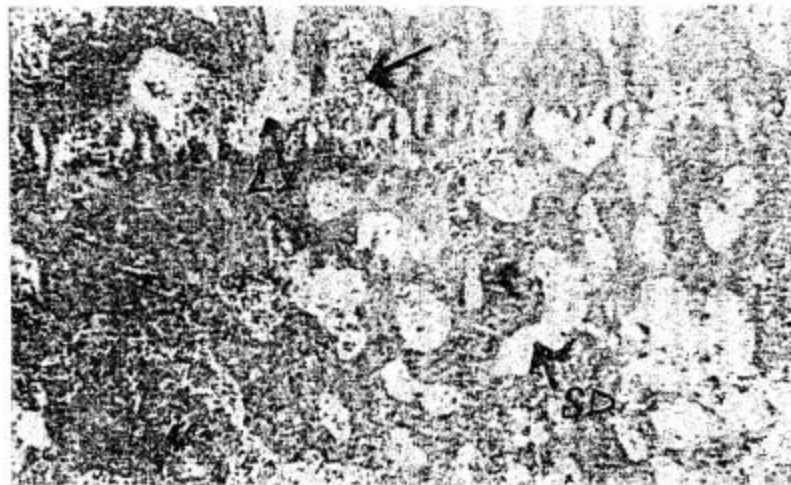


Figure 8: Histomicrograph of lymph node in mice treated with TFs (50 μ g/ml) showing focal area of necrosis associated with lymphocyte infiltration and dilatation of sinusoid. H&E 400X.

C = Congestion, CT = Capsule thickening, DLF = Depletion of lymphocyte follicle
E = Edema, FC = Fatty change, FN = Focal necrosis, H = Hemorrhage, HP = Hemosiderosis
LI = Lymphocytic infiltration, M = Megakaryocyte, P = Plasma cells,
PLC = Perivascular lymphocytic infiltration, PN = Peripheral necrosis,
SD = Sinusoidal dilatation

DISCUSSION

The present study was conducted to determine the pathological effect of HCF of sheep origin and its TFs on liver, spleen and lymph nodes of Balb/c mice, experimentally injected with HCF and its TFs. From the results obtained, it is obvious that the severity of the pathological lesions, caused by TFs, at both concentrations, is more than that caused by the HCF. This could be attributed to the nature of the TFs, which have been found to be lipid (27). They could have affected the function of the cell organelles, especially the mitochondria and the endoplasmic reticulum. This is well demonstrated in the present study by the appearance of fatty changes and coagulative necrosis. This could have led to failure of conjugation between lipids and proteins or interference with the oxidation of fatty acids, leading to the aggregation of these substances in the cytoplasm of liver cells which, in turn, cause damage to the mitochondria due to hypoxia. The result of this will, definitely, be a decrease in the energy required for the mitochondria to work properly, in addition to the loss in the formation of proteins produced by the endoplasmic reticulum which helps in the liberation of some lytic enzymes that play an important role in coagulative necrosis of liver cells. The occurrence of such lesions may help in liberation of tumor necrosis factors from kupffer cells which contribute in the attraction of lymphocytes to the affected tissue (37).

Appearance of pathological lesions in the spleen, represented by depletion of lymphocytes and replacing them by plasma cells may be due to the increasing requirement for lymphocyte, as a defense strategy, supported by proliferation of the nucleated leukocytes which could be taken into account for the occurrence of immune response, in addition to the pigmentation which indicates the occurrence of hemorrhage and liberation of hemosiderine and its precipitation in the phagocytic cells combined by the changes appeared between lymph nodes indicating the presence of subacute lymphadenitis, as an inflammatory response to liberate some chemical mediators represented by cytokines. These changes are supported by the changes occurred in the weight and organ index of liver in mice treated with TFs at 10 and 50 μ g/ml in comparison with HCF and control group. These changes could have been a result of precipitation of some chemical substances such as glycogen and lipids in the cytoplasm of the hepatocytes, in addition to the inflammatory response (see 37, 38 and 39). These changes could also be due to the injury which occurred in the cells, affecting the capability of the cells to return to normal which in turn, could be a result of a homeostatic mechanism (37). The question which needs an answer is whether these changes are reversible or not.

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