Histopathological Studies of the Liver and Kidney in Mice Fed on Smut Wheat Infected with *Tilletia*

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Abstract

The current study was designed to investigate the effect of *Tilletia* smut spores on histopathological changes in liver and kidney in mice. Twenty animals were divided into two equal groups, 10 mice each, control group fed on normal diet and the treated groups were fed on a mixture of 50% normal diet with 50% wheat infected with *Tilletia* for 30 days. Histopathological sections taken from liver and kidney treated with *Tilletia* revealed several alterations. The changes in liver included, multiple granulomatous lesions, area of coagulation necrosis, vacuolar degeneration in the cytoplasm of hepatocytes, proliferation of hepatocytes with formation of pseudolobull which initiates for procancer. Whereas in the kidney, the changes included cellular degeneration, thickness of Bowman's capsules, in other section aggregation of inflammatory cells in the interstitial tissue together with degeneration and sloughing of epithelial lining of the renal tubules.

Keyword: *PAH ,Kidney, Liver, Mice*

Introduction

Covered smut is a disease of wheat after initial plant infection causes reduced wheat yield and grains quality [1], during the development of wheat head the grains is replaced by smut balls which consist of a mass of oily foul fishy smelling grains making it unfit for milling [2]. However, smut infected wheat may be fed to all classes of livestock including poultry [3]. Breathing high concentration of smut spores may be hazardous especially to human suffering from asthmas [4].

Mycetism is a distress or poisoning resulting from the consumption of fungal organism like smut spores, the active components are made by organism and deposited within its structure, and the distress comes after the structure containing the active components or toxin [2]. Ingestion of smut of *Tilletia caries* and *T. foetida* have been considers as toxic [3] and smut spores has the characteristic smell of trimethylamine, choline, bactain, hercynin and two unidentified derivatives of trimethy-lamine, one or more of these compounds may act as the precursors of trimethylamine itself [6]. When animals ingest a certain amount of these spores they suffer from digestive dis-orders accompanied by nervous upset [7, 8, 9]. Other authors have considered that trime-thylamine generally has the toxic activity [10].

In addition, bread made from contaminated wheat contained toxic residues as detected by chicken embryos for bioassays. Nature of this toxic material was not known [11].

In Iraq a recent studies for the first time chemical analysis of wheat smut [12] revealed the presence of carcinogenic polycyclic aromatic hydrocarbons (PAH) [13], the detected concentration of which are far beyond the acceptable limit recognized internationally [14, 15].

There were few previous studies on the pathogenic effect of smut wheat fed to poultry, livestock or humans [11]. The present study performs to know the toxic effects and carcinogenicity on the internal organs as liver and kidney of mice.

Materials and Method

Animals treatment: Twenty adult albino mice were included in this study with age of [10-12] weeks with weight (c//25g), reared at animal house, the temperature ranged between 24-26^{o}C. Animal were divided randomly into two equal groups, Group1 (control) were fed on locally prepared diet, formulated from natural ingredients suitable for animal and maintenance. Group 2, treated were fed on

a mixture of 50% of locally prepared diet and 50% of smut wheat caused by *Tilletia caries* for 30 days from the ministry of trade. Drinking Tap water were drinking fluid for both groups.

The Histopathological study of Liver and kidney were dissected out after one month of treatment, organs were fixed in small con-trainers containing 20 ml of formalin 10% and dehydrated in progressively more concen-trated alcohol embedded in paraffin and cut into section 4-5 μm thickness, stained with haematoxyline and eosin (H & E) for the light microscopically examination under 400x and 200x magnification [12, 16].

Results

Liver section of control mice, presented in [Fig.1] with normal architecture of heap-tocyte's, sinusoids and portal tract. While liver the section of treated mice revealed histo-pathological changes represented by multiple granulomatous lesion active from aggregation of macrophages and lymphocytes scattered through the liver parenchyma , also areas of coagulative necrosis characterized by pyknotic or disappeared of nuclei as shown in [Fig.2]. Also a section reveals mononuclear aggre-gation around the central vein, with large area of coagulative neurosis and disappearance of nuclei with lysis of some hepatic nuclei [Fig.3A&B].

Vascular degeneration or cellular degeneration with fatty changes and cloudy swellings with mononuclear cells aggregations around the central vein [Fig.4]. The result also showed some hepatocytes with mitotic figure and proliferation of Kupffer cells [Fig.5] and congestion of the central vein with inflammatory cells in their lumen and around vein lumen [Fig.6]. In other liver section [Fig.7] proliferation of hepatocytes which is form lobules without central vein, with nuclear hyprechromasia nuclei. The increase in the number of hepatocytes in irregular form without central vein in the center of the proliferative hepatocytes that initiate the formation of pseudolobule due to the hyperplasia of hepatocytes.

The Kidney sections from the control mice presented in [Fig.8] showed normal structure.

The histopathological section showed acute cellular degeneration characterized by con-gestion of blood vessel and dilatation of renal tubules as shown in [Fig.9], there is also in-creases thickness of Bowman's capsule due to proliferation of fibrous connective tissue with mononuclear infiltration.

Tissue with mononuclear cells in the interstitial tissue [Fig.10]. In other section aggregation of inflammatory cells in the interstitial tissue together with vascular degeneration and sloughing of the epithelial lining of the renal tubules [Fig.11].

Discussions

Histopathological studies of liver demonstrated an increase in the hepatocytes which is associated with high incidence of primary Liver cancer. This result was in agreement with the finding of [12] who reported that the smut wheat is heavily contaminated with carcinogenic polycyclic aromatic hydrocarbons PAH. These results spot the light to the harmful effects of the smut wheat for the humans and livestoks [9, 10, 19], due to accumulation of toxic material of smut wheat infected with *Tilletia* had positively related to the changes in histopathology notice in Liver & Kidney [21].

Histopathological studies in Kidney revealed the acute cellular degeneration in the epithetical lining of renal tubules is due to toxic effect of smut spore toxin which may be ca-used by cell membrane injury or effect on mi-tochondria, this effect lead to depletion Ade-nosine Triphosphate or defect in sodium-po-tassium pump, this lead to fluid disturbance in and outside the cell [18, 20]. This study is in concordance with other studies [12, 13, 14] which found that smut Wheat is heavily contaminated with carcinogenic polycyclic aromatic hydrocarbons the detected concentration of which are far beyond the acceptable limit recognized internationally and controversial to others [11] who concluded that the toxic effect may be because of irritation and suffocation effect of trimethylamine only. The results demand the alteration of standard specifications for wheat, and possibly for other crops, in order to exclude the presence of PHA in wheat consumed by humans and livestoks.

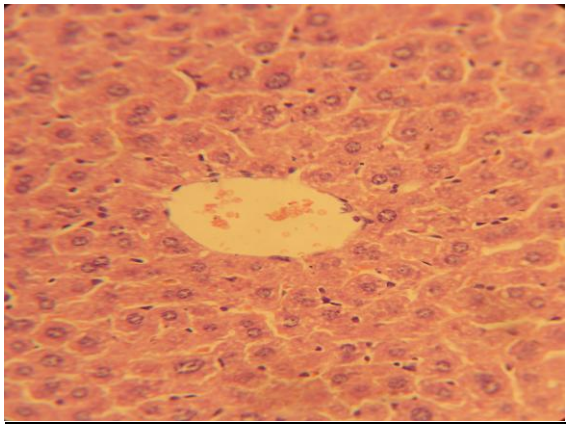


Fig.(1) Histopathological section in Liver of control mice shows normal architecture (H & E 400X).

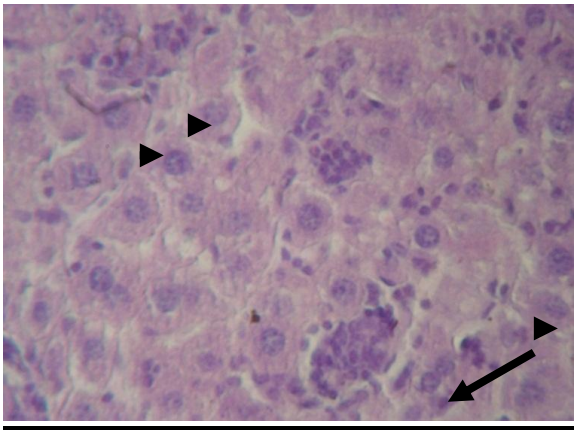
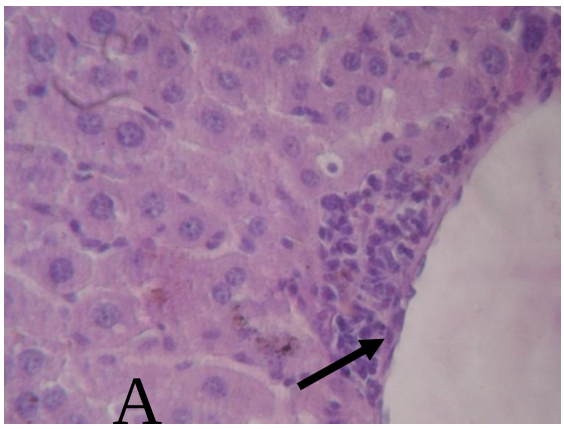


Fig.(2) Histopathological section in the liver of mouse after 30 days treated with smut wheat showing multiple granulomatous lesion (→), area of coagulative necrosis (►), (H & E 200X).



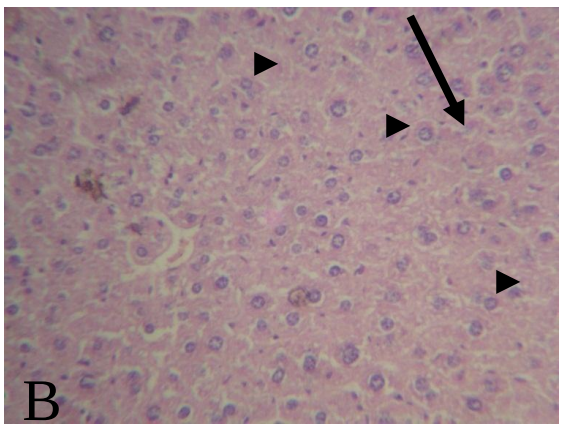


Fig.(3)A: Histopathological section in the liver of mouse after 30 days treated with smut wheat showing reveals mononuclear aggregation around the central vein (→), (H & E 400X).
Fig.(3) B: Histopathological section in the liver of mouse treated with smut wheat showing large area of coagulative neurosis and disappearance of nuclei (→), lysis of some hepatic nuclei (►), (H & E 200X).

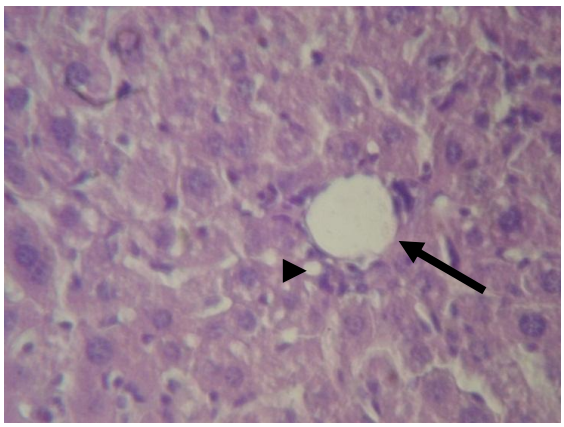


Fig.(4) Histopathological section from treated mice showing vascular degeneration in the cytoplasm of hepatocytes with fatty change (▶), cloudy swelling with mononuclear cells (→), (H & E 400X).

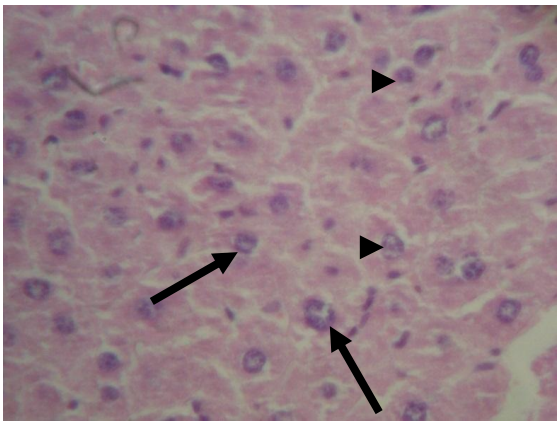


Fig.(5) Liver section from treated mice showing hepatocyte with mitotic figure (→) proliferation of Kupffer cells (▶), (H & E 400X).

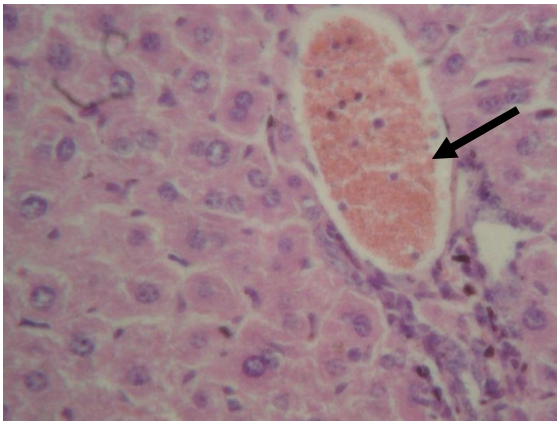


Fig.(6) Histopathological section in the liver of mouse after 30 days treated with smut wheat showing congestion of central vein with inflammatory cells in their lumen and around (→),(H & E 400X).

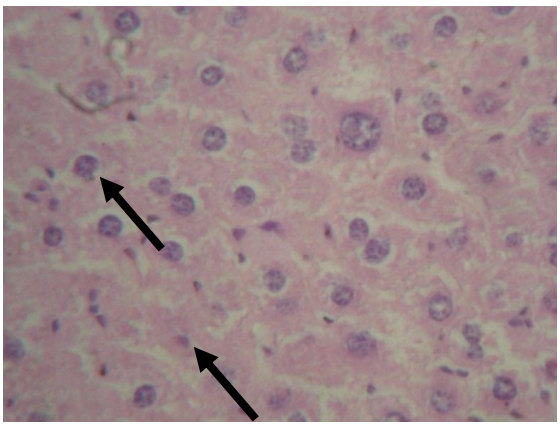


Fig.(7) Histological section of treated liver in mice showing proliferation of hepatocytes (→) hyper chromatic nuclei, (H & E 400X).

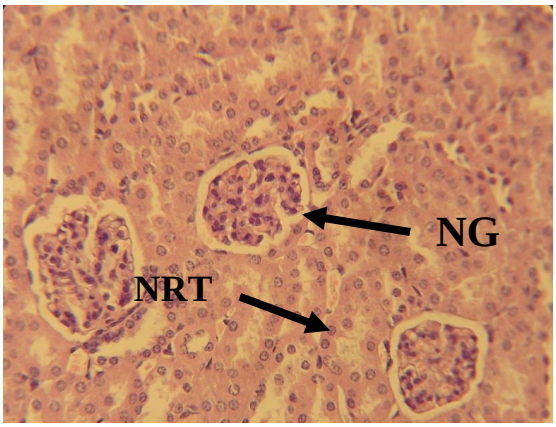


Fig. (8) Histopathological section in Kidney of mouse after 30 days treated with smut wheat showing normal glomeruli (NG), normal renal tubules (NRT),(H&E,400X).

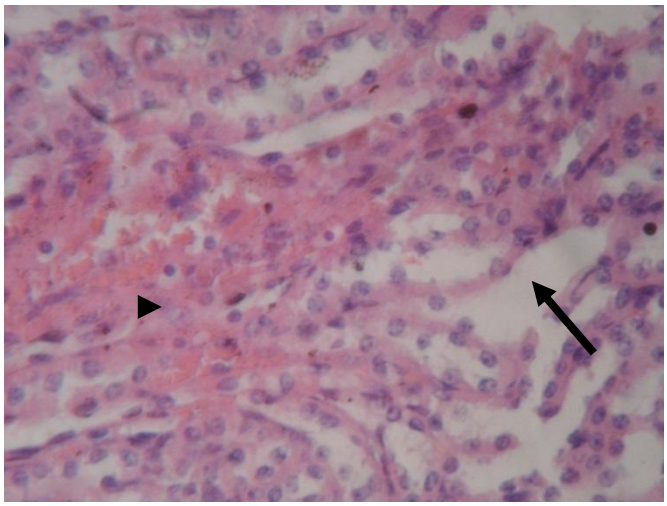


Fig.(9) Histological section of kidney showing acute cellular degeneration, (▶) dilatation of renal tubule (→),(H & E 200X).

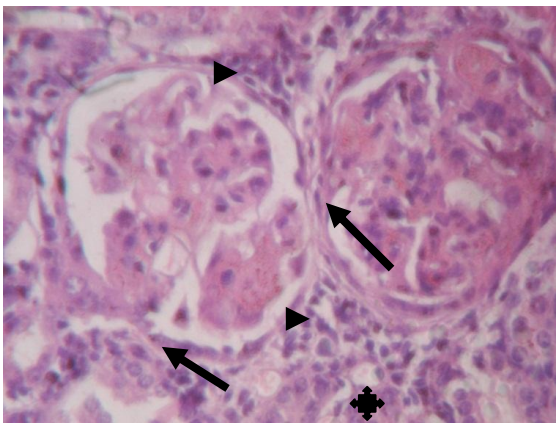


Fig.(10) Histological section of kidney showing increased thickness of Bowman's capsule (→) with mononuclear cells (►), with mononuclear cells in the interstitial tissue (✥), (H & E 400X).

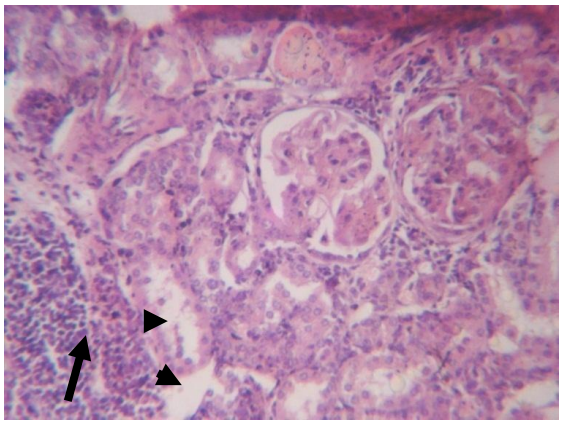


Fig.(11) Histological section of kidney showing aggregation of inflammatory cells (→), vascular degeneration and sloughing of epithelial lining of the renal tubule (►), (H & E 400X).

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الخلاصة

درست التغيرات النسجية المرضية في الأعضاء الداخلية (الكبد، الكلية) للفئران المغذاة على الحنطة المتفحمة المصابة بفطر الـــتليشيا. استخدامت عشرون فارا، قسمت إلى مجموعتين متساويتين بالعدد، غذيت المجموعة الأولى على العلف الاعتيادي بينما غذيت المجموعة الثانية على خليط من العلف مكون من 50% من علف اعتيادي و 50% من علف ملوث بفطر الـــتليشيا ولمدة ثلاثون يوما. قطعت نماذج من الكبد والكلية من كل فار من المجموعتين لعمل مقاطع نسجية في نهاية التجربة.

أظهرت النتائج تغيرات مرضية في الأكباد والكلى في الفئران المغذاة على الحنطة المتفحمة. أذ أظهرت المقاطع تغيرات مرضية في الأكباد شملت آفات كثيرة من الأورام الخبيثة, النخر التخثري، التنكس الفجوي في سايتوبلازم الخلية الكبدية, تكاثر الخلايا الكبدية مع تكوين الفصيص الكاذب والذي يشير إلى بداية تكون السرطان.

أظهرت المقاطع النسجية للكلى لتنكس خلوي ، تثخن محفظة بومان و تجمعات من الخلايا الالتهابية في النسيج الخلالي مع تحطم و انسلاخ النسيج الطلائي للانيبات الكلوية.