Effect of dietary β-glucan on histopathology of liver and gills against toxicity of copper sulfate in *Cyprinus carpio* L.

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Abstract

The objective of this study was to determine the protective effect of β-glucan against the impact of copper (Cu) toxicity on histopathological changes in common carp *Cyprinus carpio* L. which exposed to different level of Cu for 96h and also to assess the effect of addition β-glucan to the fish diet against Cu toxicity. Fish (150 ± 2g) were divided randomly into eight groups as follows: C1 were fed commercial diet; C2 were fed commercial diet with 1%β-glucan; T1 were fed diet without β-glucan and exposed to 5 mg l^{-1} to $CuSO_4$; T2 were fed diet with 1% β-glucan and exposed to 5 mg l^{-1} to $CuSO_4$; T3 were fed diet without β-glucan and exposed to 7 mgl^{-1} to $CuSO_4$; T4 were fed diet with1%t β-glucan and exposed to 7mg l^{-1} to $CuSO_4$; T5 were fed diet without β-glucan and exposed to 9 mg l^{-1} to $CuSO_4$; T6 were fed diet with1% β-glucan and exposed to 9 mg l^{-1} to $CuSO_4$ for 96 h. The main histopathological changes including: hepatic vacoulation, necrosis, accompanied with hemorrhage, congestion of blood vessels and sinusoids. While the gills lesions revealed ulceration of rakers mucosal epithelia, lifting of lamellar epithelia and aneurysms (telangiectasia) of secondary lamella and lamellar fusion caused by the filament epithelium proliferation together with mononuclear infiltration. The result of this study suggested that the addition of β-glucan in fish diet reduced Cu toxicity. Also, the result indicated that the supplementation of β-glucan provided protection against the degenerative action of Cu and increased the chance of tissue regeneration.

Keywords: Copper, histopathology, liver, gill, β- glucan.
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تأثير إضافة البيتاكلوكان ضد سمية كبريتات النحاس في التغيرات النسجية المرضية للكبد والخياشيم في اسماك الكارب الشائع

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الخلاصة

الهدف من هذه الدراسة هو تحديد التأثير الوقائي من البيتاكلوكان ضد تأثير سمية النحاس على التغيرات النسجية المرضية في اسماك الكارب الشائع المعرضة لمستويات مختلفة من كبريتات النحاس لمدة 96 ساعة وكذلك لتقييم تأثير إضافة ß-glucan إلى النظام الغذائي الأسماك ضد سمية النحاس. تراوحت أوزان الأسماك المستخدمة (150 ± 2غم) قسمت عشوائيا إلى ثماني مجموعات على النحو التالي: C1 تم تغذيتها على نظام الغذائي القاعدي. C2 تم تغذيتها على العليقة التجارية مع 1٪ بيتاكلوكان T1 . تم تغذيتها على نظام غذائي من دون اضافة البيتاكلوكان ويتعرض إلى 5 ملغم/ لتر من CuSO4. T2. تم تغذيتها العليقة التجارية مع 1٪ من البيتاكلوكان ويتعرض إلى 5 / لتر من CuSO4. T3 تم تغذيتها على العليقة التجارية دون اضافة البيتاكلوكان. وتتعرض إلى ملغم/ لتر من CuSO4. تم تغذيتها T4 تم تغذيتها على نظام غذائي ٪1 من البيتاكلوكان وتتعرض إلى 7 ملغم/ لتر من CuSO4. T5 تم تغذيتها العليقة التجارية دون إضافة البيتاكلوكان ويتعرض إلى 9 ملغم/

لتر من CuSO4. T6 تم تغذيتها على العليقة التجارية 1٪ من البيتاكلوكان ويتعرض إلى 9 ملغم/ لتر من CuSO4 ولمده 96 ساعة. اظهرت نتائج التغيرات المرضية النسجية الرئيسية تفجي ونخر الخلايا الكبدية مصحوب باحتقان الأوعية الدموية والجيبانيات. بينما في الخياشيم كشفت التغيرات في الخياشيم عن وجود تقرح في الظهارة المخاطية للأقواس الغلصمية وانفصال ظهارة الصفائح الغلصمية مع توسع الشعيرات للصفائح الثانوية فضلا عن اندماج الصفائح الثانوية مع ارتشاح للخلايا وحيدة النواة. اشارت النتائج إلى أن إضافة البيتاكلوكان إلى النظام الغذائي في عليقة الأسماك أدى إلى انخفاض في سمية النحاس. كما أشارت النتائج أن مكملات البيتاكلوكان قد اعطت الحماية ضد التغيرات التنكسية التي سببها النحاس وزاد من فرصة تجديد الأنسجة.

الكلمات المفتاحية: الكارب، التغيرات النسجية المرضية، الكبد، الخياشم، البيتاكلوكان

Introduction

Cu is plentiful in the environment and it is also one of the most commonly used metals(1). It's used as fungicide, algaecide, herbicide and in municipal water treatment system (2) and It's non-biodegradable but easily bio-accumulated (3). Cu is an essential trace metal in small concentrations for fish metabolic function. It has numerous functions in cellular biochemistry including: vital roles in cellular respiration and a cofactor for over 30 different enzyme (4). But it can exert adverse toxicological effect when present at high concentrations in water (5). Cu accumulation in tissue of animals from polluted water bodies (6) could leads to generation of free radicals which causes biochemical and morphological alterations in them (7). The effect of $CuSo_4$ on fish has been studied comprehensively and in some species have been found to be more susceptible to Cu than others (8). Gills are the first target organ of the water borne pollutants due to the constant contact with the external environment. As well as, the main place for Cu uptake (9). The liver was examined because it plays a primary role in the metabolism and excretion of xenobiotic compounds with morphological alterations occurring in some toxic conditions (10). Metals can either increase or decrease hepatic enzyme activities and can lead to histopathological hepatic changes, depending on the metal type and its concentration, fish species, length of exposure and other factors (11). β-glucan are high molecular weight substances composed of glucans as building blocks, usually isolated from cell walls of bacteria, mushroom, algae, cereal grains, yeast and fungi(12). They are mostly known as factors stimulating the immune system, having anti viral, anti microbial and anti allergic properties (13). They also have the ability to slow down the excessive cholesterol synthesis (14). As well as, show anti oxidation properties (15) and anti tumor effect (16). β-glucans further more has anti-inflammatory effect (17). On the background of above information no studies addressed the relation between the Cu toxicity and β-glucan. Hence, this study was undertaken to examine the combination between concentration of $CuSo_4$ and β-glucan on histological aspects of gills and liver of *C.carpio* as well as to investigate the protective effect of β-glucan on histopathological alterations induced by waterborne Cu toxicity.

Materials and Methods

- **Experimental Design:** A total of 160 healthy fish of *C. carpio* average weight (150±2) g were obtained from a carp farm Al-Jadeda/ Diyala Province, Iraq. Fish were acclimatized for two weeks prior to the experiment and maintained on control diet, in a two bath trough (150x80 x 50 cm). Then, fish were randomly selected and distributed into 12 tank, filled with chlorine free tap water at rate of the 10 fish per trough, two replicates were maintained for each treatment. First control (C1), T1, T3 and T5 were prepared diet without β-glucan, second control (C2), T2, T4 and T6 were mixed with β-glucan. Water quality parameters like temperature was recorded by a standard quality thermometer, Dissolved oxygen (DO) and pH were recorded

every day with digital DO and pH meter respectively. Total alkalinity was recorded by titration. Fish were fed 3% of the their body weight twice a day for 60 days. Every day trough was cleaned and water was partially changed. After determination of LC_{50} for $CuSO_4.5H_2O$ (Bio-Green Cut), $CuSO_4.5H_2O$ was added to all treatment at a dose of 5 mgL^{-1} for T1 and T2, 7 mgL^{-1} for T3 and T4, 9 mgL^{-1} for T5 and T6 for 96h. In fifth day samples of gill and liver were taken for histopathological studies.

- **Histopathological Studies:** Fish were removed from their tanks and dissected out. Gill and liver were carefully collected. Tissue samples were immediately fixed in 10% formaldehyde solution. Afterward, samples were dehydrated in a series of ethanol solutions to remove excess water: (50, 70, 80, 90, 95 and 100)% for 1, 1, 2, 2, 1.5 and 16 hours respectively. Each of the samples were then transferred in to a xylene solution for another 30 min. Then, embedded in a solution of xylene wax for 1 h. Transverse section (thickness 5-7µm) were cut using a rotary microtome (A.O Spencer, model Reicheit-Jurg 820, Leica, Germany) and quickly transferred onto a slide and kept in an water bath for 15 min. The slides were stained with haematoxylin and eosin (H & E). Slides were examined by light microscopy using an Olympus vanox-T microscope and photographed using a digital camera at total magnification of x100 and x400. According to the method described by (18).

Results and Discussion

- **Histopathology of Liver Sections:** Liver section in the control group C1 showed normal tissue architecture and there were no pathological alterations, the hepatocytes presenting a homogenous cytoplasm and a large central or sub-central spherical nucleus (Fig.1 A). While in C2 showed slight infiltration of mononuclear cells (MNCs) in liver parenchyma mainly around bile duct associated with increase number of melanomacrophage while the hepatocyte appeared normal (Fig.1B). Liver sections in T1 revealed variable degree of degenerative and necrotic changes characterized by vacuolation with presence of single cell necrosis together with cellular aggregation which consist mainly of macrophage and melano-macrophage mainly in portal area (Fig.1 C). Also, the result revealed severe dilation and congestion of sinusoids that appear more cylinder in appearance in addition to mononuclear cell infiltration in sinusoids. While in T2 there were a clear no related treated effect in the liver tissue of this group except few MNC_S aggregation around blood vessels (Fig. 1 D). The main histological changes in T3 revealed destruction in liver parenchyma which caused by necrosis associated with nuclear pyknosis of hepatocyte (Fig. 2 E). In other sections there were appearance of cystic dilation of degenerated bile duct together with cellular infiltration surrounded necrotic areas. However, T4 exhibited multifocal MNCs aggregation in liver parenchyma (Fig.2 F). The results also showed hyperplasia of bile duct epithelial lining associated with congestion of sinusoids. Similar observations to T3 were seen in T5 associated with sever hemorrhage and congestion of blood vessels and sinusoids together with wide areas of necrosis accompanied by cellular aggregations which consist mainly of neutrophil in liver parenchyma around blood vessels and in their lumen (Fig.2 G) while presence of hyperplasia of epithelial lining cells in bile duct and thickness their wall were recorded in the liver of group (T6) however, no clear lesion in (Fig.2 H).

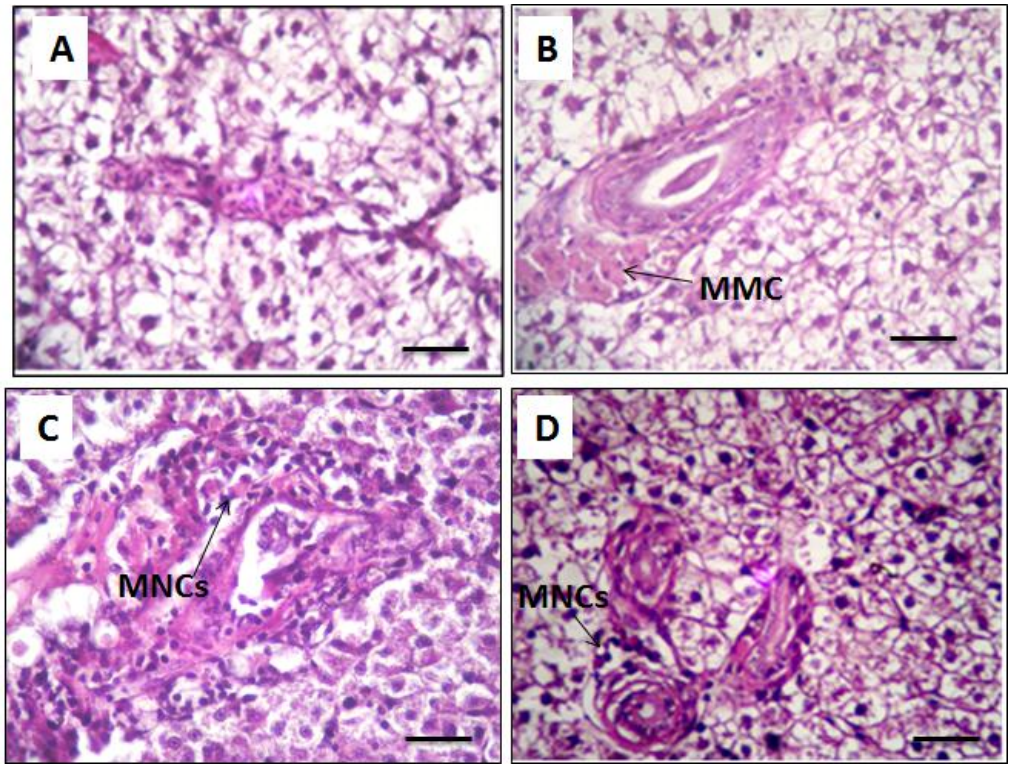

Fig. (1) Light micrograph sections showing histological structures in liver of *C. carpio* exposed to Cu 5 µm thickness. (A) control liver showing normal histology (B) C2 showing increase in number of melanomacrophage around bile duct (MMC) (C) T1 showing MNCS infiltration in portal area (D) T2 shows no clear pathological lesion in liver of this group except few MNCS aggregation around blood vessels. Scale bars: 50 µm, H&E.

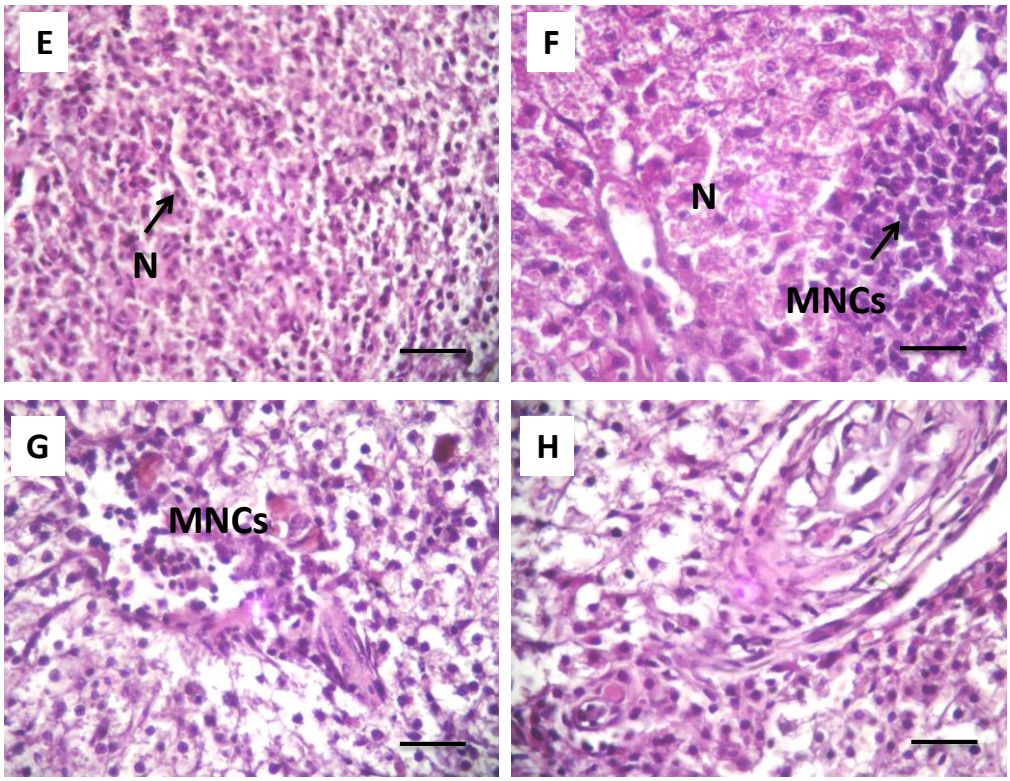

Fig. (2) Light micrograph sections showing histological structures in liver of C. carpio exposed to Cu 5 µm thickness. (E) T3 shows destruction of hepatocytes with necrostic area (N); (F) T4 shows MNCS aggregation in liver parenchyma consist mainly of macrophage and lymphocyte associated with central liver necrosis (G) T5 shows neutrophils infiltration around blood vessels and in their lumen (D) T6 shows no clear pathological lesion in liver of this group. Scale bars: 50 µm, H&E.

The present results of liver microscopy are in line with several authors who studied the effects of various contaminants in fish (19). Also, similar findings were revealed in Sleek Unicorn fish (*Naso hexcanthus*) exposed to heavy metal at contaminated site in the Red sea areas (20), also these results are in agreement with (21) who studied the effects of probiotics on cultured *Oreochromis niloticus*. Exposure to Cu is known to exert a wide range of histopathological abnormalities (19, 22). These alterations could be attributed to toxic effects of the Cu on hepatocytes, since the liver is associated with detoxification and biotransformation of all types of contaminants and toxicants (19). The aggregation of inflammatory cells (i.e., macrophages/ monocytes) may indicate the reaction of melanodiaaldehyde in oxidative stress developed by Cu exposure (22). Necrosis in some portions of the liver tissue that were observed could be resulted from the excessive work required by fish to get rid of the toxicants from its body during the pathway of detoxification and similar to the observation recorded by (23). The addition of β-glucan reduce the histopathological changes to be lower than that of copper. These changes could be indicating that β-glucan has antioxidant properties (15).

- **Histopathology of Gill Sections:** Histopathological findings of gill section in the control group C1 and C2 showed normal structure of gill filament. (Fig. 3 A & B). The characteristic feature of gill in T1 characterized by vacuolation of secondary lamellae (SL) with increase number of chloride cell (Fig 3. C), also T1 showed slight epithelial lifting of SL with micro hemorrhage in addition congestion of primary lamellae (PL) with telangiectasis of SL capillary. However, T2 showed MNC_S aggregation with presence of odema in SL in some section with hyperplasia of mucous cell that filled with the mucous substances. Also, there was epithelial lifting in secondary lamellae (Fig.3 D). T3 revealed severe destructive changes in gills which characterized by severe epithelial lifting of SL with micro hemorrhage at the tip of circling lamellae (Fig. 3 E) in another section also telangiectasia of SL were recorded with cellular aggregate of congested central venus sinus of PL. While in T4 the secondary gill filament showed slight epithelial hyperplasia accompanied with telangiectasia at the tip of SL also the result showed sever elongation of SL with cellular aggregate mainly in PL as well as congestion of central venus sinus (Fig.4 F). Treated fish of T5 showed more sever pathological changes than the T3 in which distraction of epithelia of SL with circling of survival filament (Fig. 4 G) T6 showed similar observation to the T4 in addition to present hyperplasia of mucous cell with formation of lamellar fusion also the result showing shorting of SL associated with moderate increase number of mucous cells (Fig. 4 H). With particular reference to exposure to metals (12) observed necrosis, telangiectasis, with epithelial lifted away from the basement membrane in mirror carp *C. carpio* exposed to Cu. Similar findings were also observed in Senegales sole, *Sole senegalensis* subjected to sub-lethal level of Cu for 7 days (19). The histopathological changes in gill tissue are not limited to Cu toxicity (e.g. epithelial lifting, hemorrhage, telangiectasis, congestion etc.). These changes are general defense mechanism against any stress which have been previously documented in other stressed conditions such as exposure to pesticides (24). All these alterations could represent a defense mechanism to increases the distance across waterborne pollutants must diffuse to reach the bloodstream (19). Hence, this indicates that these changes are not specifically induced by Cu or other heavy metals. Supplement of β-glucan in fish diet reduced copper toxicity, this result could be indicating that β- glucan has antioxidant properties (15). Furthermore, (17) have shown that β-glucan in Atlantic salmon has anti-inflammatory and antioxidant effects. It can be concluded that gill and hepatic alterations as a result of heavy metal exposition of fish may serve as a sensitive bio-indicator for the toxicity of sub-lethal concentrations of metals as well as other contaminants. Also, the result indicated that the supplementation of β-glucan provided protection against the degenerative action of Cu and increased the chance of tissue regeneration.

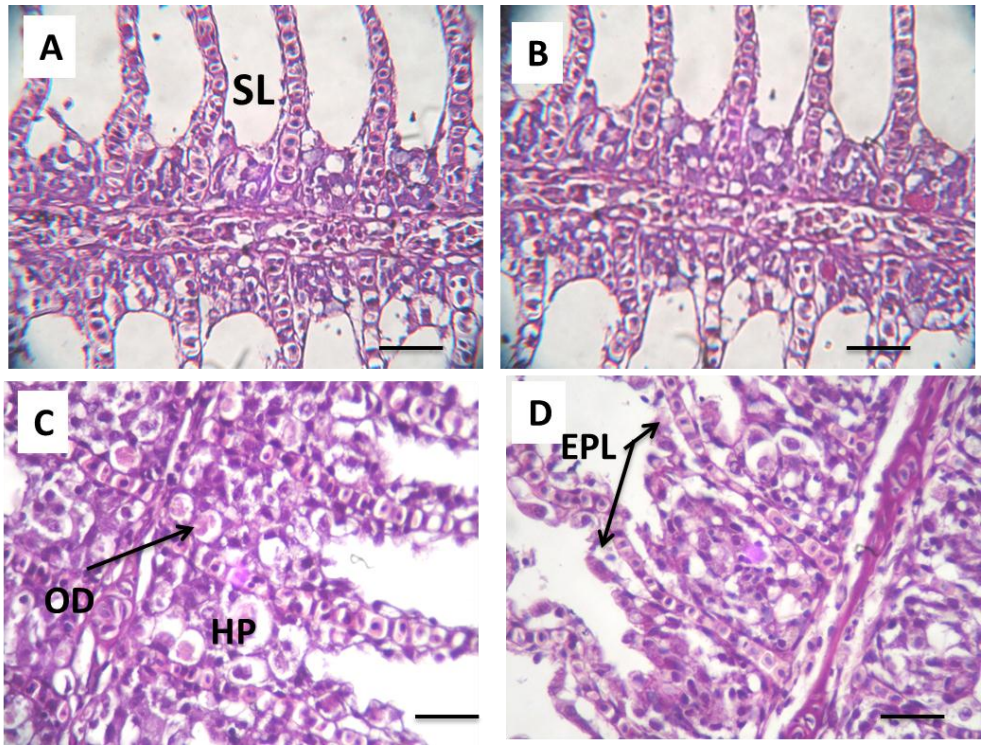

Fig. (3) Light micrograph sections showing histological structures in gill of *C. carpio* exposed to Cu and supplemented with ß- glucan. 5 µm thickness. (A&B) C1 and C2 gill showing normal histology of secondary lamellae; (C) T1 showing increase in number of chloride cells with odema (OD) and hyperplasia (HP) of mucous cells; (D) T2 showing epithelial lifting (EPL. Scale bars: 50 µm, H&E.

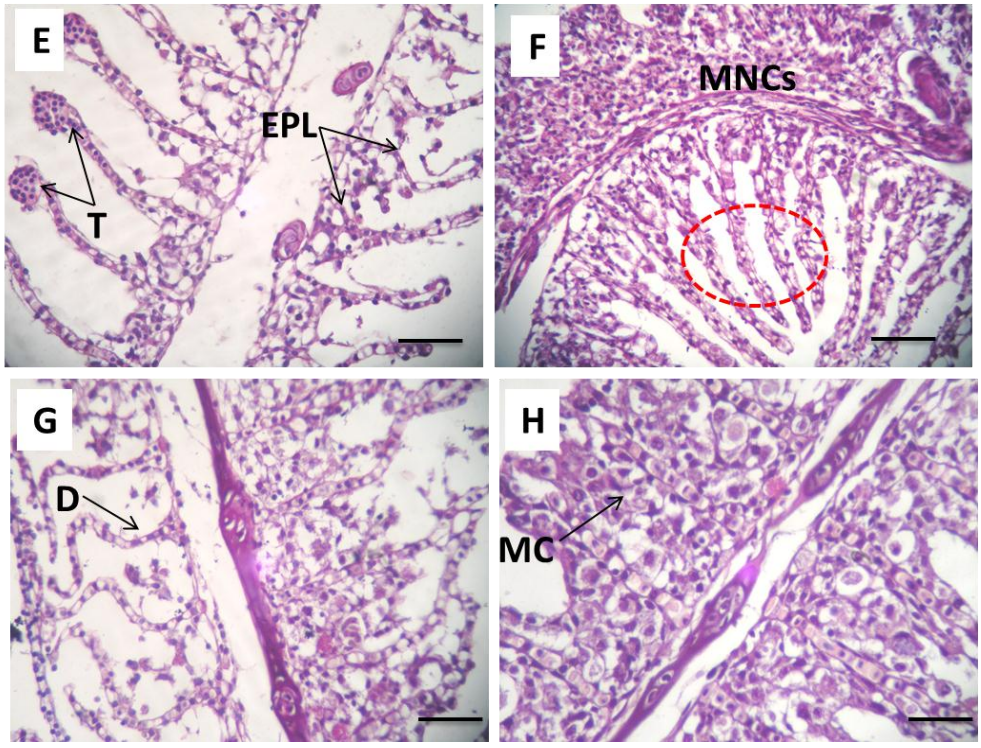

Fig. (4) Light micrograph sections showing histological structures in gill of C. carpio exposed to Cu and supplemented with ß- glucan. 5 µm thickness. (E) T3 showing telangiectasis (T) with epithelial lifting (EPL) of the secondary lamellae; (F) T4 showing sever elongation of SL with sever MNCS aggregation; (G) T5 showing distraction (D) of SL; (H) T6 showing increase in number of mucous cells (MC). Scale bars: 50 µm, H&E.

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