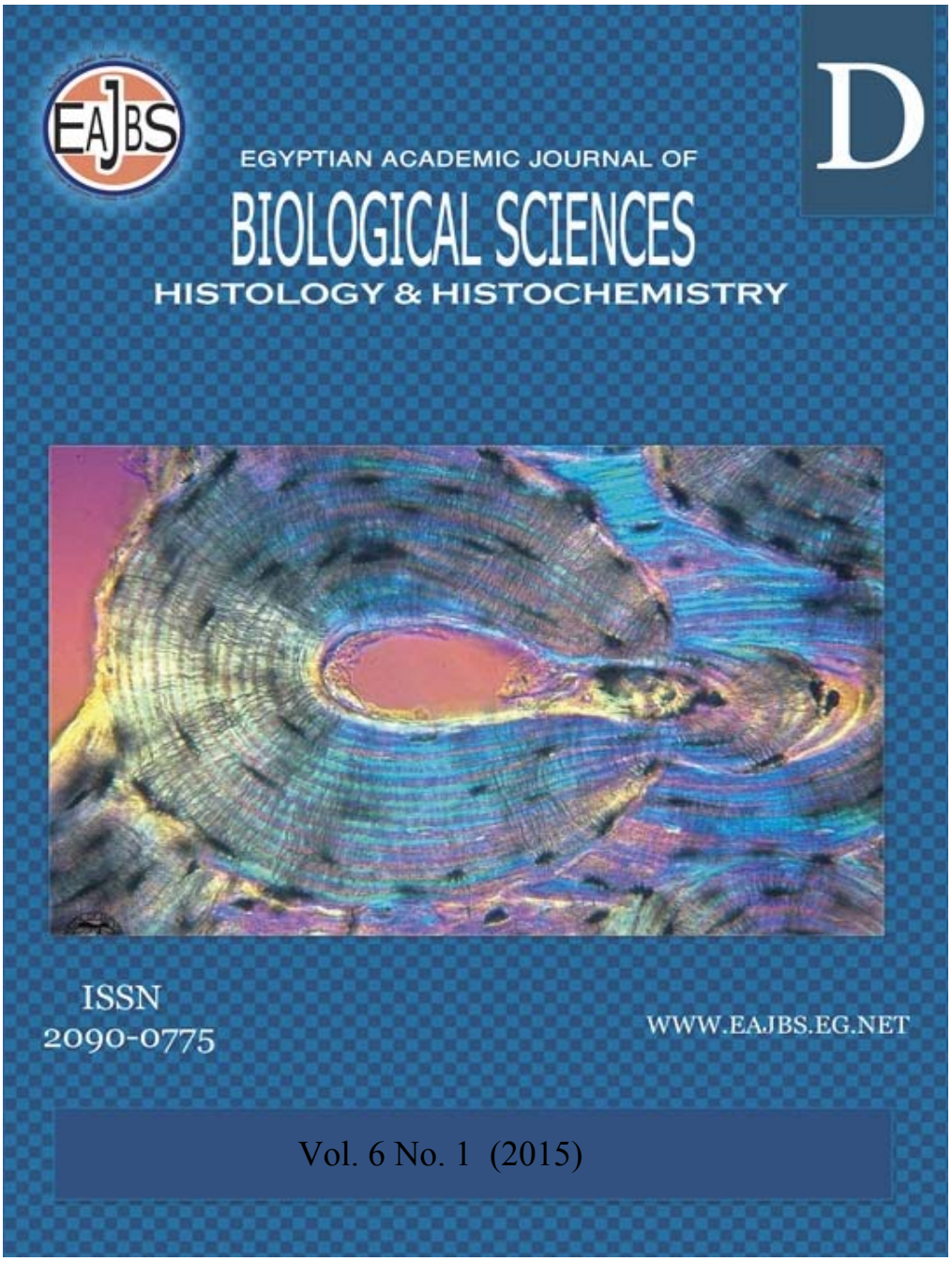

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Egypt. Acad. J. Biolog. Sci., 7(1): 19 – 32 (2015)

Egyptian Academic Journal of Biological Sciences
D. Histology & Histochemistry

2090 – 0775
www.eajbs.eg.net

Role of some functional materials in prevention of rats liver damage induced Aflatoxicosis

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ARTICLE INFO
Article History
Received: 25/1/2015
Accepted: 27/3/2015:

Keywords:
Aflatoxins
Ginseng
Whey Protein
Butylated Hydroxyl Toluene

ABSTRACT

This study was aims to investigate the ability of Aspergillus *parasiticus* NRRL 2999 on aflatoxins (AF) production on rice, as well as to detect the ability of 50, 100 and 150 mg of each functional materials (FMs) as ginseng extract (GS), whey protein (WP) and butylated hydroxyl toluene (BHT) on degradation of AF in liquid media, and its detoxification effects against 1 mg of AF on body weight and liver histopathological characterizes in growing rats after feeding for 21 days. The results showed the ability of *Aspergillus parasiticus* in production the AF after cultivation on rice at 28 ° c for 10 days, as well as the effects of 100 and 150 mg FMs from GS, WP and BHT in degradation of AF produced in malt extract broth media. The interactions between FMs and AF were confirmed by the histological study on liver of treated rats revealed congestion, kupffer cells hyperplasia, dilated sinusoids and mononuclear cells infiltration in all liver.

INTRODUCTION

Aspergillus parasiticus is a plants pathogen, which causes post-harvest disease in cereal grains and legumes. Post-harvest rot typically develops during harvest, storage, and/or transit. Many strains were able to produce significant quantities of toxic compounds known as mycotoxins, which when consumed are toxic to mammals (Agrios, 2005). Many species of *Aspergillus* are *fungus* and the most notable ones being *A. flavus* and *A. parasiticus* plus related species, *A. nomius* produced Aflatoxins (Luttfullah and Hussain, 2011). Aflatoxin-appear when a producing strain of *A. flavus* or *A. parasiticus* grows in a substrate, where environmental conditions favor their development, the genotype of each species, some biological, chemical and environmental factors determine the amount of aflatoxin produced by aflatoxicogenic strains (Richard, 2007).

Aflatoxins are toxic and among the most carcinogenic substances known (CAST, 2003), so the Aflatoxin B1 (AFB1) was appears as the most toxic of the aflatoxins types and the strongest naturally occurring chemical liver carcinogen known. Aflatoxin-B1 may be metabolized by the liver enzymes to changes for a reactive epoxide intermediate or hydroxylated derivatives compounds to become less harmful and named aflatoxin M_1 (Hudler, 1998).

Antioxidants are believed as defense the human body against the adverse effects of free radicals, and highly reactive compounds of oxygen and nitrogen (Fang, *et al.*, 2002). On the other hand, through electron transport chain (ETC), they are generated reactive oxygen species (ROS) from molecular oxygen/nitrogen cytochrome P450, and other cellular and sub-cellular functions. They are also play a key role in pathological conditions of the body and further they have been affected metabolic and cellular processes (Noori, 2012). Thus antioxidants compounds were able to reduce the risk of chronic diseases (Percival, 1998). The dietary sources, including plants, herbs, spices, vitamins and herbal extracts, play an important role to overcome the reactive metabolites production (Noori, 2012).

Therefore, the main goals of this study are to evaluate the effects of some functional materials as giseng, whey protein and butylated hydroxy toluene against AF on histological structures of liver in rats.

MARERIALS AND METHODS

Ability of functional materials on aflatoxins Degredation:

The ability of each functional materials (FMs) as JE, WP, BHT on aflatoxins degradation was tested by preparation of Malt Extract broth (MEB) media and add the JE, WP, BHT at 50, 100 and 150 mg/l of each extract according to Verma, *et al*., (2004) and modified by World Health Organization (Wagacha, *et al.,* 2008). Spore suspension for *Aspergillus parasiticus* was add to each flask containing MEB with JE, WP and BHT separately and incubated for 7 days at 28°C to allow it's for growth and AF produced. Aflatoxins concentration was detected using enzyme linked immune sorbent assay ELISA technique after extraction of aflatoxins used the chloroform solvents, ELISA kits according the manufactured information by the ShenZhenLvshiyuan Biotechnology Co. ltd (China).

Animal Finalization:

In this experiment Fifty six, 45 days old age of male Albino-Sprague Dawley Rats, were individually weighed, between 110-113 g have been obtained from the house livestock in the Faculty of Veterinary Medicine, University of Mosel. Wing banded and housed in heated battery brooders under 12 hours fluorescent lighting daily, Rats were fed the optimal formula according to NAS-NRC, 2002). The rats were randomly assigned to the following treatment groups: 1) Control with 0 mg AF, 0 mg FMs; 2) 1 mg AF/kg of diet; 3) 100mg/ WP/kg diet; 4) 100mg JE/kg diet; 5) 100mg BHT /kg diet; 6)150mg WP/kg diet; 7) 150mg JE/kg diet; 8) 150mg BHT/kg diet; 9) 100mg WP/kg diet + 1mg AF; 10) 150mg WP /kg diet + 1mg AF; 11) 100mg JE/kg diet + 1mg AF; 12) 150mg JE/kg diet+1mg AF;13)100mg BHT/kg diet + 1mg AF; 14) 150mg BHT/kg diet+ 1mg AF. Each treatment consisted of two replicates of four per replicate. Diets and water were available for ad labium consumption. At 21 days of age, all rats per pen were sacrificed. Liver dissected out. Liver were fixed in plastic containers containing 100 ml of 10 % formalin. After that were dehydrated in alcohols, then embedded in paraffin and cut into section at thickness of 4 µm and the histological examination was evaluated by estimating the morphological changes by stained with

haematoxylin and eosin H and E stain (Lanza, *et al*., 1980). Aflatoxin was prepared through inoculation of rice by *Aspergillus parasiticus* NRRL 2999 (Obtained from the Department of Food Science-Agriculture College-Tikrit University), described by Shotwell; *et al ;*(1966). Fermented rice was autoclaved and ground and the AF content measured by ELISA technique according the manufactured information by the ShenZhenLvshiyuan Biotechnology Co. ltd (China).

Data were analyzed by the ANOVA analysis, using the general linear model of the Statically Analysis System (SAS, 2004). Significant treatment differences were evaluated using Duncan's multiple-range test (Duncan, 1955). All statements of significance are based on the 0.5 level of probability.

RESULTS AND DISCUSION

Ability of Functional Materials FM on Aflatoxins AF Degradation:

The ability of *Aspergillus parasiticus* NRRL 2999 to produce AF on rice medium was analyzed quantitatively using Enzyme Linked Immune Sorbent Assay ELISA kit. The result showed that the sample of rice was contained AF, and the concentration of AF in these samples were ranged from1500 to 1700 ppm.

The ability of FMs Jensen extract (GS), Whey protein (WP), Butylated hydroxyl toluene (BHT) to degrade AFs after 7 day incubation in ME media were summarized in the Fig. 1. The results were appeared that the concentration of AFs was decreased when treated with each of FMs and there's significant ($p<0.05$) difference between the effect of GS, WP and BHT on AF.

In this result was appeared the BHT and GS as the maximum ability to degraded of AF in the medium, and the concentration of GS at 50, 100 and 150 mg/kg diet were decreased the AF concentration in medium and became at 75, 62 and 53 ppb respectively, when compared with the control treatment which at 123 ppb. This degraded ability was increased with the concentration increased from each FMs.

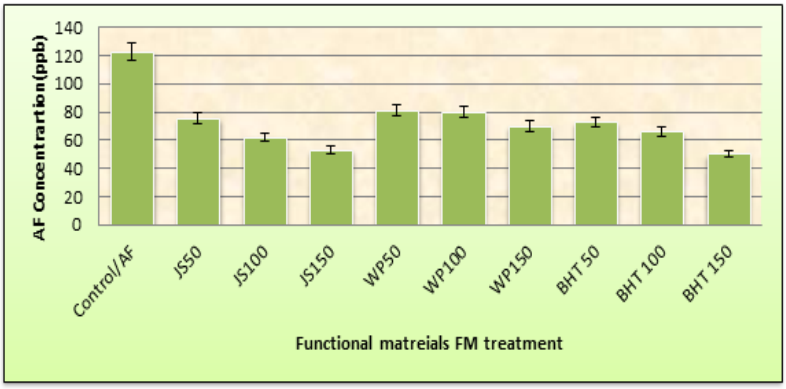

Fig. 1: Effect of functional materials on AFs degradation.

The degradable of AF when treated with BHT may be attributed to the action of BHT on target the double bond of the furfuran ring of the AF molecule and changed the bonds connected resulted as decreased the influences of the toxins on the fluorescence and mutagenicity properties (Liu *et al*., 1998). Also Alberts *et al*., (2009) reported that GS has ability function to degradation of AF at 40.45%, while the ability of WP was at 35.90%, and this function was referred for the activated compounds which these FMs were contained, such as antioxidants compounds.

Assay of Interaction Effects of FMs with AF on body weights

The effects of interaction between FMs types and aflatoxin in rat's animals Body (BW) after feeding for 21 days were illustrated in the Fig. 2. The results indicated that the feeding of AF only, showed significantly decreased ($p<0.05$) in animals body weights, while the adding of FMs with the AF were causing in prevention of the toxicity effects and efficacy of the body weights with all treatments, and the increased of FMs concentration was effected on the body weights in compination with the control group.

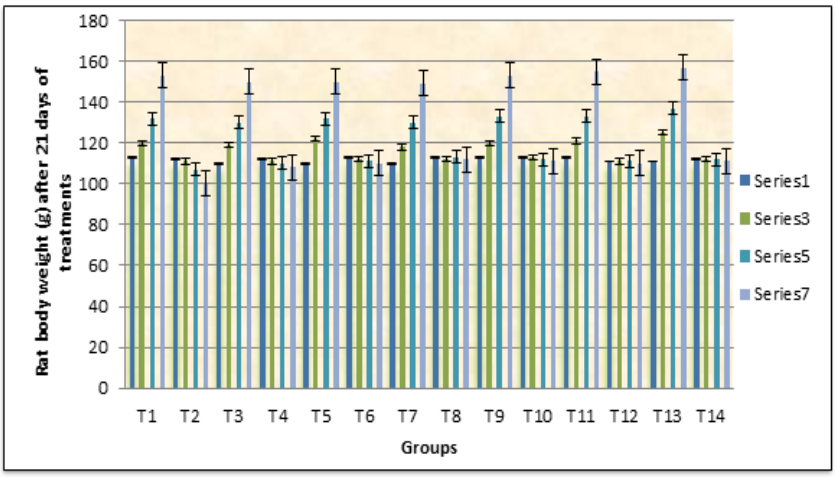

Fig. 2: Effects of interaction between FMs and AF in the Body weights of Rats after dietary for 21 days. T1:control group;T2:1mgAF/kg; T3:Gs (100mg/kg); T4:Gs 100mg+1mg AF/kg; T5:Gs 150mg/kg; T6:Gs 150mg+1mg AF/kg; T7: WP (100mg/kg); T8: WP 100 mg+1mgAF/kg; T9: WP150 mg/kg; T10: WP 150 mg+1mgAF/kg; T11: BHT100mg/kg; T12: BHT 100 mg+1mgAF/kg; T13: BHT 150mg/kg and T4:BHT 150 mg+1mgAF/kg.

The depression in growth upon feeding aflatoxin could be attributed to reduced protein synthesis as reported by Verma, *et al*., (2002) who stated that the toxicity of aflatoxins interfere with normal metabolic pathway through the inhibition of protein synthesis and enzyme system that is involved in carbohydrate metabolism and energy release.

These effects may be refer to the effects of composition contents of the FMs such as antioxidants and other activated compounds, which affects on breakdown the AF structure and became less toxicity for animals (Gibson, *et al*., 1989).

There are different changes signs observed after treated with AF lead to mycotoxicosis were loss of hair, shivering, dyspnea, redness around the neck and bristling of hair. Although of the aggressive behavior of rats, loss of activity observed at the last two weeks of treatment. All signs mentioned have referred to the action of toxin resulted from mycotoxicosis, similar morphological changes were observed in rats fed diet contaminated with AF Al-Taie, (2001) which is attributed to the mycotoxicosis.

Histopathological effects of AFs in the liver of Rats:

Liver sections from control Rats treated with PBS showed normal structure appearance of hepatocytes arranged as a thread around central vein and presence of portal area. (Fig. 3).

Histopathological examination of liver after 21 days revealed hypertrophy of the liver cell with degenerative of others was evident and the nuclei of certain cell were enlarged and the

cytoplasm was containing eosinophilic granules as in Fig. (3).

Lymphocyte infiltration in the portal area was present and also present in focal pattern between certain liver cells as in Fig. (3.b) the central veins were engorged with RBC.

Also the liver cell were present in the liver parenchyma, some of them were hypotrophies, others were hyper trophies and there was degenerated cells of liver also the degenerative cells have light cytoplasm and some of them lost its nuclei, the portal area were infiltrated with lymphocyte around the blood vessel of these area. The results obtained confirm what it referred to by many of the research in the impact of AF on the liver Eaton and Groopman, (1994); Hussein and Basel (2001); Brender, *et al*., (2005).

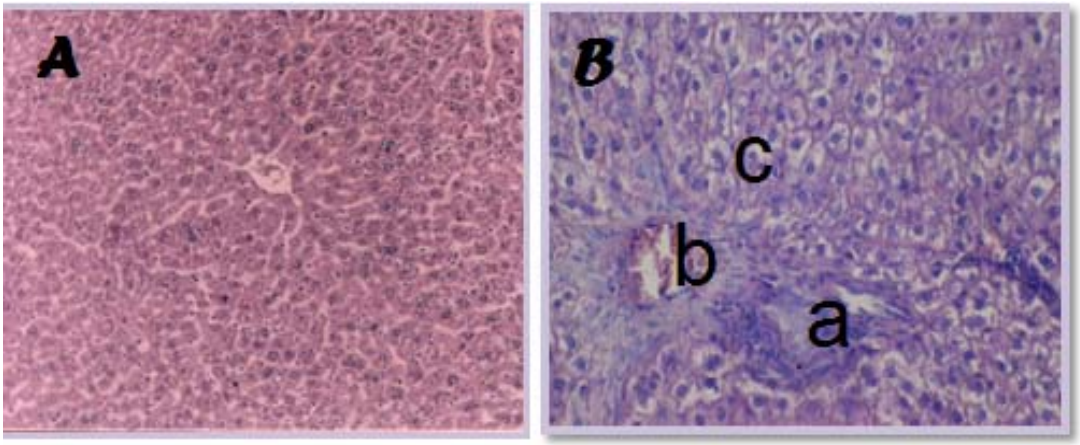

Fig. 3: A: Histological Section in the liver shows (1): A-normal structure of the cell B-the normal structure of the liver tissue B: Animal treated with AF...A-lymphocyte aggregation in the portal area B-Portal vein with RBc C- Hypertrophic liver cell (H&E stain, 40X).

The results obtained by Fareed and Inaam (2006) showed that female `albino rats treated with 80 µg/kg AF caused necrosis of the hepatocytes accompanied by lymphocyte infiltration, while Guylaine,*et al*., (2007) showed that 1807 µg/kg AF fed to pig's caused changes including moderate to extensive swollen hepatocytes, presence of enlarged nucleus and vacillation of perioral parenchyma cells. Results were reached by Shyamal, *et al*., (2010) revealed that treated of Westar rats with 1.5 mg/kg AF orally cause broad infiltration of lymphocytes and Kupffer cells, disturbed lobular architecture, fatty degenerative changes and focal necrosis, Devendran and Balasubramanian, (2011) showed that many histological changes of liver include degenerative reversible lesion, mild parenchymatous degeneration characterized by granular appearance of hepatocytes cytoplasm, severe hydrophilic and vacuolar degeneration. The vast majority of hepatocytes had significant cytoplasmic visualization with disseminated necrotic cells on rats treated with 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm AF for 8 days.

Results revealed by Helal, *et al*., (2012) showed liver histological changes in rabbits treated with AF including, congestion of central vein, sinusoids and portal blood vessel. Hepatocytes showed increase in its size with vacillation of their cytoplasm (Hydropicdegeneration), there was midzonal necrosis characterized by deeply eosinophilic cytoplasm, psychosis of nucleus, cholangiofibrosis was detected in the portal areas characterized by proliferation of fibrous connective tissue around portal triad accompanied with mononuclear cells aggregation. However results reached by Gallo *et al*., (2013) include treatment of adult rats with AF (60 µg/kg

body weight) caused hepatic necrosis, collagen fibers around portal tract and iron deposits as well as features of general cellular collapse and cirrhosis. Nabila, *et al*., (2013) showed that rats treated with 2 mg/kg AF have many liver histological changes including fatty degeneration, focal necrosis in the hepatocytes with fibrous tissues deposited in the portal tract, large fatty droplets, necrosis and vacuolar degeneration in the hepatocytes in the central area.

Histopathological picture of liver after treated with ginseng: Histopathological change of liver was appear when treated with 100 mg of ginseng for 21 days of infection showed the liver cells of the lobule mostly were arranged in radial and the liver cells were polygonal pattern and sinusoid between these cells there was hypotrophy liver cell in open place. The blood vessels of the cavities of the blood vessels of the portal areas as in Fig. (4.c). The liver cell near by the central veins were arranged in columns and were arranged in columns and were slightly swollen and between them was the blood sinusoid with kupffer cells and the central vein in the central of each lobule had a mass of blood. There was degenerative changes at the live cell in certain place associated with karyolitic of nuclei, the blood vessel of portal area were engorged with blood also.

The treated with Ginseng at 150mg/ kg for 21 days were effects on the liver cells as hyper atrophied with necrotic cytoplasm which appeared empty from any structure and its color was whitish, the cell membrane of hepatocyte were thickened and stained reddish color as in Fig. (4.d). The central veins were congested with blood, in certain place the liver cell appeared broken down to from a cavities with each other the parenchyma of the liver shown seven degenerative of the liver cell and necrosis, that the cytoplasm of the most liver cell were deficient from any cytoplasmic component except the nuclei in certain cell and others lost even these nuclei and the cytoplasmic membrane was thickened some of the central veins empty from the blood and a few of these were containing hemolysis blood.

The interactions between the Ginseng at 100mg/ kg with AFs at 1mg/ kg appeared at this treatment that the liver cells were hypertrophied and swollen and form amasses of cells appeared as cytoplasmic syncytium but its nuclei were well recognized as spherical pattern. Some of these cells were appeared as radial pattern near central vein in between there was blood sinusoid that the kupffer cells could be recognized as in Fig. (4.e) the portal area had lymphocytic infiltration around the blood vessels like the branches of portal vein and hepatic artery the other side of the section appeared the border of the liver cells were mostly not recognized well due to enlargement of cell and compact to each other to from a mass of cell, but its nuclei were spherical and normal size with basophilic stain, the blood sinusoids were not seen because of the cells enlargement of liver the most of the central veins congested with blood.

Section in the liver lobule demonstrating the radial pattern of liver cell columns of normal size and shape A- blood sinusoid B- central vein F: Histological Section in the liver shows A-portal area with congested blood inside the portal vein B- lymphocytic aggregation (H&E stain, 40X).

While the treatments with Ginseng at 150mg/ kg and AFs at 1mg/ kg show the liver cells after this treatment were hypertrophied and swollen and form a mass of cell appeared as cytoplasmic syncytium but its nuclei were well recognized as spirit pattern. Some of this cell was appeared as radial pattern near the central vein between there was blood sinusoid that the kupffer cell could be recognized as in (Fig. 4. f) the portal area

had lymphocytic infiltration around the blood vessels like the branches of portal vein and hepatic artery.

At the other side of the same section the borders of the liver cell were mostly not recognized well due to enlargements of cells and compact to each other to form amass of cell, but its nuclei were spherical and normal size with basophilic stain.

The ginseng is functional materials may be responsible for its wide pharmacological actions in clinical practice by a free radical reaction-inhibition mechanism. Therefore, the Ginseng has protective effects this effect may be related to the functional materials properties consequently decreased risk for most cancers including carcinomas liver (Dianese and Lin, 2001).

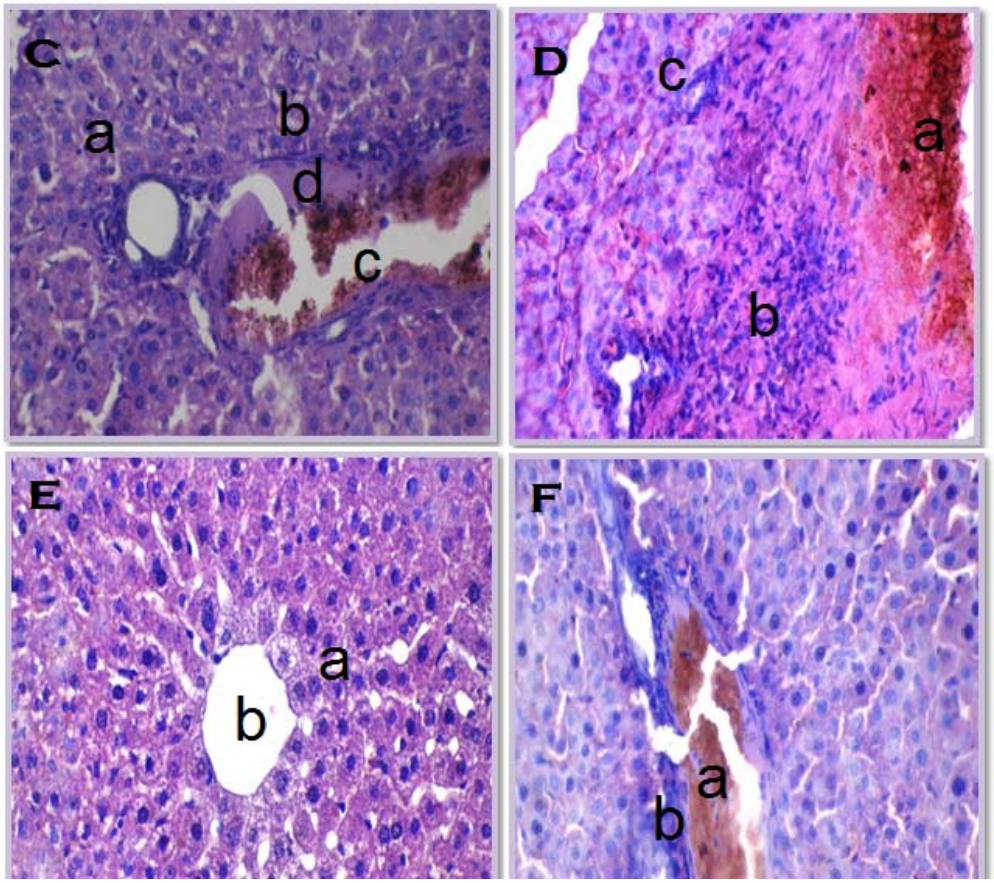

Fig. 4: C: Histological Section in the liver shows A-hypotrophy liver cell B- necrosis of liver cell C- congestion of blood in portal vein D- hemolysis of blood in the portal vein D: Histological Section in the liver shows A-sever congestion of portal vein B- lymphocytic infiltration C- blood sinusoid between liver Celle: Histological Section in the liver lobule demonstrating the radial pattern of liver cell columns of normal size and shape A-blood sinusoid B- central vein F: Histological Section in the liver shows A-portal area with congested blood inside the portal vein B- lymphocytic aggregation (H&E stain, 40X).

Likewise, the main active components of GS are ginsenoside Kim, *et al*., (1999), which have been shown to have a range of biological properties including anti-inflammatory, functional materials, and anticancer effects. In addition, ginseng extract reduces liver damage induced by certain chemicals including alcohol (Zain, 2001) or carbon tetrachloride (Reddy, *et al*., 2009).

Another study suggested that ginseng and/or ginsenoside can induce functional materials enzymes essential for maintaining cell viability by lowering

the level of oxygen radicals generated from intracellular metabolism (Cast, 2003). Ginseng was found to induce the potent protective action in rats may play a role in the prevention of hepatic cellular injury produced by aflatoxins (Abdel- Fattah, 2010). Abdel-Wahhab, *et al*.(2002)and El-Nekeety *et al*. (2007)) found that the treatment with FB1 was resulted in hepatotoxicity, apoptosis and inhibitory effect on cell proliferation, Moreover, AF treatment induced a severe cytotoxicity and inhibition of hepatocytes cell proliferation interferes with normal growth related processes and hence the disruption of normal liver homeostasis (Abdel- Wahhab *et al*., 2002).Our results agreement withYokozawa, *et al* (1994) whom show that revealed that ginseng had no harmful effects on liver tissues. However, the liver of the animals in the aflatoxins-treated groups showed severe histopathological changes may be typical to those reported in the literature. Furthermore, in this concern, Ginseng has component ginsenoside was demonstrated that ginsenoside could decrease these verity of renal injury induced by cisplatin. These authors suggested that decreased level of urea in serum in rats given Ginseng reflected the protective action of ginsenoside against the renal dysfunction (Abdel- Fattah, *et al*., 2010).

Histopathological picture of liver after treated with whey protein (WP):

The treatments of Rats group with WP at 100mg/ kg showed that the parenchyma of the liver was demonstrated with the hypertrophy of the liver cells and appeared the whole cell a great mass that liver cell were packed to each other, so the border of cells was not detected well. The nuclei of the cell were present as spherical shape as in Fig. (5.g) around the central veins could be demonstrate a cords a liver cells directed toward the central vein and around these cell there was sinusoid containing kupffer cell Fig.(5.g) There was lymphocytic focal aggregation between the liver cells and around certain central veins. Also the central veins were congested with blood which had blackish spots and lymphocytic aggregations were present in between liver cell.

Also when treated with WP at 150mg/ kg, the histopathic picture were appear as the hypertrophy of liver cell were prominent and form a mass of cell inside the tissue of liver and the most of nuclei of these cell were normal the blood sinusoid in certain place were easily demonstrated containing RBc and kupffer cell as in Fig. (5.h) and the portal areas were containing lymphocytic aggregation around the portal vein and hepatic artery Fig. (5.h).

While the treatments with the WP at 100mg/ kg and the AF at 1mg/ kg appear as histopathological changes represented by the parenchyma of the liver was containing mostly of the liver cell columns arranged in radial pattern, comb arrangement as in Fig. (5.i). These cell were polygonal shape with normal size of spherical nuclei, the blood sinusoid were present between cell with RBc also there was swelling of certain liver cell with degeneration changes in its cytoplasm Fig. (5.i) could be seen, the blood vessels of the portal areas were congested with blood.

The liver cell in the lobule were arranged in radial pattern directed with blood sinusoid between to the center of each lobule which hade the center vein these liver cell were containing eosinophilic cytoplasm and basophilic spherical nuclei. There was certain number of live cell with hypotrophy size and smaller nuclei and the hyper trophy of certain other cell also noted the central veins in certain place and hemolysis blood.

Further the treatments with the WP at 150mg/ kg and AF at 1mg/ kg were caused to Hypertrophy of liver cells was prominent at this treatment associated with light cytoplasm and the cell

membrane of these cell was demonstrated associated with thickening of this cell membrane as in Fig. (5.j) some of these cell lost its nuclei there was necrosis of the liver cell reflected by breaking down of its cell membrane and appeared as cavities there was focal aggregation of lymphocyte around the central vein Fig. (5.j) and portal area.

At the other side of section appeared breaking down of cell membrane and thickening of other membrane was recognized also the absence of certain nuclei from liver cell was seen.

Aflatoxins the major toxic metabolites of fungi which are able to induce chronic liver damages. The functional materials and hepatic-protective effects of WP. Our result Agreement with El-Kady, *et al*. (2010) they mentioned, their results indicated that both WP exhibit functional materials activity in the combined treatment showed the potential effect. Both agents showed a potential hepatic-protective effects against aflatoxins-induced liver damage and oxidative stress. Furthermore, El-Nekeety, *et al*. (2014) reported Rodents did not causes apparent adverse health effects on the kidneys and/or liver.

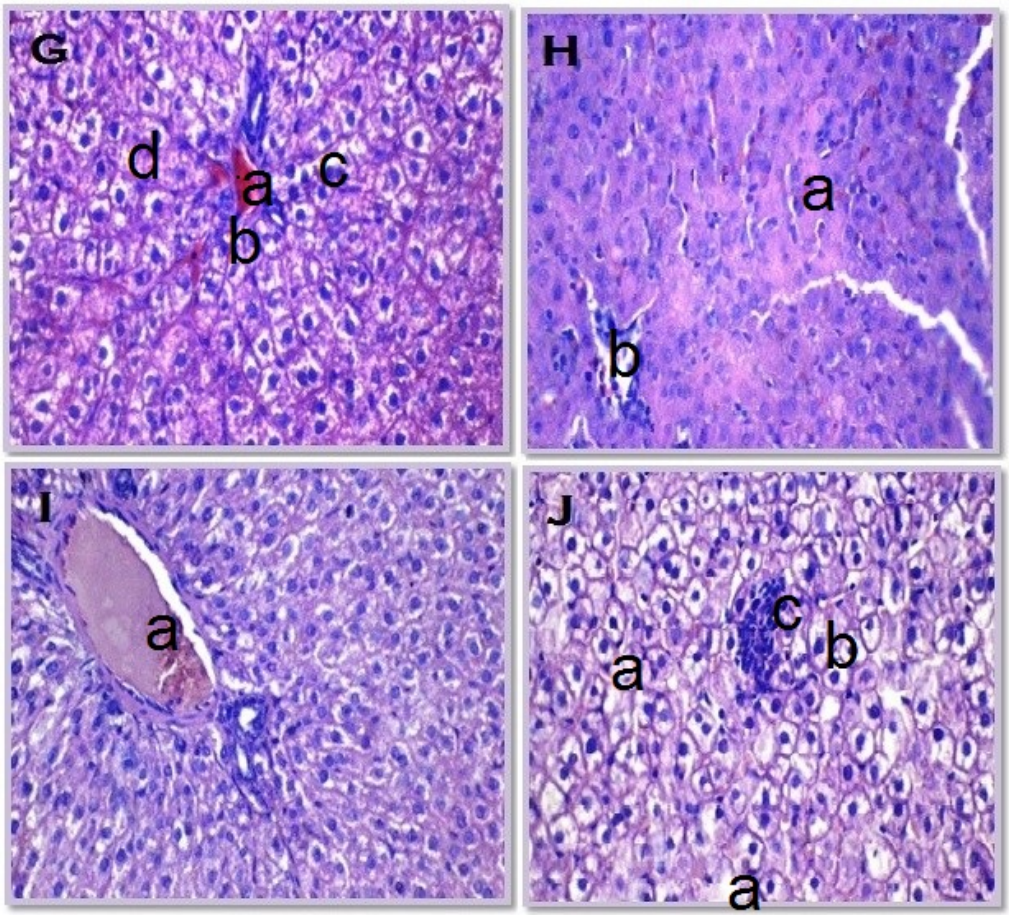

Fig. 5: G: Histological Section in the liver shows A-Congested central veinB- lymphocytic aggregation C- kupffer cells D- in the sinusoid radial pattern of liver H: Histological Section in the liver shows A-amass of hypertrophic liver cell associated with blood sinusoid with the kupffer cell B-lymphocytic aggregation I:Histological Section in the liver shows A-hemolysis the blood when was congested in the portal vein J:Histological Section in the liver shows A-necrosis of a liver cell B- hypertrophy of liver cell .C-lymphocytic aggregation.

Histopathological picture of liver after treated with butylated hydroxyl toluene (HBT):

The effects of BHT at 100mg/ kg on the liver tissues had appear a columns of the liver cell of polygonal shape and its nuclei were spherical between there was sinusoid of blood and had kupffer cell and these were directed toward the central vein as in Fig. (6.k) the regions present to the peripheral of the lobule were containing hypertrophy of the live cell and its cell membrane were thickened Fig. (6.k) the portal area had lymphocytic infiltrations around the portal vein.

Degenerative changes of the liver cell and hypertrophy of others was easily recognized and the cell was compressed to each other, so it was difficult to recognize the blood sinusoid between liver cells. The central vein was engorged with blood.

The treatment by 150mg from BHT/ kg were caused on the liver cells as hypertrophied with necrotic cytoplasm which appeared empty from any structure and its color was whitish, the cell membrane of hepatocyte were thickened and stained reddish color as in Fig. (6.l) the central veins were congested with blood, in certain place the liver cell appeared broken down to form a cavities with each other Fig. (6.l). The parenchyma's of liver cell were prominent and form a mass of cell inside the tissue of liver and the most of nuclei of these cells were normal. The blood sinusoid in certain place was easily demonstrated containing RBC and kupffer cell, the portal area were containing lymphocytic aggregation around the portal vein.

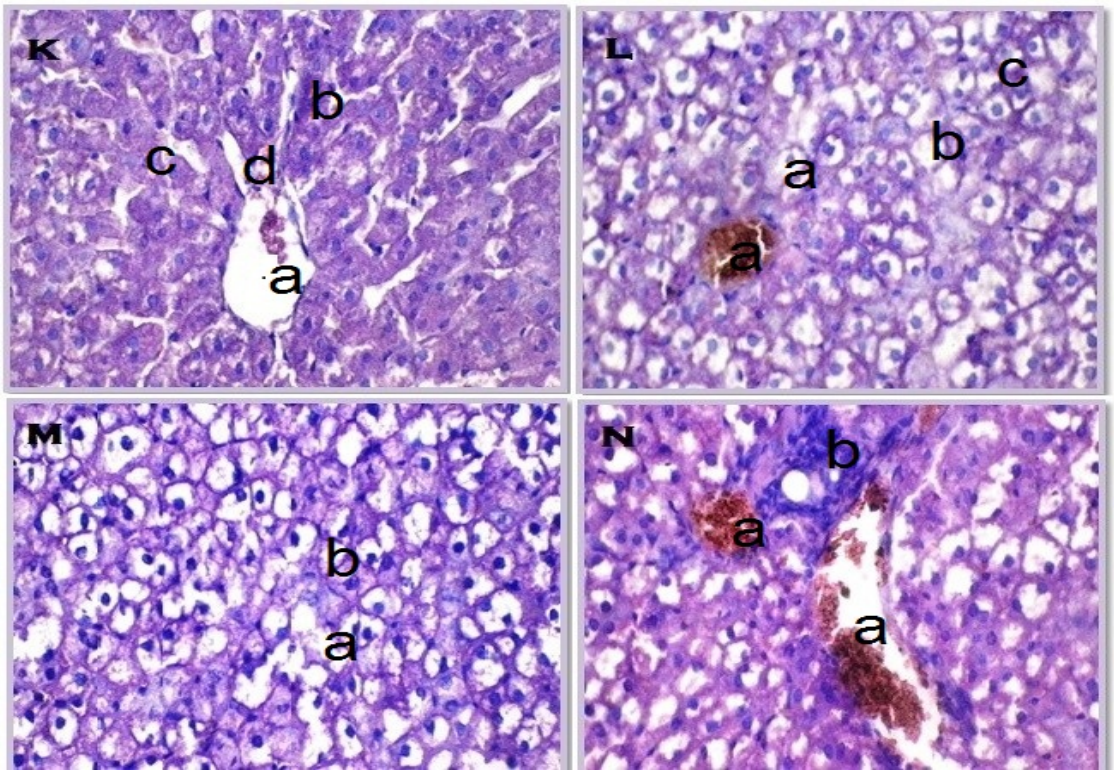

Fig. 6: K: Histological Section in the liver shows A-Congested central vein B- lymphocytic aggregation C- kupffer cells D- in the sinusoid radial pattern of liver cell L: Histological Section in the liver shows A-amass of hypertrophic liver cell associated with blood sinusoid with the kupffer cell B- lymphocytic aggregation M :Histological Section in the liver shows A-hemolysis the blood when was congested in the portal vein N: Histological Section in the liver shows A-necrosis of a liver cell B- hypertrophy of liver cell .

The interaction effects between BHT at 100mg/ kg and AF at 1mg/ kg on the histopathological picture of liver cells were appear in Fig. (6.m). the results were shows that degenerative changes of the liver cell was prominent associated with necrosis of the liver cell characterized by breaking down of the cell membrane and formation of cavities and adjacent cell, most of the liver cell had its nuclei but others were devoid for its nuclei as in the Fig. above. Lymphocytic aggregation unsheathing the blood vessel of the liver was common as in Fig. (6.m) certain blood inside these blood vessels was hemolysis.

Also the effects of 150mg of BHT/kg were actions against the AF at 100mg/ kg, and the results were investigated the effects of this treatment as sever congestion of the whole blood vessel of the liver was noted as shows in Fig. (6.n) And the congested central veins were unsheathed by lymphocytic infiltrations necrosis, hypertrophy and degeneration of the live cell was demonstrated as in Fig. (6.n) some of these cell lost its nuclei, cell membrane and its cytoplasmic white color. Extensive degeneration of the liver cell was demonstrated the nuclei of certain liver cell were psychotic and some of the cell membrane lost its boundaries so cavities were present instead of independent cell.

Our result accord with Al-Malki, (2010) He mentioned BHT treated rats liver section of CCl4 intoxication rats showed huge fatty changes necrosis and broad infiltration of the lymphocytes and loss of cellular boundaries. Also BHT treated rats showed more normal lobular pattern with mild degree of fatty changes, necrosis and lymphocyte infiltration almost comparable to control. On other hand, Martin and Eriksson, (1996) show that BHT has adverse effects in the liver, and metabolized by the cytochrome P450 system in the liver may be converted to pyro oxidative compounds during this process. Adipose tissue lacks the cytochrome P450 system. Therefore, the decreased hepatic concentration of α-tocopherol may be a consequence of a BHT-induced, free oxygen radical mediated, depletion of this functional materials.

CONCLUSION

From the results of this study we can concluded that ELISA method revealed that *Aspergillus parasiticus* strain have ability to produce AF in high concentration. So the consumed of food contaminated with aflatoxins from laboratory animals caused in physiological changes in tissue and internal organs are represented by the liver. And then the results were appear that the different concentrations of GS, WP and BHT as able to prevention against the negative effects of aflatoxins on physiological histopathological change.

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