THE POTENTIAL PROTECTIVE EFFECT OF LICORICE ON AFLATOXIN INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Aflatoxins, toxic metabolic by-products produced by Aspergillus flavus and A. parasiticus, are unavoidable food contaminant and reducing their toxicity in animals is of great interest. The potential of licorice which exhibits antioxidant and anti-inflammatory properties was evaluated for alleviating the AFB1-induced (Aflatoxin B1) hepatotoxicity in rat. Four experimental groups were used, each comprising 5 rats; control group (G1), licorice-received group (G2) (300mg/kg b.w intragastric daily for 7 days), AFB1-treated group (G3) (5 mg/kg b.w. one dose intrapretonial), and a group given licorice 7 days before AFB1 intoxication (G4) 24 hours later, the animals sacrificed. The activities of Alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were measured in rat serum as well as histopathological examination of liver. Results revealed the following: AFB1 significantly elevated the serum ALT, AST and the liver showed diffuse necrosis, bile duct hyperplasia, congestion of the central vein and portal mononuclear cells infiltrations. Rats received licorice before AFB1, showed a significant amelioration in serum enzymes and improvement in liver tissues architecture. In conclusion, licorice was found to be safe and successful agent counteracting the toxicity induced by AFB1 in the liver of rat.

Key words: Aflatoxin, licorice, Rats, ALT, AST, liver, histopathology, AFB1, hepatotoxicity.

INTRODUCTION

One of the most important problems that occur as a result of unconditioned storage of food and foodstuff is toxication caused by mycotoxins which are toxic metabolic by-products produced by fungi ***(Elik et al, 2000)***. Aflatoxins are the mostly seen mycotoxins and there are four naturally occurring aflatoxins, the most hepatotoxic being aflatoxin B1 (AFB1), and three structurally similar compounds namely aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and Aflatoxin G2 (AFG2) ***(Fink-Gremmels, 1999)***. Aflatoxins are secondary metabolites produced by Aspergillus flavus and A. parasiticus, and they have carcinogenic, mutagenic, hepatotoxic and teratogenic effects ***(Massey et al., 1995)***. Aflatoxin B1, the most potent toxic and the most common of aflatoxins ***(Abdel-Wahhab et al., 2002, Eraslan et al., 2004)***, has generated much concern due to its carcinogenicity ***(Stoloff, 1983; IARC, 1987; Phillips et al, 1994)***. Aflatoxin B, has been implicated as a factor in human liver cancer and classified as a Group 1 human carcinogen ***(IARC, 1987; Rothschild, 1992)***. The major source of exposure to aflatoxins is via the ingestion of contaminated food***(Stoloff,1983)***.Consumption of aflatoxin-contaminated food by human and animals causes important health problems together with important economical losses. All animal species are susceptible to aflatoxicosis, but outbreaks are commonly occurring in pigs, sheep, and cattle. Beef and dairy cattle are more susceptible to

aflatoxicosis than sheep or horses ***(Rasostits et al., 2000)***. Aflatoxicosis causes several determinable effects including decrease in growth rate, immunosuppression, anemia, and increase in coagulation time and deteriorated lipid, carbohydrate, and protein metabolism ***(Elik et al, 2000, Raju and Devegowda,2000)***. Several diseases are associated with the human consumption of these toxins, including toxic hepatitis and even primary hepatocellular carcinomas ***(Pitt, 2000)***. AFB1-mediated toxicity was found to be related to its pro-oxidant potential. This is because the reactive oxygen species (ROS) including superoxide anion (O-), hydrogen peroxide (H2O2), and hydroxyl radical (-OH) generated during the metabolic processing of AFB1 by liver enzymes ***(Preston and Williams, 2005; Towner, et al. 2003)***. ROS cause oxidative stress by damaging cellular membranes and components. Therefore, it can be assumed that natural components having antioxidant potential are capable of inhibiting ABF1-induced oxidative damage either by scavenging ROS or stimulating antioxidant defense systems ***(Yener et al., 2009)***. Licorice is a plant that grows in Southern Europe, Asia and Mediterranean. Its scientific name is Glycyrrhizic acid. Its components glycyrrhizin, triterpenoids, polysaccharides, and polyphenols such as liquirtin, flavones and isoflavonoids It is used for many ailments including asthma, athlete's foot, baldness, bursitis, canker sores, chronic fatigue, depression, colds and flu, coughs, dandruff, emphysema,

gingivitis and tooth decay, gout, heartburn, HIV, viral infections, fungal infections, ulcers, liver problems, menopause, psoriasis, shingles, sore throat, tendinitis, tuberculosis, ulcers, yeast infections, prostate enlargement and arthritis ***(Herbwisdom 2013)***. It had a well-documented reputation for healing ulcers. It can lower stomach acid levels, relieve heartburn and indigestion and acts as a mild laxative, can also be used for irritation, inflammation and spasm in the digestive tract. Licorice also appeared to enhance immunity by boosting levels of interferon, a key immune system chemical that fights off attacking viruses ***(Shinada et.al, 1986)***. Glycyrrhizinic acid also seemes to stop the growth of many bacteria and viruses such as influenza A ***(Herbwisdom 2013)***. The HCV replication is significantly and dose-dependently suppressed by two purified compounds, isoliquiritigenin and glycycoumarin, which were from *Glycyrrhizae* ***(Nakagawa, 2009)***. *Glycyrrhiza glabra* derived compound glycyrrhizin and its derivatives reduce hepatocellular damage in chronic hepatitis B and C ***(Arase et.al,1997)***. In hepatitis C, the risk of hepatocellular carcinoma was reduced. Licorice was used as a protective agent against Cadmium ***(Lee et.al, 2007)*** and CCL4 ***(Wang et.al, 2011)*** but there is no previous literature about the potential protective effect of licorice on AFB1induced hepatotoxicity. Thus the aim of the present study is to asses the efficiency of licorice and its amelioration of experimental aflatoxicosis in rats.

MATERIALS AND METHODS

Chemicals:

Aquos extract of Licorice prepared from Licorice root in concentration 3:1 after washing, grinding, boiling, centrifugation and filtration (Andalos Company, China).

Aflatoxin B1 was purchased from Segma Egypt company imported from USA.

Experimental Animals:

Twenty male Wistar-albino rats 4 weeks old weighing 90-100 grams were obtained from the animal house colony, National Research Center, Giza, Egypt. The animals were maintained on standard casein diet and water ad libitum and housed individually in a temperature-controlled and artificially illuminated room with relative humidity 50-6o% and on 12hr. light-dark cycle.

Experimental design:

Rats were randomly divided into four groups each consisting of 5rats. Animals were treated as follows: untreated control (G1), treated with licorice 300mg/ kg b.w. intragastric for 7days (G2), treated with AFB1 5 mg/kg b.w. intraperitoneal one dose on day 7 (G3), or treated with the licorice 300mg/ kg b.w. intragastric for 7days before AFB1 injection 5 mg/kg b.w. (G4). On the 8th day of the study, blood was collected directly from the retro-orbital venus plexus for serum separation and analysis of ALT and AST. Then, animals were sacrificed by cervical decapitation and liver samples were dissected out and processed for histopathological examination.

Determination of the serum biochemical parameters:

At the end of the experimental period, animals were sacrificed by cervical decapitation under light ether anesthesia and blood was collected on a plain centrifuge tube from the retro-orbital venus plexus, left to clot and serum samples were obtained by centrifugation at 3,000 rpm for 10 min. The clear serum was separated carefully and freezed until used for the assay of marker enzymes of liver function serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST), using the commercially available standard kits and according to manufacturer's instructions.

Histopathological examination:

For histopathological studies, samples of the liver of each animal were excised and processed for light microscopy. The processing involved fixing the tissue specimens in a 10% neutral buffered formalin solution, preparing the blocks in paraffin, cutting sections 5 μm in thickness, and stained with hematoxylin and eosin (H&E) ***(Bancroft, and Stevens, 1990)***.

Statistical analysis:

Statistical analyses were performed by one-way ANOVA followed by Tuckey's test. All data were presented as Means ±SD. Differences were considered significant when $P<0.05$. The parameters were analyzed by analysis of variance by using SPSS 10.0 for Windows.

RESULTS

Biochemical studies:

Serum biochemical parameters (ALT, AST activity):

The effect of different treatments on serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) levels in rats is presented in Table (1) and Fig.1. Rats given AFB1 showed an increase in activities of ALT (493.8) and AST (912.8), which were statistically high significant at $P<0.01$, as compared to control. In addition, On the other hand, oral administration of licorice before AFB1-intoxication caused significant amelioration in ALT and AST activities as compared to the AFB1 alone treated groups at $P<0.01$. Rats treated with licorice alone showed a non significant reduction in ALT and AST activities when compared to control at $P>0.05$.

Table (1): Effect of Licorice on some serum biochemical parameters of liver functions of AFB1-treated male rat.

Group	ALT	AST
Group 1	77.3 b	177.3 b
Group 2	74.2 b	184.4 b
Group 3	672.8 a	912.8 a
Group 4	166.6 b	296.8 b
LSD0.05	151.6	218.7
Group	**	**

-Means with different superscript letters (a, b) are significantly different (P <0.01).

Analysis of variance presented in Table (1) showed that the mean squares for Groups were highly significant (**) for ALT and AST traits.

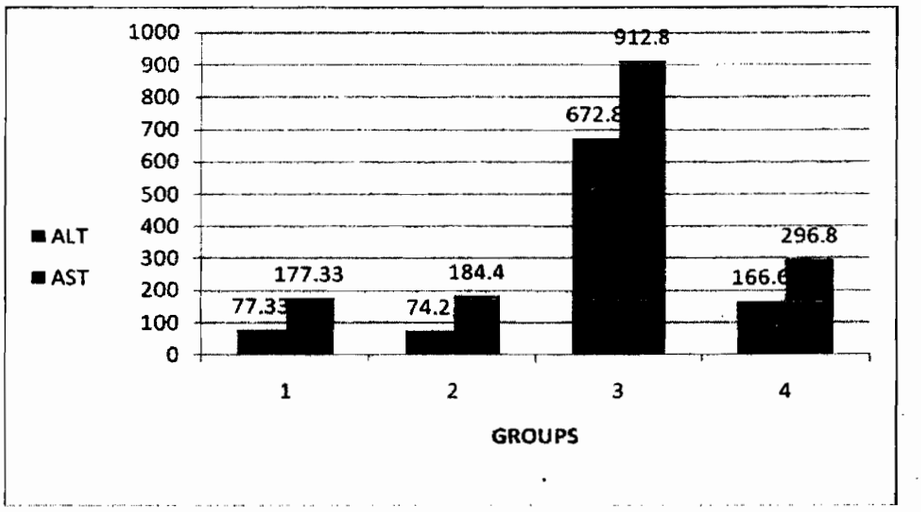


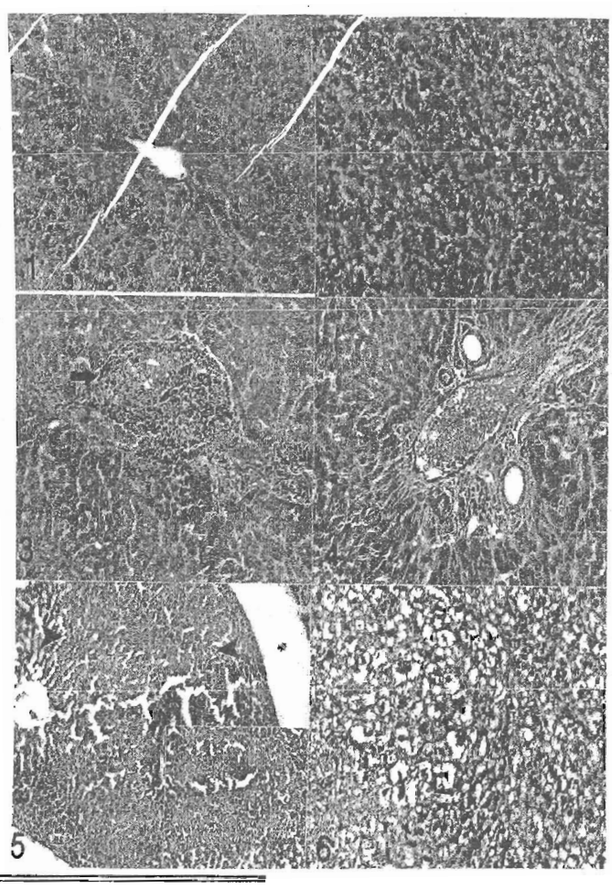
Fig. (1): Effect of Licorice on serum biochemical parameters of liver functions of AFB1-treated male rat.

Histopathological examinations of liver:

Control rats liver sections showed normal lobular architecture with central vein and radiating hepatic cell cords (Plate 1, Fig.1). No obvious histopathological changes were observed in the liver of rats treated with licorice except mild hydropic degeneration and sinusoidal cells activation (Plate 1, Fig.2). However, aflatoxin treated rats liver showed distortion of hepatic architecture with dilated and congested central vein, portal mononuclear cells infiltrations, mild bile ducts hyperplasia as well as patchy hepatic cells necrosis (Plate 1, Fig. 3, 4, 5). On other side, the combined administration with licorice and AFB1 resulted in restoration of normal hepatic architecture (Plate 1, Fig.6) however; mild hydropic degeneration along with mild mononuclear cells infiltrations was observed.

FIGURE LEGENDS

Plate 1, Fig.1, G1, liver showing normal lobular architecture with central vein and radiating hepatic cell cords. H&E. X 200. Fig.2, G2, liver showing mild hydropic degeneration of the hepatocytes. H&E. X 200. Fig.3 , G3, liver showing portal mononuclear cells infiltrations (arrow) and congestion. H&E. X 200. Fig.4, G3, liver showing bile duct hyperplasia. H&E. X 200. Fig.5, G3, liver showing marked hepatic cell necrosis (head arrow). H&E. X 100. Fig.6, G4, liver showing mild hydropic degeneration along with mild mononuclear cells infiltrations. H&E. X 200.



DISCUSSION

Aflatoxin, a potent hepatotoxic and hepatocarcinogenic mycotoxin, is associated with various diseases such as aflatoxicosis and hepatocellular carcinoma ***(Premalatha et al. 1999)***. The liver is considered the main target organ for aflatoxicosis ***(Yildırım et al. 2011)*** where most aflatoxins are bioactivated to the reactive 8, 9-epoxide form, which is known to bind DNA and proteins, causing damage to the liver as well as lipid peroxidation ***(Miazzo et al., 2005; Bailey et al., 2006; Pasha et al., 2007, Premalatha et al. 1999)***.

In the present study, hepatic lesions were observed in aflatoxinB1 treated rats including congestion of the central vein, portal mononuclear cells infiltrations, mild bile ducts hyperplasia, sinusoidal diltation as well as patchy hepatic cells necrosis. The abovementioned histopathological changes in the liver of rats exposed to AFB1 are similar to those reported in the literature on rabbits aflatoxicosis, where vascular congestion, leucocytic infiltration and degenerative changes were observed during the initial stage of toxicity. On the other hand, at its terminal stage, coagulative necrosis, perivascular and periductal fibrocellular reactions along with mononuclear-cellular infiltration and distortion of the hepatic chords were observed in the liver ***(Avinash et al., 2004)***. ***Lakkawar et al. (2004)*** observed vascular congestion (up to 30 days), areas of coagulative necrosis around the central veins, and engorged portal areas (from the 40th day onwards) in rabbits. ***Abdel-Wahhab et al. (2002)*** reported vascular dilation, congestion, and moderate hemorrhages in rats. ***Salim et al. (2011)*** reported dilated sinusoids depending on the

hepatocytes necrosis as a result of aflatoxicosis. ***Lakkawar et al. (2004)*** found that high doses of Aflatoxin cause severe hepatocellular necrosis. On the other hand, prolonged low dosage leads to liver enlargements. There is a direct correlation between the concentration of Aflatoxin, the duration of the exposure and the induced histopathological changes ***(Baptista et al., 2008)***. Aflatoxins affect primarily the cellular immunity process in most animal species ***(Oguz et al. 2003)***. In the Aflatoxin-exposed chickens, ***Yıldırım et al. (2011)*** have declared mild mononuclear cell infiltration in portal areas of the liver. In present study, we also observed a similar finding in the Aflatoxin treated group. In the present study, licorice extract significantly alleviate the histopathological changes induced by aflatoxin and resulted in restoration of normal hepatic architecture except mild hydropic degeneration along with mild mononuclear cells infiltrations observed in G4 which received licorice before aflatoxin injection.

Aflatoxin elevated the activity of hepatospecific enzymes as ALT, AST and ALP in serum indicating liver dysfunction ***(Yousef, et.al 2003)***. Several reported studies showed that Licorice extract significantly inhibited the elevated AST, ALP and ALT activities of cadmium (CdCl2, Cd) and CCl4-induced liver toxicity in rats ***(Achliya et.al, 2003; Wang et.al, 2011; Lee et.al, 2007)***. In the present study serum hepatic biomarkers, AST and ALT activities were greatly increased ($P<0.01$) in rats received aflatoxin treatment compared to control. However, rats received licorice before aflatoxin injection showed significant decrease in serum hepatic biomarkers, AST and ALT compared to aflatoxin

received rats. The increased serum levels of hepatic markers have been attributed to the liver injury. The mechanism of cellular damage caused by AFB1 had not been fully elucidated. Mead, 1976 reported that intraperitoneal route of aflatoxin for 8 days caused significant increase in lipid peroxidation in liver and kidney of aflatoxin treated rats, as compared to controls. Lipid peroxidation is regarded as one of the primary key events in cellular damage ***(Rastogi,et.al; 2001)***.

In conclusion, licorice extract significantly decreased the elevated AST, and ALT activities of aflatoxin-induced liver toxicity in rats as well as it significantly alleviated the histopathological changes induced by aflatoxin and returned the normal hepatic lobular architecture. The alleviation may be related to antioxidant and anti-inflammatory properties of licorice. licorice was found to be safe agent against AFB1induced hepatotoxicty.

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