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ZIZYPHUS SPINA-CHRISTI EXTRACT PROTECTS AGAINST AFLATOXIN B_1-INTITIATED HEPATIC CARCINOGENICITY

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Abstract

Aflatoxins (AF), a group of closely related, extremely toxic mycotoxins, produced by *Aspergillus flavus* and *A. parasiticus* can occur as natural contaminants of foods and feeds. Aflatoxins have been shown to be hepatotoxic, carcinogenic, mutagenic, and teratogenic to different animal species. *Zizyphus spina-christi* L. extract was investigated for its antifungal and antimicrobial activities. The aim of the present work was to evaluate the antioxidant activity of the methanol extract of *Z. spina-christi* L. leaves against the oxidative stress of aflatoxin in rats. Fourty male Sprague-Dawley male rats were divided into four groups including the control group, the group fed aflatoxin-contaminated diet (3 mg/kg diet) and the groups treated with *Zizyphus* extract (5 mg/kg b.w) alone or in combination with AF for 15 days. Biochemical analysis reveled that treatment with AF resulted in a significant increase in ALT, AST, cholesterol, triglycerides, uric acid, TNFa, LPO, NO and CEA, whereas it decrease significantly GPX and SOD. The histopatholgical examination of the liver, kidney and testis showed sever histological changes typical to those reported for aflatoxicosis. Animals treated with *Zizyphus* extract alone or plus AF showed a significant improvement in all biochemical parameters and histological picture of liver, kidney and testis. It could be concluded that *Zizyphus* extract have a power protective role against aflatoxicosis.

Key words: Aflatoxin, antioxidant, hepatotoxicity, *Zizyphus spina-christi*,

Introduction

Several species of the mold *Aspergillus* produce metabolites known as mycotoxins that are extremely toxic to animals and humans (Vesonder et al., 1991; Hussein and Brasel, 2001). Among the various mycotoxins, the aflatoxins are produced mainly by two species: *Aspergillus flavus* (AFB_1 and AFB_2) and *Aspergillus parasiticus* (AFB_1, AFB_2, AFG_1 and FG_2). Aflatoxins have been found in various susceptible commodities including oil seed and grains. Farm animals can retain residues of aflatoxin or metabolites in their tissues (IARC, 1993). Outbreaks of aflatoxicosis are common in tropical countries, mostly among adults in poorly nourished rural populations whose staple food is maize. Reported mortality rates in the acute phase range from 10 to 60% (Chao et al, 1991; Peraica et al, 1999).

Aflatoxins especially AFB_1 are among the most common mycotoxins to which humans are exposed. Epidemiological and experimental studies have shown that aflatoxins are hepatotoxic, hepatocarcinogenic, mutagenic and teratogenic (Groopman et al., 1996; Mayura et al, 1998; Abdel-Wahhab et al, 1998, 1999; Abdel-Wahhab and Aly, 2003, 2005). Aflatoxins cause oxidative stress by increasing lipid peroxidation and decreasing enzymatic and non-enzymatic antioxidants in aflatoxin-treated animals (Abdel-Wahhab and Aly, 2003, 2005; El-Gibaly et al., 2003). Oxidative damage in the cell or tissue occurs when the concentration of reactive oxygen species (superoxide radical, hydroxyl radical and hydrogen peroxide) generated exceeds the antioxidant

capability of the cell (Sies, 1991) or when the antioxidant capacity of the cell decreases. Levels of non-enzymatic antioxidants (glutathione, ascorbic acid) and enzymatic antioxidants (glutathione peroxidase, catalase and superoxide dismu tase) are the major determinants of the antioxidant defense mechanism of the cell.

Zizyphus spina-christi L. (Rhamnaceae) is a plant that grows into a tree with thorny branches and is used as a hedge to form defensive fences for animals. The fruit have a sweet edible pulp, the leaves are applied locally to sores, and the roots are used to cure and prevent skin diseases (Adzu et al., 2001). The infusion of the root park of the plant is used traditionally in certain African countries as a remedy for stomach pain and other gastrointestinal tract ailments (Adzu et al., 2001). It has been used in folk medicine as domulecent, depurative, emollient, stomachic, for toothaches, astringent and as mouth wash. Fruits are used to promote the healing of fresh wounds, for dysentery, bronchitis, cought and tuberculosis (Adzu, et al., 2001). Mahran et al. (1996) reported that the butanol extract of *Z. spina-christi* leaves contains four saponin glycosides: Christinin A, (jujubagenin), Christinin B, C and D. The butanol extracts of the leaves (flavonoids) and the main saponin glycoside (christinin A) improved glucose utilization in diabetic rats (Glombitza et al., 1994). It was shown to contain beutic acid and ceanothic acid, cyclopeptides, as well as saponin glycoside and flavonoids, lipids, protein, free sugar and mucilage (Adzu, et al., 2003.)

Materials and methods

Aflatoxins and chemical materials

The aflatoxin (AF) was produced through the fermentation of maize by *Aspergillus parasiticus* NRRL 2999 as described by Stubblefield et al. (1967). The fermented maize was autoclaved; ground to a fine meal, and the AFs content was measured by the use of HPLC (Hustchins and Hagler, 1983). The AFs within the maize meal consisted of 590 mg B_1, 110 mg B_2, 200 mg G_1 and 100 mg G_2 /kg. The maize meal was incorporated into the basal diet to provide the desired level of 2.5 mg of total AFs/Kg diet. The diet containing AF was analyzed and the presence of parent AFs was confirmed by HPLC. The safety measures recommended by WHO (1998) were taken when handling the AFB_1-contaminated diet.

Kits

Alanine transaminase (ALT), Aspartate transaminase (AST), Cholesterol, triglycerides and uric acid were purchased from Biodiagustic. Carcinoembryonic antigen (CEA) kit was purchased from Diagnostic Products Corporation Co., USA. Tumor necrosis factor alpha (TNF-a) kit was produced by Diaclone Research Co., France. NO kit R & D system was purchased from Gmbtt, Germany. Malondialdehyde (MDA) kit was purchased from Oxos Research™ Co. kit (USA). Glutathione peroxidase (GPX) and Superoxide dismutase (SOD) kits were purchased from Randox Laboratories Co., UK.

Plant materials

The plant was collected from Orman garden, Giza Egypt, and was identified by an agricultural engineer Badia Diwan during the period 2001-2003. The fruits of *Z. spinachristi* (500 g) were cut separated from the seeds and mixed with the least amount of water in the mixer unit for 24 h then dried using Freeze Dryer system (Dura-Dry Freeze Dryer, Model PAC-TC-V4; FTS system, Inc., Stone Ridge, NY. USA).

Experimental Animals

Ten-week-old, sexually mature male Sprague-Dawley rats weighting 140-150 g (purchased from Animal House Colony, Giza, Egypt) were maintained on standard lab diet (Protein: 16.04%; Fat: 3.63%; Fiber: 4.1%, and metabolic energy: 0.012 MJ) and water *ad libitum* at the Animal House Lab., National research Center. After an acclimation period of 1 week, animals were divided into four groups (10 rats/group) and housed in stainless steel cages housed in a temperature-controlled ($23 \pm 1^{O}C$) and artificially illuminated (12 hr dark/light cycle) room free from any source of chemical contamination. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of Al-Azhar University, Egypt.

Experimental Design

Animals in four groups were treated for 15 successive days as follows: (1) untreated, (2) fed aflatoxin-contaminated diet (2.5 mg/kg feed); (3) treated orally with *Zizyphus* extract (5mg/kg b.w.); and (4) fed aflatoxin-contaminated diet and treated orally with Zizyphus extract.

At the end of the treatment period, all animals were fasted for 12 hr. Blood samples were collected from the retro-orbital venous plexus under diethyl ether anesthesia and left to clot. The sera were separated using cooling centrifugation and stored at $-20\ ^{O}C$ until analysis. The activities ALT and AST were determined according to the method recommended by The Committee on Enzymes of the Scandinavian Society for Clinical

Chemistry and Clinical Physiology (1974). Cholesterol, triglycerides and uric acid in serum were determined successively according to Charles and Richmond (1974), Wahlefeld (1974), and Haisman and Muller (1977). Quantitative measurement of carcinoembryonic antigen (CEA) in serum was carried out by immunoradiometric assay (Begent, 1984). Quantitative determination of tumor necrosis factor alpha (TNF-a) was done by ELISA method (Corti et al., (1992). Serum nitrate concentration as a stable end product of nitric oxide (NO) was estimated by the Griess reaction after quantitative conversion of nitrate to nitrite by nitrate reductase (Moshage, 1995). Hepatic glutathione peroxidase activity was determined by spectrophotemetric method using reduced glutathione and cumene hydroperoxide as substrate using 20 µl diluted liver homogenate according to the modified method of Paglia and Valentine (1967). Hepatic superoxide dismutase activity was assayed spectrophometerically by red formazan dye reduction procedure (Suttle, 1986). Hepatic lipid peroxidation was estimated by the measurement of malondialdehyde (MDA) by spectrophotometeric method (Esterbauger et al., 1991)

After blood samples were collected, all animals were killed and the liver tissue of each animal was dissected, weighed and homogenized in phosphate buffer (pH 7.4) to give 20% w/v homogenate (Lin et al., 1998). This homogenate was centrifuged at 1700 rpm and $4^{O}C$ for 10 min and the supernatant was stored at –70 ^{O}C to the next day until analysis. This supernatant (20%) was used for the determination of lipid peroxidation in the liver tissue and it was further diluted with phosphate buffer solution to give 2% and 0.5% dilutions for the determination of glutathione peroxidase (2%), superoxide dismutase (0.5%) activities in the liver tissues. Lipid peroxidation in liver homogenate was estimated by the measurement of malondialdehyde (MDA) by spectrophotometeric method (Esterbauger et al., 1991). The level of lipid peroxides was expressed as *u*mol MDA/mg protein. The protein content of liver tissue was measured as described (Lowry et al., 1951). Glutathion peroxidase activity in liver homogenate was determined by spectrophometeric method using reduced glutathione and cumene hydroperoxide as substrate using 20 *u*l diluted homogenate by the modified method of Paglia and Valentine (1967). Superoxide dismutase activity in the liver was assayed spectrophotometerically by red formazan dye reduction procedure (Suttle, 1986) using 50 *u*l diluted homogenate. The specific activities of glutathione peroxidase and superoxide dismutase were expressed as units/mg protein.

All rats were subjected to post mortem examination, liver, kidney and testes from all rats within different groups were removed and fixed in 10% neutral buffered formalin, dehydrated in alcohol, cleared in xylol and embedded in paraffin. 4u thick sections were prepared and stained with hematoxilen and Eosin (H&E) for general histological investigations and bromophenol blue stain for total protein contents. Further histochemical evaluation of total protein content was done using Qwin Leica image processing and analysis system (Cambridge, UK). Total protein optical measurement was done by measuring the mean gray of the bromophenol blue reaction.

Statistical analysis

All data were statistically analyzed using the General Linear Model Procedure of the Statistical Analysis System (SAS, 1982). The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio (Waller and Duncan, 1969). All statements of significance were based on probability of $P \leq 0.05$.

Results

The effects of *Zizyphus* extract and aflatoxin on serum biochemical parameters are depicted in Table 1. Animals treated with aflatoxin showed a significant increase in ALT, AST, cholesterol, triglycerides and uric acid. *Zizyphus* extract alone caused significant decrease in AST, cholesterol, triglycerides and uric acid while it was comparable to control regarding ALT. Animals treated with the combined treatment showed a significant improvement in all tested parameters towards the control values although there was a significantly different with the controls.

Table 2 indicated that treatment with aflatoxin resulted in a significant increase in TNF- a, CEA and NO whereas these parameters were in the normal levels of the control in the animals treated with *Zizyphus* extract. The combined treatment resulted in a significant improvement in these parameters although it was still differing significantly from the controls. The effects of different treatment on the oxidant-antioxidant status of liver are depicted in table 3. Aflatoxin induced an oxidative stress as indicated by the significant increase in LPO level accompanied with the significant decrease in GPX and SOD. No significant difference was observed in the animal treated with the extract alone. Treatment with extract plus aflatoxin were comparable to the controls regarding GPX and SOD whereas, a significant improvement was found in LPO level although it still differs than the controls.

The histological examination of the liver of the control animals or those treated with *Zizyphus* extract alone showed normal central vein and hepatic cords separated with blood sinusoids (Figure 1A). Whereas the

livers of animals treated with aflatoxin alone showed congested central vein, damaged bile ducts and massive vacuolar degeneration with abnormal nuclei shrunken (S) and pyknotic (P) (Figure 1B). The livers of animals treated with aflatoxin plus *Zizyphus* extract showed more or less normal hepatocytes compared to the controls (Figure 1C).

The histological studies of the control kidney (Figure 2A) showed a normal structure of proximal convoluted tubules and malpighian capsules in kidney tissue of control or *Zizyphus* extract treated rats. Treatment with aflatoxin alone resulted in the presence of hylin casts in proximal and distal tubules, lumen and cellular debris and intertubular mononuclear cellular infiltration (Figure 2B). The kidney of animals received the combined treatment of aflatoxin plus *Zizyphus* extract showed normal tubules and glomeruli. The nuclei of the epithelial cells lining tubules revealed different degrees of mitotic division (arrow) (Figure 2C).

The histological examination of testes of the control rats or those treated with *Zizyphus* extract alone showed normal structure of spermatocytes and spermatids (Fig. 3A), whereas the testes of animals treated with aflatoxin alone showed sever damage in all stages of spermatogenesis, intermixed with remnants of mitotic figure and spermatic tails (Fig. 3B). The testes of animals treated with the *Zizyphus* extract plus aflatoxin showed marked recovery in most of the seminiferous tubules, spermatogenic cells and spermatids (Fig. 3C).

The optical density of total protein contents were performed using image analysis system and revealed the histochemical investigation of protein reaction in liver, kidney and testes of rats within different treatment groups showed a significant increase or decrease in total protein content (Table 4)

Discussion

The current study deals with the chemopreventive effects of *Zizyphus spinachristi* extract against AFB_1-induced oxidative stress and tumor initiation in rats. The selected dose of AFB_1 was based on our previous work Abdel-Wahhab and Aly, 2003), whereas, the selected dose of *Zizyphus spinachristi* extract was literature based (Adzu, et al., 2001). The activity of ALT and AST are sensitive indicators of acute hepatic necrosis (Kaplan, 1987; Abdel-Wahhab, et al., 2002). In the present study, aflatoxin-contaminated feed was found to cause a significant increase in ALT, AST, cholesterol, triglycerides and uric acid levels. Generally, these results may indicate degenerative changes and hypofunction of liver and kidneys (Abdel-Wahhab and Aly 2003; Farombi et al., 2005). The increased levels of uric acid may indicate protein catabolism and/or kidney dysfunction (Abdel-Wahhab et al., 1998, 1999, 2002). These results clearly showed that aflatoxin has a harmful and stressful influence on the hepatic and renal tissue and consistent with those reported of aflatoxicosis (Miller and Willson, 1994).

The hepatic antioxidants represent the major defense against toxic liver injury, and they act as anti-apoptosis. In the current study, the levels of oxidative stress indices, LP and NO, significantly increased while the levels of antioxidants GPX and SOD in aflatoxin-treated group were significantly decreased comparing to their levels in controls. These results were in agreement with those reported by (Meki et al., 2004; Abdel-Wahhab and Aly, 2005).

The increased level of LPO with the decreased level of GPx and SOD indicated that LPO is one of the most pronounced factors in aflatoxin-toxicity and carcinogenicity (Rastogi et al., 2001). Moreover, Abdel-Wahhab et al. (2005) stated that both GPx and SOD are considered to be enzymatic free-radical scavengers in cells. Thus, the decrease of both enzymes leading to an indirect increase in oxidative DNA damage and suggesting that SOD plays a role in the suppression of oxygen free-radical formation and the decrease of NO generation.

The histological results reported in the current study confirmed the biochemical results and indicated that aflatoxin induced severe histological changes in both the hepatic, renal and testicular tissues. The histological changes observed in the liver and kidneys induced by aflatoxin have been documented previously (Mayura, et al., 1998; Abdel-Wahhab et al., 1998). We also observed congested central vein, damaged bile ducts and massive vacuolar degeneration with abnormal nuclei shrunken and pyknotic. Although the principal target organ for aflatoxin is the liver, necrosis and hemorrhage may also occur in other organs (i.e., kidney, heart, spleen, and pancreas) depending on factors such as animal species, dose, route, and treatment protocol (Newberne and Rogers, 1981). Few reports have demonstrated renal damage in rats (Mayura et al., 1998; Abdel-Wahhab, et al., 1998). Arora et al. (1978) reported that the kidneys also excreted AFB_1 and that the renal medulla was quite sensitive to this mycotoxin. In our studies, the kidney from rats fed aflatoxin-contaminated diet showed pathological changed typical to those reported in the previous reports and confirmed the changes of the biochemical parameters of kidney functions reported herein. The effects of aflatoxin on testis have been studied previously (Hinshelwood, et al., 2003, 2002; Agnes and Akbarsha, 2003; Verma and Nair, 2002) . Ortatatli et al. (2002) reported degeneration and desquamation in the epithelium and decrease in the size and thickness of the germinative layer of the seminiferous tubules, and lowered plasma testosterone levels in adult roosters. Although androgen concentrations were not determined in AF-treated rats in the present study,

Table 1: Effects of *Zizyphus* extract on some serum biochemical parameters in rats fed aflatoxin-contaminated diet (means ± SE)

Parameters	Control	AF	*Zizyphus* extract	AF + *Zizyphus* extract
ALT (u/L)	29.00± 0.71[a]	71.20± 2.08[b]	27.83± 0.71[a]	48.80± 1.71[c]
AST (u/L)	52.00± 1.58[a]	104.80±1.93[b]	42.55 ± 0.71[c]	76.40± 2.84[d]
Cholesterol(mg/dl)	66.40± 1.21[a]	79.60± 2.29[b]	51.58± 1.08[c]	54.80± 1.98[c]
Triglycerides(mg/dl)	55.20± 1.71[a]	90.40± 2.66[b]	37.80± 1.36[c]	61.80± 2.08[d]
Uric acid(mg/dl)	0.46± 0.18[a]	1.06± 0.03[b]	0.38± 0.02[c]	0.62± 0.02[d]

Within each raw, means superscript with the same letter are not significantly difference (P≤ 0.05)

Table 2: Effects of *Zizyphus* extract on tumor markers and Nitric Oxide in serum of rats fed aflatoxin-contaminated diet (means ± SE)

Parameters Treatments	TNF- α Pg/ml	CEA ng/ml	NO µmol/L
Control	55.9± 1.7[a]	0.83± 0.05[a]	32.2± 1.3[a]
AF	94.6± 3.2[b]	2.99± 0.2[b]	66.9± 2.7[b]
Zizyphus extract	53.6± 1.2[a]	0.78± 0.04[a]	31.2± 0.7[a]
AF+ *Zizyphus* extract	67.2± 1.3[c]	1.05± 0.07[c]	39.6± 2.4[c]

Within each column, means superscript with the same letter are not significantly difference (P≤ 0.05).

Table 3: Effects of *Zizyphus* extract on the oxidant-antioxidant status of liver of rats fed aflatoxin-contaminated diet (means ± SE)

Parameters Treatments	LPO µmol/mg	GPX µg/mg	SOD µg/mg
Control	31.7± 1.8[a]	0.99± 0.02[a]	103.2± 2.6[a]
AFB_1	67.5± 1.3[b]	0.49± 0.01[b]	68.7± 1.8[b]
Zizyphus extract	31.8± 1.4[a]	0.98± 0.01[a]	115.3± 5.2[a]
Zizyphus extract + AFB_1	38.7± 1.6[c]	0.96± 0.02[a]	99.6± 4.3[a]

Within each column, means superscript with the same letter are not significantly difference (P≤ 0.05).

Table 4: Effects of different treatments on total protein contents in liver, kidney and testes tissues of rats (means ± SE)

Organ	Control	aflatoxin	*Zizyphus* extract	*Zizyphus* extract + aflatoxin
Liver	0.426± 0.008[a]	0.361± 0.005[b]	0.505± 0.023[a]	0.522± 0.006[a]
Kidney	0.429± 0.01[a]	0.286± 0.006[b]	0.488± 0.023[a]	0.368± 0.009[a]
Testis	0.478± 0.025[a]	0.396± 0.013[b]	0.520± 0.026[c]	0.560± 0.016[d]

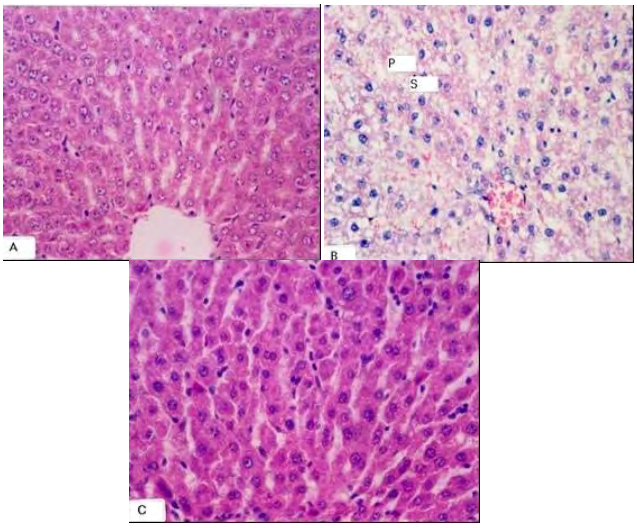

Figure 1: Sections of rat liver showing **(A)** control liver demonstrating a central vein and hepatic cords separated with blood sinusoids; (**B**) aflatoxin-treated group showing congested central vein, damaged bile ducts and massive vacuolar degeneration with abnormal nuclei (shrunken; S and pyknotic; P); (**C**) aflatoxin plus *Zizyphus* extract treated group showing more or less normal hepatocytes. (H&E X 300)

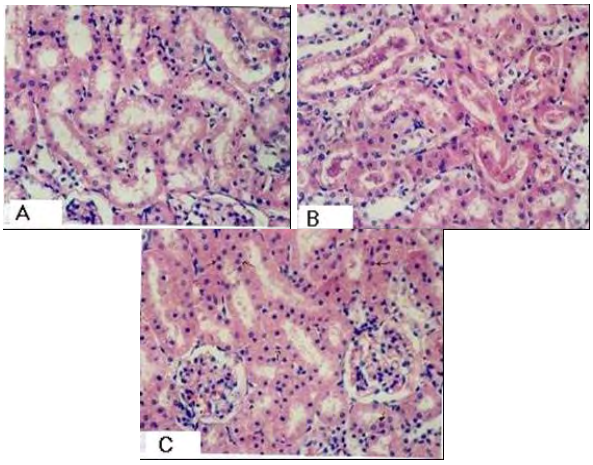

Figure 2: Section of rat kidneys showing **(A)** control kidney demonstrating normal proximal convoluted tubules and malpighian corpuscles; **(B)** aflatoxin-treated group showing hyaline casts in proximal and distal tubules lumen and cellular debris. Intertubular mononuclear cellular infiltration also was noticed; **(C)** aflatoxin plus *Zizyphus* extract treated group showing normal tubules and glomeruli. The nuclei of the epithelial cells lining tubules revealed different degrees of mitotic division (arrows). (H&E X 300)

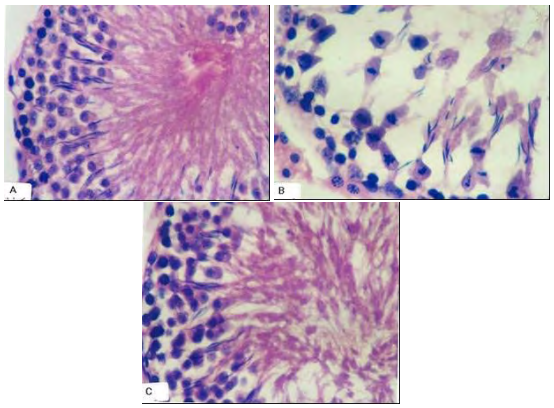

Figure 3: Sections of rat testis showing: **(A)** control testes showing spermatocytes and spermatids; **(B)** aflatoxin-treated rats showing severe damage of mitotic figures and spermatic tails; **(C)** aflatoxin plus *Zizyphus* extract treated rats showing marked recovery in most of the seminiferous tubules, spermatogenic cells and spermatids. (H&E X 3

aflatoxin treatment is known to result an impairment of Leydig cell function and a hypoandrogen status in rats (Egbunike, 1982; Agnes and Akbarsha, 2001)). In the testis of AF-treated rats, pathological changes were found in the Leydig cells (data not shown) and thus it may be inferred that hypoandrogen status is a probable mechanism of action of AF in bringing about the development of different pathological changes. However, a direct effect of AFB_1 or its metabolites on the epididymal epithelium cannot be ruled out.

Low cholesterol level in the animals treated with extracts alone or in combination with aflatoxin suggest that these extracts may have antiatherosclerotic properties and may protect against the development of coronary diseases (Morcos, 1997). It is well documented that *Zizyphus spina christi* extract is enriched in flavonoid compounds. These flavonoids compounds were found to have the ability to reduce the production of reactive oxygen species (ROS), the inhibition of protein and DNA synthesis and the apoptosis caused by aflatoxin and showed good scavenging power, in accordance with the observed inhibition of NO production (Guerra e al., 2005) and suggesting that the extract attenuated the aflatoxin-mediated decrease in the activities of GPX and SOD.

These compounds also are proton donors which act as inhibitors for the radical chain reaction (Lean and Mohamed, 1999). Abobaker et al., (1994) and Abdel-Wahhab and Aly, (2005) reported that phenolic compounds have a role in the activation and detoxification processes and hence in modulating carcinogenicity of AFB_1. According to Galvano et al., (2001), phenolic compounds at the 0.5% level caused a marked decrease in the ability of liver microsomes to metabolise AFB_1 thus preventing its activation toward epoxides and DNA adduct formation. Moreover, it was also found to induce cytosolic glutathione S-transferase activity, in turn increasing the formation of AFB_1-glutathione conjugates. Natural phenolics have the ability to reduce the enzyme activity consequent to AFB_1 treatment (Mistry et al., 1997; Abdel-Wahhab and Aly, 2005)), accordingly, the suppression of protein kinase C activity by phenolic compounds could by a way to control AF carcinogenecity. Moreover, the increasing in glutathione S-transferase activity induced by *Z. spina- christi* extract may be another way in the prevention of AF carcinogenecity through the increasing of AF-glutathione conjugates and consequently decreased the ability of hepatic microsomes to metabolise AF and preventing the formation of epoxides and DNA adduct

Lee et al. (2001) reported that flavonoids were shown to be potent inhibitors of aflatoxin B_1-8,9-epoxide formation. They found that addition of the flavonoids and flavone strongly inhibited mouse liver microsomal conversion of aflatoxin B_1 to aflatoxin B_1-8,9-epoxide, a metabolically activated mutagenic product.

Our results demonstrated that the extract of the *zizyphus* has a specific inhibitory effect on CYP1A1/2 among CYP enzymes involved in AFB_1 metabolism by rat microsomes. The protection of the extract may be due to the decreased DNA damage and hepatocarcinogenesis induced by aflatoxin B_1 by activating the phase II enzymes glutathione S-transferase (GST) and GSH peroxidase (GSH-Px). These results suggest, that piperine is capable of counteracting AFB_1 toxicity by suppressing cytochromes P-450 mediated bioactivation of the mycotoxin.

Conclusion

Aflatoxin induced oxidative stress in rats as indicating by the severe elevation of biochemical parameters, tumor markers, nitric oxide and the decrease in antioxidant enzyme activities as well as the histological changes in liver, kidney and testis. *Z. spina-christi* extract has a protective effect against aflatoxicosis and may be promising as a rich source of antioxidants.

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