EXCLI Journal 2008;7:119-131 – ISSN 1611-2156
Received: March 28, 2008, accepted: May 04, 2008, published: May 08, 2008

Original article:

EFFECT OF SOME NATURAL ANTIOXIDANTS ON AFLATOXIN B_1-INDUCED HEPATIC TOXICITY

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ABSTRACT

Aflatoxins are potent hepatotoxic and hepatocarcinogenic agents. This hepatotoxicity is thought to be mediated by their ability to generate reactive oxygen species and cause peroxidative damage. In the present investigation we assessed the ability of some natural antioxidants namely, vitamin E and Se, β-carotene, silymarin and coenzyme Q_{10} on aflatoxin B_1 (AFB_1)-induced hepatotoxicity in a rat model. Alanine and aspartate aminotransferases and alkaline phosphatase (ALP) were found to be significantly increased in the serum of AFB_1 administered (250 µg/kg body weight/day for 2 weeks) rats, suggesting hepatic damage. There was a marked increase in the lipid peroxide levels and a concomitant decrease in the hepatic reduced glutathione (GSH) and serum protein thiol (PrSHs) along with a nearly two-fold increase in hepatic glutathione-S-transferase (GST) activity. The significant increase in GST may be attributed to its being a phase II enzyme that predominately participates in the detoxification of the ultimate electrophilic metabolite AFB_1-8, 9 epoxide. On the other hand, no significant change was detected in the activities of glutathione peroxidase (GPx), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G-6-PDH), cytochrome c-reductase and levels of DNA and RNA in the hepatic tissue of AFB_1 administered rats. Results also revealed that cotreatment with studied antioxidants offered substantial hepatoprotective effects in the AFB_1 administered rats. Moreover, results revealed that vitamin E and selenium combination and β-carotene are more efficient than coenzyme Q_{10} and silymarin in modulating the liver antioxidant enzymatic system.

Keywords: Aflatoxin B_1, antioxidants, vitamin E, selenium, β-carotene coenzyme Q_{10}, silymarin, rats, liver

INTRODUCTION

The aflatoxins are difuranocoumarins that are metabolites of a certain strain of Aspergillus Flavus (Wogan, 1973). The major metabolite AFB_1 is hepatotoxic and hepatocarcinogenic in several animal species at very low doses. The aflatoxins are constant contaminants of the human food supply in many parts of the world and epidemiologically they are linked to an increased incidence of liver cancer in both Africa and Asia (Linsell and Peers, 1977; Liu et al., 1988). AFB_1 and AFM_1 were detected in 1.1 % of the food samples representing 22 different food types that were collected from several localities in the Alexandria province in Egypt. Thirty two percent of fungal isolates were aflatoxins producers (El-Gohary, 1995). The mechanism of cellular damage caused by AFB_1 has not been fully elucidated. A variety of data suggests a role for oxidative stress, including lipid peroxidation, in the pathogenesis of aflatoxicosis (Shen et al., 1994; Towner et al., 2002; Sohn et al., 2003; Abdel-Wahhab et al., 2006; Umarani et al., 2008).

AFB_1 itself is not mutagenic nor does it bind covalently to macromolecules such as deoxyribonucleic acid (DNA) in the absence of a bio-activation system (P450) (Massey et al., 1995). AFB_1 is subject to extensive metabolism in the liver and the consequence of exposure to the toxin is determined largely by the capacities of the competing activation and detoxification biotransformation pathways (McLeod et al., 1997). AFB_1 requires oxidation of the 8, 9 double bond to yield the biologically active AFB_1 8,9 epoxide which can react with DNA (Gallagher et al., 1994; Guarisco et al., 2007).

Chemoprevention of toxicoses and/or cancer using nutrients is the subject of intense study. Among the many compounds examined, antioxidants are being investigated because of their ability to reduce disease formation by either induction or inhibition of key enzyme systems (Guarisco et al., 2007). The present study was therefore conducted in order to evaluate the effect of the hepato-protective agents: β-carotene, vitamin E, selenium, silymarin and coenzyme Q_{10} on AFB_1-induced hepatic injury in rats.

MATERIALS AND METHODS

Chemicals

Aflatoxin B_1, β-carotene and vitamin E were purchased from Sigma-Aldrich Chemicals Co., St. Louis, USA. All other chemicals were of analytical grade.

Experimental protocol

Adult male Wistar rats weighing 160 ± 30 g obtained from the National Research Center Lab. (Cairo, Egypt) were allowed 4-5 days for acclimatization before the experiments. Rats were divided into 6 groups; control group, received an oral daily dose of 0.5 % xanthan gum for 2 weeks. The AFB_1 group received an oral daily dose of 250 µg AFB_1/kg body weight for 2 weeks (suspended in xanthan gum) (Kensler et al., 1987). The remaining 4 groups of rats received the same previous dose of AFB_1 for the same period simultaneously with an oral daily dose of β-carotene 200 mg/kg body weight (Muto and Moriwaki, 1984); silymarin 150 mg/Kg body weight (Favari et al., 1997) or coenzyme Q_{10} 60 mg/kg body weight (Maruyama et al., 1995). The last group received vitamin E (300 mg/kg body weight and selenium 2 mg/kg body weight of selenium that corresponds to that taken as 6 ppm in drinking water (Chen et al., 1982; Shen et al., 1994). Rats in all groups were fed by intragastric tube.

At the end of 2 weeks, all animals were fasted over night (16-18 hours) with free access to water only. The animals were sacrificed by decapitation and the blood of each animal was collected into a dry centrifuge tube. Serum was separated by centrifugation at 3000 r.p.m. for 15 min and was used to determine alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities and protein thiol levels (PrSHs).

The liver was removed, rinsed with ice-cold saline and blotted dry. A portion of liver was homogenized in redistilled water using potter elvehjem glass homogenizer and divided into five portions. Two portions of the homogenate were mixed with 3 % m.phosphoric and 2.3 % KCL acid, and were centrifuged at 3000 r.p.m for 15 min to obtain the supernatants used for assay of liver glutathione (GSH) and malondialdehyde (MDA) contents, respectively. The third portion of the homogenate was mixed with Tris-EDTA buffer, pH 7.6 and centrifuged at 105,000 xg for 30 min at 4°C to isolate the cytosolic fraction used for assay of glutathione-S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G-6-PDH) activities. The fourth portion was mixed with 0.25 M sucrose solution, centrifuged at 15,000 xg at 4°C for 20 min for preparing the fraction used for cytochrome-c reductase estimation. Finally, the fifth portion was mixed with sucrose/Tris buffer, pH 7.5, for preparing the fraction used for DNA and ribonucleic acid (RNA) estimation.

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The activities of serum ALT, AST and ALP were estimated using commercially available kits supplied by bio-Merieux, France. Serum levels of PrSHs, hepatic GSH and lipid peroxidation (MDA) were determined according to the method of Koster et al. (1986), Beutler et al. (1963) and Uchiyama and Mihara (1978), respectively. Measurement of hepatic activities of GST (Habig et al., 1974; Gawai and Pawar, 1984), GPx (Paglia and Valentine, 1967), GR (Long and Carson, 1961), G-6-PDH (Glock and McLean (1954) and cytochrome-c reductase (Edelhoch et al., 1952; Mahler et al., 1952) were carried out. Hepatic levels of RNA and DNA were estimated according to method of Blobel and Potter (1968) and Burton (1956) as modified by Giles and Myers (1965), respectively. Protein content in liver fractions was measured by the method of Lowry et al. (1951).

Statistical analysis

Data were expressed as the mean ± S.E.M. Means were compared by one-way analysis of variance (ANOVA) followed by Duncan test, which was used to identify differences between groups. A value of $P < 0.05$ was accepted as significant.

RESULTS

Data are presented in Table 1, 2 and 3.

Aflatoxin B_1

AFB_1 intake caused significant increase of serum ALT, AST and ALP and hepatic GST activities amounting to 124.6 %, 108.8 %, 129.5 % and 176.9 %, respectively as compared with control group whereas no change in hepatic GPx, GR, G-6-PDH and cytochrome-c reductase activities was observed. On the other hand, a significant decrease and increase in hepatic levels of GSH and MDA was observed which amounted to 81.5 % and 202.25 % from the control group. A significant reduction in serum level of protein thiols that reached 70.4 % in the control group was demonstrated whereas no significant change in hepatic levels of DNA and RNA was observed after AFB_1 intake.

Vitamin E and selenium combination

Vitamin E and Se normalized the elevated activities of serum AST and ALP and lowered ALT significantly. The change in ALT reached 92.1 % as compared to the AFB_1 group. Co-administration of vitamin E and Se induced significant increase in hepatic GST, GR and G-6-PDH that reached 121 %, 130 % and 119.5 %, respectively from the AFB_1 intake group whereas no significant increase was observed in GPx and cytochrome-c reductase activities as compared with the AFB_1 group. In addition, vitamin E and Se intake caused restoration of diminished hepatic GSH level and a significant decrease in the elevated hepatic MDA level that reached 175 % as compared with the AFB_1 group. On the other hand, no change in serum protein thiols, liver DNA and RNA levels was observed in the vitamin E+ Se group.

β-Carotene

β-carotene was found to normalize serum ALP and to lower the elevated ALT activity significantly. The change in ALT activity was 90.4 % whereas no change in serum AST was observed as compared to the AFB_1-group. Liver GST activity showed no significant increase as compared to AFB_1-group. In addition, a significant increase was observed in hepatic GPx, cytochrome-c reductase and GR activities that reached 184.7 %, 176.9 % and 132 %, respectively as compared to AFB_1 group. The results revealed that β-carotene intake restored levels of hepatic GSH, RNA and serum protein thiols relative to control animals whereas no significant change of MDA and DNA levels and G-6-PDH activity in liver was observed in the β-carotene group.

Silymarin

Silymarin caused normalization of ALT and ALP relative to the control group whereas a significant decrease of AST activity amounted to 86 % was observed as

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compared to AFB_1 group. Moreover, no significant change was observed concerning hepatic GST, GR, G-6-PDH and cytochrome-c reductase activities. No significant increase was detected in the activity of hepatic GPx, levels of hepatic GSH, and DNA along with serum protein thiols. In addition, normalization of the hepatic levels of MDA and RNA was detected relative to the control animals.

Coenzyme Q_{10}

Coenzyme Q_{10} resulted in normalization in the activities of serum ALT and AST relative to the control group whereas significant decrease was demonstrated in ALP that amounted to 54.8 % as compared to AFB_1 group. Significant increase was observed in hepatic GST,GPx and GR activities that reached 130.6 %, 136.9 % and 127.4 %, respectively as compared with the AFB_1 group. Moreover, normalization of hepatic level of MDA was achieved relative to the control group. On the other hand, no significant change in the diminished hepatic GSH level, serum level of protein thiols, activities of cytochrome c-reductase, G-6-PDH activity, and levels of DNA and RNA in liver was observed as compared to the AFB_1 group.

Table 1: Effect of AFB_1 alone and in combination with vitamin E and selenium, β-carotene, silymarin or coenzyme Q_{10} on serum ALT, AST and ALP activities

	ALT (unit/ml)	AST (unit/ml)	ALP (U/100 ml)
Control	52.68 ± 0.8 (33)	93.1 ± 1.3 (34)	40.7 ± 3 (25)
AFB_1	65.85 ± 1.3 [a] (10)	101.39 ± 1.4[a] (11)	52.7 ± 4.3[a] (13)
AFB_1+vitamin E+Se	60.69 ± 1.68 [ab] (11)	94.19 ± 1.6 [b] (8)	32.76 ± 2.2 [b] (7)
AFB_1+β-carotene	59.5 ± 3.2 [ab] (9)	102.6 ± 0.99[a] (8)	35.56 ± 2.98 [b] (5)
AFB_1+silymarin	55.7 ± 1.4 [b] (9)	87.5 ± 1.2 [ab] (8)	35.2 ± 1.5 [b] (8)
AFB_1+coenzymeQ_{10}	53.6 ± 1.7 [b] (9)	91.53 ± 1.6 [b] (8)	28.9 ± 3 [ab] (6)

Values represent mean ± S.E.M. Comparisons are made as follows: (a) with the control group (b) with AFB_1group. Values are statistically significant at $P< 0.05$. Number of observations in each group is given between parentheses.

Table 2: Effect of AFB_1 alone and in combination with vitamin E and selenium, β-carotene, silymarin or coenzyme Q_{10} on serum PrSHs, liver contents of GSH and MDA as well as activities of GPx and GR

	Serum PrSHs (µmol/L)	GSH (mg/g liver)	Liver MDA (nmoles/mg protein)	GPx (U/mg protein)	GR (U/mg protein)
Control	444.39 ± 20.99 (34)	2.9 ± 0.009 (24)	2.22 ± 0.18 (15)	180.19 ± 9.1 (24)	68.82 ± 3.46 (20)
AFB_1	312.88 ± 25.29 [a] (10)	2.38 ± 0.1 [a] (10)	4.49±0.4 [a] (8)	174.96 ± 10.5 (9)	69.66 ± 3.7 (12)
AFB_1+ vitamin E+Se	409.29 ± 64.59 (6)	2.9 ± 0.1 [b] (8)	0.786 ± 0.004[ab] (8)	204.38 ± 12.89 (8)	90.75 ± 6 [ab] (8)
AFB_1+ β-carotene	493.6 ± 25.3 [b] (9)	3 ± 0.1 [b] (8)	4.15 ± 0.3 [a] (7)	323.29 ± 22.8 [ab] (8)	91.96 ± 4.8 [ab] (7)
AFB_1+ silymarin	400.6 ± 39.55 (7)	2.38 ± 0.1 [a] (7)	2.44 ± 0.39 [b] (8)	204.39 ± 12.66 (8)	70.18 ± 2 (8)
AFB_1+ coenzymeQ_{10}	327 ± 41.4 [a] (8)	2.47 ± 0.1 [a] (7)	2.84 ± 0.45 [b] (10)	239.6 ± 11.7 [ab] (9)	88.74 ± 4.6 [ab] (7)

Values represent mean ± S.E.M. Comparisons are made as follows: (a) with the control group; (b) with AFB_1group. Values are statistically significant at $P < 0.05$. Number of observations in each group is given between parentheses.

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Table 3: Effect of AFB_1 alone and in combination with vitamin E and selenium, β-carotene, silymarin or coenzyme Q_{10} on hepatic GST, G-6-PDH and cytochrome c-reductase activities as well as DNA and RNA levels

	GST (U/mg protein)	G-6-PDH (U/mg protein)	Cytochrome c-reductase (U/mg protein)	DNA (mg/g liver)	RNA (mg/g liver)
Control	231.49 ± 12 (26)	60.66 ± 3.55 (16)	16.29 ± 0.9 (18)	2.16 ± 0.22 (21)	1.88 ± 0.14 (28)
AFB_1	409.68 ± 16.19 [a] (12)	61.2 ± 4.1 (13)	15.89 ± 0.9 (18)	1.77 ± 0.11 (18)	1.6 ± 0.1 (20)
AFB_1+vitamin E+Se	491.19 ± 38.5 [ab] (8)	73.17 ± 3.77 [ab] (7)	13.4 ± 0.6 (11)	1.35 ± 0.1 (11)	1.6 ± 0.15 (9)
AFB_1+β-carotene	459.1 ± 21.16 [a] (9)	60.44 ± 3.2 (8)	28.1 ± 1.8 [ab] (8)	1.77 ± 0.165 (8)	2.07 ± 0.1 [b] (9)
AFB_1+silymarin	411.48 ± 16.57 [a] (8)	60.89 ± 2.8 (7)	15.8 ± 2.21 (8)	2.36 ± 0.2 (8)	2.2 ± 0.16 [b] (8)
AFB_1+coenzymeQ_{10}	535.15 ± 33.3 [ab] (7)	71.1 ± 4.9 (7)	14.37 ± 1 (10)	1.65 ± 0.25 (9)	1.9 ± 0.19 (9)

Values represent mean ± S.E.M. Comparisons are made as follows: (a) with the control group; (b) with AFB_1group. Values are statistically significant at $P < 0.05$. Number of observations in each group is given between parentheses.

DISCUSSION

Results of the present study revealed significant increase in hepatic GST activity in the AFB_1-received group. This finding is in agreement with Kensler et al. (1992) who reported that repetitive treatment with AFB_1 alone induced hepatic GST activity and that the specific activity of GST was doubled after one week of exposure, remaining elevated throughout the AFB_1 dosing period. The increase in hepatic GST activity is due to GSTs being a family of dimeric proteins that possess a multitude of functions including the enzymatic conjugation of GSH to electrophilic xenobiotics (Primiano et al., 1995). Indeed, GST belongs to phase II enzymes that in contrast to phase I, which can participate in both metabolic activation and inactivation, predominately participate in the detoxification of xenobiotics (Wang et al., 1990). It has been known that the harmful effects of AFB_1 are a consequence of its being metabolized to AFB_1-8, 9 epoxide, the ultimate electrophilic metabolite that serves as an alkylating agent and mutagen (Hayes et al., 1991a). In many mammalian species, including rats, AFB_1-8, 9 epoxide is efficiently conjugated with reduced glutathione (Degan and Neumann, 1978), a reaction that is catalyzed by GST (Neal and Green, 1983). A significant increase in hepatic MDA, measured as an index of liver lipoperoxidation was observed in AFB_1-treated group as shown in Table 2. This finding is found to be in agreement with other previous studies (Shen et al., 1994; Ip et al., 1996; Premalatha et al., 1997; Souza et al.,1999; Meki et al., 2001; Umarani et al., 2008). The increase in hepatic MDA level may be attributed to the fact that AFB_1 is metabolized by the cellular cytochrome P450 enzyme system to form the reactive intermediate, AFB_1-8,9-epoxide, which in turn reacts with macromolecules such as lipid and DNA. This leads to lipid peroxidation and cellular injury (Stresser et al., 1994). In the present study a significant reduction in the levels of hepatic GSH and serum protein thiols were observed in the AFB_1 group. This was demonstrated previously and the depletion of liver GSH may be attributed to its conjugation with the electrophilic metabolites (Mitchell et al., 1973; Liu et al., 1999; Meki et al., 2001; Umarani et al., 2008). Similarly to another report (Wang et al., 1991), the present study found no significant change in hepatic GPx and GR activities in the AFB_1-received group. Siegers et al. (1982) demonstrated that the depletion of hepatic GSH below a

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Received: March 28, 2008, accepted: May 04, 2008, published: May 08, 2008

critical threshold-concentration allowed the enhancement of lipid peroxidation evoked by exogenous promotors, while the GPx remains unaffected. Therefore, this insignificant change in hepatic GPx may be attributed to the reduction of hepatic GSH level.

The significant increase in serum activities of ALT, AST and ALP that is observed in AFB_1-treated group (Table 1) is an indicator of the toxic liver damage induced by AFB_1. This result is in agreement with Moundipa and Domngang (1991). Similar results have also been reported in other previous studies (Pelissier et al., 1992; Liu et al., 2001; Preetha et al., 2006).

The apparent decrease in the hepatic levels of DNA and RNA observed in the AFB_1 – treated group is similar to the observation of Liu et al. (1988) and Abdel-Wahhab et al. (2006). Several processes seem to contribute to this apparent decrease. One possibility is the loss of AFB_1-DNA adducts from the liver by cell death. Indeed, after in vivo or in vitro activation by cytochrome P450, AFB_1 binds preferentially to physiologically active regions of rat liver nuclear chromatin (Yu, 1983). There is subsequent depression of nucleic acid synthesis (Neal and Ghabral, 1980) causing fixation of molecular lesions, which ultimately leads into cancer cells (Essigmann et al., 1982). Moreover, the N^7 position of guanine appears to be the only site of hepatic AFB_1-DNA adduct formation in humans as well as in experimental animals (Wogan, 1992). Attachment at this position of the purine base in DNA labializes both the imidazole ring and glycosyl bond rendering it unstable (Groopman et al., 1981).

Regarding the effect of vitamin E and selenium supplementation on AFB_1-induced liver injury, this study showed that vitamin E and selenium significantly decreased the elevated level of hepatic MDA and increased the reduced level of hepatic GSH level. Moreover, our results showed normalization of hepatic GSH level in the vitamin E and Se-treated group, and increase in the activities of both hepatic GR and G-6-PDH. Our results are consistent with that of Cassand et al. (1993) and Shen et al. (1994) who demonstrated that treatment with vitamin E and selenium, both antioxidants, significantly inhibited lipid peroxidation as well as liver cell damage. In addition, the apparent increase in hepatic GPx activity shown in the current study can be attributed to the fact that vitamin E and selenium are reinforcing each other in their actions. Vitamin E reduces selenium requirement by preventing loss of selenium from the body, which is explained by selenium as an integral part of the enzyme glutathione peroxidase (Rotruck et al., 1973). Cellular exposure to xenobiotics and antioxidants leads to coordinated induction of a battery of genes encoding detoxifying enzymes including GST (Venugopal et al., 1997). The increase in hepatic GST activity in the vitamin E and selenium group revealed in this study can therefore be responsible for the reduction of AFB_1 toxicity because GST has been shown to play a critical role in preventing the binding of AFB_1 to DNA (Hayes et al., 1991b).

Moreover, normalization of serum AST, ALP, and decrease in ALT activities were achieved relative to control and AFB_1 groups, respectively in the vitamin E + selenium-treated group. The decrease in the elevated ALT and AST activities may be attributed to the decrease in oxidative stress through the antioxidant properties of vitamin E and selenium whereas the decrease in serum ALP may be attributed to a reduction in bile duct proliferation by vitamin E (Zamora et al., 1991; Souza et al., 1999). On the other hand, treatment with vitamin E and selenium did not modulate the hepatic levels of DNA and RNA. Vitamin E, a lipid soluble inhibitor of lipid peroxidation, may not be able to prevent the binding of AFB_1 metabolites to DNA during the initiation process of cellular injury, which occurs in the hydrophilic intracellular compartment (Stresser et al., 1994).

Regarding the effect of β-carotene intake on the AFB_1-induced hepatic injury, the present study revealed normalization of hepatic GSH and serum of protein thiols levels relative to control group in the β-

carotene-treated group. In addition, there was significant increase in the hepatic GPx and GR activities as compared to the AFB_1-treated group that may be explained by the antioxidant property of β-carotene. β-carotene may play a role in trapping peroxy free radicals in tissues and by functioning as a scavenger of singlet oxygen at low oxygen partial pressures (Burton and Ingold, 1984). A further explanation of the carotenoid action is their conversion into retinoids. Indeed, since β-carotene has the highest provitamin A activity, this hypothesis is attractive and is supported by the demonstration that vitamin A and β-carotene reversed oral leukoplakias in tobacco chewers but that canthaxanthin (a carotenoid without provitamin A activity), was without effect (Stich et al., 1984). In consequence, the present study showed that β-carotene intake resulted in a decrease of ALT and normalization of ALP in serum relative to AFB_1 and control groups, respectively.

The current study showed no significant change in the elevated level of hepatic MDA in the β-carotene-treated group. This might be explained by the ability of β-carotene to inhibit transformation, and their ability to up-regulate junctional communication without inhibiting the formation of MDA (taken as an index of lipid peroxidation) (Zhang et al., 1991). The observed increase in hepatic GST and cytochrome c-reductase activities in the β-carotene-treated group is related to the conversion of β-carotene to vitamin A derivatives, and the ability of vitamin A to induce P450 enzymatic system including cytochrome c-reductase (Bhattacharya et al., 1989; Koch et al., 1990).

Normalization in the hepatic level of RNA in the β-carotene- treated group could be due to the action as a protective agent of DNA nucleophilic sites and thus could influence the AFB_1-DNA formation. It has also been reported that vitamin A readily forms epoxides, such as 5,6-epoxyretinoic acid, which can compete with mutagenic epoxides in reacting with DNA (De Flora, 1988). Moreover, retinol binding to chromatin may change the accessibility of DNA to xenobiotics and intercalation of AFB_1 epoxide on DNA (Harris et al., 1989).

This study showed that silymarin a standard hepato-protective, restored the elevated level of hepatic MDA and apparently increased hepatic GPx activity. The is due to the inhibition of lipid peroxidation caused by silymarin free radical-scavenging properties (Dehmlow et al., 1996; Rastogi et al. 2001; Farghali et al., 2000). This hepato-protective antioxidant property of silymarin was shown in the present study to consequently restore ALT and ALP and to decrease AST activities in serum relative to the control and AFB_1 groups, respectively. Silymarin was reported to improve the functional markers of liver damage (Lang et al., 1990; Preetha et al., 2006) and silymarin and silybinin have been demonstrated to act as cell membrane stabilizers (Fraschini et al., 2002).

Silymarin also produced significant increase in the hepatic level of RNA as compared to the AFB_1-treated group. The increase in the hepatic level of RNA is in agreement with reports that silymarin has a stimulating influence on proliferation, RNA synthesis and protein synthesis in liver cells (Sonnenbichler and Zetl, 1987). Silymarin increases the rate of RNA synthesis through stimulation of nucleolar polymerase 1 (Fraschini et al., 2002). Normalization of the hepatic RNA level was also observed in the silymarin group as compared to the control group.

This study showed that coenzyme Q_{10} restored the elevated hepatic MDA level and increased the activities of hepatic GPX, GR and GST as compared to the control and AFB_1-treated groups, respectively. This decrease in hepatic MDA level and the consequent increase in hepatic GPX and GR activities may be attributed to the antioxidant property of coenzyme Q_{10} (Mellors and Tappel, 1966). Both the oxidized and reduced form of coenzyme Q_{10} is found in all cellular membranes where they have been suggested to protect membrane phospholipids and proteins against oxidative damage (Ernster and Dallner, 1995). Leibovitz et al.

(1990) suggested that the increase in hepatic GPx is attributed to the presence of an inverse relationship between tissue GPx and TBARS released. Consequently, normalization in the serum activities of ALT and AST along with a significant decrease in ALP has been observed in the coenzyme Q_{10}-treated group relative to the control and AFB_1 groups, respectively. This effect may be related to the effectiveness of coenzyme Q_{10} in suppressing the increased lipid peroxidation and destruction of the hepatocyte membrane in the regenerating liver cells of rats as reported previously by Okuyama et al. (1991). Moreover, coenzyme Q_{10} exhibits protection against liver damage by lowering TBARS and alanine release leading to decrease in serum enzyme activities (Zamora et al., 1991). The liver also represents the target organ for coenzyme Q_{10} on investigating the distribution of coenzyme Q_{10} into different rat tissues as reported previously by Scalori et al. (1990).

It can be concluded that the hepatotoxicity induced by aflatoxin B_1 seemed to be modulated effectively by the simultaneous use of antioxidants: vitamin E and selenium combination, β-carotene, silymarin or coenzyme Q_{10}. Moreover, vitamin E and selenium combination and β-carotene are more efficient than coenzyme Q_{10} and silymarin in modulating the liver antioxidant enzymatic system. It is highly recommended to eat well-balanced and nutritious diets that contain sufficient amounts of natural antioxidants as a way to counteract the deleterious effects of the environmental hepatotoxins, including aflatoxin B_1. These dietary antioxidants typically boost the liver's health and can minimize any excess damage done to the liver and may even expedite liver recovery.

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