

## Original article:

# EFFECT OF SOME NATURAL ANTIOXIDANTS ON AFLATOXIN B<sub>1</sub>-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,  $\beta$ -carotene, silymarin and coenzyme Q<sub>10</sub> on aflatoxin B<sub>1</sub> (AFB<sub>1</sub>)-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<sub>1</sub> administered (250  $\mu$ g/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<sub>1</sub>-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<sub>1</sub> administered rats. Results also revealed that cotreatment with studied antioxidants offered substantial hepatoprotective effects in the AFB<sub>1</sub> administered rats. Moreover, results revealed that vitamin E and selenium combination and  $\beta$ -carotene are more efficient than coenzyme Q<sub>10</sub> and silymarin in modulating the liver antioxidant enzymatic system.

**Keywords:** Aflatoxin B<sub>1</sub>, antioxidants, vitamin E, selenium,  $\beta$ -carotene coenzyme Q<sub>10</sub>, silymarin, rats, liver

## INTRODUCTION

The aflatoxins are difuranocoumarins that are metabolites of a certain strain of *Aspergillus Flavus* (Wogan, 1973). The major metabolite AFB<sub>1</sub> 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<sub>1</sub> and AFM<sub>1</sub> were de-

tected 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> requires oxidation of the 8, 9 double bond to yield the biologically active AFB<sub>1</sub> 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:  $\beta$ -carotene, vitamin E, selenium, silymarin and coenzyme Q<sub>10</sub> on AFB<sub>1</sub>-induced hepatic injury in rats.

## MATERIALS AND METHODS

### *Chemicals*

Aflatoxin B<sub>1</sub>,  $\beta$ -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  $\pm$  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<sub>1</sub> group received an oral daily dose of 250  $\mu$ g AFB<sub>1</sub>/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<sub>1</sub> for the same period simulta-

neously with an oral daily dose of  $\beta$ -carotene 200 mg/kg body weight (Muto and Moriwaki, 1984); silymarin 150 mg/Kg body weight (Favari et al., 1997) or coenzyme Q<sub>10</sub> 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.

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 (Edelhoc 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  $\pm$  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<sub>1</sub>**

AFB<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> intake group whereas no significant increase was observed in GPx and cytochrome-c reductase activities as compared with the AFB<sub>1</sub> 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<sub>1</sub> group. On the other hand, no change in serum protein thiols, liver DNA and RNA levels was observed in the vitamin E+ Se group.

#### **$\beta$ -Carotene**

$\beta$ -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<sub>1</sub>-group. Liver GST activity showed no significant increase as compared to AFB<sub>1</sub>-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<sub>1</sub> group. The results revealed that  $\beta$ -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  $\beta$ -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

compared to AFB<sub>1</sub> 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<sub>10</sub>

Coenzyme Q<sub>10</sub> resulted in normalization in the activities of serum ALT and AST relative to the control group whereas sig-

nificant decrease was demonstrated in ALP that amounted to 54.8 % as compared to AFB<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> group.

**Table 1:** Effect of AFB<sub>1</sub> alone and in combination with vitamin E and selenium, β-carotene, silymarin or coenzyme Q<sub>10</sub> 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 <sub>1</sub>	65.85 ± 1.3 <sup>a</sup> (10)	101.39 ± 1.4 <sup>a</sup> (11)	52.7 ± 4.3 <sup>a</sup> (13)
AFB <sub>1</sub> +vitamin E+Se	60.69 ± 1.68 <sup>ab</sup> (11)	94.19 ± 1.6 <sup>b</sup> (8)	32.76 ± 2.2 <sup>b</sup> (7)
AFB <sub>1</sub> +β-carotene	59.5 ± 3.2 <sup>ab</sup> (9)	102.6 ± 0.99 <sup>a</sup> (8)	35.56 ± 2.98 <sup>b</sup> (5)
AFB <sub>1</sub> +silymarin	55.7 ± 1.4 <sup>b</sup> (9)	87.5 ± 1.2 <sup>ab</sup> (8)	35.2 ± 1.5 <sup>b</sup> (8)
AFB <sub>1</sub> +coenzymeQ <sub>10</sub>	53.6 ± 1.7 <sup>b</sup> (9)	91.53 ± 1.6 <sup>b</sup> (8)	28.9 ± 3 <sup>ab</sup> (6)

Values represent mean ± S.E.M. Comparisons are made as follows: (a) with the control group (b) with AFB<sub>1</sub> group. Values are statistically significant at  $P < 0.05$ . Number of observations in each group is given between parentheses.

**Table 2:** Effect of AFB<sub>1</sub> alone and in combination with vitamin E and selenium, β-carotene, silymarin or coenzyme Q<sub>10</sub> 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 <sub>1</sub>	312.88 ± 25.29 <sup>a</sup> (10)	2.38 ± 0.1 <sup>a</sup> (10)	4.49 ± 0.4 <sup>a</sup> (8)	174.96 ± 10.5 (9)	69.66 ± 3.7 (12)
AFB <sub>1</sub> + vitamin E+Se	409.29 ± 64.59 (6)	2.9 ± 0.1 <sup>b</sup> (8)	0.786 ± 0.004 <sup>ab</sup> (8)	204.38 ± 12.89 (8)	90.75 ± 6 <sup>ab</sup> (8)
AFB <sub>1</sub> + β-carotene	493.6 ± 25.3 <sup>b</sup> (9)	3 ± 0.1 <sup>b</sup> (8)	4.15 ± 0.3 <sup>a</sup> (7)	323.29 ± 22.8 <sup>ab</sup> (8)	91.96 ± 4.8 <sup>ab</sup> (7)
AFB <sub>1</sub> + silymarin	400.6 ± 39.55 (7)	2.38 ± 0.1 <sup>a</sup> (7)	2.44 ± 0.39 <sup>b</sup> (8)	204.39 ± 12.66 (8)	70.18 ± 2 (8)
AFB <sub>1</sub> + coenzymeQ <sub>10</sub>	327 ± 41.4 <sup>a</sup> (8)	2.47 ± 0.1 <sup>a</sup> (7)	2.84 ± 0.45 <sup>b</sup> (10)	239.6 ± 11.7 <sup>ab</sup> (9)	88.74 ± 4.6 <sup>ab</sup> (7)

Values represent mean ± S.E.M. Comparisons are made as follows: (a) with the control group; (b) with AFB<sub>1</sub> group. Values are statistically significant at  $P < 0.05$ . Number of observations in each group is given between parentheses.

**Table 3:** Effect of AFB<sub>1</sub> alone and in combination with vitamin E and selenium, β-carotene, silymarin or coenzyme Q<sub>10</sub> 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 <sub>1</sub>	409.68 ± 16.19 <sup>a</sup> (12)	61.2 ± 4.1 (13)	15.89 ± 0.9 (18)	1.77 ± 0.11 (18)	1.6 ± 0.1 (20)
AFB <sub>1</sub> +vitamin E+Se	491.19 ± 38.5 <sup>ab</sup> (8)	73.17 ± 3.77 <sup>ab</sup> (7)	13.4 ± 0.6 (11)	1.35 ± 0.1 (11)	1.6 ± 0.15 (9)
AFB <sub>1</sub> +β-carotene	459.1 ± 21.16 <sup>a</sup> (9)	60.44 ± 3.2 (8)	28.1 ± 1.8 <sup>ab</sup> (8)	1.77 ± 0.165 (8)	2.07 ± 0.1 <sup>b</sup> (9)
AFB <sub>1</sub> +silymarin	411.48 ± 16.57 <sup>a</sup> (8)	60.89 ± 2.8 (7)	15.8 ± 2.21 (8)	2.36 ± 0.2 (8)	2.2 ± 0.16 <sup>b</sup> (8)
AFB <sub>1</sub> +coenzymeQ <sub>10</sub>	535.15 ± 33.3 <sup>ab</sup> (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<sub>1</sub> group. 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<sub>1</sub>-received group. This finding is in agreement with Kensler et al. (1992) who reported that repetitive treatment with AFB<sub>1</sub> alone induced hepatic GST activity and that the specific activity of GST was doubled after one week of exposure, remaining elevated throughout the AFB<sub>1</sub> 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<sub>1</sub> are a consequence of its being metabolized to AFB<sub>1</sub>-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<sub>1</sub>-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<sub>1</sub>-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<sub>1</sub> is metabolized by the cellular cytochrome P450 enzyme system to form the reactive intermediate, AFB<sub>1</sub>-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<sub>1</sub> 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<sub>1</sub>-received group. Siegers et al. (1982) demonstrated that the depletion of hepatic GSH below a

critical threshold-concentration allowed the enhancement of lipid peroxidation evoked by exogenous promoters, 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<sub>1</sub>-treated group (Table 1) is an indicator of the toxic liver damage induced by AFB<sub>1</sub>. 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<sub>1</sub> – 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<sub>1</sub>-DNA adducts from the liver by cell death. Indeed, after *in vivo* or *in vitro* activation by cytochrome P450, AFB<sub>1</sub> 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<sup>7</sup> position of guanine appears to be the only site of hepatic AFB<sub>1</sub>-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<sub>1</sub>-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<sub>1</sub> toxicity because GST has been shown to play a critical role in preventing the binding of AFB<sub>1</sub> to DNA (Hayes et al., 1991b).

Moreover, normalization of serum AST, ALP, and decrease in ALT activities were achieved relative to control and AFB<sub>1</sub> 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<sub>1</sub> 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  $\beta$ -carotene intake on the AFB<sub>1</sub>-induced hepatic injury, the present study revealed normalization of hepatic GSH and serum of protein thiols levels relative to control group in the  $\beta$ -

carotene-treated group. In addition, there was significant increase in the hepatic GPx and GR activities as compared to the AFB<sub>1</sub>-treated group that may be explained by the antioxidant property of  $\beta$ -carotene.  $\beta$ -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  $\beta$ -carotene has the highest provitamin A activity, this hypothesis is attractive and is supported by the demonstration that vitamin A and  $\beta$ -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  $\beta$ -carotene intake resulted in a decrease of ALT and normalization of ALP in serum relative to AFB<sub>1</sub> and control groups, respectively.

The current study showed no significant change in the elevated level of hepatic MDA in the  $\beta$ -carotene-treated group. This might be explained by the ability of  $\beta$ -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  $\beta$ -carotene-treated group is related to the conversion of  $\beta$ -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  $\beta$ -carotene-treated group could be due to the action as a protective agent of DNA nucleophilic sites and thus could influence the AFB<sub>1</sub>-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 chro-

matin may change the accessibility of DNA to xenobiotics and intercalation of AFB<sub>1</sub> 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. This 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<sub>1</sub> 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<sub>1</sub>-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<sub>10</sub> restored the elevated hepatic MDA level and increased the activities of hepatic GPX, GR and GST as compared to the control and AFB<sub>1</sub>-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<sub>10</sub> (Mellors and Tappel, 1966). Both the oxidized and reduced form of coenzyme Q<sub>10</sub> 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<sub>10</sub>-treated group relative to the control and AFB<sub>1</sub> groups, respectively. This effect may be related to the effectiveness of coenzyme Q<sub>10</sub> 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<sub>10</sub> 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<sub>10</sub> on investigating the distribution of coenzyme Q<sub>10</sub> into different rat tissues as reported previously by Scalori et al. (1990).

It can be concluded that the hepatotoxicity induced by aflatoxin B<sub>1</sub> seemed to be modulated effectively by the simultaneous use of antioxidants: vitamin E and selenium combination,  $\beta$ -carotene, silymarin or coenzyme Q<sub>10</sub>. Moreover, vitamin E and selenium combination and  $\beta$ -carotene are more efficient than coenzyme Q<sub>10</sub> 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<sub>1</sub>. 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.

## REFERENCES

Abdel-Wahhab MA, Ahmed HH, Hagazi MM. Prevention of aflatoxin B<sub>1</sub>-initiated hepatotoxicity in rat by marine algae extracts. *J Appl Toxicol* 2006;26:229-38.

Beutler E, Duran O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963;61:882-8.

Bhattacharya RK, Prabhu AL, Aboobaker VS. In vivo effect of dietary factors on the molecular action of aflatoxin B<sub>1</sub>: role of vitamin A on the catalytic activity of liver fractions. *Cancer Lett* 1989;44:83-8.

Blobel G, Potter VR. Distribution of radioactivity between the acid-soluble pool and the pools of RNA in the nuclear, non-sedimentable and ribosome fractions of rat liver after a single injection of labelled orotic acid. *Biochem Biophys Acta* 1968;166:48-57.

Burton GW, Ingold KU. Beta-carotene: an unusual type of lipid antioxidant. *Science* 1984;224:569-73.

Burton K. A study of the conditions and mechanism of diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J* 1956;62:315-23.

Cassand P, Decoudu S, Leveque F, Daubeze M, Narbonne JF. Effect of vitamin E dietary intake on in vitro activation of AFB<sub>1</sub>. *Mutat Res* 1993;319:309-16.

Chen J, Goetchius MP, Campbell TC, Comb GF Jr. Effects of dietary selenium and vitamin E on hepatic mixed-function oxidase activities and in vivo covalent binding of aflatoxin B<sub>1</sub> in rats. *J Nutr* 1982;112:324-31.

De Flora S. Problems and prospects in anti-mutagenesis and anticarcinogenesis. *Mutat Res* 1988;202:279-83.

Degan GH, Neumann HG. The major metabolite of aflatoxin B<sub>1</sub> in the rat is a glutathione conjugate. *Chem Biol Interact* 1978;22:239-55.

Dehmlow C, Erhard J, De Groot H. Inhibition of kupffer cell functions as an explanation for the hepatoprotective properties of silibinin. *Hepatology* 1996;23:749-54.



- Edelhoc H, Hayaishi O, Teply LJ. The preparation and properties of a soluble diphosphopyridine nucleotide cytochrome. *J Biol Chem* 1952;197:97-104.
- El-Gohary AH. Study on aflatoxins in some food stuffs with special reference to public health hazard in Egypt. *J Anim Sci* 1995;8:571-75.
- Ernster L, Dallner G. Biochemical, physiological and medical aspects of ubiquinone function. *Biochim Biophys Acta* 1995;1271:195-204.
- Essigmann JM, Croy RG, Bennett RA, Wogan GN. Metabolic activation of aflatoxin B<sub>1</sub>: patterns of DNA adduct formation, removal, and excretion relation to carcinogenesis. *Drug Metab Rev* 1982;13:581-602.
- Farghali H, Kamenikova L, Hynie S, Kmonickova E. Silymarin effects on intracellular calcium and cytotoxicity: a study in perfused rat hepatocytes after oxidative stress injury. *Pharmacol Res* 2000;41:231-7.
- Favari L, Soto C, Mourelle M. Effect of portal vein ligation and silymarin treatment on aspirin metabolism and disposition in rats. *Biochem Drug Dispos* 1997;18:53-64.
- Fraschini F, Demartini G, Esposti D. Pharmacology of silymarin. *Clin Drug Invest* 2002;22:51-65.
- Gallagher EP, Wienkers LC, Stapleton PL, Kunze KL, Eaton DL. Role of human microsomal and human complementary DNA-expressed cytochromes P 4501A2 and P4503A4 in the bioactivation of aflatoxin B<sub>1</sub>. *Cancer Res* 1994;54:101-8.
- Gawai KR, Pawar SS. Purification and characterization of glutathione S-transferase from liver cytosol of phenobarbital-treated rabbits. *Xenobiotica* 1984;14:605-7.
- Giles KW, Myers A. An improved diphenylamine method for the estimation of deoxyribonucleic acid. *Nature* 1965;206:93-4.
- Glock GE, McLean P. Levels of enzymes of the direct oxidative pathways of carbohydrate metabolism in mammalian tissues and tumour. *Biochem J* 1954;56:171-5.
- Groopman JD, Croy RG, Wogan GN. In vitro reactions of aflatoxin B<sub>1</sub>-adducted DNA. *Proc Natl Acad Sci USA* 1981;18:5445-9.
- Guarisco JA, Hall JO, Coulombe RA Jr. Mechanisms of butylated hydroxytoluene chemoprevention of aflatoxicosis-inhibition of aflatoxin B(1) metabolism. *Toxicol Appl Pharmacol* 2008;227:339-46.
- Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase: the first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974;249:7130-9.
- Harris TM, Stone MP, Goplakrishnan S. Aflatoxin B<sub>1</sub> epoxide, the ultimate carcinogenic form of aflatoxin B<sub>1</sub>: synthesis and reaction with DNA. *J Toxicol Toxin Rev* 1989;8:111-20.
- Hayes JD, Judah DJ, McLellan LI, Neal GE. Contribution of glutathione S-transferase to the mechanisms of resistance to aflatoxin B<sub>1</sub>. *Pharmacol Ther* 1991a;50:443-72.
- Hayes JD, Judah DJ, McLellan LI, Kerr LA, Peacock SD, Neal GE. Ethoxyquin-induced resistance to aflatoxin B<sub>1</sub> in the rat is associated with the expression of a novel alpha-class glutathione S-transferase subunit Yc2, which possesses high catalytic activity for aflatoxin B<sub>1</sub>-8,9-epoxide. *Biochem J* 1991b;279:385-98.
- Ip SP, Mak DH, Li PC, Poon MKT, Ko KM. Effect of a lignan-enriched extract of *Schisandra Chinensis* on aflatoxin B<sub>1</sub> and cadmium chloride-induced hepatotoxicity in rats. *Pharmacol Toxicol* 1996;78:413-6.

- Kensler TW, Egner PA, Dolan PM, Groopman JD, Roebuch BD. Mechanism of protection against aflatoxin tumorigenicity in rats fed 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz) and related 1,2-dithiol-3-thiones and 1,2-dithiol-3-ones. *Cancer Res* 1987;47:4271-7.
- Kensler TW, Groopman JD, Eaton DL, Curphey TJ, Roebuch BD. Potent inhibition of aflatoxin-induced hepatic tumorigenesis by the non-functional enzyme inducer 1,2-dithiol-3-thione. *Carcinogenesis* 1992;13:95-100.
- Koch B, Antignac E, Garcin JC, Pascal G, Narbonne JF. In vitro effects of retinoids on spectral and catalytic properties of rat liver cytochrome P-450. In: Ingelman-Sundberg M, Gustafsson JA, Orrenius S, eds: *Drug metabolizing enzymes: genetics, regulation and toxicology*. Proc. of the VIII<sup>th</sup> International Symposium on Microsomes and Drug Oxidations, pp 111ff. Stockholm: Karolinska Institutet, 1990.
- Koster JF, Biemond P, Swaak AJ. Intracellular and extracellular sulphhydryl levels in rheumatoid arthritis. *Ann Rheum Dis* 1986;45:44-66.
- Lang I, Nekam K, Deak G, Muzes G, Gonzales-Cabello R, Gergely P, Csomos G. Immunomodulatory and hepatoprotective effects of in vivo treatment with free radical scavengers. *Ital J Gastroenterol* 1990;22:283-7.
- Leibovitz B, Hu ML, Tappel AL. Dietary supplements of vitamin E,  $\beta$ -carotene, coenzyme Q<sub>10</sub> and selenium protect tissues against lipid peroxidation in rat tissue slices. *J Nutr* 1990;120:97-104.
- Linsell CA, Peers FG. Field studies on liver cell cancer. In: Hia HH, Watson JD, Winsten JA, eds: *Origins of human cancer*, pp 549-56. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1977.
- Liu J, Yang CF, Lee BL, Shen HM, Ang SG, Ong N. Effect of *Salvia miltiorrhiza* on aflatoxin B<sub>1</sub>-induced oxidative stress in cultured rat hepatocytes. *Free Radic Res* 1999;31:559-68.
- Liu J, Yang CF, Wasser S, Shen HM, Tan CE, Ong CN. Protection of *salvia miltiorrhiza* against aflatoxin-B<sub>1</sub>-induced hepatocarcinogenesis in Fischer 344 rats dual mechanisms involved. *Life Sci* 2001;69:309-26.
- Liu YL, Roebuck BD, Yager JD, Groopman JD, Kensler TW. Protection by 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz) against the hepatotoxicity of aflatoxin B<sub>1</sub> in the rat. *Toxicol Appl Pharmacol* 1988;93:442-51.
- Long WK, Carson PE. Increased erythrocyte glutathione reductase activity in diabetes mellitus. *Biochem Biophys Res Commun* 1961;5:394-9.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin reagent. *Biol Chem* 1951;193:265-75.
- Mahler HR, Sarkar NK, Vernon LP. Studies on diphosphopyridine nucleotide-cytochrome c reductase II: purification and properties. *J Biol Chem* 1952;199:585-97.
- Maruyama H, Furukawa K, Onda M. Effect of coenzyme Q<sub>10</sub> on endotoxin-induced hepatocytes injury modulation of endotoxin-activated polymorphonuclear neutrophils. *Nippon-Ika-Daigaku-zasshi* (The Journal of Nippon Medical School) 1995;62:271-82.
- Massey TE, Stewart RK, Daniels JM, Liu L. Biochemical and molecular aspects of mammalian susceptibility to aflatoxin B<sub>1</sub> carcinogenicity. *Proc Soc Exp Biol Med* 1995;208:213-27.

McLeod R, Ellis EM, Arthur JR, Neal GE, Judah DJ, Manson MM, Hayes JD. Protection conferred by selenium deficiency against aflatoxin B<sub>1</sub> in the rat is associated with the hepatic expression of an aldo-keto reductase and glutathione S-transferase subunit that metabolize the mycotoxins. *Cancer Res* 1997;57:4257-77.

Meki AR, Abdel-Ghaffar SK, El-Gibaly I. Aflatoxin B<sub>1</sub> induces apoptosis in rat liver: protective effect of melatonin. *Neuroendocrinol Lett* 2001;22:417-26.

Mellors A, Tappel AL. The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol. *J Biol Chem* 1966;241:4353-6.

Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie B. Acetaminophen-induced hepatic necrosis. *J Pharmacol Exp Ther* 1973;187:211-7.

Moundipa PF, Domngang FM. Effect of the leafy vegetable solanum nigrum on the activities of some liver drug-metabolizing enzymes after aflatoxin B<sub>1</sub> treatment in female rats. *Br J Nutr* 1991;65:81-91.

Muto Y, Moriwaki H. Antitumor activity of vitamin A and its derivatives. *J Natl Cancer Inst* 1984;73:1389-93.

Neal GE, Ghabral JRP. Effect of partial hepatectomy on the response of rat liver to aflatoxin B<sub>1</sub>. *Cancer Res* 1980;40:4739-43.

Neal GE, Green JA. The requirement for glutathione S-transferase in the conjugation of activated aflatoxin B<sub>1</sub> during aflatoxin hepatocarcinogenesis in the rat. *Chem Biol Interact* 1983;45:259-75.

Okuyama Y, Shinzawa H, Ukai K, Ono K, Togashi H, Wakabayashi H, Yamada N, Nakamura T, Takahashi T, Ishikawa M. Changes of Cu, Zn-SOD and LPO in the regenerating liver after partial hepatectomy-effect of coenzyme Q<sub>10</sub> administration. *Nippon-Shokakibyō-Gakkai-Zasshi (Journal of Japanese Society of Gastroenterology)* 1991;88:1208-15.

Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967;70:158-69.

Pelissier MA, Frayssinet C, Boisset M, Albrecht R. Effect of phenoclor DP6 on enzyme-altered foci and lipid peroxidation in livers of aflatoxin B<sub>1</sub>-initiated rats. *Food Chem Toxicol* 1992;30:133-7.

Preetha SP, Kanniappan M, Selvakumar E, Nagaraj M, Varalakshmi P. Lupeol ameliorates aflatoxin B<sub>1</sub>-induced peroxidative hepatic damage in rats. *Comp Biochem Physiol C Toxicol Pharmacol* 2006;143:333-9.

Premalatha B, Muthulakshmi V, Vijayalakshmi T, Sachdanandam P, Premalatha B. Semecarpus anacardium nut extract induced changes in enzymic antioxidants studied in aflatoxin B<sub>1</sub> caused hepatocellular carcinoma bearing wistar rats. *Pharm Biol* 1997;35:161-6.

Primiano T, Egner PA, Sulter TR, Kelloff GJ, Roebuck BD, Kensler TW. Intermittent dosing with oltipraz: relationship between chemoprevention of aflatoxin-induced tumorigenesis and induction of glutathione S-transferases. *Cancer Res* 1995;55:4319-24.

Rastogi R, Srivastava AK, Rastogi AK. Long term effect of AFB<sub>1</sub> on lipid peroxidation in rat liver and kidney. *Phytother Res* 2001;15:307-10.

- Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. *Science* 1973;179:588-90.
- Scalori V, Alessandri MG, Giovannini L, Bertelli A. Plasma and tissue concentrations of coenzyme Q<sub>10</sub> in the rat after intravenous, oral, and topical administration. *Int J Tissue React* 1990;12:149-54.
- Shen HM, Shi CY, Lee HP, Ong CN. Aflatoxin B<sub>1</sub>-induced lipid peroxidation in rat liver. *Toxicol Appl Pharmacol* 1994;127:145-50.
- Siegers CP, Hubscher W, Younes M. Glutathione S-transferase and GSH-peroxidase activities during the state of GSH-depletion leading to lipid peroxidation in rat liver. *Res Commun Chem Pathol Pharmacol* 1982;37:163-9.
- Sohn DH, Kim YC, Oh SH, Park EJ, Li X, Lee BH. Hepatoprotective and free radical scavenging effects of *Nelumbo nucifera*. *Phytomedicine* 2003;10:165-9.
- Sonnenbichler J, Zetl I. Stimulating influence of a flavonolignan derivative on proliferation, RNA synthesis and protein synthesis in liver cells. In: Okolicsanyi L, Csomos G, Crepaldi G, eds: *Assessment and management of hepatobiliary disease*, pp. 265-72. Berlin: Springer-Verlag, 1987.
- Souza MF, Tome AR, Rao VS. Inhibition by the bioflavonoid ternatin of AFB<sub>1</sub>-induced lipid peroxidation in rat liver. *J Pharm Pharmacol* 1999;51:125-9.
- Stich HF, Stich W, Rosen MP, Vallejera MO. Use of the micronucleus test to monitor the effect of vitamin A,  $\beta$ -carotene and canthaxanthin on the buccal mucosa of betel nut/tobacco chewers. *Int J Cancer* 1984;34:745-50.
- Stresser DM, Bailey GS, Williams DE. Indole-3-carbinol and  $\beta$ -naphthoflavone induction of aflatoxin B<sub>1</sub> metabolism and cytochrome P450 associated with bioactivation and detoxication of aflatoxin B<sub>1</sub> in the rat. *Drug Metab Dispos* 1994;22:383-91.
- Towner R, Mason R, Reinke L. In vivo detection of aflatoxin-induced lipid free radicals in rat bile. *Biochim Biophys Acta* 2002;1573:55-62.
- Uchiyama M, Mihara M. Determination of malondialdehyde precursor in tissue by thiobarbituric acid method. *Anal Biochem* 1978;86:271-8.
- Umarani M, Shanthi P, Sachdanandam P. Protective effect of Kalpaamruthaa in combating the oxidative stress posed by aflatoxin B(1)-induced hepatocellular carcinoma with special reference to flavonoid structure-activity relationship. *Liver Int* 2008;28:200-13.
- Venugopal R, Joseph P, Jaiswal AK. Gene expression of DT-diaphorase in cancer cells. In: Forman HJ, Cadenas E, eds: *Oxidative stress and signal induction*, pp. 441ff. New York: Chapman & Hall, 1997.
- Wang CJ, Wang SW, Shiah HS, Lin JK. Effect of ethanol on hepatotoxicity and hepatic DNA-binding of aflatoxin B<sub>1</sub> in rats. *Biochem Pharmacol* 1990;40:715-21.
- Wang CJ, Wang SW, Lin JK. Suppressive effect of geniposide on the hepatotoxicity and hepatic DNA binding of aflatoxin B<sub>1</sub>. *Cancer Lett* 1991;60:95-102.
- Wogan GN. Aflatoxins as risk factors for hepatocellular carcinoma in humans. *Cancer Res* 1992;52:2114s-18s.
- Wogan GN. Aflatoxin carcinogenesis. In: Bush H, ed: *Methods in cancer research*, Vol. 7, pp. 309-44. New York: Academic Press, 1973.

Yu FL. Preferential binding of aflatoxin B<sub>1</sub> to the transcriptionally active regions of rat liver nuclear chromatin in vivo and in vitro. *Carcinogenesis* 1983;4:889-93.

Zamora R, Hidalgo FJ, Tappel AL. Comparative antioxidant effectiveness of dietary  $\beta$ -carotene, vitamin E, selenium and coenzyme Q<sub>10</sub> in rat erythrocytes and plasma. *J Nutr* 1991;121:50-6.

Zhang LX, Conney RV, Bertram JS. Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: relationship to their cancer chemopreventive action. *Carcinogenesis* 1991;12:2109-14.