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β-carotene Protects the Physiological Antioxidants Against Aflatoxin-B_1 Induced Carcinogenesis in Albino Rats

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Abstract: To study the effects of β-carotene on the body growth and physiological antioxidants, male weanling rats were fed with low and high amount of β-carotene before four weeks and after six months of Aflatoxin-B_1 (AFB_1) treatment (0.5 mg kg^{-1} body wt., on alternate days, total 10 doses, i.p). The results were compared with animals treated with AFB_1. The final body weight of AFB_1 treated animals was significantly reduced in the normal group (NVE). Plasma vitamin E was reduced significantly in NVE group whereas vitamin C levels decreased significantly in NVE and low β-carotene (LBE) fed group. The maximum reduction was found in NVE group. Plasma GSH levels were increased significantly in animals in high β-carotene (HBC) fed group. Liver protein showed significant reduction in NVE group. Liver lipid peroxidation was increased significantly in NVE and LBE groups. Liver vitamin A showed dose dependent increased levels in animals fed with high amount of β-carotene. Vitamin E was decreased significantly in NVE group. Liver antioxidative enzymes glutathione peroxidase, catalase and glutathione-S-transferase levels were reduced significantly in the treated animals of the NVE group. Results obtained indicated that β-carotene supplementation elevated the levels of vitamin C, glutathione and glutathione related enzymes which act as a free radical scavenger and reduced the toxicity effect of AFB_1 in rats.

Key words: Aflatoxin-B_1 (AFB_1), β-carotene, antioxidants, lipid peroxidation

INTRODUCTION

Aflatoxin B_1 (AFB_1) has the highest potency as a toxin and is classified as group I carcinogen by International Agency for Research on Cancer (Anonymous, 1993). Aflatoxin contamination in animal feed and food stuffs has been reported world wide (Abdelhamid, 1990). High risk agricultural commodities for aflatoxin contamination include cereals, oilseeds, groundnut, corn, wheat, rice, etc. which not only form a major component of the animal feeds but also of human diet (Anonymous, 1988). Epidemiological studies have established that contamination of foods with AFB_1, a mycotoxin, is one to the important risk factors responsible for human liver cancer (Wogan, 1992). Among laboratory animals, rat is the most susceptible to AFB_1 hepatocarcinogenesis (Newberne and Butler, 1969). AFB_1 requires metabolic activation to express its carcinogenicity. Cytochromes P450 (CYP) are primarily responsible for activation of AFB_1 to the ultimate carcinogen AFB_1-8,9-epoxiede (AFBO) (Eaton and Gallagher, 1994; Groopman *et al*., 1988). AFBO may be conjugated enzymatically with glutathione by glutathione S-transferase (GST), which is a critical pathway for AFB_1 detoxification (Buetler *et al*., 1996). However, relatively little is known about the mechanisms of its hepatotoxicity. It was discovered that AFB_1 was able to induce chromosomal damage through the release of free oxygen radicals in human lymphocytes (Manson *et al.,* 1997). In another study, it was reported that *in vitro* enzymatic and non-enzymatic production of free radicals from AFB_1 (Hayes *et al.,* 1991; Amsted *et al*., 1984). These findings suggest that oxidative damage might contribute to the cytotoxic effects of AFB_1. One of manifestations of AFB_1-induced toxicity is oxidative stress (Kodama *et al.,*1990). Aflatoxins cause oxidative stress by increasing lipid peroxidation and decreasing enzymatic and non-enzymatic antioxidants in aflatoxin-treated animals (Shen *et al*., 1995; Souza *et al*.,1999). 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 when the antioxidant capacity of the cell decreases (Abdel-Wahhab and Aly, 2003).

The damaging effects of oxidative stress are believed to be nullified in part by a variety of dietary antioxidants when they are administrated prior to or concomitantly

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with the radical inducing xenobiotics. β-carotene is a lipid soluble dietary pigment and act as an physiological antioxidant. β-carotene is a potent quencher of singlet oxygen. *In vitro* studies using radical generating systems have documented the capacity of β-carotene to quench free radicals by mechanisms that include addition of the radical to the carotenoids, hydrogen abstraction, and/or electron transfer (Sies, 1991; Niki *et al*., 1995). β-carotene can regenerate the antioxidant form of vitamin E but not vitamin C (Zhang and Omaye, 2000). In lipoproteins, β-carotene protects vitamin E from oxidation and is oxidized before vitamin E Of great importance are the data that show the synergistic action of β-carotene, vitamin E and vitamin C in the protection of lipids in membranes (Zhang and Omaye, 2000; Tsuchihashi *et al*., 1995). The antioxidant activity of β-carotene is higher at low oxygen pressure found within the human body (Zhang and Omaye, 2000). The cooperative free radicals scavenging of β-carotene and vitamin E reduce the potential for oxidation products to be formed as a result of β-carotene's scavenging. A large body of observational epidemiologic studies has consistently demonstrated that individuals eating more fruits and vegetables (which are rich in carotenoids) and people having higher serum β-carotene levels have a lower risk of cancer, particularly lung cancer (Mayne, 1996). Moreover, a number of animal and laboratory studies have shown that β-carotene can block certain carcinogenic processes and inhibit tumor cell growth (Deflora *et al.,* 1999; Burton and Ingold, 1984).

With a view to studying the central role of the antioxidant defense mechanism in hepatocarcinogenesis induced by AFB_1 and during β-carotene supplementation, the following experiment has been performed and the results are presented in this communication.

MATERIALS AND METHODS

Chemicals: Aflatoxin-B_1, β-carotene, glutathione reductase (GR), pyrogallol, thiobarbituric acid (TBA) and {3-(Tris-9-hydroxymetyl) methyl) amino) propane sulfonic acid} (TAPS) were purchased from sigma chemical company, U.S.A. Dimethylsulfoxide(DMSO) was purchased from Fluka chemical Co., England. All other chemicals (analytical grade) were purchased from Indian company.

Animals: Thirty young male albino rats in the weight range of 54-67 g of *Charles foster* strain were used and they matched for their weight at the start of the experiment. The animals were divided in to 6 treatment groups consisting five animals in each and they were housed individually in galvanized cages in the departmental animal house. The animals of group-I and II were fed with low amount of β-carotene (120 μg 100 g^{-1} diet). The animals of group-III and IV were fed with normal diet containing normal amount of retinol (control animals). In group-V and VI animals were fed with high amount of β-carotene (1200 μg of β-carotene 100 g^{-1} diet). The basal diet contains Corn Starch-69.5 g, Casein-12.5 g, Cellulose-5.0 g, groundnut oil-10.0 g, salt mixture-2.0 g (Hawk and Osar, 1964) and vitamin mixture-1.0 g (Osar and Osar, 1956) per 100 g of diet. All fat soluble vitamins and β-carotene were added to groundnut oil. The animals were provided *ad libitum* food and drinking water throughout the period of the experiment. All animals were fed above mentioned diets for four weeks. From 29th day, level of β-carotene was reduced to 300 μg 100g^{-1} of diet in group V and VI. The details of control and experimental animals are given in Table 1.

Table 1: β-carotene supplementation and carcinogen treatment given to different groups

Group No	Treatment	β-carotene/ Vitamin A added to groundnut oil (μg or IU /100 g diet)	Carcinogen treatment
I	Low β-carotene treated group(LBC).	120	Not given
II	Low β-carotene treated group toxicated with AFB_1 (LBE).	120	AFB_1
III	Normal vitamin A treated group (NVC).	200 I.U	Not given
IV	Normal vitamin A treated group toxicated with AFB_1 (NVE).	200 I.U	AFB_1
V	High β-carotene treated group (HBC).	1200*	Not given
VI	High β-carotene treated group toxicated with AFB_1 (HBE).	1200*	AFB_1

*- This level of β-carotene was reduced to 300 μg 100 g diet after four week of feeding.

Carcinogen Treatment: From 29th day carcinogen (AFB_1) was started. The experimental animals (Group-II, IV and VI) were injected i.p with AFB_1 (0.5 mg kg^{-1} body weight) 10 doses on alternate days while the control animals (Group-I, III and V) were injected the same amount of dimethylsulfoxide that served as the placebo. AFB_1 was dissolved in dimethylsulfoxide. The animals were fed with their respective diets for another six months.

Autopsy procedure and tissue processing: At the end of experimental period (after six month of the last dose of carcinogen) the rats (fasted for 16h.) were weighed and sacrificed under mild anesthesia. Blood was collected from the heart directly, and centrifuged under refrigeration to obtain plasma. Liver tissue was excised and blotted free of blood and tissue fluids on a filter paper, cleared of

extraneous tissues. A known amount of liver tissue was homogenized with phosphate buffer (pH 7.0) and volume was made up to 10% tissue homogenate. This homogenate was used for the analysis of liver protein, vitamin C, glutathione, vitamin A and vitamin E (stored at -20°C until analysis). Small portion of the tissue homogenate was centrifuged at 8000 rpm for 20 min under refrigeration. The supernatant obtained was used for the analysis of antioxidative enzymes.

Bio-Chemical assays: Standard methods cited in literature were used for the estimation of plasma and liver vitamin E (Desai, 1984), plasma and liver vitamin A (Neeld and Pearson, 1963), plasma and liver vitamin C (Roe and Kuether, 1955), plasma glutathione (Griffith, 1980), liver protein (Lowry *et al.*, 1951), liver glutathione (Ellman, 1959) and liver lipid peroxidation (Ohkawa *et al.*,1979). The liver superoxide dismutase (SOD) was measured by monitoring the inhibition of autoxidation of pyrogallol (Marklund and Marklund, 1974). One unit of SOD was designated as the amount giving half inhibition of change in absorption at 320 nm. Liver glutathione peroxidase (GSHPx) was estimated by continuous monitoring of GSSG formation (Wenck *et al.*, 1972). The one unit of enzyme, activity was calculated using following formula. U = 0.868 x A x Vi/Vs, where Vi- volume of incubation mixture and Vs- Volume of sample. Liver catalase was assayed by the method of Aebi (1983). One unit of enzyme decomposes 1.0 μM of H_2O_2 per min at pH 7.0 at 25°C. Liver glutathione S- transferase (GST) was measured by the method given by Habig *et al.* (1974). A unit of enzyme activity is defined as the amount of enzyme that catalyzed the formation of 1.0 μM of 2,4- dinitrophenyl glutathione per minute at 30°C using 1 mM GSH concentration and CDNB. Liver glutathione reduetase (GR) was determined by the method given by Racker (1955). A unit of GR activity is defined as the amount of enzyme that catalyzed the reduction of 1 μM of NADPH per minute.

Statistical analysis: Results are expressed as means ± SEM. Student "t" test and regression analysis were calculated using "Microsta" software and the levels of significance was accepted with $p<0.05$. Comparison was carried out between AFB_1 treated animals with their respective control. The control animals of Group-I and Group-V were compared with normal retinol supplemented animals (Group-III).

RESULTS

The final body weights of control and experimental groups are shown in Fig 1. Plasma antioxidant vitamins

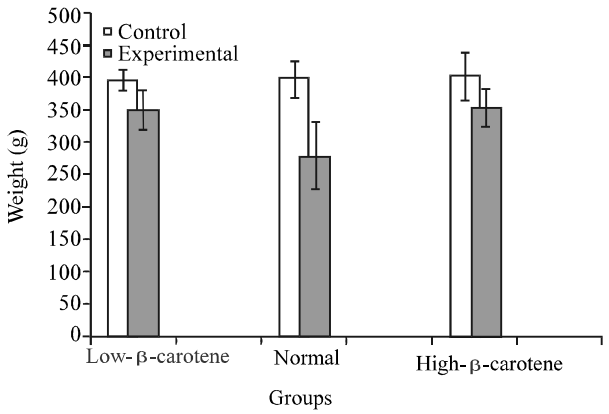

Fig. 1: Final body weight of control and experimental animal fed different levels of β-carotene levels

(vitamin E, vitamin A, vitamin C and glutathione (GSH) are presented in Table 2. Plasma antioxidant vitamins showed decreasing trend in all the AFB_1 treated groups and vitamin E was reduced significantly in NVE whereas vitamin C was found significantly lower in the LBE and NVE. Plasma GSH was reduced non-significantly in AFB_1 treated groups.

The results of liver protein, lipid peroxidation and antioxidants (vitamin E, vitamin A, vitamin C and GSH) are summarized in Table 3. Liver protein was reduced significantly in NVE group. Liver lipid peroxidation has shown significant increase in the NVE and LBE groups and the highest increase was found in NVE group (37.81%) compared to LBE (20.12% and HBC (4.79%). All the antioxidant levels were found to be decreased in AFB_1 induced cancer bearing animals. Antioxidative enzymes (SOD, GSHPx, catalase, GR and GST) levels are depicted in Table 4. Among control groups, liver catalase levels were increased significantly in LBC and HBC whereas liver GSHPx was increased significantly only in LBC compared to NVC group. Liver catalase was reduced significantly in all the three groups, liver GST was reduced in LBE and NVE groups whereas GSHPx was decreased significantly only in NVE group and non-significantly in LBE.

DISCUSSION

The liver is the target organ for AFB_1. Ingestion of this mycotoxin, is known to be capable of inducing acute poisoning, aflatoxicosis, and is believed to be implicated in development of primary liver cancer (Neal, 1995). In the present study, Aflatoxin-B_1 intoxication causes a significant reduction in body weight in NVE group and was reduced non-significantly in LBE and HBE groups.

Table 2: Plasma Vitamin E, Vitamin A, Vitamin C, and GSH of rats fed different levels of β-carotene and treated with and without AFB_1

	Low-β-carotene		Normal		High-β-carotene	
Parameter	LBC	LBE	NVC	NVE	HBC	HBE
Vitamin E (mg %)	1.28±0.10	1.07±0.09	1.32±0.10	1.06*±0.09	1.15±0.04	1.13±0.04
Vitamin A (μg %)	20.1±2.0	14.0±1.1	29.4±4.4	21.5±5.6	40.7±3.7	35.8±3.2
Vitamin C (mg %)	1.89ª±0.06	1.47*±0.13	1.63±0.06	1.10*±0.23	2.04ª±0.09	1.73±0.08
GSH (mg %)	3.10±0.16	2.97±0.21	2.71±0.16	2.92±0.15	3.42ª±0.19	3.24±0.18

Values are mean ± SEM (N=5).
*- indicates the significant difference at $p<0.05$ compared to their respective control.
ª- indicates the significant difference at $p<0.05$ compared to NRC.

Table 3: Liver protein, GSH, lipid peroxidation, vitamin E, Vitamin A and Vitamin C of rats fed different levels of β-carotene and treated with and without AFB_1

	Low-β-carotene		Normal		High-β-carotene	
Parameter	LBC	LBE	NVC	NVE	HBC	HBE
Protein(g%)	12.8±0.5	12.5±0.6	14.0±0.6	10.9± 0.6	12.9±0.8	11.0±0.5
GSH(mg/g tissue)	2.03±0.22	1.31±0.19	1.93±0.20	1.64±0.33	1.91±0.4	1.91±0.22
Lipid Peroxidation (μg/g protein)	15.9±0.9	19.1±2.8	13.6±1.4	18.8*±2.9	14.6±1.5	15.3±0.4
Vitamin E (μg/g tissue)	31.0±1.7	23.4±4.1	33.3±2.0	24.6*±5.2	30.1±2.5	27.6±2.6
Vitamin A (μg/g tissue)	0.4±1.1	6.8±1.3	10.4±1.5	7.6±1.1	61.4ª±5.1	53.1*±5.4
Vitamin C (mg %)	32.1±0.7	26.4±0.9	31.3±2.3	23.8±5.2	37.8±2.8	30.2±5.1

Values are mean ± SEM (N=5).
*- indicates the significant difference at $P<0.05$ compared to their respective control.
ª- indicates the significant difference at $P<0.05$ compared to NRC.

Table 4. Liver SOD, GSHPx, catalase, GR and GST of rats fed different levels of β-carotene and treated with and without AFB_1 .

	Low-β-carotene		Normal		High -β-carotene	
Parameter	LBC	LBE	NVC	NVE	HBC	HBE
SOD(U/mg protein)	60.3±4.6	53.3±3.1	55.6±2.2	47.2±3.1	59.1±4.7	63.3±4.6
GSHPx (U/mg protein)	18.1±0.5	14.5ª±0.6	15.5±0.9	11.8*±0.6	18.3±1.3	16.0±0.3
Catalase (U/mg protein)	390.1±34.1	318.5*±35.9	396.4±29.1	255.1*±32.3	495.1ª±32.3	403.5*±29.0
GR (U/mg protein)	0.21±0.01	0.24±0.02	0.23± 0.01	0.25±0.03	0.25±0.02	0.24±0.04
GST(U/mg protein)	921.26±116.1	705.5*±58.3	820.9±48.9	565.5*± 64.6	910.1±51.9	885.1±92.6

Values are mean ± SEM (N=5).
*- indicates the significant difference at $P<0.05$ compared to their respective control.
ª- indicates the significant difference at $P<0.05$ compared to NRC.

The reduction in body weight in AFB_1 treated animals may be due to the impaired liver functions due to development precancerous lesions in liver. The histopathological studies showed the precancerous lesions in experimental animals (results are not presented here). Lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids (PUFA). Its occurrence in biological membranes causes impaired membrane function, impaired structural integrity (Gutteridge and Halliwell, 1988), decrease in fluidity, and inactivation of a number of membrane bound enzymes and protein receptors. Lipid peroxidation is an autocatalytic free-radical process and could be responsible for DNA damage (Shirali *et al.*, 1994). A significant increase in lipid peroxidation was observed in AFB_1 treated animals fed with normal and low-β-carotene diet. Similar results were reported by Choudhary and Verma (2005) and Meki *et al.* (2004). The increased lipid peroxidation in the present study could be attributed to the reduction in detoxifying hyperperoxides in AFB_1 induced hepatocellular carcinoma (HCC). The lower increase in lipid peroxidation values in β-carotene supplemented groups confirmed the antioxidant function of β-carotene against ROS produced in response to AFB_1 AFB_1 induced (HCC). In the present study, lipid peroxidation has been negatively and significantly correlated with liver SOD ($p<0.04$), GSHPx ($p<0.02$) and catalase ($p<0.0006$) which suggest that the enzymatic antioxidant levels are very much involved in scavenging free radicals produced in cancerous tissue.

To protect themselves against free radicals, cells have developed antioxidant defenses and repair systems which prevent the accumulation of oxidatively damaged molecules. The antioxidant defense systems include enzymes like superoxide dismutase (SOD), catalase, glutathione peroxidase (GSHPx), glutathione reductase (GR) and glutathione-S-transferase, as well as small molecules such as vitamin E, β-carotene, ascorbic acid, reduced glutathione and uric acid (Chow, 1989; Cadenas, 1989). Vitamin E is among the most potent antioxidants from natural sources. Several experimental and epidemiological studies suggest that vitamin E may

reduce the risk of cancer (Bertram *et al.,* 1987). It is the major lipid soluble peroxyl radical scavenger, which can limit lipid peroxidation by terminating chain reactions initiated in the membrane lipids (Wiseman, 1996). Plasma vitamin E levels were seen to be decreased in AFB_1 treated groups and were reduced significantly in NVE group, but the decrease was found minimum in HBE group. Here author is unable to explain the exact mechanism by which the plasma vitamin E is suppressed but it could be due to (I) excessive conversion of vitamin E to it's quinone form when it reacts with free radicals produced in response of AFB_1 and/or (ii) reduction in lipoprotein and total lipid levels in plasma may be a result of AFB_1 induced liver damage and as lipoproteins are vitamin E carriers, plasma vitamin E levels may be reduced. These results are in agreement with the results of Harvey *et al.* (1994) in AFB_1 induced toxicity in swine and Mitsuo (1994) who reported decreases plasma vitamin E levels in liver cirrhosis. Decreased vitamin E in plasma in AFB_1 induced hepatocaricnoma in rats was also reported (Premalatha *et al*., 1999). Plasma vitamin A showed positive relation with dietary-β-carotene in control and experimental animals. Plasma vitamin A in AFB_1 treated groups showed decreasing trend compared to their respective controls. Reduced plasma vitamin A level was attributed with reduced plasma protein in the study. A significant correlation between serum vitamin A and serum protein was reported by Frankul (1989) and suggested that albumin might be a carrier of vitamin A in the blood. Unfortunately plasma albumin was not estimated in this study but total protein values were reduced in AFB_1 intoxicated animals in this experiment. These results support the reduced vitamin A in the plasma. A low serum retinol level has been associated with the development of various human cancers including HCC (Yu *et al.,* 1995). Plasma vitamin C was significantly increased in control animals fed with low and high amount of β-carotene compared to the normal control (NVC). The exact mechanism for the same is not known but it may be possibly be due to the presence of β-carotene in the body which is very good antioxidant and also scavenges free radicals. This may preserve physiological vitamin C and therefore increased plasma vitamin C levels were observed in β-carotene supplemented animals. Plasma vitamin C was also found to be low in AFB_1 intoxicated animals and NVE and LBE groups showed significant reduction. This may be due to the utilization of vitamin C for scavenging the ROS produced by the cancerous cells and AFB_1 metabolism. Vitamin C can protect biomembranes against lipid peroxidation damage by eliminating peroxyl radicals in the aqueous phase before the later can initiate peroxidation (Frei *et al.,* 1989). Vitamin C is effective against superoxide, hydroxyl radical, hydrogen peroxide, peroxyl radical and singlet oxygen (Sies *et al.,* 1992). Vitamin C might have played a role in reducing the tocopheroxyl radical, thereby restoring the radical scavenging activity of vitamin E (Niki, 1987). The reduced vitamin C in AFB_1 carcinoma in rats was reported (Premalatha *et al*., 1999). Glutathione plays a critical role in important cellular functions, which includes the maintenance of thiol status of proteins, the destruction of H_2O_2, lipid peroxides, free radicals, translocation of amino acids across cell membrane, the detoxification of foreign compounds and biotransformation of drugs (James and Harbison, 1982). The decreased level of GSH in AFB_1 treated animals may be due to it's utilization by excessive amount of free radical by cancerous cells.

The results of liver antioxidant compounds showed similar trend as plasma antioxidant compounds. Reduction in liver vitamin E in AFB_1 treated groups was supported by the result of (Kanematsy *et al.*,1989) who reported lower hepatic vitamin E in tissue with tumor. Decreasing trend of liver vitamin A may be due to leakage of retinyl esters and a reduction in liver storage of vitamin A or it may be due to the disturbance of parenchymal and fat storing cells of the liver as reported by (Mobarhan *et al.,* 1986). Low level of vitamin A in experimental groups may also be attributed to the presence of cancerous cells in the tissue which contains less number of vitamin A storing cells (Takashi et *al.* , 1989). A reduced level of retinol binding protein (RBP) reported in human HCC compared to the adjacent tissue (Muto and Omori, 1981). Liver vitamin C reduction may be due to the utilization of vitamin C in the scavenging of ROS in the body or it's synthesis in body may be affected by the damaged liver in response of AFB_1 treatment. All enzymes were found almost equal in control animals except liver GSHPx which was higher in β-carotene supplemented groups, suggested that β-carotene is a good determinant for liver GSHPx .The results of these enzymes showed decreasing trend in almost all AFB_1 treated groups with few exception. Liver GSHPx was significantly reduced in LBE and NVE groups whereas liver catalase and GST were significantly decreased only in NVE group. The SOD levels were increased non-significantly in LBE and HBE groups. The reduction in liver SOD is possibly due to a continuous higher production of superoxide radical by the mitochondria of the damaged liver cells and loss of Mn-SOD from mitochondria of the cancerous cells. A diminished amount of Mn SOD in tumor cells was reported (Oberley and Buettner, 1979). A reduced level of liver SOD in AFB_1 induced toxicity in rats after 10 days of toxin treatment was reported (Pelissier *et al.,* 1992).

Liver GSHPx and catalase levels were decreasing in AFB_1 intoxicated animals. These may due to continuous overload of ROS particularly H_2O_2 produced by the catalytic reaction of superoxide by SOD. The GSHPx performs its activity in cytosolic fraction whereas catalase is performing its activity in microbodies. Both the enzymes are involved in the detoxification of H_2O_2, which is formed from superoxide radicals. Higher superoxide formation is may be due to the presence of cancerous cells in liver. These results are in agreement with the results of Meki *et al.* (2004) who reported significant reduction in GSHPx and GR activities in liver after 10 days of AFB_1 treatment.

Liver GR is an enzyme responsible for the conversion of glutathione disulphide (GSSG) back to reduced GSH. GSSG is formed during the detoxification of H_2O_2 by GSHPx. GSH is a component of three detoxifying enzymes namely GSHPx, GR and GST. It is also taking part in the detoxification of carcinogen or it's metabolites by conjugation. Liver GR was reduced in LBE and NVE groups confirmed the higher production of GSSG due to the higher rate of detoxification of H_2O_2 by GSHPx. Similar results were reported in hepatotoxicity in rats (Cascales *et al.,* 1991). Liver GST eliminates the reactive epoxide of AFB_1 by conjugating with GSH. The values showed significant reduction in normal group and non-significant change in β-carotene supplemented groups indicate that β-carotene plays important role in maintenance of GST activities in liver in control and experimental animals.

From these results it is concluded that AFB_1 had induced liver carcinogenicity at a given dose and time. The results also confirmed the oxygen toxicity in AFB_1 induced HCC that had imbalanced the physiological antioxidant status. From the obtained results it would appear that the supplementation of β-carotene protects the physiological antioxidants and prevents the ROS damage to the liver and also reduces the liver carcinogenesis, this confirms the antioxidant activity of β-carotene *in vivo* and proved to be dietary chemopreventive agent. Therefore the presence of β-carotene in the diet could be important in prevention of liver damage induced by ROS and thereby helps in reducing the risk of Aflatoxin -B_1 induced carcinogenicity.

REFERENCES

Abdelhamid, A.M., 1990. Occurrence of some mycotoxins (aflatoxins, ochratoxin A, citrunin, zearalenone and vomitoxin) in various egyptian feeds. Arch. Tieremahr., 40: 647-664.

Abdel-Wahhab, M.A. and S.E. Aly, 2003. Antioxidants and free radical scavenging properties of vegetable extracts in rats fed aflatoxin contaminated diet. J. Agric. Food Chem., 51: 2409-2414.

Aebi, H., 1983. Catalase. In: Methods of Enzymatic Analysis, Ed: Bergmeyer, H.U. Weinheim and Academic Press., pp : 227-282.

Amsted, P., A. Levy, I. Emerit and P. Cerutti, 1984. Evidence for membrane-mediated chromosomal damage by AFB_1 in human lymphocytes. Carcinogenesis, 5: 719-723.

Anonymous, 1988. Global Environmental Eonitoring System. Assessment of Chemical Contaminants in Food. Report on the Results of the UNEP/FAO/WHO Programme on Health Related Environmental Monitoring. 1988; FAO, Rome, pp: 50-53.

Anonymous, 1993. Monographs on the evaluation of carcinogenic risk to humans: some naturally occurring substances. In: Food Items and Constituents Heterocyclic Aromatic Amines and Mycotoxins. No. 56. IARC, Lyon, France, pp: 245-395

Bertram, J,S., L.N. Kolonel and F.L. Meyskens, 1987. Rational and Strategies for Chemoprevention of Cancer in Humans. Cancer Res., 47: 3012-3031.

Buetler, T.M., T.K. Bammler, J.D. Haye and D.L. Eaton, 1996. Oltipraz mediated changes in aflatoxin B_1 biotransformation in rat liver: Implications for human chemointervention. Cancer Res., 56: 2306-2313.

Burton, G.W. and K.U. Ingold, 1984. β-carotene : an unusual type of lipid antioxidant. Science, 224: 569-573.

Cadenas, 1989. Biochemistry of oxygen toxicity. Am. Rev. Biochem., 58:79-110.

Cascales, M., P. Martin-Sanz, D.G. Craciunescu, I. Mayo, A. Aguilar, E.M. Robles- Chillida and C. Cascales, 1991. Alterations in hepatic peroxidation mechanisms in thioacetamide-induced tumors in rats. Effect of a rhodium(III) complex. Carcinogenesis, 12: 233-240.

Choudhary, A. and R.J. Verma, 2005. Ameliorative effects of black tea extract on aflatoxin induced lipid peroxidation in the liver of mice. Food Chem. Toxicol., 43: 99-104.

Chow, C.K., 1989. Interrelationships of cellular antioxidant defense systems. In: Cellular Antioxidant Defense Mechanisms (Chow CK. Ed) CRC Press, Boca Raton. pp: 217-237.

Deflora, S., M. Bagnasco and H. Vainio, 1999. Modulation of genotoxic and related effects by carotenoids and vitamin A in experimental models: Mechnistic issues. Mutagenesis, 14: 153-172.

Desai, I.D., 1984. Vitamin E Analysis in animal tissue. Methods in Enzymology, 105: 138-144.

Eaton, D.L. and E.P. Gallagher, 1994. Mechanisms of aflatoxin carcinogenesis. Annu. Rev. Pharmacol. Toxicol., 34: 135-172.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys., 82: 70-77.

Frankul, W., 1989. The Vitamin A content in the liver in the serum of acute experimental protein malnutrition in the force-fed rat. J. Faculty of Medicine, Baghdad, 31: 135-143.

Frei, B., L. England and B.N. Ames, 1989. Ascorbate is an outstanding antioxidant in human blood plasma. Proc. Natl. Acad. Sci., 86: 6377-6381.

Griffith, O.W., 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal. Biochem., 106: 207-212.

Groopman, J.D., L.G. Cain and T.W. Kensler, 1988. Aflatoxin exposure in human populations: measurements and relationship to cancer. Crit. Rev. Toxicol., 19: 113-145.

Gutteridge, J.M. and B. Halliwell, 1988. Oxygen radicals and tissue injury. FASEB J., 13:9-19.

Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferase: the first enzymatic step in mercapturic acid formation. J. Biol. Chem., 246: 7130-7139.

Harvey, R.B., L.F. Kubena and M.H. Elissalde, 1994. Influence of vitamin E on aflatoxicasis in growing swine. Am. J. Vet. Res., 55: 572-577.

Hawk, P.B. and B.L. Osar, 1964. Hawk's Physiological Chemistry, 14th Edn., Tata Megrraw Hill Book Company, New Dalhi, pp: 1376.

Hayes, J.D., D.J. Judah, L.I. McLellan and G.E. Neal, 1991. Contribution of the glutathione S-transferases to the mechanisms of resistance to aflatoxin B_1. Pharmacol. Ther., 53: 3887-3894.

James, R.C. and R.D. Harbison, 1982. Hepatic glutathione and hepatotoxicity. Biochemical Pharmacology, 31: 1829-1835.

Kanematsy, T., T. Kawano, K. Takenaka, T. Matsumata, K. Sugimachi and M. Kuwano, 1989. Levels of vitamin A and cellular retinol binding protein in human hepatocullar carcinoma and adjacent normal tissue. Nut. Cancer, 12: 311-319.

Kodama, M., F. Enoue and M. Akao, 1990. Enzymatic and non-enzymatic formation of free radicals from aflatoxin B_1. Free Rad. Res. Commun., 10: 137-142.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J.Biol.Chem., 193: 265-275.

Manson, M.M., H.W. Ball, M.C. Barrett, H.L. Clark, D.J. Judah, G. Williamson and G.E. Neal, 1997. Mechanism of action of dietary chemo protective agents in rat liver: Induction of phase I and II drug metabolizing enzymes and aflatoxin B_1 metabolism. Carcinogenesis, 18: 1729-1738.

Marklund, S. and G. Marklund, 1974. Involvement of SOD anion radical the autooxidation of pyrogallol and convenient assay for SOD. Euro. J. Biochem., 47: 469-474.

Mayne, S.T., 1996. β-carotene, carotenoids and disease prevention in humans. FASEB J., 10: 690-701.

Meki, A.R.M.A., E.E.D.F. Esmail, A.A. Hussein and H.M. Hassanein, 2004. Caspase-3 and heat shock protein-70 in rat liver treated with aflatoxin B1: Effect of melatonin Toxicon, 43: 93-100.

Mitsuo, A., 1994. Vitamin A status in liver patients with various liver disease. Osaka Ika Diagaku Zasshi, 53: 13-19

Mobarhan, S., T.J. Layden, H. Friedman, A. Kunigk and P. Donahue, 1968. Depletion of liver and esophageal epithelium vitamin A after chronic moderate ethanol consumption in rats: Inverse relation to zinc nutriture. Hepatology, 6: 615-621.

Muto, Y. and M. Omori, 1981. A novel cellular Retinoid-binding protein, F-type , in hepatocelluar carcinoma. Ann. NY Acad. Sci., 359:91-103.

Neal, G.E., 1995. Genetic implications in the metabolism and toxicity of mycotoxins. Toxicol.Lett., 82: 861-867.

Neeld, J.B. and W.N. Pearson, 1963. Micro method for the determination of serum beta-carotene and vitamin A using trifluoroacetic acid (TFA). J. Nutr., 79: 454-462.

Newberne, P.M. and W.H. Butler, 1969. Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals: A review. Cancer Res., 29: 236-250.

Niki, E., 1987. Interaction of ascorbate and α-tocopherol. Ann. NY. Acad. Sci., 498: 186-199.

Niki, E., N. Noguchi, H. Tsuchihashi and N. Gotoh, 1995. Interaction among vitamin C, Vitamin E and β-carotene. Am. J. Clin. Nutr., 62: 1322-1326.

Oberley, L.W. and G.R. Buettner, 1979. Role of superoxide dismutase in cancer: A review. Cancer Res., 39: 1141-1149.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Analy. Biochem., 95: 351-358.

Osar, B.L. and M. Osar, 1956. Hawk's Physiological chemistry, 14th Edn., Tata Megrraw Hill Book Company,New Dalhi, pp: 1373.

Pelissier, M.A. C. Frayssinet, M. Boisset and R. Albrecht, 1992. Effect of phenoclor DP6 on enzyme-altered c foci and lipid peroxidation in livers of Aflatoxin B_1-initiated rats. Fd. Chem. Toxic., 30: 133-137.

Premalatha, B. and P. Sachdanandam, 1999. *Semecarpus anacardium* L. nut extract administration induces the *in vivo* antioxidant defence system in aflatoxin-B_1 mediated hepatocellular carcinoma. J. Enthopharmacol., 66: 131-139.

Racker, E., 1955. Glutathione reductase from baker's yeast and beef liver. J. Biol. Chem., 217: 855-866.

Roe, J.H. and C.A. Kuether, 1955. Determination of Ascorbic acid in whole blood and plasma by 2,4 dinitrophynyl hydrazine method. J. Biol. Chem., 147: 399.

Shen, H.M., C.N. Ogn and C.Y. Shi, 1995. Involvement of reactive oxygen species in aflatoxin B_1- induced cell injury in cultured rat hepatocytes. Toxicology, 99: 115-123.

Shirali, P., E. Teissier, T. Marez, H.F. Hildebrand and J.M. Haguenoer, 1994. Effect of alpha Ni3S2 on arachidonic acid metabolites in cultured human lung cells (L132 cell line). Carcinogenesis, 15: 759-762.

Sies, H., W. Stahl and A.R. Sundquist, 1992. Antioxidant functions of vitamins: vitamin E and C, β-carotene and other carotenoids. Ann. NY. Aca. Sci., 669: 7-20.

Sies, H., 1991. Oxidative stress: Oxidants and Antioxidants. Academic Press, San Diego, California.

Souza, M.F., A.R. Tome and V.S. Rao, 1999. Inhibition by the bioflavanoid ternatin of aflatoxin B_1-induced lipid peroxidation in rat liver. J. Pharm. Pharmacol., 51: 125-129.

Takashi, K., K. Toyokazu, T. Kenji, M. Takashi, S. Keizo and K. Michihiko, 1989. Levels of vitamin A and cellular retinol binding protein in human hepatocellular carcinoma and adjacent normal tissue. Nutr. Cancer, 12: 311-319.

Tsuchihashi, H., M. Kigoshi, M. Iwatsuki and E. Niki, 1995. Action of β-carotene as an antioxidant against lipid peroxidation. Arch. Biochem. Biophys., 323: 137-147.

Wenck, H., E. Schwabe, F. Schneider and L. Flohe, 1972. A comparative study of some physico-chemical methods for the quantitative determination of SH-compounds of different structure. Fresenius Zeitsch. Anal. Chem., 258: 267-272.

Wiseman, H., 1996. Dietary influences on membrane function: Importance in protection against oxidative damage and disease. J. Nut. Biochem., 7:2-15

Wogan, G.N., 1992. Aflatoxins as risk factors for hepatocellular carcinoma in humans. Cancer Res. Suppl., 52: 2114-2119.

Yu, M.W., H.H. Hsieh, W.H. Pan, C.S. Yang and C.J. Chen, 1995. Vegetable consumption, serum retinol level and risk of hepatocellular carcinoma. Cancer Res., 55: 1301-1305.

Zhang, P. and S. Omaye, 2000. β-carotene and protein oxidation: effects of ascorbic acid and β-tocopherol. Toxicology, 146: 37-47.