

RESEARCH COMMUNICATION

Anti-Mutagenic and Anti-Carcinogenic Potential of the Carotenoid *Meso-Zeaxanthin*

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Abstract

Meso-zeaxanthin was investigated for antimutagenic and anticarcinogenic activity, using the Ames test (*Salmonella typhimurium* strains TA 98, TA 100, TA 102 and TA 1535) with direct acting mutagens like sodium azide (NaN₃) (5 µg/ plate), nitro-*o*-phenyldiamin (NPD) (20 µg/ plate), N-methyl- N'-nitro-N-nitrosoguanidine (MNNG) (1µg/ plate) and tobacco extract 50 mg/ plate) and with a mutagen needing microsomal activation, acetamidofluorene (AAF) (20 µg/ plate). The carotenoid was found to inhibit the mutagenicity induced by NaN₃, NPD and MNNG in a concentration dependent manner, as well as that with AAF and the tobacco extract. Concentrations needed for 50 % inhibition was found to be 50 µg/ plate for the chemical mutagens and 100 µg/ plate for tobacco extract. Using specific resorufin derivatives as substrates *in vitro*, the concentration of *meso-zeaxanthin* needed for 50 % inhibition of CYP1A2 (7-methoxyresorufin-O-demethylase) was 5 µg/ml, for CYP2B 1/2 (7- pentoxyresorufin-O-depentylyase) was 8 µg/ml and for CYP1A1 (7-ethoxyresorufin-O-deethylase) was 12 µg/ml, while that of CYP 2E1 (aniline hydroxylase) was 7µg/ml and for CYP 1A, 2A, 2B, 2D and 3A (aminopyrene-N-demethylase) was 10.5 µg/ml. Evaluated using nitroso diethyl amine (NDEA) induced hepatocellular carcinoma in rats, treatment with *meso-zeaxanthin* reduced the tumor incidence when compared to the control group. The activity of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and alkaline phosphatase was drastically elevated in both serum and liver tissue of NDEA alone treated control animals and *meso-Zeaxanthin* pretreated animals showed significant decrease to normal levels, in line with histopathological findings.

Keywords: Anticarcinogenicity - antimutagenicity - carotenoid - *meso-zeaxanthin* - CYP450 enzymes

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Introduction

Carotenoids are one of the most widespread groups of naturally occurring pigments, imparting red, yellow and orange colours to fruits and vegetables. More than 600 carotenoids have been discovered thus far. Of these only about a dozen have been studied closely, though all the carotenoids have their own benefits. A voluminous body of literature including *in vitro* studies, animal studies, human observational studies, and clinical trials has suggested a multiplicity of health effects of carotenoids in human. Major health benefits of carotenoids include antioxidant activity, immuno enhancement, inhibition of mutagenesis and transformation, regression of premalignant lesions etc (Krinski, 1993).

Cancer is one of the leading causes of death worldwide. The initiation of many tumours results from damage to DNA by electrophilic carcinogen metabolites or by reactive oxygen species that arise during carcinogen metabolism or endogenous cellular process. Modulation of the metabolism of carcinogens is one of the most effective and well established strategies for protecting

animals and their cells against the toxic and neoplastic effects of carcinogens.

Hepato cellular carcinoma is considered the fifth most common pathology worldwide and the most common type of liver cancer, representing up to 83% of all cases (Oliff, Gibbs and McCormick, 1996). N-nitrosodiethylamine (NDEA) is a N-nitroso compound found to produce hepatotoxicity in experimental animals after repeated administration (Anna et al., 1994). NDEA is present in variety of food stuffs like cheese, soybeans, smoked, salted and dried fish, cured meat and alcoholic beverages etc (Froment et al., 1994). NDEA becomes metabolically active by the action of cytochrome p450 enzymes to produce reactive electrophiles, which increase oxidative stress level leading to cytotoxicity, mutagenicity and carcinogenicity (Telliez et al., 1995). Oxidative stress is considered as critical mechanism contributing to NDEA induced hepatotoxicity, and the use of anti-oxidants reduced the liver damage.

meso-zeaxanthin (MZ), a xanthophyll carotenoid present in the fovea centralis of retina along with lutein and zeaxanthin. MZ is a powerful antioxidant owing to its

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extensive conjugated double bonds and facilitates a wider range of blue light filtration. At an anatomic level, MZ is more closely related to vulnerable photoreceptors than either lutein or zeaxanthin, and is therefore ideally located to protect against free radical damage (Krinsky, 1989).

A typical diet does not contain MZ. It is generated in the body from lutein. However, recent research has shown that MZ supplementation will ensure its bioavailability in a way that is not dependent on conversion of lutein (Thurnham, 2007). In the present study we tried to explore anti mutagenicity, inhibition of CYP450 enzymes and anti carcinogenic potential of this carotenoid.

Materials and Methods

Chemicals

Nutrient broth was purchased from Hi-media laboratories, Mumbai. Agar agar, L- Histidine, biotin, NADP, glucose-6-phosphate, dimethyl sulphoxide and sodium azide (NaN_3) were obtained from Sisco Research Laboratories, Mumbai, N-Methyl- N'-nitro-N-nitrosoguanidine (MNNG), 4-Nitro-O-phenylenediamine (NPD), 2-Acetaminofluorene (AAF) and Nitroso diethyl amine (NDEA) were purchased from Sigma chemicals (St. Louis, MO, USA). Resorufin, 7-ethoxy resorufin (ER), 7- pentoxyresorufin (PR), 7-Methoxy resorufin (MR) were obtained from Sigma-Aldrich Inc., U.S.A. Phenobarbitone (Gardenal[®] 60, Batch No.B03007) was purchased from Nicholas-Piramel India Ltd, Gujarat. Nicotinamide adenine dinucleotide phosphate reduced (NADPH) was obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Tobacco was purchased from local market. All other chemicals were of analytical grade.

Sample

meso-Zeaxanthin (MZ) was supplied by Omni Active Health Technologies Pvt. Ltd, Mumbai. MZ was dissolved in DMSO for invitro studies. For in vivo studies 5% suspension made in sunflower oil was used. The structure of MZ shown in Figure 1.

Bacterial strains

Salmonella typhimurium strains TA 98, TA 100 and TA 1535 were supplied by Institute of Microbial Technology, Chandigarh, India. After subculturing in nutrient broth was dispensed in small vials and stored in -70°C in presence of DMSO (9%). during the experiment bacterial culture was freshly inoculated in nutrient broth and grown for 12 h at 37°C . TA 102 strain was a gift from Dr. Padma Ambalam, Sourashtra University, Gujarat, India. All experiments were done after initial genotoxicity as per the original instructions (Moron and Ames, 1983).

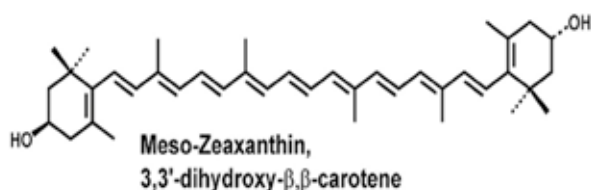


Figure 1. Structure of MZ

Animals

Male Wistar rats (155 g) were used in the study. They were purchased from Small Animal Breeding Station, Mannuthy, Kerala, India and were housed in well ventilated cages under controlled conditions of light and humidity and provided with normal mouse chow (Sai Durga Food and Feeds, Bangalore, India) and water ad libitum. All the animal experiments were done as per the instructions prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India, and implemented through the Institutional Animal Ethical Committee of Amala Cancer Research Centre.

Preparation of aqueous extract of tobacco

100 g of tobacco was cut into small pieces and boiled in 500 ml water for 1 h and the extract of the tobacco used for the study.

Determination of anti mutagenic potential of MZ

(a) Direct acting mutagens. Anti mutagenicity of MZ was tested using Salmonella typhimurium strains TA 98, TA 100, TA 102 and TA 1535. Non toxic concentration of MZ was incorporated into minimal agar plate containing 0.5 mM Histidine/Biotin. Plates were spread with solutions of mutagens: sodium azide ($2.5 \mu\text{g/plate}$, dissolved in distilled water), MNNG ($1 \mu\text{g/plate}$, in distilled water), NPD ($20 \mu\text{g/plate}$, dissolved in DMSO), acetamino fluorine ($20 \mu\text{g/plate}$, in distilled water) or aqueous tobacco extract (50 mg/plate). Plates were inoculated with $100 \mu\text{l}$ bacteria ($1-2 \times 10^9$ cells/ml) in 2 ml of molten agar and incubated for 48 h at 37°C and the revertant colonies formed in presence and absence of mutagens were counted using a colony counter. Anti mutagenicity was expressed as the difference in the number of colonies from the control and that treated with MZ. Each data was the mean of 3 plates with standard deviation.

(b) Mutagens needing microsomal activation Male Wistar rats (160-170g) were given 0.1% solution of the sodium salt of phenobarbitone for 4 consecutive days in drinking water to induce liver microsomal enzymes. On the fifth day the liver was excised aseptically and microsomal fraction (S9) was prepared.

Anti mutagenicity of MZ was tested in *S. typhimurium* strains TA 98 and TA 100 against AAF ($25 \mu\text{g/plate}$) as mutagen by plate pre- incubation method. For that S9 mix (0.5ml) was mixed with mutagen, 0.1 ml of bacterial culture ($1-2 \times 10^9$ cells/ml) and various concentrations of MZ and was incubated for 30 minutes at 37°C . Further it was overlaid on minimal glucose agar plates and incubated for 48 h at 37°C and revertant colonies were counted using a colony counter (Garner, Miller and Miller, 1972).

(c) Against tobacco extract. Anti mutagenicity of MZ was tested in *S. typhimurium* strain TA 102 against the aqueous extract of tobacco by the plate incorporation method. Fresh bacterial culture (0.1 ml, $1-2 \times 10^9$ cells/ml) was mixed with 2 ml of top agar containing histidine

and biotin, various concentrations of drugs and tobacco extract (50 mg/ plate). Further it was poured on to minimal glucose agar plate and incubated for 48 h at 37°C. After incubation, revertant colonies were counted using colony counter.

The percentage of inhibition of mutagenicity was calculated using the formula $[(R1-SR)-(R2-SR)] \times 100 / (R1-SR)$ where R1 is the number of revertants in the presence of mutagen alone, R2 is the number of revertants in the presence of drugs and SR is the spontaneous revertants (Sukumaran and Kuttan, 1995).

Inhibition of different isoforms of microsomal cytochrome P450 enzymes by MZ

Rats were administered with phenobarbitone continuously for 4 days (60mg/kg b. wt., intra peritonally, once daily). They were sacrificed 24 h after the last dose of phenobarbitone. The livers of all the animals were excised quickly and washed thoroughly in ice-cold saline and kept at -70°C. Liver homogenate (25%) was prepared in cold phosphate buffer (pH 7.4, 0.1M). Homogenate was initially centrifuged at 14000 g for 20 min in a cold centrifuge (Remi) and supernatant was then further centrifuged at 10,5000 g for 1 h in an ultracentrifuge (Sorvall) and microsomes obtained were washed and resuspended in cold phosphate buffer (pH 7.4, 0.1 M) and used for the analysis of various CYP450 enzymes.

To study the effect of MZ on CYP450 isoenzymes in vitro, various concentrations of MZ (5-20µg/ml) were incubated with microsomal fraction. The effect of MZ on the dealkylation of methoxy resorufin by 7-methoxyresorufin-O-demethylase (MROD), CYP1A2, pentoxy resorufin by 7-pentoxyresorufin-O-depenthylase (PROD), CYP2B1/2 and ethoxy resorufin by 7-ethoxyresorufin-O-deethylase (EROD), CYP1A1 were studied (Pohl, Fouts, 1980; Nerurkar et al., 1993)

Assay mixture contained sodium phosphate buffer (0.1M, PH 7.4), 6.25 mM MgSO₄, 60µM EDTA, 5µM ER, MR or PR, 100µg microsomal protein, 100µm NADPH and various concentrations of MZ in a final volume of 1ml. The reaction time was 5 min with a preincubation period of 5min without the addition of NADPH at 370C. The reaction was stopped by the addition of 2ml of chilled methanol. The precipitated protein was centrifuged down and supernatant was used for the estimation of enzyme activity using a fluorescent spectrophotometer (Hitachi F-2500) at the excitation wavelength of 550 nm and the emission wavelength of 585 nm. Blanks were set without any NADPH. The percentage inhibition was calculated by the following formula.

$$C-T/C \times 100$$

Where C is the optical density of control without MZ, T is the optical density with MZ.

Inhibition of aniline hydroxylase activity (an indicator of CYP 2E1 activity) and aminopyrene-N-demethylase activity (an indicator of CYP 1A, 2A, 2B, 2D and 3A activity) was measured by the method of Mazel.

Anti carcinogenic activity of MZ against NDEA induced hepatocellular carcinoma

Male Wistar rats were divided into following 4 groups.

Group I: Normal (n=6), Group II: NDEA Control (n=8), Group III: NDEA+ vehicle (n=8), Group IV: NDEA+ 50mg/kg b. wt MZ (n=8) and Group V: NDEA+2 50mg/kg b. wt MZ (n=8).

NDEA (0.02%) was prepared fresh in distilled water every day and animals from group II-V were fed with NDEA (2-5ml/rat/dose) 5 days in week for consecutive 20 weeks through oral gavage. This dosage was found to produce liver cancer in rats within 20 weeks (Jose, Joy and Kuttan, 1999). Various concentrations of MZ (50 and 250 mg/kg, body weight) were administered (orally) once daily for 5 days in a week for consecutive 20 weeks. Administration of NDEA and drug were stopped at 20th week and animals were kept under observation for another 9 weeks. At the end of 29th week all the animals were sacrificed under light ether anesthesia. Gross necropsy of animals was made to see any visible morphological changes of the organs. Blood was collected from each animal through heart puncture into heparinised and non-heparinized tubes.

Survival rate of animals in each group was monitored every day. Livers from each animal were excised after sacrifice, washed in ice-cold saline (0.9%) and observed for tumor nodules and other morphological abnormalities. Weight of each liver was recorded and was expressed as liver weight/100g body weight. Marker enzymes of hepatic damage such as gamma-glutamyl transpeptidase, ALP, GPT, GOT in both serum and liver tissue. Histopathological analysis- A small portion of liver was fixed in 10% formalin for histopathological analysis.

Statistical analysis

The values were expressed as mean ± standard deviation (SD). Statistical evaluation of the data was done by one way ANOVA followed by Tukey test (post-hoc) using Instat 3 software package.

Results

Antimutagenicity of MZ against different mutagens

(a) Sodium Azide (NaN₃). MZ significantly reduced the revertants induced by NaN₃ in TA 100, TA 102 and TA 1535 strains in a dose dependent manner. The inhibition of mutagenicity was found to be 46.70, 64.50 and 97 % for TA 100 strain, 44, 86.6 and 93.80 % for TA 102 strain and 46.40, 68.50 and 79.80 % for TA 1535 at concentrations 50, 100 and 250 µg MZ / plate (Table 1).

(b) 4-Nitro-O-phenylenediamine (NPD). MZ showed significant anti mutagenic activity against NPD induced mutagenicity. Percentage of inhibition increased with an increase in the concentration of MZ, which is 48, 62.7 and 65 % for TA 98 and 49.1, 57.3 and 62.8 % for TA 100 at concentrations of 50, 100 and 250 µg / plate of MZ (Table 2).

(c) N-Methyl- N'-nitro-N-nitrosoguanidine (MNNG). MZ inhibited mutagenicity produced by MNNG to S. typhimurium strains TA 100 and TA1535. The inhibition of mutagenicity was 39, 51.94 and 56.8 % for TA 100 and 14.9, 32.5 and 41.8 % for TA 1535 using 50, 100 and 250

Table 1. Anti Mutagenic Activity of MZ on Mutagenicity Induced by NaN₃ in *S. typhimurium* Strains TA100 TA 102 and TA1535

	TA 100	TA 102	TA 1535
Concentration of MZ ($\mu\text{g}/\text{plate}$)	Average number of revertants/ plate	Average number of revertants/ plate	Average number of revertants/ plate
NaN ₃	979 \pm 49.6	608 \pm 51.7	847 \pm 48.7
NaN ₃ +DMSO	633 \pm 37.8	517 \pm 31.6	756 \pm 48.7
NaN ₃ +50 μg	557 \pm 37.4*** (46.70)	368 \pm 33.1*** (44.00)	461 \pm 42.4*** (46.40)
NaN ₃ +100 μg	396 \pm 29.6*** (64.50)	136 \pm 27.3*** (86.60)	277 \pm 19.0*** (68.50)
NaN ₃ +250 μg	102 \pm 19.4*** (97.00)	97 \pm 17.3*** (93.80)	168 \pm 21.0*** (79.80)
SR	75 \pm 12.6	63 \pm 9.6	15 \pm 8.0

The values are mean \pm SD of 6 different determinations. ***P < 0.001; SR is spontaneous reversion. Values in parentheses indicated percentage inhibition. Percentage inhibition was calculated from NaN₃ alone treated group

Table 2. Anti Mutagenic Activity of MZ on Mutagenicity Induced by NPD in *S. typhimurium* Strains TA98 and TA100

	TA 98	TA 100
Concentration of MZ ($\mu\text{g}/\text{plate}$)	Average number of revertants/ plate	Average number of revertants/ plate
NPD	1110 \pm 43.6	790 \pm 52.9
NPD+DMSO	1082 \pm 34.2	786 \pm 61.2
NPD+50 μg	589 \pm 48.7 (48.00)	459 \pm 32.4*** (49.1)
NPD+100 μg	429 \pm 26.3 (62.70)	404 \pm 25.8*** (57.30)
NPD+250 μg	404 \pm 31.3 (65.00)	367 \pm 26.4*** (62.80)
SR	22 \pm 6.2	116 \pm 14.6

The values are mean \pm SD of 6 different determinations. ***P < 0.001; SR is spontaneous reversion. Values in parentheses indicated percentage inhibition. Percentage inhibition was calculated from NPD alone treated group

Table 3. Anti Mutagenic Activity of MZ on Mutagenicity Induced by MNNG in *S. typhimurium* Strains TA100 and TA1535

	TA 100	TA 1535
Concentration of MZ ($\mu\text{g}/\text{plate}$)	Average number of revertants/ plate	Average number of revertants/ plate
MNNG	742 \pm 18.9	520 \pm 13.6
MNNG+DMSO	713 \pm 27.0	509 \pm 19.7
MNNG+50 μg	500 \pm 24.0*** (39.00)	446 \pm 17.5* (41.90)
MNNG+100 μg	420 \pm 26.6*** (51.94)	358 \pm 20.0*** (32.50)
MNNG+250 μg	396 \pm 26.8*** (56.80)	312 \pm 16.9*** (41.80)
SR	122 \pm 7.8	22 \pm 5.4

The values are mean \pm SD of 6 different determinations. ***P < 0.001, * P < 0.05; SR is spontaneous reversion. Values in parentheses indicated percentage inhibition. Percentage inhibition was calculated from MNNG alone treated group

$\mu\text{g}/\text{plate}$ of MZ (Table 3).

(d) Acetaminofluorene (AAF)

MZ inhibited mutagenicity induced by AAF, which needs microsomal (S9) activation. The inhibition of mutagenicity in *S. typhimurium* strain TA 98 was 45.4, 63.7 and 77.4% and in TA 100 strain was 47.7, 66.7

and 86.2 % at concentrations 50, 100 and 250 $\mu\text{g}/\text{plate}$ (Table 4).

(e) Tobacco extract

Earlier studies have reported that 50 mg of tobacco extract/plate produced maximum mutagenic response to Salmonella tester strain TA 102. MZ showed significant anti mutagenic activity against tobacco induced mutagenicity. The inhibition of mutagenicity by MZ in *S.*

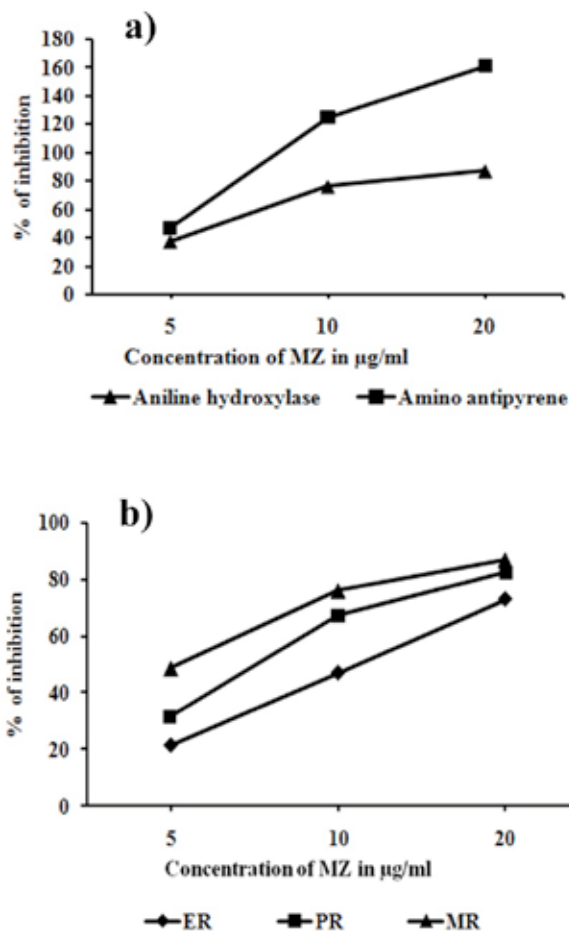


Figure 2. Effect of MZ on the Inhibition of Various CYP450 Isoforms. (a) aniline hydroxylase and aminopyrene-N-demethylase activity. (b) The effect of MZ on the dealkylation of methoxy resorufin by 7-methoxyresorufin-O-demethylase (MROD), CYP1A2, pentoxy resorufin by 7-pentoxyresorufin-O-depentylase (PROD), CYP2B1/2 and ethoxy resorufin by 7-ethoxyresorufin-O-deethylase (EROD), CYP1A1.

Table 4. Anti Mutagenic Activity of MZ on Mutagenicity Induced by AAF in *S. typhimurium* Strains TA 98 and TA 100 in the Presence of S9 Mix

	TA 98	TA 100
Concentration of MZ ($\mu\text{g}/\text{plate}$)	Average number of revertants/ plate	Average number of revertants/ plate
AAF	515 \pm 18.9	603 \pm 21.3
AAF+50 μg	300 \pm 6.7*** (45.40)	415 \pm 11.0*** (47.70)
AAF+100 μg	213 \pm 12.4*** (63.70)	341 \pm 11.9*** (66.70)
AAF+250 μg	148 \pm 13.0*** (77.40)	265 \pm 9.9*** (86.20)
SR	41 \pm 3.0	211 \pm 6.1

The values are mean \pm SD of 6 different determinations. ***P < 0.001; SR is spontaneous reversion. Values in parentheses indicated percentage inhibition. Percentage inhibition was calculated from AAF alone treated group

Table 5. Anti Mutagenic Activity of and TA 98 on Mutagenicity Induced by Tobacco Extract in *S. typhimurium* Strain TA 102

Concentration of drug ($\mu\text{g}/\text{plate}$)	Average number of revertants/ plate
Tobacco extract	712 \pm 34.6
Tobacco extract+DMSO	699 \pm 37.5
Tobacco extract+50 μg	585 \pm 26.3*** (19.80)
Tobacco extract+100 μg	412 \pm 17.3*** (46.80)
Tobacco extract+250 μg	204 \pm 10.8*** (79.40)
SR	72 \pm 17.5

The values are mean \pm SD of 6 different determinations. ***P < 0.001; SR is spontaneous reversion. Values in parentheses indicated percentage inhibition. Percentage inhibition was calculated from tobacco extract alone treated group

Table 6. NDEA Survival Rate and Liver Weight of NDEA Induced HCC Harboring Animals (at 29th week)

Groups	No of animals Survived	Liver wt (g)/100g b. wt
Normal	8/8	3.1 \pm 0.9
Control	5/8	6.2 \pm 3.2
50 mg/kg b.wt	8/8	5.2 \pm 2.3
250 mg/kg b.wt	8/8	3.8 \pm 1.1

Table 7. Effect of MZ on Serum Parameters After NDEA Induced Hepatic Carcinoma

	SGOT (IU/l)	SGPT (IU/l)	ALP (KA)	Bilirubin (mg/dl)	GammaGGT (U/l)
Normal	31 \pm 4	35 \pm 4	28 \pm 2	0.6 \pm .7	29 \pm 3
Control	427 \pm 31***	290 \pm 18***	66 \pm 1***	2.7 \pm .2***	106 \pm 10***
50mg/kg b.wt MZ	226 \pm 14***	130 \pm 10***	49 \pm 2***	1.6 \pm .1***	68 \pm 4***
250mg/kg b.wt MZ	92 \pm 10***	78 \pm 12***	34 \pm 3***	1.1 \pm .1***	43 \pm 6***

***P < 0.001, significant (one-way ANOVA followed by Dunnett multiple comparison test); against control groups

Table 8. Effect of MZ on Liver Parameters After NDEA Induced Hepatic Carcinoma

	GOT (U/mg protein)	GPT (U/mg protein)	ALP (KA/mg protein)	Gamma GGT (U/mg protein)	Bilirubin (mg/mg protein)
Normal	41.3 \pm 4.3	32.6 \pm 1.5	15.3 \pm 4.7	.695 \pm .1	.4 \pm .11
Control	320.7 \pm 16***	171 \pm 33***	58 \pm 4***	5 \pm 2***	1.1 \pm .1
50mg/kg.b.wt MZ	131 \pm 25***	67 \pm 8***	28 \pm 3***	1.3 \pm .2***	.65 \pm .2***
250mg/kg.b.wt MZ	45 \pm 5***	45 \pm 3***	19 \pm 2***	.66 \pm .04***	.52 \pm .3***

***P < 0.001, significant (one-way ANOVA followed by Dunnett multiple comparison test); against control groups

typhimurium strain TA 102 was found to be 19, 46 and 79.4 % at concentrations 50, 100 and 250 $\mu\text{g}/\text{plate}$ (Table 5).

2. Inhibition of different CYP 450 isoforms by MZ

Effect of MZ on the inhibition of various CYP450 enzymes in vitro studied by using microsomal preparation from phenobarbitone (a CYP450 inducer) treated animals. It was found that all the CYP450 enzymes were significantly inhibited by MZ in a concentration dependent manner (Figure 2). Concentration needed for 50 % inhibition (IC₅₀) of CYP1A2 (MROD) was 5 $\mu\text{g}/\text{ml}$, CYP2B 1/2 (PROD) was 8 $\mu\text{g}/\text{ml}$ and CYP1A1(EROD) was 12 $\mu\text{g}/\text{ml}$ while that of CYP 2E1 (aniline hydroxylase) was 7 $\mu\text{g}/\text{ml}$ and CYP 1A, 2A, 2B, 2D and 3A (aminopyrene-N-demethylase) was 10.5 $\mu\text{g}/\text{ml}$.

3. Effect of MZ on NDEA induced hepatocellular carcinoma

MZ inhibited NDEA induced hepatocellular carcinoma in a dose dependent manner. Treatment of NDEA 5 days/week for 20 weeks induced hepatocellular carcinoma in all the control group animals. The number of tumors and percentage of incidence was reduced significantly in animals administered with 50 and 250 mg/ kg b.wt MZ (Table 6 and Figure 3). The activity of GOT, GPT and ALP was drastically elevated in both serum and liver tissue of NDEA alone treated control animals. MZ pretreated animals showed significant decrease in these levels to normal levels. The elevated activity of GGT in both serum and liver tissue of control group animals were also found to be decreased to normal levels by MZ pretreatment (Tables 7 and 8).

Histopathology of liver tissue of NDEA alone treated control group animals showed carcinomatous growth composed of groups of large pleomorphic polyhedral or oval cells having hyperchromatic nuclei. There were many degenerating cells and mitotic cells. Stroma showed areas of necrosis and inflammatory reaction. Many congested blood vessels and areas of hemorrhage were seen. All these carcinomatous changes were found to be significantly decreased in the MZ pretreated animal groups.

Discussion

Ames test serves as a quickest way to analyze the

carcinogenic potential of a compound. In this test *Salmonella typhimurium* strains carrying mutations in the histidine operon is being used, hence are histidine dependent. Addition of mutagens, bacteria reverse back to histidine independent and form colonies in histidine deficient medium. So addition of anti mutagenic agents considerably reduces reverse mutation capability of mutagens.

Different bacterial strains used here detect different mutagens. TA 1535 and TA 100 detect mutagens causing base pair substitutions, TA 98 detects frame shift mutagens and TA 102 detects oxidative mutagens. Mutagens may be either direct acting or requiring microsomal activation. Direct acting mutagens interact directly with DNA to produce mutation. In this study direct acting mutagens like NaN₃, NPD, MNNG were used and able to reverse the mutation of bacteria to form colonies in minimal glucose agar plates. Addition of MZ significantly reduced the number of revertant colonies.

Mutagens like acetaminofluorine (AAF) require microsomal activation to induce mutation. Since bacteria do not have this metabolic capacity, an exogenous mammalian organ activation system (S9 fraction from phenobarbitone treated rats) was added. Addition of MZ markedly reduced the number of revertant colonies produced by the addition of AAF.

Phase I (cytochrome p450, CYP) drug metabolising enzymes are usually involved in biotransformation of a procarcinogen into ultimate carcinogen. Thus, compounds that could regulate either mRNA transcript level or activity of CYP are thought to be important in the prevention of chemical-induced carcinogenesis. In the present study MZ showed significant inhibitory effect towards various isoforms of CYP450 at very low concentrations (5-20 µg/ml) in a concentration dependent manner indicating the chemo preventive potential of MZ.

NDEA is an indirect acting carcinogen and requires metabolic activation by CYP450 enzymes to form ethyl radical (ultimate carcinogen). Ethyl radical so formed attack the DNA and produce genetic changes which in turn results in carcinogenesis (Boiter, 1995). So compounds that inhibit CYP450 enzymes will have good anticarcinogenic activity. In this study we checked anticarcinogenic effect of MZ against NDEA induced hepatocellular carcinoma. In the NDEA alone treated animals there was a drastic elevation in the levels of hepatic marker enzymes like AST, ALT and ALP in both serum and liver tissue and were significantly reduced to normal levels by MZ treatment. Treatment of MZ also reduced the tumor incidence when compared to the control group. MZ a rare stereoisomer of zeaxanthin, naturally present in certain turtles, shrimp, yellow skin of fish etc (Maoka et al., 1986). This carotenoid has extended conjugated double bonds accounting for its profound antioxidant activity. Toxicity studies on this compound have shown that it is nontoxic (Chang, 2006). *In vitro* and *in vivo* antioxidant studies on MZ have revealed that it has good antioxidant potential (Firdous et al., 2010). Its multiple inhibitory properties-antimutagenic, inhibition of CYP450 isoforms, anticarcinogenic activities-make it a welcome addition to the class of chemoprevention drugs.

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