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Research Article

Apigenin protects 7, 12-dimethylbenz(a)anthracene–induced chromosomal abnormalities and DNA damage in the bone marrow cells of golden Syrian hamsters

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ABSTRACT

The present study has investigated the protective effect of apigenin on 7, 12-dimethylbenz(a)anthracene (DMBA)-induced chromosomal abnormalities and DNA damage in the bone marrow cells of golden Syrian hamsters. The protective effect of apigenin was examined by analyzing the frequency of micronucleated polychromatic erythrocytes (MnPCEs), chromosomal aberrations and DNA damage in hamsters treated with DMBA. We noticed an increase in MnPCEs frequency, chromosomal aberrations and DNA damage in hamsters treated with DMBA alone. Oral pretreatment of apigenin for 5 days to DMBA-treated hamsters significantly decreased the frequency of MnPCEs, chromosomal aberrations and DNA damage. The present study thus suggests that apigenin has the ability to prevent 7, 12-dimethylbenz(a)anthracene–induced chromosomal abnormalities and DNA damage in the bone marrow cells of golden Syrian hamsters.

Keywords: Apigenin; Chromosomal aberrations; DNA damage; DMBA; MnPCEs

INTRODUCTION

7, 12-dimethylbenz(a)anthracene (DMBA), a potent carcinogenic and immunosuppressive agent, is present in the environment as a product of incomplete combustion of complex hydrocarbon (Guerin., 1981). It has been reported that intraperitoneal administration of DMBA to mice caused substantial reduction in bone marrow cellularity. Profound studies demonstrated the clastogenic potential of DMBA in the bone marrow cells of rodents. DMBA mediates its clastogenic or genotoxic effects through its active metabolite dihydrodiol epoxide, which binds and cause damage to DNA (Manoharan et al., 2006; Bhuvaneswari et al., 2004). DNA damaging and mutagenic effects of DMBA in experimental animal models have been well documented (Baer-Dubowska et al., 1990). Also, excessively generated reactive oxygen species (ROS) during the metabolic activation of DMBA, induces strand breaks and causes oxidative modification of DNA bases. ROS has been implicated in the pathogenesis of several disorders including cancer, if they are excessively generated in the system. However, the host has an array of sophisticated antioxidant defense system (enzymatic and non-enzymatic) to combat the deleterious effects of ROS (Miyata et al., 2001).

Liver plays a prominent role in the metabolic activation and detoxification of DMBA. Assay of phase I and phase II detoxification enzymes in the liver could thus help to investigate the antigenotoxic potential of the test compound (Wilkinson et al., 1997). Profound studies pointed out that phytochemicals interfere with the role of carcinogenic agent by stimulating the activities of detoxification enzymes, especially glutathione-S-transferase (GST), which detoxifies carcinogens either by destroying their reactive center or facilitating their excretion by conjugation process (Bhuvaneswari et al., 2005). Micronucleus arises as a result of lagging chromosome or whole chromosomes that fail to migrate with one of the two daughter nuclei formed during mitosis. Any substances that cause chromosome breakage or affect the spindle apparatus could induce the formation of micronuclei (MacGregor et al., 1980). The frequency of micronuclei has therefore been used to assess the clastogenic potential of chemicals and xenobiotics. Chromosomal instability, the gain and/or loss of whole chromosomes or chromosomal segments, is a common feature of neoplastic cells. Any defects in chromosomal segregation, cellular checkpoints, telomere stability, and the DNA damage response lead to numerical and structural chromosomal instability. Accumulation of such defects in the genome prone the normal cell into malignant phenotype (Nersesion et al., 1993; Fenech et al., 1999).

Comet (single cell gel electrophoresis) assay is an attractive, uncomplicated and sensitive test for investi-

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gating the genotoxicity of the environmental mutagens. Comet assay detects DNA breaks, alkaline-labile sites, cross links and transient DNA-strand breaks arising due to DNA repair processes. Moreover, the assay is rapid, simple, and relatively inexpensive and samples can be prepared and evaluated very quickly by visual scoring or by using image analysis systems (Collins et al., 1993; Henderson et al., 1998).

Apigenin, a member of the flavones family of the flavonoid compounds, is widely distributed in many vegetables and fruits including apple, guava, tomato and broccoli. Apigenin is also present in tea and wine (Miean et al 2001). Apigenin possesses diverse pharmacological effects including antioxidant and anticarcinogenic potential. Topical application of apigenin significantly reduced the number of UV-induced benign papillomas and squamous cell carcinomas in SENCAR mice. Apigenin inhibited the growth of several types of cancer cells under in vivo and in vitro conditions (Lepley et al, 1997; Patel et al, 2007).

The present study focuses the antigenotoxic and anticlastogenic potential of apigenin in hamsters treated with DMBA. In the present study, DMBA-induced DNA damage and chromosomal abnormalities were measured at the cellular level using single cell gel electrophoresis (comet assay) and cytogenetic studies (chromosomal aberrations and micronuclei frequency) respectively.

MATERIALS AND METHODS

Animals

Twenty-four male golden Syrian hamsters, 8 weeks old, weighing 80–120 g, were obtained from National Institute of Nutrition, Hyderabad, India and maintained in Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. The animals were housed in polypropylene cages and provided standard pellet diet and water *ad libitum*. The animals were maintained under controlled conditions of temperature and humidity with a 12 h light–dark cycle.

Chemicals

DMBA, colchicine, Giemsa and May-Grunwald's stains were purchased from Sigma-Aldrich Chemical Pvt. Ltd., Bangalore, India. Apigenin was purchased from Shaanxi Sciphar Biotechnology Co. Ltd., China. All other chemicals used were of analytical grade.

Experimental design

The Institutional animal ethics committee (Reg. no.: 160/1999/ CPCSEA), Annamalai University, Annamalainagar, approved the experimental design. A total number of 24 animals were divided into four groups and each group contained six animals. Group 1 animals were served as control. Group 3 animals were pretreated with apigenin (2.5 mg/kg b.w. *p.o*.) for 5 days and were intraperitonealy injected with DMBA (30 mg/kg b.w.) on 5th day after 2 h of administration of apigenin. Group 2 animals were given intraperitoneal injection of DMBA (30 mg/kg b.w.) on 5th day. Group 4 animals were pretreated with apigenin (2.5 mg/kg b.w. *p.o*.) alone for 5 days and did not receive DMBA. All the animals were sacrificed after 24 h of DMBA injection by cervical dislocation for the assessment of micronucleus frequency, chromosomal aberrations and DNA damage.

Assessment of chromosomal aberrations

Assessment of chromosomal aberrations in bone marrow was carried out according to the procedure of Kilian, et al., 1977. The femur bones were removed from control and experimental animals injected with 0.1% colchicine (1 ml/100 g b.w. *i.p*.), 90 min before sacrificing the animals. The bone marrow contents were flushed into 5 ml of physiological saline and centrifuged at 500 X g for 5 min. The sediments obtained were resuspended in 6 ml of hypotonic KCl (0.075 M) and incubated at 37^0C for 25 min. The pellets were then fixed using methanol: acetic acid (3:1, v/v) fixative and stained with Giemsa stain. One hundred well-spread metaphase cells were scored for each animal and structural chromosomal aberrations were observed and recorded (Gulkac MD, et al., 2004).

Bone marrow micronucleus test

Bone marrow micronucleus test was carried out according to the method of Schmid W, 1975. The femur bones removed from the golden Syrian hamsters were cleaned and the content was flushed into tube containing 1 ml of calf serum and was centrifuged at 500 X g for 10 min. The obtained pellet was suspended with few drops of fresh serum and slides were prepared and air-dried for 18 h. After drying, the slides were stained with May-Grunwald stain followed by Giemsa stain. The frequency of micronucleated polychromatic erythrocytes (MnPCEs) in each group was calculated by scoring 2500 polychromatic erythrocytes (PCEs) per animal (Abraham SK,et.al., 2001).

Single-cell gel (comet) assay

The single-cell gel (comet) assay, a rapid, simple, and reliable technique, was used to assess the DNA damage in bone marrow cells (Tice RR, et.al. 2000). The femur bone marrow cells were flushed into Hank's balanced salt solution (HBSS) and then filtered through a 50 µm nylon filter. The cells were counted and diluted to arrive a final suspension of 50,000–1, 00,000 cells/ml. The mixture of 10 ml bone marrow cells and 200 ml of 0.5% low melting point agarose was layered on to precoated slides, which contain 1% normal melting point agarose and then covered with a cover slip. The slides were placed in the chilled lysing solution contain in 2.5 M NaCl, 100 Mm Na2+ EDTA, 100 mM Tris-HCl, pH 10 and 1% DMSO, 1% Triton X 100 and 1% sodium sarcosinate for 1 h at 40C and followed by alkaline buffer (pH> 13) for 20 min. The electrophoresis was carried out for 20 min, at 25 V and 300 mA. The slides were

Table 1: Frequency of MnPCEs in control and experimental hamsters in each group

Groups	Parameters	MnPCEs/2500 PCEs	PCEs/NCEs	PCE (%)[#]
1	Control	5.75 ± 0.42[a]	1.13 ± 0.08[a]	51.73
2	DMBA	41.09 ± 4.96[b]	0.76 ± 0.05[b]	41.33
3	DMBA + Apigenin	16.61± 1.56[c]	0.90 ± 0.08[c]	47.36
4	Apigenin alone	5.71 ± 0.47[a]	0.99 ± 0.07[a]	49.80

Values are expressed as mean ± SD (n = 6; 2500 PCEs were scored per hamsters). Values that do not share a common superscript letter in the same column differ significantly at $p < 0.05$ (DMRT).

[#] Percentage of polychromatic erythrocytes was calculated as follows. (PCEs / (PCEs + NCEs) × 100)

stained with 50 µl of ethidium bromide (20µg/ml) and analysed under fluorescence microscope. The images (25 cells/slide) were viewed under high-performance Nikon camera.

DNA damage

DNA damage, as reflected by % DNA in tail (tail intensity), tail length, tail moment (product of tail DNA/total DNA by the center of gravity) and olive tail moment (the product of the distance between the barycenters of the head and tail and the proportion of DNA in the tail) of the stored images, was investigated from 25 cells per treatment using CASP software. (http://casp.sourceforge.net).

Biochemical estimations

Blood samples were collected into heparinized tubes. Plasma was separated by centrifugation at 1000 X g for 15 min. The buffy coat was removed and the packed cells were washed three times with physiological saline. Liver tissues from animals were washed with ice-cold saline and homogenized using appropriate buffer in an all-glass homogenizer with Teflon pestle and used for biochemical estimations.

Lipid peroxidation was estimated as evidenced by the formation of thiobarbituric acid reactive substances (TBARS). TBARS in plasma were assayed by the method of Yagi K, 1987. Plasma was deproteinised with phosphotungstic acid and treated with thiobarbituric acid at 90 ^{o}C for 1 h. The pink color formed gives a measure of TBARS, which was read at 530 nm. Tissue lipid peroxidation was done by the method of Ohkawa et al. 1979. The color formed by the reaction of thiobarbituric acid with breakdown products of lipid peroxidation was measured colorimetrically at 532 nm. Superoxide dismutase activity in plasma and liver was assayed by the method of Kakkar et al. 1984, based on the 50% inhibition of formation of NADH-phenazine methosulfate nitro-blue tetrazolium (NBT). The color developed was read at 520 nm. One unit of the enzyme is taken as the amount of enzyme required to give 50% inhibition of NBT reduction. The activity of glutathione peroxidase (GPx) in plasma and liver was determined using the method of Rotruck et al., 1973 based on the utilization of reduced glutathione (GSH) by the enzyme. One unit of the enzyme is expressed as micromoles of GSH utilized per minute. The activity of catalase in plasma and liver was assayed by the method of Sinha, 1972 based on the utilization of H_2O_2 by the enzyme. The color developed was read at 620 nm. One unit of the enzyme is expressed as micromoles of H_2O_2 utilized per minute.

The GSH level in liver was determined by the method of Beutler and Kelley,1963.The technique involves protein precipitation by meta-phosphoric acid and spectrophotometric assay at 412 nm of the yellow derivative obtained by the reaction of the supernatant with 5-5'dithiobis-2-nitrobenzoic acid. The levels of cytochrome P_{450} and b5 in liver were determined according to the method of Omura and Sato, 1964. Cytochrome P_{450} was measured by the formation of pigment on reaction between reduced cytochrome P_{450} and carbon monoxide. The pigment was read with an absorbance maximum at 450 nm. The difference spectrum between reduced and oxidised cytochrome was used as an index to measure the level of cytochrome b_5. The activity of GST in liver tissue homogenate was assayed by the method of Habig et al., 1974. GST activity was measured by incubating the tissue homogenate with the substrate 1-chloro-2,4-dinitrobenzene (CDNB). The absorbance was followed for 5 min at 540 nm after the reaction was started by the addition of GSH. Glutathione reductase (GR) activity in liver tissue homogenate was assayed by the method of Carlberg and Mannervik, 1985. The enzyme activity was assayed by measuring the formation of GSH when the oxidized glutathione (GSSG) is reduced by reduced nicotinamide adenine dinucleotide phosphate (NADPH).

Statistical analysis

The data are expressed as mean ± SD. Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT). The results were considered statistically significant if the P values were 0.05 or less.

RESULTS

Tables 1 and 2 shows the frequency of MnPCEs and chromosomal aberrations respectively in control and experimental hamsters in each group. Hamsters treated with DMBA alone(group 2) showed highest frequency of MnPCEs and chromosomal aberrations (chromosomal gap, chromatid break, chromosomal break, fragment, and minute) as compared to control hamsters(group 1). Oral pretreatment of apigenin to DMBA treated hamsters significantly decreased the frequency of MnPCEs and protected chromosomal ab-

Table 2: Mitotic index and chromosomal abnormalities in control and experimental hamsters in each group

Groups	Parameters	Mitotic index (%)[A]	Chromosomal aberrations[B] hamsters $^{-1}$					Total aberration hamsters^{-1}	Abnormal metaphase hamsters^{-1}
			G*	B'	B''	F	M		
1	Control	4.45 ± 0.41[a]	0.49 ± 0.02[a]	1.07 ± 0.06[a]	0[a]	1.28 ± 0.11[a]	0.34 ± 0.02[a]	2.73 ± 0.22[a]	1.54 ± 0.13[a]
2	DMBA	1.83± 0.16[b]	11.91 ± 0.98[b]	5.97 ± 0.45[b]	2.38 ± 0.19[b]	5.97 ± 0.71[b]	2.37 ± 0.22[b]	16.65 ± 1.62[b]	13.96 ± 1.72[b]
3	DMBA + Apigenin	3.13 ± 0.23[c]	3.73 ± 0.29[c]	2.87 ± 0.18[c]	0.65 ± 0.07[c]	3.06 ± 0.19[c]	0.92 ± 0.07[c]	8.21 ± 0.88[c]	6.28 ± 0.61[c]
4	Apigenin alone	4.38 ± 0.44 [a]	0.45 ± 0.05[a]	1.01 ± 0.08[a]	0[a]	0.93 ± 0.07[a]	0.29 ± 0.01[a]	2.62 ± 0.25[a]	1.60 ± 0.27[a]

Values are expressed as mean ± SD; n = 6. Values that do not share a common superscript letter in the same column differ significantly at $p < 0.05$ (DMRT).

G-Gap, B'-Chromatid Break, B''-Isochromatid Break, F-Fragment, M-Minute. A-Mitotic index has been calculated by analyzing 1000 cells/animal (for a total of 6000 cells / treatment) and percentage of the mitotic cells calculated for each treatment group.

B-Frequency per 100 cells. Each chromosomal aberration has been counted by analyzing 100 cells/animal (6 animals/group, for a total of 6000 cells / treatment) and the mean ± SD were calculated per treatment group.

* Gaps were not included in total chromosomal aberration

normalities in their structure. Oral pretreatment of apigenin alone to hamsters displayed no significant differences in MnPCEs frequency and chromosomal aberrations as compared to control hamsters.

Figure 1 shows the extent of DNA damage (% DNA in tail, tail length, tail moment, olive tail moment (table 3)) in the bone marrow cells of control and experimental hamsters in each group. Extensive DNA damage as reflected by an increase in DNA tail length, tail moment, % DNA in tail, and olive tail moment was noticed in hamsters treated with DMBA alone. Oral pretreatment of apigenin significantly protected DNA damage in DMBA treated hamsters. Oral pretreatment of apigenin alone to hamsters showed similar pattern of comet, observed in control hamsters.

The status of TBARS and enzymatic antioxidants in the plasma and liver and detoxification enzymes in the liver of control and experimental hamsters in each group is shown in table 4. TBARS levels, enzymatic antioxidants and detoxification agents activities were increased in hamsters treated with DMBA alone as compared to control hamsters (group 1). Oral pretreatment of apigenin significantly decreased the levels of TBARS, antioxidants and detoxification agents activities in hamsters treated with DMBA (group 3). No significant difference in the status of TBARS, antioxidants and detoxification agents was observed between control hamsters (group 1) and apigenin alone (group 4) treated hamsters.

The activities of detoxification agents (phase I (Cyt P_{450} and Cyt b_5) and II detoxification enzymes (GST and GR) and reduced glutathione (GSH)) in liver of control and experimental hamsters in each group. The activities of detoxification agents were significantly increased in DMBA alone treated hamsters (group 2) as compared to control hamsters (group 1). Oral pretreatment of DMBA-treated hamsters with apigenin brought back the status of detoxification agents to near normal range in group 3. No significant difference was observed between control hamsters (group 1) and apigenin alone (group 4) treated hamsters.

DISCUSSION

Somatic cell mutation due to carcinogenic or mutagenic substances has been implicated in the pathogenesis of several diseases including cancer. It has been well documented that unrepaired DNA damage and misreplication could lead to abnormalities in the chromosomal structure and function. Dihydrodiol epoxide, the ultimate carcinogenic metabolite of DMBA, mediates carcinogenesis and oxidative DNA damage by causing chromosomal abnormalities and mutations in key growth regulatory genes (Shimada et al., 2003; Bhuvaneswari et al., 2004). Accumulated evidences suggested that DMBA treatment caused over expression of C-erB and its product, the epidermal growth factor receptor and H-ras mutation in DMBA induced hamster buccal pouch carcinogenesis (Hussain et al., 1989). Assay of micronucleus frequency and chromosomal aberrations is commonly employed to investigate the anticlastogenic potential of natural products. Micronucleus assay is a valuable complement to detect chromosomal aberration since the presence of one or more MnPCEs in peripheral erythrocytes is an accepted marker of chromosomal breakage or that occurred prior to the extrusion of the nucleus during erythrocyte differentiation (Venkatesh et al., 2007). Altered structural or numerical chromosomal aberrations are usually observed in metaphase-blocked cells using conventional microscopy. An increase in bone marrow micro-

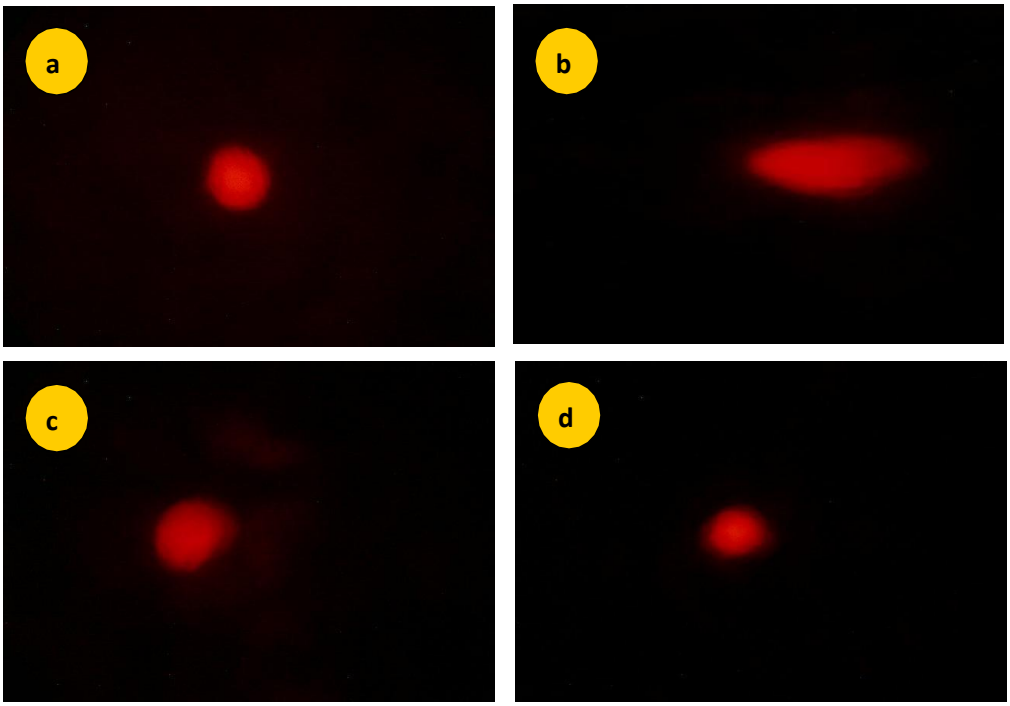

Figure 1: Representative photographs depict the extent of DNA damage in control hamsters

(a), DMBA-treated hamsters (b), DMBA+apigenin-treated hamsters (c) and apigenin alone treated hamsters (d). (40x magnification)

Table 3: Changes in the levels of DNA damage (% DNA in tail, Tail length, Tail moment and olive tail moment) in the bone marrow cells of control and experimental hamsters in each group

Groups	Parameters	(% DNA in tail)	Tail length	Tail moment	olive tail moment
1	Control	0.17 ± 0.01^{a}	3.01 ± 0.26^{a}	0.02 ± 0.003^{a}	0.04 ± 0.003^{a}
2	DMBA	21.9 ± 1.40^{b}	69.02 ± 4.60^{b}	8.58 ± 0.50^{b}	6.34 ± 0.42^{b}
3	DMBA + Apigenin	2.74 ± 0.36^{c}	26.2 ± 1.20^{c}	5.59 ± 0.29^{c}	4.08 ± 0.37^{c}
4	Apigenin alone	0.18 ± 0.01^{a}	3.06 ± 0.24^{a}	0.03 ± 0.004^{a}	0.03 ± 0.002^{a}

Values are expressed as mean ± SD; n = 6. Values that do not share a common superscript letter in the same column differ significantly at $p < 0.05$ (DMRT).

nucleus frequency and chromosomal aberrations is considered as hallmarks of cancer development (Miyamoto et al., 2007). Higher MnPCEs frequency and abnormalities in chromosome structure, numbers and functions were reported in hamster treated with DMBA (Panjamurthy et al., 2008).

Agents that reduce the frequency of MnPCEs and chromosomal aberration in somatic cells are identified as potent anticlastogenic agent. Anticlastogenic compound exert their protective role probably by interfering with covalent interactions of carcinogens with DNA and through antioxidant potential in DMBA-induced clastogenesis. Oral pretreatment of apigenin significantly reduced the frequency of MnPCEs and abnormalities in chromosome structure, which suggest that apigenin has potent anticlastogenic potential during DMBA, induced clastogenesis. The single cell gel electrophoresis (comet) has wide spread application in genotoxicity testing, which measures DNA damage against environmental mutagens. Oral administration of apigenin to DMBA treated hamsters at a dose of 2.5mg/kg bw significantly inhibited the amount of DNA that migrates out of immobilized cells nuclei that are subjected to electrophoresis, as evidenced by the absence of comet appearance. (reduction in % tail DNA, tail movement etc in the comet). The present results suggest that apigenin significantly protected DNA damage (antigenotoxic potential) in DMBA treated hamsters.

Several studies demonstrated increased activities of phase I and phase II detoxification agents in DMBA treated hamsters (Chandra Mohan et al., 2003; Bhuvaneswari et al., 2005). Our results are in line with these findings and suggest that the detoxification cascade in stimulated to detoxify the carcinogenic agent DMBA in DMBA treated hamsters. Apigenin scavenges free radicals and stimulated phase II detoxification enzymes in *in vivo* tumour model. Apigenin stimulated the activity of GST in Wistar rat heart (Lindenmeyer et al., 2001). Oral pretreatment of apigenin to DMBA treated hamsters brought back the status of phase I and phase II detoxification agents, which suggests that apigenin could probably attenuated the metabolic activation of DMBA. Over production of ROS as well as ROS me-

Table 4: TBARS and antioxidants (plasma and liver) and detoxification agents (liver) in control and experimental hamsters in each group

Parameters	Control	DMBA	DMBA + apigenin	Apigenin alone
PLASMA				
TBARS (nmol/ml)	2.10 ± 0.15[a]	3.24 ± 0.36[b]	2.48 ± 0.12[c]	2.02 ± 0.13[a]
GPx (U^A/L)	112.40 ± 10.1[a]	139.38 ± 13.2[b]	124.05 ± 11.05[c]	114.63 ± 9.48[a]
SOD (U^B/ml)	2.66 ± 0.21[a]	4.54 ± 0.40[b]	3.28 ± 0.28[c]	2.69 ± 0.24[a]
CAT (U^C/ml)	0.83 ± 0.08[a]	1.67 ± 0.17[b]	1.09 ± 0.15[c]	0.87 ± 0.09[a]
LIVER				
TBARS (nmol/100 mg protein)	51.25 ± 3.01[a]	85.60 ± 8.80[b]	64.60 ± 6.35[c]	50.75 ± 4.25[a]
GPx (U^A/g protein)	4.53 ± 0.34[a]	9.69 ± 0.99[b]	6.10 ± 0.59[c]	4.44 ± 0.36[a]
SOD (U^B/mg protein)	3.75 ± 0.24[a]	6.03 ± 0.49[b]	4.67 ± 0.38[c]	3.70 ± 0.26[a]
CAT (U^C/mg protein)	21.55 ± 1.90[a]	37.40 ± 2.94[b]	28.40 ± 2.62[c]	20.65 ± 2.10[a]
GSH (nmol ⁄mg protein)	3.50± 0.24[a]	5.21 ± 0.49[b]	4.02 ± 0.40[c]	3.52 ± 0.26[a]
GST (nmol of CDNB conjugate formed ⁄min ⁄mg protein)	135.92± 10.87[a]	182.07± 15.57[a]	156.32± 10.97[a]	137.42±11.07[a]
GR (nmol of NADPH oxidised ⁄ min ⁄mg protein	32.68± 2.34[a]	53.31 ± 5.79[b]	39.57 ± 3.87[c]	31.70 ± 3.05[a]
Cyt P450 (U^X ⁄ mg protein)	0.45± 0.03[a]	0.80 ± 0.09[b]	0.58 ±0.04[c]	0.43 ± 0.03[a]
Cyt b5 (U^X ⁄ mg protein)	0.94± 0.13[a]	1.71 ± 0.09[b]	1.17 ± 0.16[c]	0.95 ± 0.09[a]

Values are expressed as mean ± SD for hamsters in each group. Values that do not share a common superscript letter in the same column differ significantly at $p < 0.05$ DMRT). A - Micromoles of glutathione utilized/min.; B - The amount of enzymes required to inhibit 50% nitroblue-tetrazolium (NBT) reduction; C - Micromoles of H_2O_2 utilized/second; X – Micromoles of cytochrome per gram of tissue.

diated lipid peroxidation occurred during the metabolic activation of carcinogenic or mutagenic substance could lead to genotoxicity and cell death in somatic cells. ROS that are excessively generated during DMBA treatment could induce oxidative stress and DNA damage contributing to mutagenesis and carcinogenesis (Pugalendhi et al., 2009; Ray and Husain 2002). In the present study the status of both lipidperoxidation byproducts (TBARS) and the activities of enzymatic antioxidants are significantly increased in DMBA treated hamsters. Present results suggest that DMBA might have induced oxidative stress in hamsters by generating excess reactive oxygen species. Increase in antioxidant activities in DMBA treated hamsters is probably due to host compromised antioxidant defense mechanism to combat the deleterious effects of ROS. It has been reported that apigenin attenuated oxidative stress by enhancing the intracellular levels of glutathione. Apigenin eliminated the hydroxyl radicals, superoxide radicals or singlet oxygen that are produced during chronic cyclosporine treatment. The antioxidant potential of apigenin lies in the H^+ -donating potential of its aromatic OH-group (Nagaraja et al., 2009). Oral administration of apigenin significantly reduced the levels of TBARS and activities of enzymatic antioxidants, which suggests that apigenin has potent antioxidant function during DMBA induced genotoxicity. Although the exact mechanism of antigenotoxic or anticlastogenic potential of apigenin is unclear, its antioxidant potential and modulating effect on metabolic activation of DMBA as evidenced by the decreased activities of phase I and II detoxification enzymes could play a possible role.

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