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Research Article

Lupeol protects DNA damage against 7,12-dimethylbenz(a)anthracene induced genotoxicity

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ABSTRACT

The present study has investigated the protective effect of lupeol on 7,12-dimethylbenz (a)anthracene (DMBA) induced genotoxicity in the bone marrow cells of golden Syrian hamsters. DNA damage was assessed in the bone marrow cells using single cell gel electrophoresis (comet assay) in experimental hamsters. Lipid peroxidation and antioxidants status in plasma was utilized as biochemical endpoints to assesses the antigenotoxic effect of lupeol during DMBA induced genotoxicity. Extensive DNA damage with marked abnormalities in the status of lipid peroxidation and antioxidants was noticed in hamsters treated with DMBA alone. Oral pretreatment of lupeol to DMBA-treated hamsters not only protected DNA damage, but also restored the status of lipid peroxidation and antioxidants. The present study thus suggests that lupeol exhibited antigenotoxic potential against DMBA-induced genotoxicity, which is probably due to its antioxidant property.

Keywords: Lupeol; DMBA; Genotoxicity; Lipid peroxidation; Antioxidants; Comet assay

INTRODUCTION

Genotoxicity assays are commonly employed to assess the genotoxic potential of carcinogens as well as the antigenotoxic potential of natural products (Rampal, et al., 2010). Single cell gel electrophoresis (Comet assay) a rapid, simple and reliable technique, is widely used to identify the genotoxicity of harmful drugs and chemicals. Comet assay detects DNA damage based on the appearance of comet. This assay can detect carcinogen induced DNA single strand breaks, cross links and alkali labile sites (Wen, et al., 2011).

7,12-dimethylbenz(a)anthracene (DMBA), a potent site specific procarcinogen , is converted into its ultimate carcinogenic metabolite, dihydrodiol epoxides in the liver. During metabolic activation of DMBA reactive oxygen species are excessively generated, which can cause oxidative base damage and strand breaks in DNA (Manoharan, et al., 2010b). DMBA induced DNA damage and mutations has been well documented in experimental animal models (Manoharan, et al., 2010a). DMBA induced mutagenic response has been shown both *in vitro* and *in vivo* mutation assay systems (Ashby, et al., 1993). Extensive studies also demonstrated DMBA-induced DNA damage in the bone marrow cells of golden Syrian hamsters (Balakrishnan, et al., 2008; Sindhu and Manoharan, 2010). DMBA-induced H-ras and N-ras mutations have been shown in experimental carcinogenesis (Vairaktaris, et al., 2007; Wei, et al., 2002).

Reactive oxygen species at physiological concentrations play vital role in various signaling pathways and in phagocytosis. Human body has an endogenous antioxidant [superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT)] defense system to combat the deleterious effects of excessively generated reactive oxygen species during pathological conditions. Any disturbances in the homeostasis between oxidant and antioxidant status leads to a condition known as oxidative stress, which has been implicated in the pathogenesis of almost all diseases including cancer (Silvan, et al., 2011).

Lupeol (figure 1), a bioactive pentacyclic triterpene, is found in vegetables (white cabbage, pepper, cucumber, tomato), fruits (mango, red grapes, strawberry) and in several medicinal plants (*Tamarindus indica, Bombax ceiba* etc). Lupeol has diverse pharmacological and biochemeical effects, which include anti-inflammatory, anticancer, antidiabetic, hepatoprotective and antioxidant properties (Prasad, et al., 2007). Lupeol exhibited antimutagenic potential both under *in vivo* and *in vitro* conditions (Saleem, et al., 2008). Oral administration of lupeol to animals at a dose of 2g/kg b.w produced no adverse effects and mortality (Patocka, 2003). Lupeol has thus gained wide attention of the researchers due to its diverse pharmacological and therapeutic effects.

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The present study was designed to focus the antigenotoxic potential of lupeol in DMBA-induced genotoxicity, due to the reason that there were no scientific studies on the antigenotoxic effect of lupeol against DMBA-induced genotoxicity in the bone marrow cells of golden Syrian hamsters.

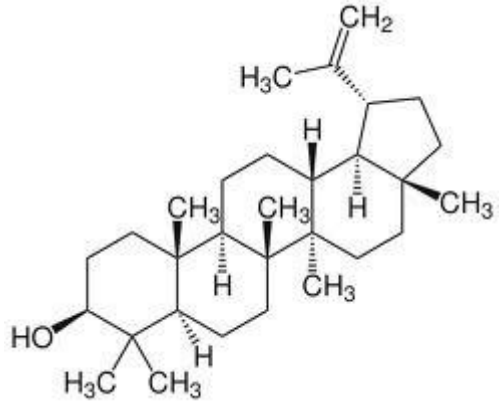

Figure 1: Chemical structure of lupeol

MATERIALS AND METHODS

Animals

Twenty-four male golden Syrian hamsters, 8 weeks old, weighing 80-120g, 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 a12 h light–dark cycle.

Chemicals

DMBA, Lupeol and Colchicine were purchased from Sigma Aldrich Chemical Pvt. Ltd., Bangalore, India. All other chemicals and reagents 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 hamsters were divided into four groups and each group contained six hamsters. Group 1 hamsters were served as control. Group 3 hamsters were pretreated with lupeol (50 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 lupeol. Group 2 hamsters were given intraperitoneal injection of DMBA (30 mg/kg b.w.) on 5th day. Group 4 hamsters were pretreated with lupeol (50 mg/kg b.w. p.o.) alone for 5 days and did not receive DMBA. All the hamsters were sacrificed after 24 h of DMBA injection by cervical dislocation for the assessment of micronucleus frequency and DNA damage.

Single cell gel electrophoresis assay/ Comet assay

The single cell gel electrophoresis (comet) assay, a rapid, simple, and reliable technique, was used to assess the DNA damage in the bone marrow cells (Tice, 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 µl bone marrow cells and 200 µl of 0.5% low melting point agarose was layered onto pre-coated 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 stained with 50 µl of ethidium bromide (20 µg/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).

Table 1: Changes in the levels of DNA damage (% DNA in tail, tail length, tail moment and olive tail moment) in the hamsters bone marrow cells

Group	Parameters	(% DNA in tail)	Tail length	Tail moment	Olive tail moment
1	Control	0.15 ± 0.04^{a}	3.49 ± 0.23^{a}	0.04 ± 0.003^{a}	0.06 ± 0.007^{a}
2	DMBA	11.85 ± 1.02^{b}	53.25 ± 4.49^{b}	6.78 ± 0.47 ^{b}	6.11 ± 0.56^{b}
3	DMBA+ lupeol	2.96 ± 0.12^{c}	15.89 ± 1.65^{c}	2.35 ± 0.17 ^{c}	3.51 ± 0.61^{c}
4	Lupeol alone	0.21 ± 0.01^{a}	3.96 ± 0.25^{a}	0.03± 0.002^{a}	0.09 ± 0.008^{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)

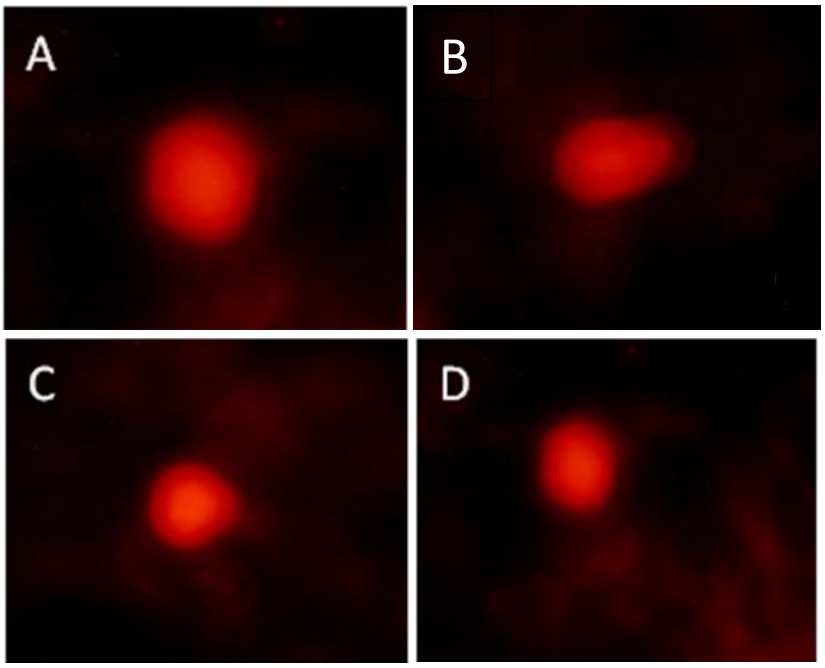

Figure 2: Representative photographs depict the extent of DNA damage

in control (a), DMBA treated hamsters (b), DMBA + Lupeol treated hamsters (c) and Lupeol alone treated hamsters (d) (× 40 magnification).

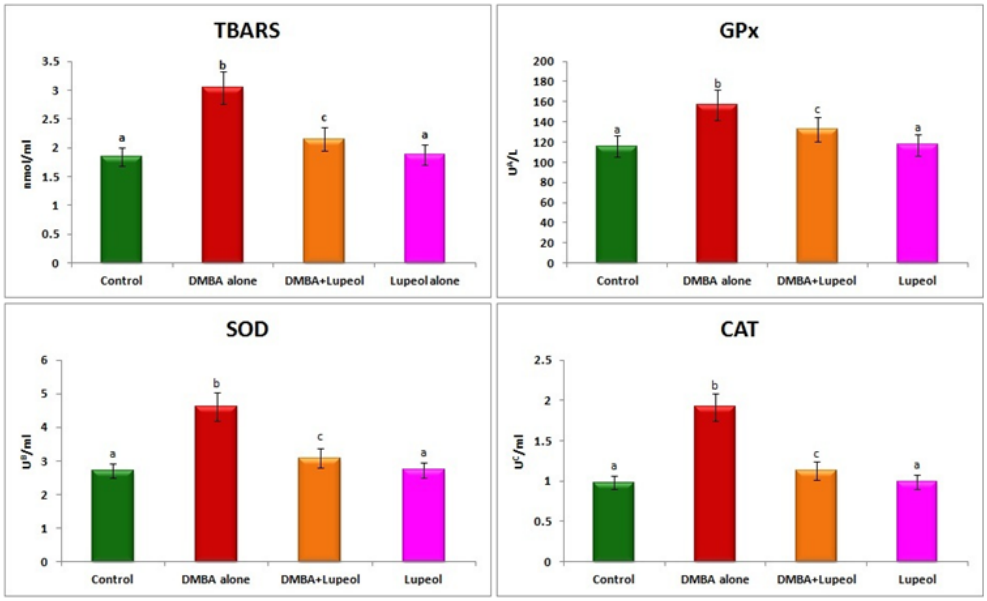

Figure 3: Plasma TBARS and antioxidants in control and experimental hamsters in each group

Values are expressed as mean ± SD for 6 animals 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.

Biochemical estimations

Blood samples were collected into heparinized tubes. Plasma was separated by centrifugation at 1000 × g for 15 min. The buffy coat was removed and the packed cells were washed three times with physiological saline. Lipid peroxidation was estimated as evidenced by the formation of TBARS. TBARS in plasma was assayed by the method of Yagi (1987). The activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) in plasma was determined according to the methods of Kakkar et al (1984), Rotruck et al (1973) and Sinha (1972) respectively.

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

Table 1 shows the extent of DNA damage (% DNA in tail, tail length, tail moment, olive tail moment) in the bone marrow cells of control and experimental hamsters in each group. We observed an increase in DNA tail length, tail moment, % DNA in tail and olive tail moment in hamsters treated with DMBA alone (group 2). Oral pretreatment of lupeol significantly protected DNA damage in DMBA treated hamsters. Hamsters treated with lupeol alone and control hamsters displayed no significant difference in % DNA in tail, tail length, tail moment and olive tail moment as compared to control hamsters.

The appearance of comet during single cell gel electrophoresis in control and experimental hamsters is shown in figure 2. We noticed a comet head with tail in hamsters treated with DMBA alone (group 2). The length of tail was significantly smaller in DMBA + lupeol treated hamsters (group 3). Hamsters treated with lupeol alone (group 4) and control hamsters (group 1) displayed similar pattern of comet appearance.

Figure 3 shows the status of TBARS and enzymatic antioxidants (SOD, CAT, GPx) in the plasma of control and experimental hamsters in each group. TBARS and enzymatic antioxidants were increased in hamsters treated with DMBA alone (group 2) as compared to control hamsters. Oral pretreatment of lupeol significantly decreased the levels of TBARS and enzymatic antioxidants activities in hamsters treated with DMBA (group 3). No significant difference was observed between control hamsters (group 1) and lupeol alone treated hamsters (group 4).

DISCUSSION

Natural triterpenoids, also known as phytosterols, are important components of human diets. A number of triterpenoids exhibited anti-cell proliferative effect against various cancer cell lines (He, et al., 2011). A potent antigenotoxic agent should have the property to inhibit DNA adduct formation and stimulate DNA repair mechanism and to scavenge excessively generated reactive oxygen species (Nigam, et al., 2007). In the present study, oral pretreatment of lupeol at a dose of 50mg/kg bw to hamsters treated with DMBA not only prevented DNA damage in the bone marrow cells, but also inhibited lipid peroxidation and improved the antioxidant defense mechanism. Lupeol inhibited chemical-induced DNA damage under *in vitro* conditions (Sultana, et al., 2003). It has also been reported that lupeol prevented DMBA-induced DNA strand breaks in the mouse skin (Nigam, et al., 2007).

The present study has observed a comet head with long tail in hamsters treated with DMBA alone. The results of the present study thus suggest that the DNA is extensively damaged in DMBA treated hamsters as evidenced by increase in the DNA migration upon electrophoresis. Oral pretreatment of lupeol to DMBA treated hamsters significantly inhibited the migration of DNA from the comet head, as evidenced by short DNA tail formation upon electrophoresis. The present study thus suggests that lupeol exhibited pronounced antigenotoxic effect during DMBA-induced genotoxicity.

Reactive oxygen species are among the most intracellular modifiers of DNA, if they are excessively generated. Extensive studies have shown an increase in TBARS and enhanced antioxidants activities during DMBA-induced genotoxicity (Baskaran, et al., 2011; Pugalendhi, et al., 2009). Our results are in line with these findings. Overproduction of lipid peroxidation by-products accompanied by compromised increase in antioxidants activity confirm oxidative stress in hamsters treated with DMBA alone (Manoharan, et al., 2010a). Oral administration of lupeol restored the status of lipid peroxidation and improved the antioxidant defense mechanism in DMBA treated hamsters, which suggests its free radical scavenging potential during DMBA-induced genotoxicity. The present study thus demonstrates the antigenotoxic potential of lupeol against DMBA-induced genotoxicity. The antigenotoxic potential of lupeol is probably due to its free radical scavenging property.

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