

·Complementary Medicine·

Protective effects of lupeol and mango extract against androgen induced oxidative stress in Swiss albino mice

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Abstract

Aim: To investigate antioxidant potential of lupeol/mango pulp extract (MPE) in testosterone induced oxidative stress in prostate of male Swiss albino mice. **Methods:** Oral treatment of lupeol (1 mg/animal) and MPE (1 mL [20% w/v]/ animal) was given separately to animals along with subcutaneous injection of testosterone (5 mg/kg body weight) consecutively for 15 days. At the end of the study period, the prostate was dissected out for the determination of reactive oxygen species (ROS) levels, lipid peroxidation and antioxidant enzymes status (catalase, superoxide dismutase, glutathione reductase, glutathione-S-transferase). **Results:** In testosterone treated animals, increased ROS resulted in depletion of antioxidant enzymes and increase in lipid peroxidation in mouse prostate. However, lupeol/MPE treatment resulted in a decrease in ROS levels with restoration in the levels of lipid peroxidation and antioxidant enzymes. **Conclusion:** The results of the present study demonstrate that lupeol/MPE are effective in combating oxidative stress-induced cellular injury of mouse prostate. Mango and its constituents, therefore, deserve study as a potential chemopreventive agent against prostate cancer. (*Asian J Androl 2008 Mar; 10: 313–318*)

Keywords: mango pulp extract; lupeol; testosterone; oxidative stress; antioxidant enzymes; prostate cancer

1 Introduction

Pro-oxidants or free radicals are generated in our body during the normal metabolic processes and during exposure to adverse pathophysiological conditions [1]. Because of their high chemical reactivity, free radicals are able to induce cellular damage in a variety of ways. The most deleterious effects of free radicals are damage to DNA [2], which is associated with the process of carcino-

genesis. Cells have multiple protective mechanisms against oxidative stress and can prevent cell damage [3]. These protective mechanisms act through antioxidant enzymes (e.g. superoxide dismutase [SOD], catalase [CAT], glutathione reductase [GR] and glutathione S-transferase [GST]). SOD and CAT are considered to be primary antioxidant enzymes because they involve direct elimination of free radicals and reactive oxygen species (ROS). GR and GST are secondary antioxidant enzymes, which help in detoxification of ROS and by decreasing peroxide levels or by maintaining a steady supply of metabolic intermediates, such as glutathione [4].

Many dietary constituents, ranging from antioxidant vitamins and minerals to food additives, are important sources of antioxidants. Mango is a fruit consumed worldwide. The beneficial health effects of mango pulp

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are the antilithiatic and free radical scavenging properties, which reduce lipid peroxidation and enhance antioxidant enzymes (SOD and CAT) against isoproterenol (reported in kidney and heart of rats [5]). Antimutagenic properties of mango extract were investigated using mutagenicity assay with *Salmonella typhimurium* strain TA1538 against the heterocyclic amine 2-amino-3 methylimidazole (4,5-f) quinoline (IQ) [6]. Chemical analysis of mango pulp extract (MPE) has shown that it contains vitamins, organic acids, carbohydrates, amino acids, polyphenols and certain volatile compounds [7]. Lupeol (Lupa-21, 20 [29] dien 3 beta-ol) is a naturally occurring pentacyclic triterpene present in mango pulp and other fruits that exhibits strong anti-inflammatory, anti-arthritic, antimutagenic and anti-malarial activity. Lupeol has been also shown to possess antitumor promoting effects in mouse skin tumorigenesis [8]. The oral administration of lupeol can change the tissue redox system induced by cadmium exposure by scavenging the free radicals and by improving the antioxidant status of the rat liver [9]. Lupeol/MPE supplementation has been shown to effectively influence dimethylolbutanoic acid induced oxidative stress in liver, characterized by restored antioxidant enzyme activities and decreases in lipid peroxidation [10].

Androgens are the key factors in the initiation or progression of prostate cancer by inducing oxidative stress. Therefore, the present study was designed to evaluate the effect of pretreatment with MPE and lupeol on testosterone-induced oxidative stress. The alteration in lipid peroxidation, status of the antioxidant enzymes (SOD, CAT, GST, GR) and the level of ROS were used as intermediate biomarkers of chemoprevention in the prostate of Swiss albino mice.

2 Materials and methods

2.1 Chemicals

Testosterone, lupeol, dichlorodihydrofluorescein diacetate dye (DCFH-DA), phenazine methosulfate, 1-chloro-2, 4-dinitrobenzene (CDNB), 2-thiobarbituric acid, 1,1,3,3-tetramethoxy propane (TMP), nitro blue tetrazolium, nicotinamide adenine dinucleotide phosphate reduced, nicotinamide adenine dinucleotide reduced, reduced glutathione (GSH) and oxidized glutathione were obtained from Sigma Chemical Company (St. Louis, MO, USA). The rest of the chemicals used in the present study were of analytical grade and procured locally.

2.2 Preparation of mango pulp extract

The pulp (20 g) of ripened mango fruit was homogenized with 100 mL of distilled water. The resulting homogenate was filtered through four layered muslin cloth and then centrifuged at $4\,000 \times g$ for 5 min at room temperature to collect the supernatant as the crude extract of mango pulp.

2.3 Animal and treatment

Male, Swiss albino mice (25 ± 2 g body weight) were taken from Industrial Toxicology Research Centre (ITRC) animal colony of India and acclimatized for 1 week. They were randomly divided into six groups, each consisting of 6 animals. Animals were kept under standard condition ($25 \pm 2^\circ\text{C}$, relative humidity $57\% \pm 2\%$ and 12 h:12 h light : dark phase) and were fed with synthetic pellet diet (Ashirwad, Chandigarh, India) and water *ad libitum*. Mice in group I were fed with normal drinking water whereas animals in group III and V were given lupeol (1 mg/mouse, dissolved in minimal amount of ethanol and diluted in corn oil) orally through gavage for 15 consecutive days. Animals of group IV and VI were given MPE (1 mL/mouse) orally through gavage for 15 consecutive days. Testosterone (5 mg/kg body weight dissolved in ethanol and diluted in corn oil) was given in 200 μL of corn oil to animals of groups II, III and IV subcutaneously. The feeding regimen was followed for 15 days. Animals from all the groups were examined every day for gross morphological changes during the entire study period. On day 16, all the animals were sacrificed humanly by cervical dislocation. Prostate from each animal were excised, weighed and immediately washed with ice cold saline. The tissues were homogenized in ice cold phosphate buffer (pH 7.4) containing 0.15 mol/L KCl and taken as enzyme source.

2.4 Biochemical estimation

Cu, Zn-SOD was analyzed as per the protocol of Kakkar *et al.* [11]. The SOD activity was expressed as specific activity in units/min/mg protein. One unit of enzyme activity is defined as the quantity of Cu, Zn-SOD required to inhibit 50% of reaction. The activity of CAT was analyzed according to the method of Sinha [12] using H_2O_2 as substrate. The enzyme activity measured following the disappearance of H_2O_2 at 570 nm and expressed as μmol of H_2O_2 consumed/min/mg protein. GR activity was determined by the procedure of Carlberg and Mannervic [13]. The activity was expressed as nmol

NADPH consumed/min/mg protein. GST activity was analyzed by the method of Habig *et al.* [14]. The activity was expressed as nmol CDNB-GSH conjugate/min/mg protein. Lipid peroxidation was analyzed by the method of Ohkawa *et al.* [15]. The peroxides were expressed as nmol of thiobarbitric acid reactive substance (TBARS)/h/mg of tissue protein using TMP as standard. The protein content of the tissue was determined by the method of Lowry *et al.* [16] using bovine serum albumin as standard at 660 nm.

2.5 Flowcytometric analysis of ROS level

ROS production was monitored by flow cytometry (BD-LSR II, San Jose, CA, USA) using DCFH-DA dye as described by Degli Esposti and McLennan [17]. The fluorescence, increased due to the hydrolysis of DCFH-DA to dichlorodihydrofluorescein (DCFH) by some non-specific cellular esterases and its subsequent oxidation by peroxides, was measured. Values were given in terms of mean fluorescence intensity (MFI) using software 'cell quest'.

2.6 Statistical analysis

Significance difference of variance in antioxidant level data between positive control (group II) and experimental groups (groups III-VI) was analyzed using paired *t*-test and $P < 0.05$ was considered significant.

3 Results

On the basis of the results obtained, we reported that lupeol/MPE possess protective effects against testosterone induced alterations in mouse prostate. In testosterone alone treated animals (group II), the mean prostate weight was significantly ($P < 0.05$) increased by (46.4 mg) approximately twice to normal animals (23.8 mg) having no treatment (group I) (Figure 1). Both lupeol (group III) and MPE (group IV) significantly ($P < 0.05$) reduced the growth of prostate by (34.2 mg) ~ 1.35 and (37.1 mg) ~ 1.25 times respectively in comparison to testosterone alone treated group II (Figure 1). As expected, lupeol and MPE alone showed no significant alterations in any parameters studied, when compared with untreated controls indicating their non-toxic nature at the doses given here.

3.1 Level of antioxidant enzymes

Testosterone treatment (group I) significantly (P

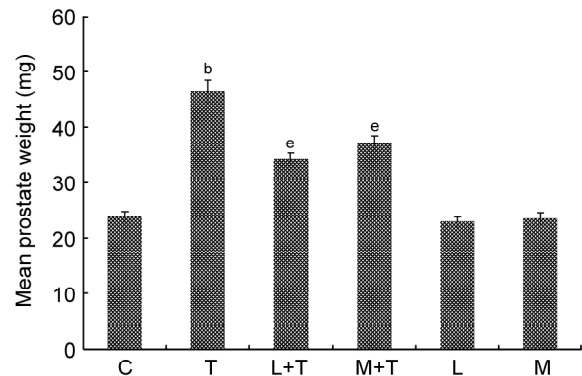


Figure 1. Effects of lupeol/mango pulp extract (MPE) on prostate enlargement. Testosterone treatment increased the mean prostate weight which was decreased by supplementation of lupeol/MPE (untreated control [C], testosterone alone [T], MPE [M], lupeol [L]). Data are mean \pm SE of six animals. ^b $P < 0.05$, compared with control; ^e $P < 0.05$, compared with testosterone treated group.

< 0.05) lowered the antioxidant enzymes Cu, Zn-SOD, CAT, GR and GST levels by 51.3%, 32.5%, 41.2% and 39.0%, respectively, in comparison to untreated control group I (Figure 2). Both lupeol and MPE were found to effective in restoration of antioxidant enzymes. Lupeol significantly restored antioxidant enzymes Cu, Zn-SOD, CAT, GR and GST levels by 58.3%, 30.5%, 32.6% and 50.3%, respectively, and MPE by 55.8%, 22.4%, 22.4% and 45.8%, respectively, in comparison to testosterone treated group II (Figure 2).

3.2 Status of lipid peroxidation

Testosterone treatment significantly ($P < 0.05$) increased the levels of lipid peroxidation by 90.1% in mouse prostate when compared to untreated group I (Figure 3). However, supplementation of lupeol (group III) and MPE (Gr. IV) depleted the lipid peroxidation by 36.6% and 23.1%, respectively, in comparison to testosterone treated group II (Figure 3).

3.3 Level of ROS

The intracellular ROS level was determined in terms of mean fluorescence intensity (MFI) of 2,7'-dichlorofluorescein by using flow cytometry. In mouse prostate, testosterone treatment (group II) significantly ($P < 0.05$) increased the ROS level (MFI 84.12) in comparison to untreated control group I (MFI 40.94) (Figure 4). This increased levels of ROS was reduced significantly ($P < 0.05$) by both lupeol (MFI 57.40) and MPE (MFI 66.48)

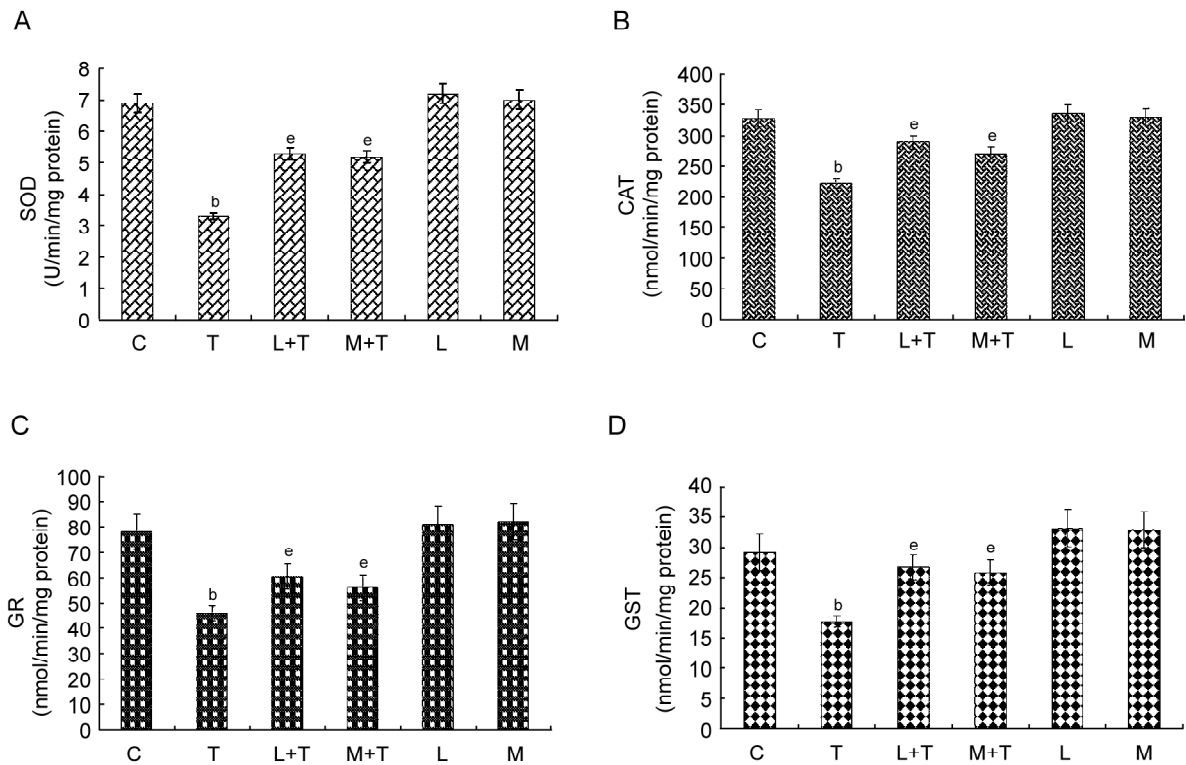


Figure 2. Biochemical analysis of testosterone induced inhibition of antioxidant enzymes. (A): superoxide dismutase (SCD), (B): catalase (CAT), (C): glutathione reductase (GR) and (D): glutathione-S-transferase (GST). Treatment of lupeol/mango pulp extract (MPE) significantly restored the antioxidant enzymes in mouse prostate. Vertical axis represents the activity and horizontal axis shows different control and treated group. Values are expressed as mean \pm SE of six animals. ^b*P* < 0.05, compared with untreated control group; ^e*P* < 0.05, compared with testosterone treated group. C, untreated control; T, testosterone (5 mg/kg body weight); L, lupeol; M, MPE (1 mL 20% [w/v]/mouse).

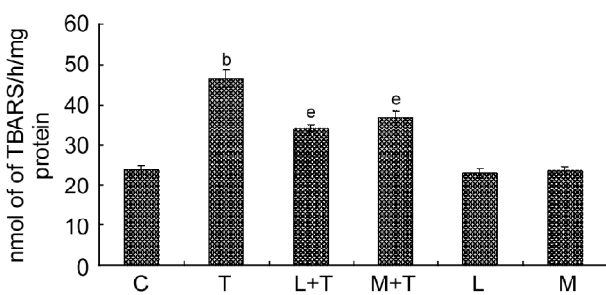


Figure 3. Biochemical analysis of testosterone induced increase of lipid peroxidation. Supplementation of lupeol/MPE significantly restored the TBARS level in mouse prostate. Vertical axis represents the level of TBARS and horizontal axis shows different control and treated group. Values are expressed as mean \pm SE of six animals. ^b*P* < 0.05, compared with untreated control group; ^e*P* < 0.05, compared with testosterone treated group. C, untreated control; T, testosterone (5 mg/kg body weight); L, lupeol; M, MPE (mango pulp extract, 1 mL 20% [w/v]/mouse).

supplementation (group V) in prostate (Figure 4) when compared with testosterone treated group II. However, lupeol (MFI 37.56)/MPE (MFI 38.02) alone showed no significant change in ROS level in comparison to control (group I).

4 Discussion

Benign prostate hyperplasia (BPH) and prostate cancer are considered problems of public health. The present study demonstrated that lupeol/MPE reduced prostate weight in adult male Swiss albino mice in which BPH has been induced by testosterone. Finasteride, an elective drug for BPH, and red maca extract has been shown to reduce prostate size in male rats in which BPH had been induced by testosterone enanthate [18]. Because of this, lupeol/MPE could become an important alternative for the treatment of BPH.

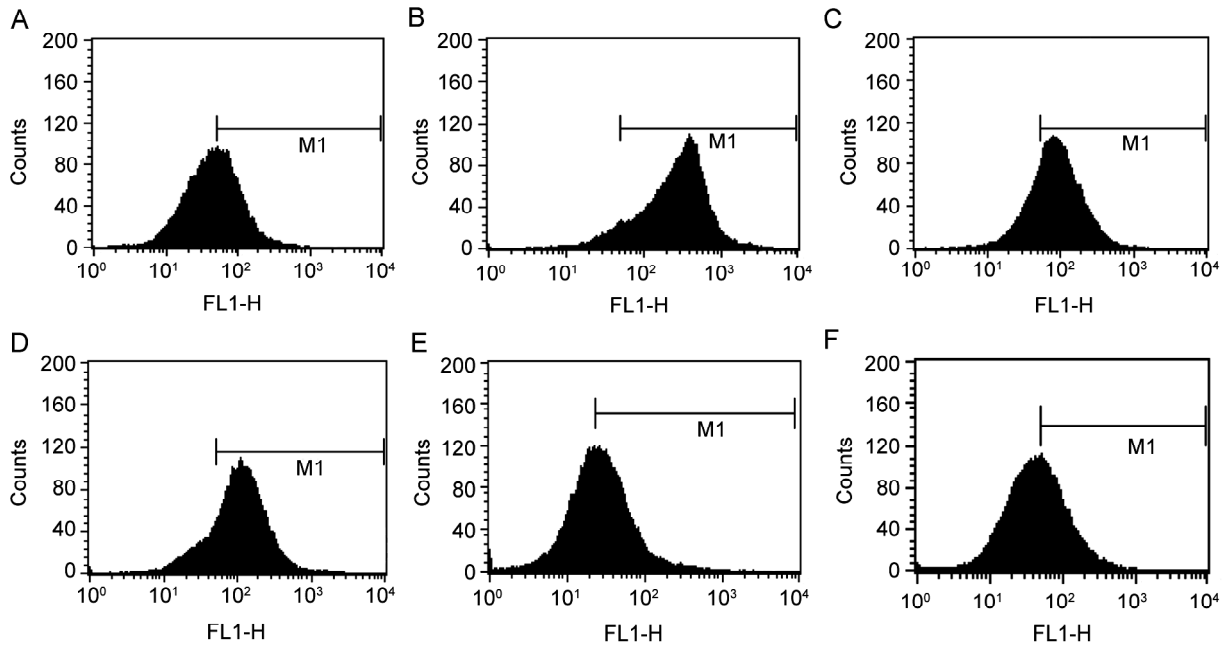


Figure 4. A flow cytometric analysis of reactive oxygen species (ROS) generation showing testosterone induces significant production of ROS (B) in comparison to untreated control (A), which was quenched by lupeol/mango pulp extract (MPE) (C and D) in mouse prostate. Treatment of lupeol/MPE alone (E and F) causes no significant change in production of ROS in comparison to untreated control. The horizontal axis represents dichlorodihydrofluorescein diacetate dye (DCFH-DA) fluorescence. M1, marker 1; FL1-H, filter 1H.

Oxidative stress due to overproduction of radical non-radical ROS is inactivated by SOD, CAT, GR and GST [19]. SOD converts superoxide radicals into hydrogen peroxide, which in turn has to be removed by CAT and GR [20]. Here we observed a significant ($P < 0.05$) decline in the levels of antioxidant enzymes and enhancement of lipid peroxidation after testosterone administration due to its oxidative stress. However, pretreatment with both MPE and lupeol effectively reduced the frequency of occurrence of testosterone induced lipid peroxidation and enhanced the level of antioxidant enzymes SOD, CAT, GR and GST in prostate. GST perform function ranging from catalyzing the detoxification of electrophilic species including the metabolites of genotoxic and nongenotoxic compounds via a spontaneous enzyme catalyzing conjugation reaction to protect the cells against peroxidative damage [21]. The reduced activity of GST observed in present study may be partly due to the lack of its substrate GSH, as it plays an important role in the detoxification of xenobiotic compounds and scavenging of ROS. This reduction of GST level with testosterone administration was prevented by supplementation of lupeol and MPE in our study.

Lipid peroxidation is one of the main manifestations of oxidative damage initiated by ROS and it has been linked to the altered membrane structure and enzyme inactivation. It is initiated by the abstraction of a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane [22] are considered to enhance the process carcinogenesis. The present data shows that testosterone administration produced marked oxidative impact as evidenced by significant ($P < 0.05$) decrease in antioxidant enzymes and increase in lipid peroxidation, which may in all probability to increased production of free radicals. Both lupeol and MPE treatment, in the present study significantly lowered the lipid peroxidation, free radical generation and increased antioxidant enzymes. Results of the present study indicate protective effects of both lupeol and MPE against testosterone-induced oxidative stress. By increasing the GPx and SOD activity, that removes peroxides and superoxides [23], both lupeol and MPE may prevent the accumulation of ROS in our study by trapping them. These modulations in the antioxidant enzymes system which upregulate the host detoxification process may be associated with reduced risk of prostate cancer.

Thus, these findings may open novel prospective in cancer chemoprevention. Taken together, this study has demonstrated that lupeol/MPE have ROS scavenging property. The data also imply that ROS and antioxidant enzymes can be used as targets for studies on prevention of different type of cancer and that lupeol/MPE merits further investigations for developing strategies against carcinogenesis.

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