CHAPTER 5

ANTIGENOTOXIC EFFECT OF LUPEOL USING CANCER INDUCED MOUSE MODEL

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).

Techniques employed to assess mutagenic and DNA damaging effects include, the Salmonella mutagenecity assay (Ames test) and the single cell electrophorosis assay (SCE or Comet assay). Using known mutagenic compounds as “positive controls”, it is possible to study whether specific dietary components can reduce DNA damage.

Comet assay is a simple technique used for the detection of DNA damage at the level of eukaryotic cell. It is useful for the evaluation of DNA damage / repair, biomonitoring and genotoxicity testing. In the present study the therapeutic potential of lupeol was evaluated in cancer induced mouse model.

Materials and Methods

Antigenotoxic effect of Lupeol using Comet Assay

Animals

Twenty-four male golden Syrian hamsters, 8 weeks old, weighing 80–120g, were obtained from National Institute of Nutrition, Hyderabad, India. 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**

7, 12-dimethylbenz (a)anthracene (DMBA), Hank’s balanced salt solution (HBSS), EDTA, Ethidium bromide, DMSO, melting point agarose, were purchased from Sigma Aldrich Chemical Pvt. Ltd., Bangalore, India. All other chemicals used were of analytical grade.

**Experimental design**

The 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 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 2hrs of administration of Lupeol. Group 2 animals were given intraperitoneal injection of DMBA (30 mg/kg b.w.) on 5th day. Group 4 animals were pretreated with Lupeol (50 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 and DNA damage.

**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 et al., 2000). The femur bone marrow cells were flushed into Hank’s balanced salt solution (HBSS) and then filtered through a 50 mm 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 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 containing in 2.5 M NaCl, 100 mM Na$^{2+}$ EDTA, 100 mM Tris-HCl, pH 10 and 1% DMSO, 1% Triton X 100 and 1% sodium sarcosinate for 1hr at 4°C 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 mL of ethidiumbromide (20 mg/mL) and analysed under fluorescence microscope. The images (25 cells/slide) were viewed under high performance Nikon camera.

**Result and discussion**

Fig.52 (A-D) showed the extent of DNA damage (% DNA in tail, tail length, tail moment olive tail moment (Table 14) in bone marrow cells of control and experimental animals 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 [1B]. Oral pretreatment of Lupeol significantly protected DNA damage in DMBA treated hamsters [1C]. Oral pretreatment of alone [1D] to hamsters showed similar pattern of comet, observed in control hamsters [1A].

Present study emphasized the antigenotoxic effects of lupeol in DMBA treated hamsters. DMBA induced pronounced mutagenic response in several *in vivo* and *in vitro* mutation assay systems. DMBA produced H-ras and N-ras mutations in experimental carcinogenesis. In the present study the amount of DNA liberated from the tail of the comet was used to assess the extent of DNA damage. A clear comet was noticed with head and tail in hamsters treated with DMBA alone, which suggested that DNA was
extensively damaged in the bone marrow cells of DMBA treated hamsters. Present study suggested that an imbalance in oxidant-antioxidant status could account for increased DNA damage in the bone marrow cells of DMBA treated hamsters.

Oral pretreatment of Lupeol to DMBA treated hamsters significantly suppressed the appearance of tail in the comet and percentage of DNA in tail. The results suggested the potent antigenotoxic effect of lupeol, as evidenced by less damaged DNA, in the bone marrow cells of DMBA treated hamsters (i.e.) The cancerous changes induced by DMBA was suppressed by lupeol.

“Percentage DNA in tail” was used as the indicator of DNA damage by Mastaloudis et al., 2004. Using this technique, the DNA damage in leucocytes, induced by the extreme exercise of an ultramarathon, increased transiently at midrace but returned to normal after 2hrs, indicating the exercised induced non-persistent DNA damage. Interestingly using comet assay it was proved that endurance exercise resulted in DNA damage and antioxidants seemed to enhance recovery in women but not in men.

Comet assay is useful in many DNA damage related studies. Process which introduce single-strand gaps in the DNA, such as incomplete excision repair events, are readily detectable, in addition to direct DNA damage.
Table – 14: 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 animals in each group

<table>
<thead>
<tr>
<th>Groups</th>
<th>% DNA in Tail</th>
<th>Tail length</th>
<th>Tail moment</th>
<th>Olive tail moment</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.11 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.08 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.01 ± 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.03 ± 0.002&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>DMBA alone</td>
<td>18.26 ± 1.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.59 ± 2.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.39 ± 0.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.18 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DMBA + Lupeol</td>
<td>2.37 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.14 ± 2.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.34 ± 0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.60 ± 0.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lupeol alone</td>
<td>0.16 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02 ± 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02 ± 0.002&lt;sup&gt;a&lt;/sup&gt;</td>
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Values are expressed as mean ± SD (n = 6).

Values that are not sharing a common superscript letter in the same column differ significantly differ at p < 0.05 (DMRT).
Fig. 52. [A-D] - Representative photographs depict the extent of DNA damage in control and experimental animals in each group.

(A) Control, (B) DMBA, (C) DMBA + Lupeol, (D) Lupeol Alone