



## RESEARCH ARTICLE

## Antigenotoxic effect of lupeol isolated from sesbaniagr and ifloraleaves using cancer induced mouse model

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### Abstract

*Sesbaniagrandidiflora* leaves were collected from maruthuvalmalai in kanyakumari district (Tamil Nadu, India) and identified by a plant taxonomist. Isolation of chemical components of methanol extract was done. The isolated compounds were identified as lupeol (C<sub>30</sub>H<sub>50</sub>O), kemferol (C<sub>15</sub>H<sub>10</sub>O<sub>6</sub>) and gallic acid (C<sub>7</sub>H<sub>6</sub>O<sub>5</sub>). The compound Lupeol is subjected to Genotoxicity and Comet assay. In the present study the therapeutic potential of lupeol was evaluated in cancer induced mouse model. The results reveal that cancerous changes induced was suppressed by lupeol.

### Keywords

Lupeol

Genotoxicity

Gallic acid

Comet assay

### Introduction

*Sesbaniagrandidiflora* is a loosely branched tree grows up to 15 m height. Its leaves are pinnately compound up to 30 cm long with 20-50 leaflets in pairs, dimensions 12.44 x 5.15 mm and oblong to elliptical in shape. Flowers are large, white, yellowish, rose pink or red with a calyx of 15-22 mm long, the standard dimensions up to 10.5 x 6 cm. Pods are long (20 – 60 cm) and thin (6-9 mm) with broad sutures containing 15-50 seeds.

It is well adapted to hot, humid environments and does not grow well in the subtropics particularly in areas with a cool season minimum temperature of below 10°C. It has the ability to tolerate water logging

and is ideally suited to seasonally water logged or flooded environments. When flooded, they initiate floating adventitious roots and protect their stems, roots and nodules with spongy, arenchyma tissue. *Sesbaniagrandidiflora* is adapted to rain fall conditions of 2000-4000 mm but grow well in areas receiving only 800 mm. Another interesting feature is its extraordinary tolerance to saline, alkaline as well as to highly acidic soils. Cutting management has a very important influence on the productivity of perennial *Sesbania* species. *Sesbaniagrandidiflora* cannot survive by repeated cutting. Farmers used to cut only the side branches of trees for fodder leaving the main growing stem untouched. The trees are grown on rice paddy walls at 1.5-2 m intervals and forage is harvested in this manner for 3-4 years, yielding up to 2 kg dry matter per harvest per tree. When the foliage is no longer within easy reach the trees are cut and the long straight poles are used as firewood or for construction purposes.

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The tree is grown as an ornamental shade tree, and as a fast growing plant used for reforestation also. The tree is extensively used as a pulp source. *Pepper* vines (*Piper nigrum*) are sometimes grown on and in the shade of the Agati. It is a suitable plant for agro forestry, capable of growing in paddy fields, where trees are not normally grown. However, botanist's quote three undesirable features i.e. (i) short lived (ii) shallow rooted and subject to wind throw, and (iii) being prolific seeder, the pods are often considered as a litter. In India, *Sesbania* has a long history of agricultural use primarily as green manure and as a source of forage. Bark, leaves, gums, and flowers are used for medicinal purpose.

In Yunani the tonic of leaves of *Sesbaniagrandiflora* are used in biliousness, fever and nyctalopia. The juice of leaves is used for headache and nasal catarrh, mixed with stramonium. Malayans apply crushed leaves to sprains. They gargle the leaf juice to cleanse the mouth and throat. In Java leaves are chewed to disinfect the mouth and throat. The tender leaves, green fruit, and flowers are eaten alone as vegetable or mixed into curries or salads. The dried leaves of both *Sesbaniagrandiflora* and *Sesbaniasesban* are used in some countries as tea and are considered to have antibiotic, anti-helminthic, anti tumor and contraceptive properties. *Sesbaniagrandiflora* leaves were collected and identified by a plant taxonomist. As the methanol extract of the plant leaves was found to be rich in important phytochemicals, further pharmacological evaluations were done using this extract only. Isolation of chemical components of methanol extract was done using Sephadex LH 20 column and preparative TLC was used to check individual compounds. Seven chemical components are present and were separated, but three components have remarkable biological activities which were characterized by FTIR and NMR ( $^1\text{H}^{13}\text{C}$  and 2D NMR). The isolated compounds were identified as lupeol ( $\text{C}_{30}\text{H}_{50}\text{O}$ ), kemferol ( $\text{C}_{15}\text{H}_{10}\text{O}_6$ ) and gallic acid ( $\text{C}_7\text{H}_6\text{O}_5$ ) and their structures were elucidated based on the spectral data [1].

Genotoxicity assays are commonly employed to assess the genotoxic potential of carcinogens as well as the antigenotoxic potential of natural products [2]. Techniques employed to assess mutagenic and DNA damaging effects include, the *Salmonella* mutagenicity assay (Ames test) and the single cell electrophoresis 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.

## Experimental

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

## 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.

## 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 intraperitoneally injected with DMBA (30 mg/kg b.w.), on 5<sup>th</sup> day after 2 h of administration of Lupeol. Group 2 animals were given intraperitoneal injection of DMBA (30 mg/kg b.w.) on 5<sup>th</sup> 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 [3]. The femur bone marrow cells were flushed into 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  $\text{Na}^{2+}$  EDTA, 100 mM Tris-HCL, pH 10 and 1% DMSO, 1% Triton X 100 and 1% sodium sarcosinate for 1 h at 4°C and followed by alkaline buffer (pH > 13) for 20 min. The electrophoresis was carried out for 20 min, at 25 V 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.

## Results and discussion

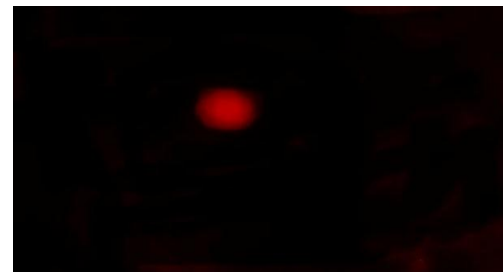
**Fig 1** (a-d) showed the extent of DNA damage (% DNA in tail, tail length, tail moment olive tail moment (**Table 1**) in bone marrow cells of control and experimental animals in each group. Extensive DNA damage as reflected by an increase in DNA tail length, tail moment, % DNA in tail, and olive tail moment was noticed in hamsters treated with DMBA alone [b]. Oral pretreatment of Lupeol significantly protected DNA damage in DMBA treated hamsters [c]. Oral pretreatment of alone [d] to hamsters showed similar pattern of comet, observed in control hamsters [a].

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. The cancerous changes induced by DMBA was suppressed by lupeol.

“Percentage DNA in tail” was used as the indicator [4] of DNA damage. 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 2 h, 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.

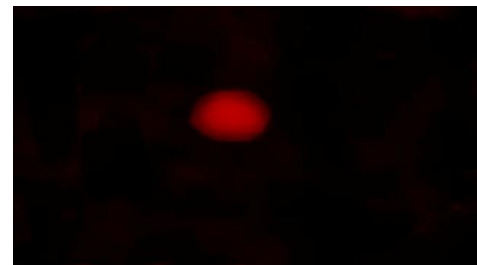
Comet assay is useful in many DNA damage related studies. Processes which introduce single-stranded gaps in the DNA, such as incomplete excision repair events, are readily detectable, in addition to direct DNA damage.



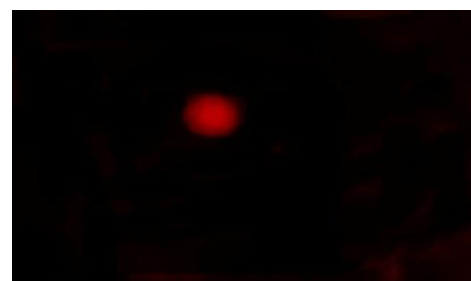
(a) Control



(b)DMBA



(c) DMBA+Lupeol



(d) Lupeol Alone

**Fig 1** [a-d] Representative photographs depict the extent of DNA damage in control and experimental animals in each group.

**Table 1** 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

Groups	% DNA in Tail	Tail length	Tail moment	Olive tail moment
Control	0.11 ± 0.01 <sup>a</sup>	1.08±0.17 <sup>a</sup>	0.01±0.001 <sup>a</sup>	0.03±0.002 <sup>a</sup>
DMBA alone	18.26±1.60 <sup>b</sup>	27.59±2.76 <sup>b</sup>	6.39 ± 0.40 <sup>b</sup>	4.18±0.36 <sup>b</sup>
DMBA + Lupeol	2.37 ± 0.27 <sup>c</sup>	3.14 ± 2.17 <sup>c</sup>	2.34 ± 0.13 <sup>c</sup>	1.60±0.19 <sup>c</sup>
Lupeol alone	0.16 ± 0.01 <sup>a</sup>	1.03 ± 0.08 <sup>a</sup>	0.02 ± 0.001 <sup>a</sup>	0.02±0.002 <sup>a</sup>

Values are expressed as mean ± SD (n = 6).

Values that are not sharing a common superscript letter in the same column differ significantly at  $p < 0.05$  (DMRT).

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