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# Role of *Syzygium Cumini* Seed Extract in Preventing the 7, 12-Dimethylbenz (a) anthracene Induced Skin Carcinogenesis

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## Research Article

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**Keywords:** 7,12-dimethyl benz(a)anthracene (DMBA), carcinogenesis, anti-oxidant, swiss albino mice.

**ABSTRACT**

Investigation of cancer chemopreventive and anti-oxidative property of *S.cumini* seed extract (SCE) on 7,12-dimethyl benz(a)anthracene (DMBA) induced skin carcinogenesis in mice. Animals were divided into four groups on the basis of their respective treatments wherein mice of Group I & II served as vehicle treated and SCE treated controls respectively. For induction of skin tumors, mice of Group III and IV were applied topically with 7,12-dimethylbenz(a)anthracene (DMBA) followed 2 weeks later by repeated application of croton oil (1% in acetone three times a week) and continued till the end of the experiment (i.e. 16 weeks). Mice of Group IV were administered *S.cumini* seed extract (SCE) at peri- & post-initiational stage. The results of the study revealed a significant decrease in incidence, cumulative number of papillomas, tumor yield and tumor burden in mice of Group IV as compared with DMBA alone at the end of experiment. A significant reduction in tumor weight and tumor volume was also observed. Reduction in the incidence and number of papilloma, the preneoplastic lesions, was considered to be the mean of assessment. Significant ($p<0.01$) decrease in the level of lipid peroxidation and significant ($p<0.01$-$p<0.001$) enhancement in the activity of antioxidants (GSH, SOD, Vitamin-C) and total proteins levels were recorded in Group IV. SCE was also found potential in reducing the histopathological lesions induced in tumors by DMBA. From the present study, it can be inferred that *Syzygium cumini* possesses has chemopreventive and anti-oxidative potential for chemical induced carcinogenesis. Such chemopreventive activity may be linked with the antioxidant/free radical-scavenging constituents present in the extract.

## INTRODUCTION

Despite the efforts of innumerable researchers worldwide to ameliorate the dismal outcomes of cancer, it stills continues to be a huge burden on mankind. Cancer originates in our own cells, but several factors, both intrinsic and external to the body, which influence our daily life, can add to the life time cancer risk. While cancer, as such, is not infectious, some infections can act as a stimulus to induce and promote cancer development. In addition, environmental pollutants like many chemicals, industrial effluents, some therapeutic drugs, and mutagenic agents, including ionizing radiation, can increase the incidence of cancer. About 50% of all cancers are attributed to life style, eg. diet, tobacco habits and alcohol consumption, and exposure to industrial toxins.

Chemical and UV radiation-induced carcinogenesis in murine skin and possibly human skin is a stepwise process of at least three distinct stages: initiation, promotion, and progression [1]. Tumors can be initiated by a single dose of carcinogen such as 7, 12-dimethylbenz (a) anthracene (DMBA) followed by a repetitive application of a tumor promoter such as TPA [2]. Increasing skin cancer morbidity and mortality is alarming and expensive, in both human and economic terms. New strategies are needed to combat this disease. The goals of cancer chemo-prevention are to reduce the incidence, morbidity and mortality due to cancer through the identification and elimination of precancerous lesions termed intraepithelial neoplasias and/or the early detection of minimally invasive cancers.

Herbal medicines are in great demand in the developed medicines offers a host of problems. To solve this as well as developing countries for primary healthcare problem, first and foremost task is the selection of the because of their wide biological

activities, higher safety right kind of plant material which is therapeutically margins and lesser costs. Ayurveda, a traditional Indian medicine of plant drugs has been successful from very early times in using these natural drugs and preventing or suppressing various tumors using various lines of treatment. Recently, phytochemicals and their effects on human health have been intensively studied. In particular, a search for antioxidants, hypoglycemic agents, and anticancer agents in vegetables, fruits, teas, spices and medicinal herbs has attracted great attention.

Some of the useful plant drugs with medicinal properties include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, capscicine, allicin, curcumin, artemesinin and ephedrine among others.[3] In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, investigations on both mixture of traditional medicine and single active compounds are very important [3].

*S. cumini* (L.) Skeels has been attributed in the Indian folklore medicine system to possess several medicinal properties [4]. The bark of the plant is astringent, sweet, refrigerant, carminative, diuretic, digestive, anti-helminthic, febrifuge, constipating, stomachic and antibacterial. The fruits and seeds are used to treat diabetes, pharyngitis, spleenopathy, urethrorrhea and ringworm infection. The leaves have been extensively used to treat diabetes, constipation[5], leucorrhoea, stomachalgia, fever, gastropathy, strangury and dermopathy[4], and to inhibit blood discharges in the faeces [5].

Hence, the present study is designed to evaluate the effectiveness of seed extract of *Syzygium cumini* in modifying the carcinogenic process, as well as oxidative stress in DMBA- induced skin carcinogenesis.

## MATERIALS AND METHODS

The animal care and handling was approved by ethical committee of our institution and was done according to guidelines set by the World Health Organization, Geneva, Switzerland, and the Indian National Science Academy, New Delhi, India. The inhibition of tumor incidence by *Syzygium cumini* seed extract was evaluated on two-stage skin carcinogenesis, induced by a single application of DMBA (initiator), and two weeks later, promoted by repeated application of croton oil (promoter) thrice per week, following the protocol for 16 weeks [2].

### Animals

The study was conducted on random-breed male Swiss albino mice of 7-8 weeks old with 24 ± 2 g body weight. These animals were housed in polypropylene cages in the animal house under controlled conditions of temperature (25°C ± 2°C) and light (14 light:10 dark). The animals were fed a standard mouse feed procured from Aashirwad Industries, Chandigarh (India), and water *ad libitum*. Eight animals were housed in one polypropylene plastic cage containing saw dust (procured locally) as bedding material. For precaution against infections, tetracycline hydrochloride water was given to these animals once each fortnight.

### Chemicals

7, 12-Dimethyl Benz (a) anthracene (DMBA) and croton oil was procured from Sigma Chemical Co., USA. DMBA was dissolved at a concentration of 100 µg/ 100µl in acetone. Croton oil was mixed in acetone to give a solution of 1% dilution.

### Plant Material and Extract Preparation

The fruit of *Syzygium cumini* L. was collected locally after proper identification. The identification of the plant *Syzygium cumini* L. (Family: Myrtaceae) was done by a botanist (Voucher Specimen No: RUBL- 20425) from Herbarium, Department of Botany, University of Rajasthan, Jaipur, Rajasthan (India). The pulp was removed from the fruit and the seed were washed properly and shade dried, then after fruit was powdered in a mixture and the hydro-alcoholic extract was prepared by refluxing with the double distilled water (DDW) and alcohol (3:1) for 36 (12 x 3) hrs at 40°C. The extract was cooled and concentrated by evaporating its liquid contents. The prepared *Syzygium cumini* extract (SCE) was stored at low temperature until its further use and it was redissolved in DDW prior for the oral administration in mice.

### Experimental Design

The dorsal skin (2 cm diameter) of Swiss albino mice was shaved 2 days before chemical treatment and animals in the resting phase of growth cycle were selected for the experiment. Mice selected from inbreed colony were grouped into following five groups:

**Group I: Vehicle treated control**

Animals of this group received topical application of acetone (100 µl/ mouse) on the shaven dorsal skin and double distilled water equivalent to SCE (100 µl/ mouse/ day) by orally for 16 weeks.

**Group II: Drug (SCE) treated control**

These animals received SCE (125 mg/kg b. wt./animal/day) alone orally during the entire experimental period (i.e., 16 weeks).

**Group III: Carcinogen treated control**

A single dose of 100 µg of DMBA in 100 µl of acetone was applied topically over the shaven area of the skin of the mice. Two weeks later croton oil (1% croton oil in acetone) was applied three times per week until the end of the experiment (i.e., 16 weeks).

**Group –IV: SCE treated experimental**

Animals of this group were administered SCE (125 mg/kg b. wt./animal/day) orally starting from 7 days before of DMBA application and continued until the end of experiment (i.e., 16 weeks) and served as peri- and post- initiational group.

**The Following Morphological Parameters Were Studied**

**Morphological Parameters**

*Tumor incidence*: The number of mice carrying at least one tumor expressed as a percentage incidence.
*Tumor yield*: The average number of papillomas per mouse.
*Tumor burden*: The average number of tumors per tumor bearing mouse.
*Diameter*: The diameter of each tumor was measured.
*Weight*: The weight of the tumors of each animal at the termination of each experiment was measured.
*Body weight*: The weight of the mice was measured weekly.
*Average latent period*: The time lag between the application of the promoting agent and the appearance of 50% of tumors was determined. The average latent period was calculated by multiplying the number of tumors appearing each week by the time in weeks after the application of the promoting agent and dividing the sum by total number of tumors.

$$\text{Average Latent Period} = \frac{\sum FX}{n}$$

Where F is the number of tumors appearing each week, X is the numbers of weeks, and n is the total number of tumors.

**Biochemical Study**

The following biochemical parameters were estimated in the liver and skin of mice.

*Lipid peroxidation (LPO)*

The level of LPO was estimated spectrophotometrecally by thiobarbituric acid reactive substances (TBARS) method as described by Ohkhawa *et al.* (1979) [6]. Briefly, thiobarbituric acid (0.6%), sodium dodecyl sulphate (0.1%), and trichloroacetic acid (20%) were added to 200 µl of the tissue homogenate (10%) prepared as described above. This mixture was heated for 60 minutes, cooled, and extracted with N butanol- pyridine (15:1), the optical density (OD) was recorded at 532 nm and the contents were expressed as nmol/mg of tissue.

*Reduced glutathione (GSH)*

The level of GSH was estimated as total nonprotein sulphahydryl group by the method of Moron *et al.* (1979) [7]. The homogenate was immediately precipitated with 100 µl of 25% trichloroacetic acid (TCA) and the precipitate was removed after centrifugation. Free endogenous-SH was assayed in a total volume of 3 ml by the addition of 200 µl of 0.6 mM 5, 5′ dithio-bis (2-nitrobenzoic acid) dissolved in 0.2 M phosphate buffer (pH 8.0) to 100 µL of the supernatant and the absorbance was recorded at 412 nm using a UV-VIS Systronics spectrophotometer. Reduced GSH was used as a standard and the levels of GSH were expressed as µmol/gm of tissue.

*Catalase*

The catalase activity was assayed by the method of Aebi [8]. The change in absorbance was followed spectrophotometrically at 240 nm after the addition of $H_2O_2$ (30 mM) to 100 µL of the supernatant (10% of skin homogenate prepared in 50 mM phosphate buffer and centrifuged for 10 min.) in 50 mM phosphate buffer (pH 7).The activity of the enzyme is expressed as U/mg of tissue, where U is µmol of $H_2O_2$ disappearance/min.

*Total Proteins*

Total Proteins were estimated by the method of Lowery *et al.*,[9] using bovine serum albumin as a standard and the level was expressed as mg/ gm.

*Superoxide Dismutase*

SOD was determined by the method of Marklund and Marklund [10] by quantification of pyrogallol auto oxidation inhibition and the results are expressed as units/mg protein. Auto oxidation of pyrogallol in Tris-HCL buffer (50 mM, pH-7.5) was measured by increase in absorbance at 420 nm.

*Vitamin-C*

For this, tissue, the fresh organs were weighed, homogenized in acetate buffer (20 mg/ ml) extracted with cold 4 per cent trichloroacetic acid, centrifuged, and filtered. Ascorbic acid was determined by the method of Roe and Kuether[11].

**Histopathological Study**

Tumors and normal skin were removed from the sacrificed animals and immediately fixed in 10% formalin fixative for 24h. The tissues were then dehydrated in ascending series of alcohol, embedded in paraffin wax and 5 micron thick sections were cut and these were deparaffinized in xylene, rehydrated in descending concentrations of ethanol, stained with hematoxylin and eosin. and viewed under light microscope.

**Statistical Analysis**

Data from different experimental groups were analyzed and expressed as mean $\pm$ SD. The significant level of difference between Carcinogen treated control and SCE treated experimental groups were statistically analyzed using Student's t-test.

## RESULTS

No tumor development was recorded in vehicle treated and SCE treated control (Group I and II) respectively. Papillomas appeared on skin of all the animals of carcinogen treated control (Group-III), where a single topical application of DMBA followed 2 weeks later by repeated application of croton oil, i.e. 100 % incidence was recorded in the Group III (Fig.1). The cumulative number of papillomas as induced during the entire experimental period was 68 (Fig. 2). The average number of tumors per tumor bearing mouse (tumor burden) and tumor yield was found to be as 8.5 (Fig. 3 and 4), and the average latent period was 7.88 weeks (Fig. 5).

Mice of Group IV that received a continuous treatment of *S.cumini* extract orally at peri- and post- initiational phase, showed a significant reduction in the tumor incidence (62.5%) as compared with the carcinogen treated control (Group I) (Fig.: 1). The cumulative number of papillomas produced during the observation period (i.e., 16 weeks) were considerably lesser (76.47%) than the carcinogen treated control (Fig. 2).

Oral administration of SCE during peri- and post- initational stage (Group IV) of DMBA-induced papillomagenesis reduced the tumor burden and tumor yield to 3.2 (62.35%) and 2(76.47%) respectively (Fig. 3 and 4) The time lag between the application of the promoting agent and the appearance of 50% of tumors i.e., average latent period was documented as 11.75 (49.11%) weeks in Group IV where SCE was administered 7 days before DMBA application and continued throughout the experimental period (Fig. 5). The average weight of tumors was also significantly reduced to 45.5 mg (67.23%) in SCE treated animals than the carcinogen treated control (138.87 mg) (Table 1).

The levels of TBARS, total proteins and antioxidants (GSH, SOD, CAT and vitamin- C), in liver and skin of animals of each group are shown in Figs. 7-12. The concentration of TBARS was significantly ($p<0.001$) increased, whereas the status of antioxidants and the total proteins was significantly ($p<0.001$) decreased in animals of carcinogen treated control (Group III), as compared to vehicle treated control (Group I).

**Table 1 Effect of *Syzygium cumini* on weight gain profiles, tumor size and tumor weight**

| Treatment groups | Body weight (gm) (mean ± S.E.) | | Tumor size | | Tumor weight (mg) |
|---|---|---|---|---|---|
| | Initial | Final | 2-5 mm | 6-9 mm | |
| Vehicle treated control | 25.50 ±1.10 | 37.64 ±0.87 | - | - | - |
| Drug (SCE) treated control | 24.50± 0.98 | 37.97±0.76 | - | - | - |
| Carcinogen treated control | 25.50±1.10 | 31.33±0.87 | 30 | 38 | 138.87 |
| SCE treated experimental | 25.88±1.16 | 35.50±1.23 | 13 | 3 | 45.5 |

*Treatment schedule of the groups is specified in materials and methods.

Lipid peroxidation in liver and skin, expressed in terms of TBARS, was found to be significantly ($p<0.001$) reduced at the peri- and post- initiational stage (Group IV) to that measured in the carcinogen treated Control (Group III). Oral administration of SCE to DMBA-painted animals (Group IV) at the peri- and post- initiational stage significantly revert back the status to near normal concentration of TBARS.

**Figure 1: Modulatory effect of *S.cumini* extract on Tumor incidence during the DMBA induced skin carcinogenesis**

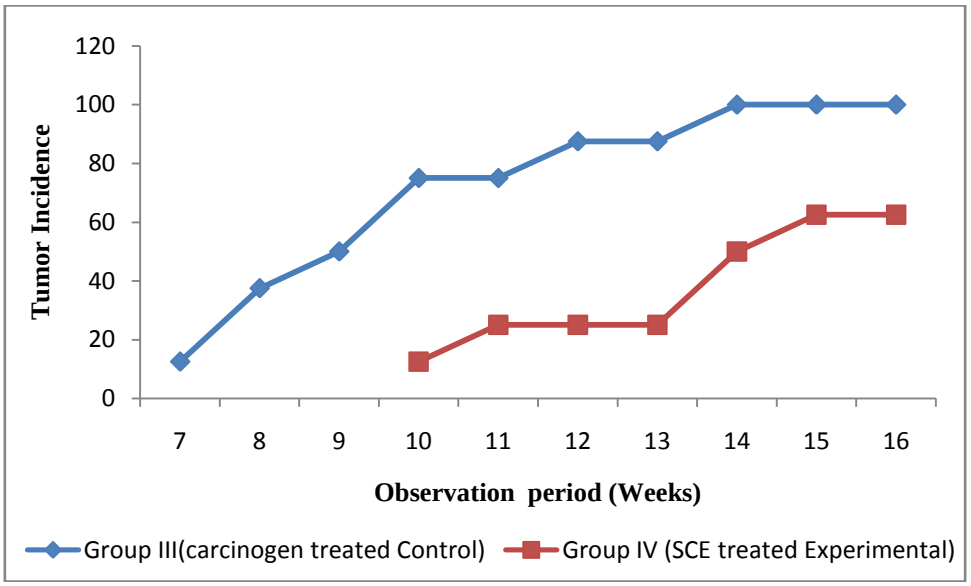



**Figure 2: Modulatory effect of *S.cumini* extract on Cumulative number of papillomas during the DMBA induced skin carcinogenesis**

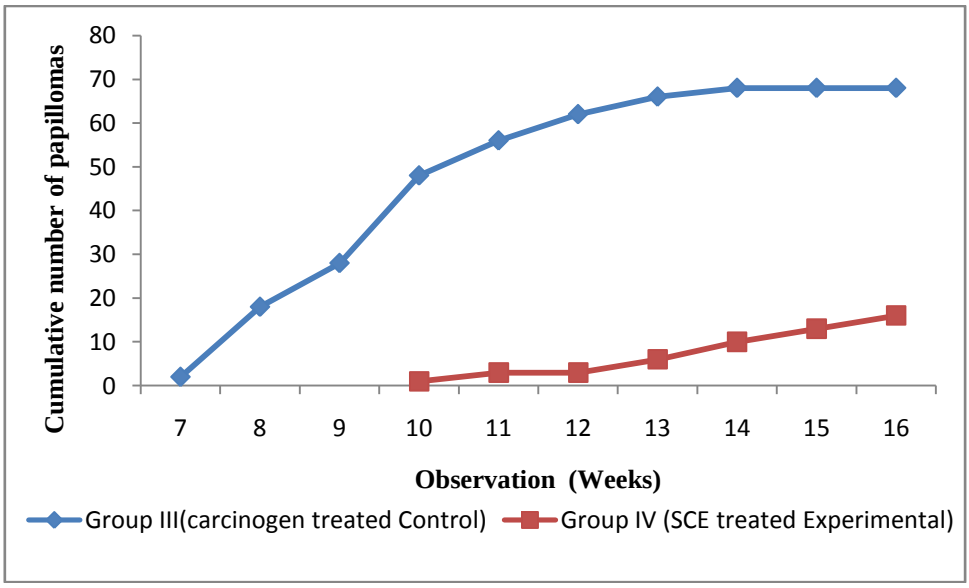


Figure 3: Modulatory effect of *S.cumini* extract on Tumor burden during the DMBA induced skin carcinogenesis

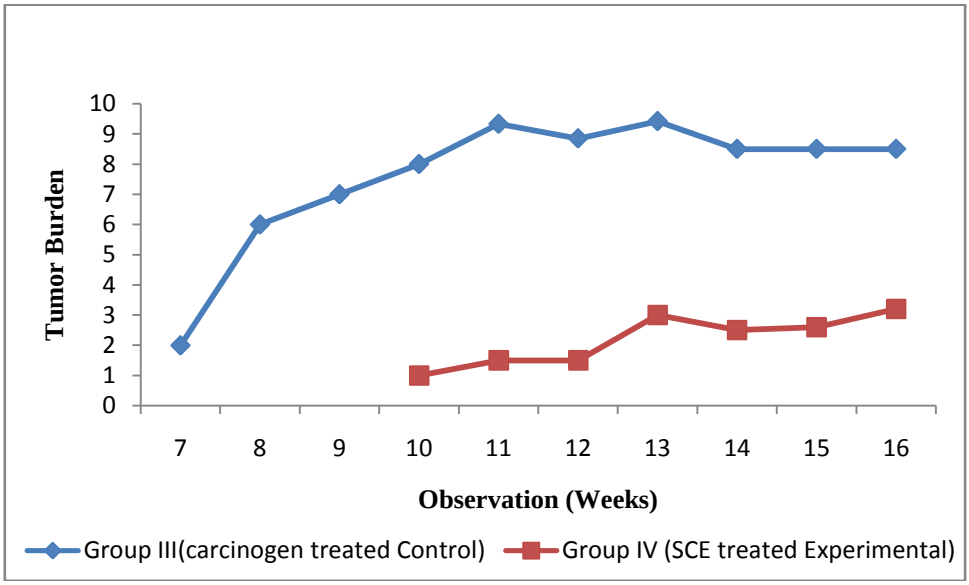



Figure 4: Modulatory effect of *S.cumini* extract on Tumor yield during the DMBA induced skin carcinogenesis

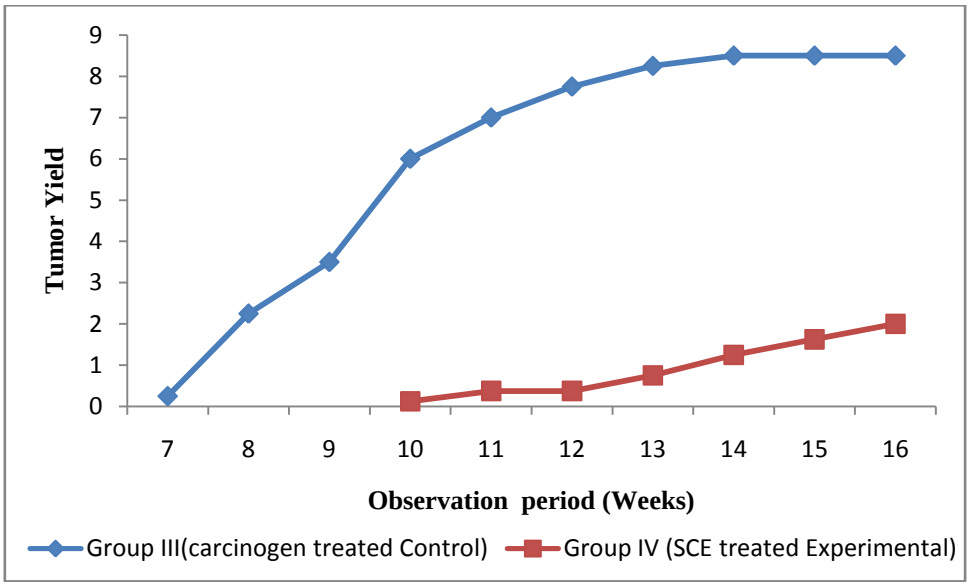



Figure 5: Modulatory effect of *S.cumini* extract on Average latent period during the DMBA induced skin carcinogenesis

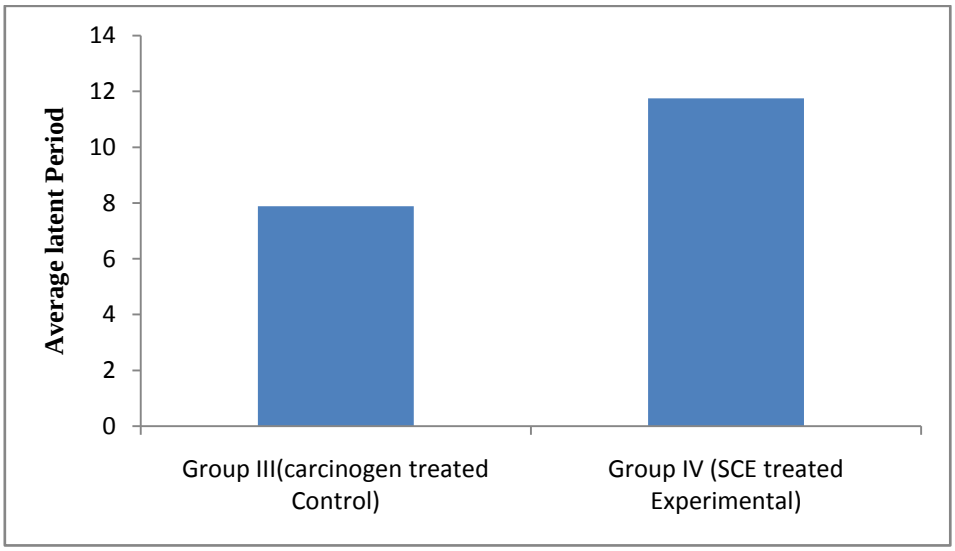
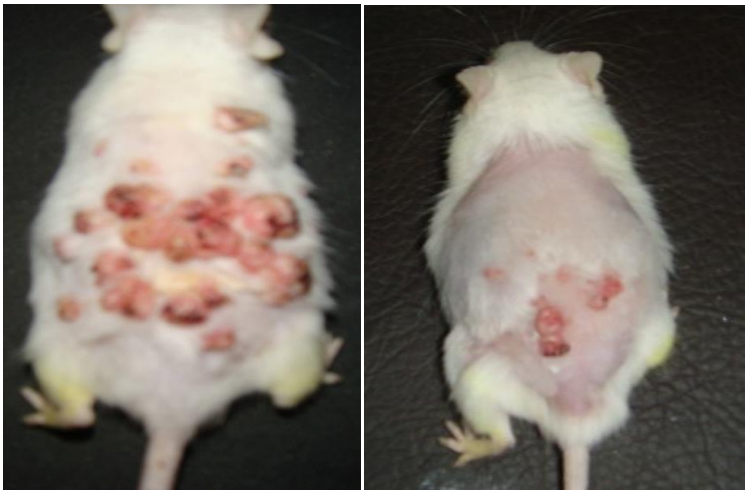
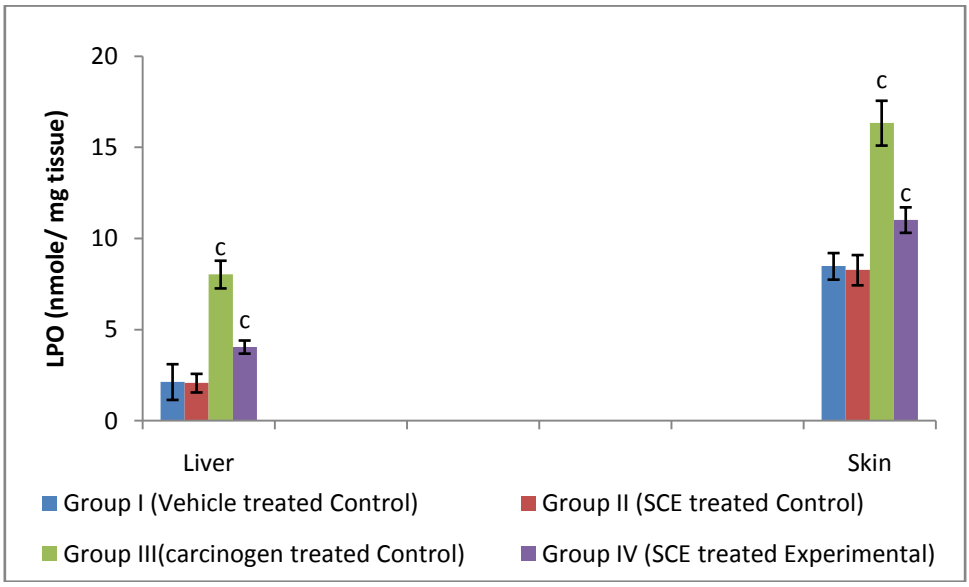


Figure 6: Modulatory effect of *S.cumini* extract (SCE) on mouse skin papilloma Growth



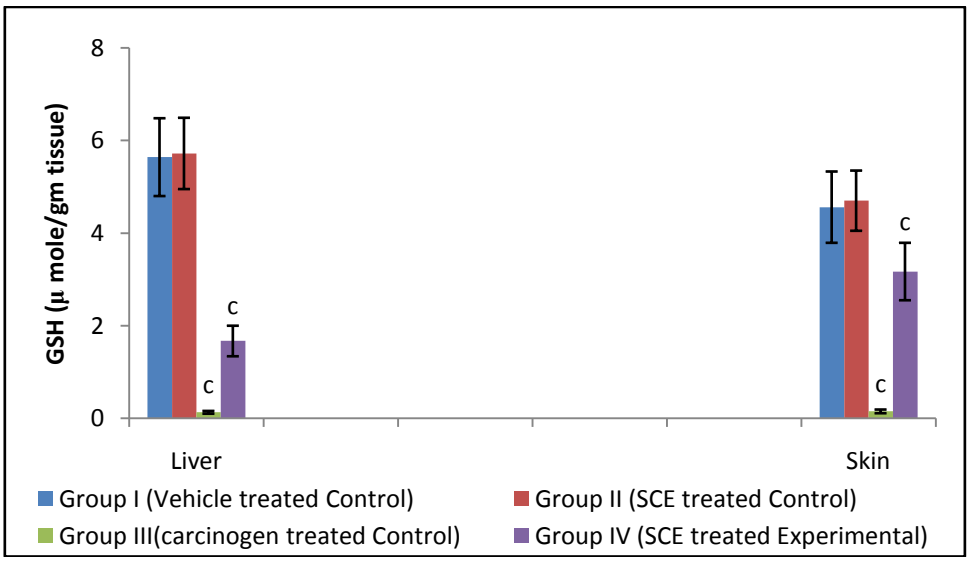
Group III Group IV

Figure 7: Modulatory effect of *S.cumini* extract on LPO level (mean±S.E) during the DMBA induced skin carcinogenesis



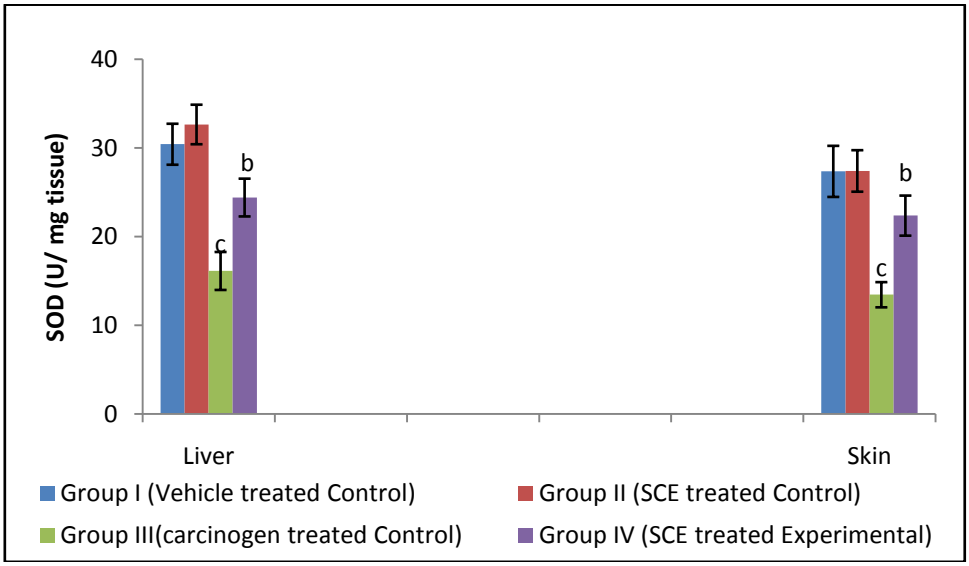

Statistical comparison Normal v/s Control; Control v/s Experimental. Significance levels $^{c}p \leq 0.001$

Figure 8: Modulatory effect of *S.cumini* extract on GSH level (mean±S.E) during the DMBA induced skin carcinogenesis



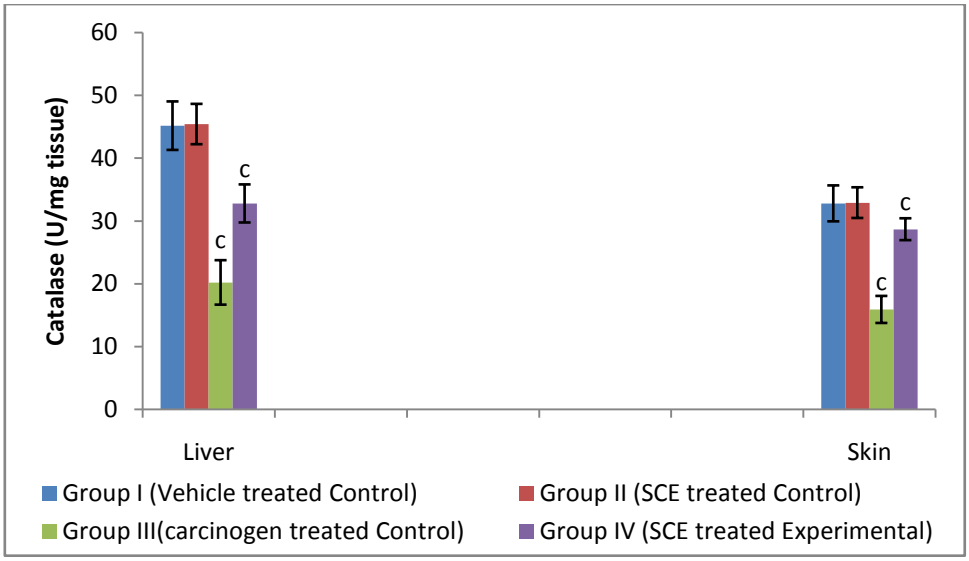

Statistical comparison Normal v/s Control; Control v/s Experimental. Significance levels $^{c}p \leq 0.001$

**Figure 9: Modulatory effect of *S.cumini* extract on SOD activity (mean±S.E) during the DMBA induced skin carcinogenesis**



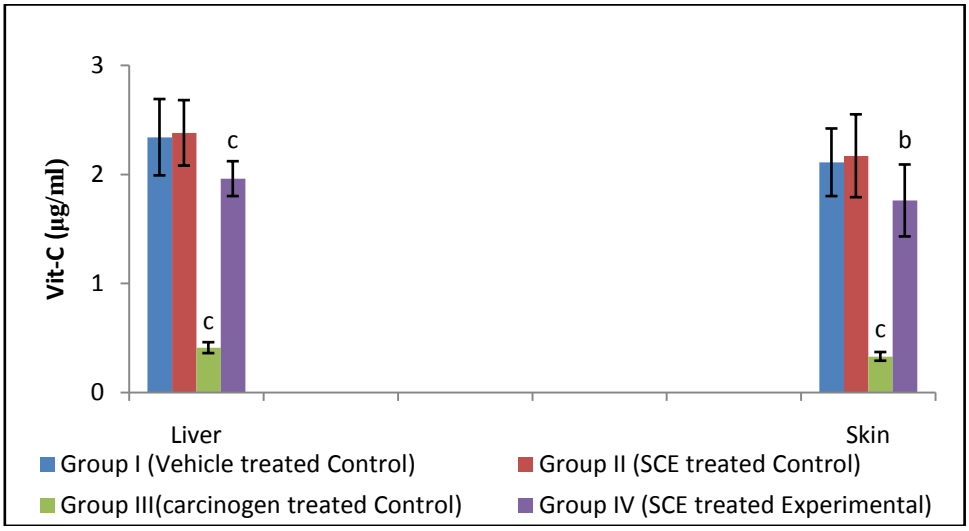

Statistical comparison Normal v/s Control; Control v/s Experimental. Significance levels $^{b}$ $p \leq 0.01$, $^{c}p \leq 0.001$

**Figure 10: Modulatory effect of *S.cumini* extract on catalase activity (mean±S.E) during the DMBA induced skin carcinogenesis**



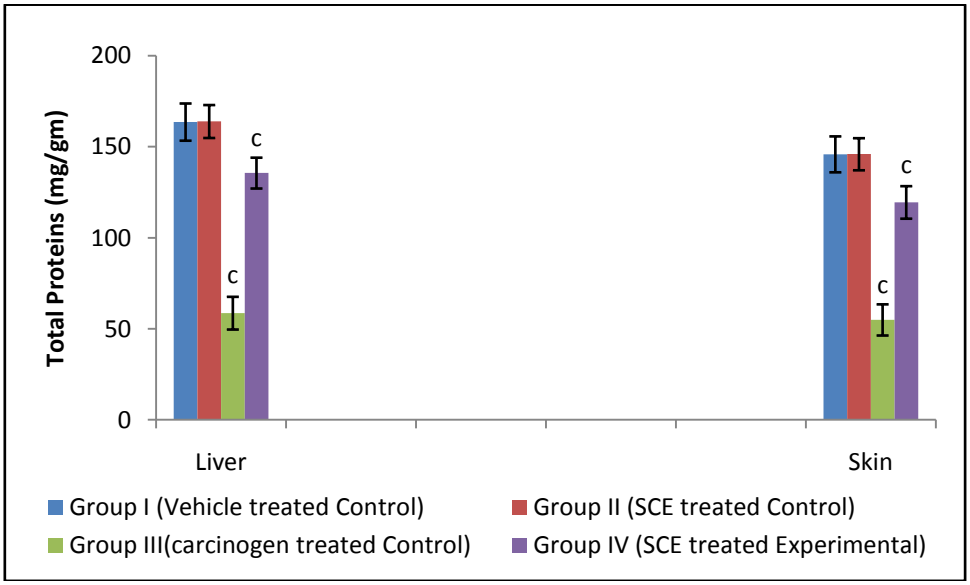

Statistical comparison Normal v/s Control; Control v/s Experimental. Significance levels $^{c}p \leq 0.001$

**Figure 11: Modulatory effect of *S.cumini* extract on vit-c level (mean±S.E) during the DMBA induced skin carcinogenesis**




Statistical comparison Normal v/s Control; Control v/s Experimental. Significance levels $^{b}$ $p \leq 0.01$, $^{c}p \leq 0.001$

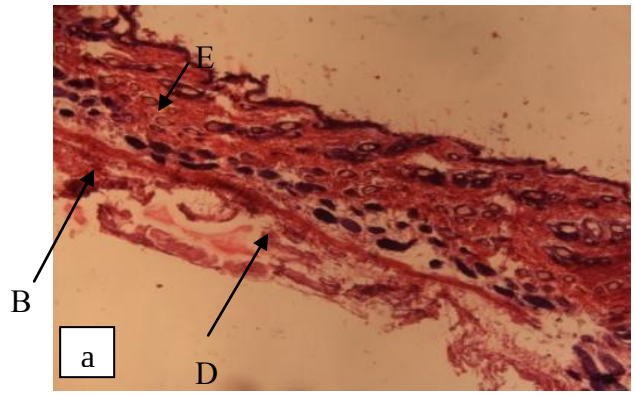
**Figure 12: Modulatory effect of *S.cumini* extract on total protein level (mean±S.E) during the DMBA induced skin carcinogenesis**

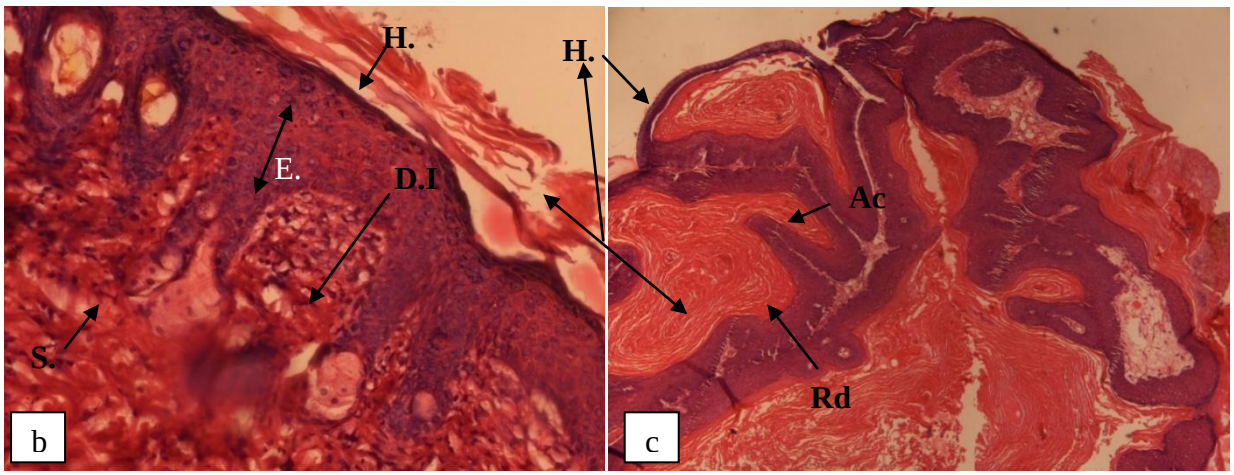



Statistical comparison Normal v/s Control; Control v/s Experimental. Significance levels $^{c}p \leq 0.001$

SCE treatment during the peri- and post- initiational stage (Group IV) significantly ($p<0.01$–$p<0.001$) elevated the hepatic as well as skin antioxidants and total proteins levels when compared with carcinogen treated control (Group III).

Mice treated with *S.cumini* extract alone (Group II) showed no significant difference in TBARS, proteins and antioxidants status when compared to vehicle treated control animals (Group I).



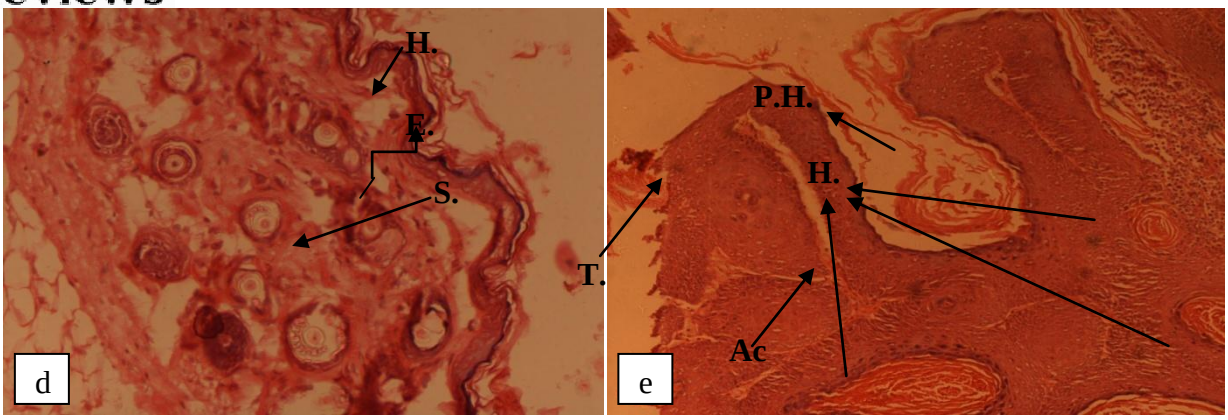



**Figure 13 (a-e)**

(a) Photomicrograph showing histological section of skin of normal mice (Group I).
(b) Photomicrograph showing histological section of DMBA/TPA induced mice skin (Group II)
(c) Photomicrograph showing histological section of DMBA/TPA induced skin tumor in mice (Group III)
(d) Photomicrograph showing histological section of DMBA/TPA induced skin in mice administered with SCE (Group IV).
(e) Photomicrograph showing histological section of DMBA/TPA induced skin tumor in mice administered with SCE (Group IV).

E- epidermis, D- dermis, B- basal Lamina, H.K- Hyperkeratosis E.H- epidermal hyperplasia, D.I.- Dermal invasion, S.G- sebaceous gland, Ac- Acnathosis, H.C- Horny cyst, P.H.C. Pseudo horny cyst, Rd- reduced stroma with lymphocytes, T.N.-Tumor Nest

The histopathological examination of the skin of carcinogen treated control animals exhibited at the cells with atypical (enlarged and hyperchromatic) nuclei at all the strata of the epidermis, severe epidermal hyperplasia and dermal invasion of epidermis. All of these symptoms were found to be minimal in SCE treated experimental animals (Group IV) (Fig. 13. a-e). Tumor of carcinogen treated control (Group III) showed well differentiated squamous cell carcinoma with severe acanthosis, reduced stroma with lymphocytes and an obvious number of horny pearls, which were larger in size, whereas *S.cumini* extract administration at the peri- and post- initiational stage (Group IV) showed poorly differentiated papilloma with lesser number and small sized horny pearls.

## DISCUSSION

7,12-Dimethylbenz[a]anthracene (DMBA) is the best studied polycyclic aromatic hydrocarbon (PAH) and widely used in experimental carcinogenesis [12, 13]. Metabolic activation by P450 through AhR is the first step in the long road of PAH carcinogenesis. The metabolism of DMBA is well characterized [14,15,16]. Briefly, P450 oxidizes DMBA to the 3,4-epoxide followed by hydrolysis by mEH to the proximate form, 3,4-diol. This metabolite is again oxidized by P450 to the ultimate form, 3,4-diol-1,2-epoxide that is capable of binding covalently to DNA and causing gene mutation.

The role of polycyclic aromatic hydrocarbons (PAH) is clearly implicated in the process of carcinogenesis especially 7,12-dimethylbenz[*a*]anthracene (DMBA), which is one of the most potent skin and breast carcinogens known [17].

In the present investigation, of toxic effects of DMBA there was a significant decrease in the body weight and increase in tumor size of carcinogen treated control mice. Such weight loss and tumor size was rectified by the administration of SCE at the peri- and post- initiational stage. Topical application of DMBA considerably increased the incidence, cumulative number of papillomas, tumor yield and tumor burden whereas it decreased average latent period in animals of carcinogen treated control (Group III) because DMBA, after metabolic activation, has been found to induce cancer through an oxidatively mediated genotoxicity by incorporating diolepoxide and other ROS into DNA [18,19].

Oxidative damage to cellular macromolecules may occur through the overproduction of ROS and faulty antioxidants and/or DNA repair mechanisms that result in cancer [20]. Several transcriptional factors such as nuclear factor kappa B (NFκB) and AP-1 are induced by ROS and are known to have a direct impact on inflammation, cellular proliferation, and apoptosis, thus making ROS instrumental in tumor promotion [21]. Therefore, administration of antioxidants may retard this process.

Oral administration of *S.cumini* extarct at peri- and post-initiational stage (Group IV) significantly reduced the occurrence of skin papilloma, induced by DMBA-croton oil after 16 weeks of experimental period. Moreover, during the entire experimental period, the cumulative number of papilloma, tumor yield and tumor burden were found to be considerably decreased in Group IV than the carcinogen treated control (Group III).

The inhibition in tumor genesis and reduction in oxidative stress during present experimentation might be because of the presence of several phytochemicals in this plant extract such as acetyl oleanolic acid, triterpenoids, ellagic acid, isoquercitin,

quercetin, kaempferol and myricetin [22,23]. Most of these compounds have been reported to possess antioxidant and free radical scavenging activities [24].

These results suggests an anti-tumorigenic potential of SCE against DMBA-croton oil induced skin carcinogenesis, which might be due to the phyto-constituents (i.e., polyphenols, flavanoids (quercetin, myrecetin), saponins and tannins) present in the seed of *S.cumini*. Myrecetin inhibits the phorbol ester-induced upregulation of COX-2 protein [25]. As reported earlier that chronic inflammation, predisposes to malignancy[26] and curumin exerts it's both anti-inflammatory and chemopreventive effects by inhibiting the phorbol ester-induced expression of COX-2 protein [27]. Quercetin is one of the most abundant dietary flavonoids that arrest the cell cycle at the point where damage occurs and thereby inhibits the initiation of carcinogenesis which clearly reflects in the present study.

These findings were supported by several other finding also. Several studies have indicated that Chinese medicinal plants contain a wide variety of natural antioxidants such as phenolic acids, flavonoids and tannins, which possess more potent antioxidant activity than common dietary plants [28,29]. In recent years, a group of plants under the 'heat-clearing' category according to the classification of Chinese medicinal plants have attracted much attention because they have demonstrated significant activities in anti-inflammatory, anti-tumor, anti-allergic, anti-viral and antibacterial assays [30,31].

Histopathological investigations showed a normal architecture of skin in vehicle treated (Group I) and SCE treated control (Group II). DNA is a target molecule of DMBA, and metabolically activated DMBA can bind DNA to form adducts [32], cause mutations [33], and then induce tumor formation [34]. Transversion from A to T on codon 61 of the H-ras oncogene is a highly consistent finding in DMBA/ TPA-induced mouse skin papilloma [35].

Skin of mice of carcinogen treated control (Group III) showed severe epidermal hyperplasia, dermal invasion and hyperkeratosis and tumor histology exhibited well differentiated squamous cell papilloma with hyper chromatic nuclei, reduced stroma with large number of lymphocytes. On the other hand, poorly differentiated papillomas and the extent of all these lesions were observed to be of relatively lesser in the tumors and skin of mice that received SCE treatment at the peri- and post- initiational stage (Group IV).

Increase in epidermal proliferation can lead to the establishment and development of a mutated clone which ultimately can result in papillomas. Cell death is a part of normal physiology for most metazoan species. During development, unwanted cells are removed through programmed cell death, making important contributions to morphogenesis, organogenesis, and other processes. [36] Defects in the cell death machinery, which prevents the programmed turnover of cells, can increase undesired cell accumulation, genetic instability, enhance cell longevity and permit cells to survive in a suspended state. Thus, defective apoptosis may indirectly promote cancer. Reduction in all the DMBA induced lesions in Group IV by *S.cumini* seed extract might be because of inhibition of cell proliferation and induction of apoptosis at the target site which is initiated by DMBA.

The formation of malondialdehyde is considered as an index of lipid peroxidation that causes cell injury. Elevation of lipid peroxides, as indicated by increased MDA and decrease in total proteins were observed in liver and skin of tumor bearing animals of carcinogen treated control (Group III). DMBA induces critical oxidative damage in the liver *in vivo* [37,38]. Significant increase in LPO in carcinogenic process may be due to abnormal levels of reactive oxygen species (ROS). ROS production in excess of cellular antioxidant capacity, may result in damage to lipids, proteins, RNA and DNA or other effects [39].

A significant decrease in the activity of antioxidants i.e., SOD, CAT and vitamin-C were documented in liver and skin of carcinogen treated control mice when compared to vehicle treated control. These results are in collaboration with the findings of others [40,41].

SOD acts as an anti-carcinogen inhibitor during initiation and promotion/transformation stages of carcinogenesis [42]. Superoxide radicals may be reduced by the enzyme superoxide dismutase to form $H_2O_2$ and oxygen. Catalase is an enzyme which converts $H_2O_2$ to neutral products like $O_2$ and $H_2O_2$. The antioxidant effect of SCE extract was observed by significant increase in the antioxidant activity of SOD, CAT and vitamin-c and by significant decrease in lipid peroxidation in liver and skin of animals of Group IV when compared to carcinogen treated control (Group III).

The antioxidant activity of *Eugenia jambolana* could be attributed due to the presence of phenolic compounds as they are believed to be the major phytochemicals responsible for antioxidant activity of plant materials [43, 44]. Phenolic compounds such as flavonoids, phenolic acid and tannins possess diverse biological activities such as anti-inflammatory and anti-atherosclerotic activities, which might be related to their antioxidant potential [45].

A significant decreased level of vitamin C was observed in liver and skin of carcinogen treated control. This decreased level of vitamin C may be due to increased utilization in trapping the oxyradicals which were generated by DMBA. Vitamin C is a key antioxidant which particularly protects lipids from per-oxidative damage by aqueous solution, thereby blocking the initiation of carcinogenesis [46]. *S.cumini* extract treatment boost up the level of vitamin-c in liver as well as in skin of SCE treated animals (Group

IV), which might be helpful in neutralizing the DMBA induced free radical and protect the cells from oxidative damage. Sharma *et al.*[47] have proposed that several medicinal plants and their constituents probably exert their chemopreventive effect, by scavenging reactive oxygen species and improving the antioxidant defence systems.

## CONCLUSIONS

From the present study, it can be inferred that *Syzygium cumini* possesses anti-carcinogenic and anti-oxidative potential against chemical induced carcinogenesis in mammals. Such chemopreventive activity may be linked with the antioxidant/free radical-scavenging constituents present in the extract.

## REFERENCES


1. Agarwal R, Mukhtar H. Cutaneous chemical carcinogens In: Pharmacology of the skin. (ed. H. Mukhtar); Boca Raton, FL: CRC Press; 1991. pp. 371-387.
2. Slaga TG, Budunova IV, Gimenez, Conti IB, Aldaz CM. The mouse skin chemical carcinogenesis model. J Investig Dermatol Symp Proc. 1996; 1: 151-156.
3. Doble B, Dwivedi Sumeet, Dubey K, Joshi H. Pharmacognostical and antimicrobial activity of leaf of Curcuma angustifolia Roxb. Int J Drug Disc Herbal Res. 2011;1(2): 46-49.
4. Warrier PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plants. Orient Longman Ltd.:Hyderabad, India; 1996; 5: 225-228.
5. Bhandary MJ, Chandrashekar KR, Kaveriappa KM. Medical ethnobotany of the siddis of Uttara Kannada district, Karnataka, India. J Ethnopharmacol. 1995; 47: 149-158.
6. Ohkhawa H, Ohishi N, Yogi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Analyt Biochem. 1979; 95: 351-358.
7. Moron MA, Depierre JW, Mannervick B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochem Biophys Acta. 1979; 582:67-78.
8. Aebi H. Catalase: in vitro. In: *Method in Enzymology* (eds Colowick SP, Kaplan NO); New York ; Academic Press 1984; 105: 121-126.
9. Lowry OH, Rosenbrough NJ, Farr AL, Landall AJ. Protein estimation with Folin phenol reagent. J Bio Chem. 1951; 193:265.
10. Marklund S and Marklund G. Involvement of the superoxide anion radical in autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974; 47: 469-474.
11. Roe JH, Kuether CA. The Determination of Ascorbic Acid in Whole Blood and Urine through the 2,4-Dinitrophenylhydrazine Derivative of Dehydro ascorbic Acid. J Biol Chem. 1943; 147:399-407.
12. IARC, Polynuclear Aromatic Hydrocarbons. Part 1: Chemical, Environmental and Experimental Data, IARC Scientific Publications, IARC, Lyon, 1983.
13. Ide F, Kitada M, Sakashita H, Kusama K. Reduction of p53 dosage renders mice hypersensitive to 7,12-dimethylbenz[a]anthracene-induced salivary gland tumorigenesis. Anticancer Res. 2003; 22: 201-204.
14. FJ Gonzalez, S Kimura. Study of P450 function using gene knockout and transgenic mice. Arch Biochem Biophys. 2003; 409: 153-158.
15. Miyata M, Kudo G, Lee YH, Yang TJ, Gelboin HV, Fernandez-Salguero P, et al. Targeted disruption of the microsomal epoxide hydrolase gene. Microsomal epoxide hydrolase is required for the carcinogenic activity of 7,12- dimethylbenz[a ]anthracene, J Biol Chem. 1999; 274: 23963-23968.
16. Shimada T, Fujii-Kuriyama Y. Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochrome P450 1A1 and 1B1. Cancer Sci. 2004; 95: 1-6.
17. Lakshmi B, Sheena N, Janardhanan KK. Prevention of mammary adenocarcinoma and skin tumour by *Ganoderma lucidum*, a medicinal mushroom occurring in South India. Curr Sci. 2009; 97: 11-10.
18. Boffetta P, Jourenkova N, Gustavsson P. Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. Cancer Causes Control. 1997; 8: 444- 472.
19. Dipple A, Pigott M, Moschel RC, Costantino N. Evidence that binding of 7,12-dimethylbenz(*a*)anthracene to DNA in mouse embryo cell cultures results in extensive substitution of both adenine and guanine residues. Cancer Res. 1983; 43: 4132-4135.
20. Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. Toxicol Pathol. 2010; 38: 96- 109.
21. Bickers DR, Athar M. Oxidative stress in the pathogenesis of skin disease. J Invest Dermatol. 2006; 126: 2565- 2575.
22. Rastogi, RM Mehrotra, BN. Compendium of Indian medicinal plants. Central Drug Research Institute: Lucknow, India. 1990; 1: 388-389.
23. Yang LL, Lee CV, Yen KY. Induction of apoptosis by hydrolysable tannins from *Eugenia Jambos* on human leukemia cells. Cancer Lett 2000; 157: 65-75.
24. Tanaka M, Kuei, CW, Nagashima Y, Taguchi T. Application of antioxidative maillard reaction products from histidine and glucose to sardine products. Nippon Sui Gakk. 1998; 54: 1409-1414.
25. Lee KM, Kang NJ, Han JH, Lee KW, Lee HJ. Myricetin down-regulates phorbol esterinduced cyclooxygenase-2 expression in mouse epidermal cells by blocking activation of nuclear factor kappa B. J Agric Food Chem. 2007; 55: 9678-9684.

26. Bennett A. The production of prostanoids in human cancers, and their implications for tumor progression. Prog Lipid Res. 1986; 25: 539-542.
27. Chun Kyung-Soo, Keum Young-Sam, Han Seong Su, Song Yong-Sang, Kim Su-Hyeong and Surh Young-Joon. Curcumin inhibits phorbol ester-induced expression of cyclooxygenase-2 in mouse skin through suppression of extracellular signal-regulated kinase activity and NF-kB activation. Carcinogenesis. 2003; 24(9):1515-1524.
28. Cai YZ, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 Chinese medicinal plants associated with anticancer. Life Sci. 2004; 74: 2157-2184.
29. Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. Several culinary and medicinal herbs are important sources of dietary antioxidants. J Nutr. 2003; 133: 1286-1290.
30. Cai YM, Ren YR, Wang L, & Zhang GT. (1999). Pharmacology and clinical application of traditional Chinese medicine. Beijing: Huaxia Press.
31. Shen YJ. Pharmacology of traditional Chinese medicine. Beijing: People's Health Press; 2000.
32. Cheng SC, Prakash AS, Pigott MA, Hilton BD, Lee H, Harvey RG ET AL. Metabolite of the carcinogen 7.12-dimethylbenz(fl lanthracene that reacts predominantly with adenine residues in DNA. Carcinogenesis. 1988; 9: 1721-1723.
33. Chakravarti D, Felling JC, Cavalieri EL, Rogan EG. Relating aromatic hydrocarbon-induced DNA adducts and c-H-raj mutations in mouse skin papillomas: the role of apurinic sites. Proc Nati Acad Sci USA. 1995; 92: 10422-10426.
34. Slaga TJ, Gleason GL, Di Giovanni J, Sukumaran KB and Harvey RG. Potent tumor-initiating activity of the 3.4-dihydrodiol of 7.12-dimethylbenz(a)anthracene in mouse skin. Cancer Res. 1979; 39: 1934-1936.
35. Sasaki K, Bertrand O, Nakazawa H, Fitzgerald DJ, Mironov N, and Yamasaki H. Cell type-specific ras mutations but no microsatellite instability in chemically induced mouse skin tumors and transformed 3T3 cells. Cancer Res. 1995; 55: 3513-1526.
36. Vaux D, Korsmeyer S. Cell death in development. Cell. 1999; 96: 245-54.
37. Letchoumy PV, Chandra Mohan KV, Kumaraguruparan R, Hara Y and Nagini S. Black tea polyphenols protect against 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis. Oncol Res. 2006; 16: 167-178, 2006.
38. Manikandan P, Murugan RS, Abbas H, Abraham SK and Nagini S: *Ocimum sanctum Linn*. (Holy Basil) ethanolic leaf extract protects against 7,12-dimethylbenz(a)anthracene-induced genotoxicity oxidative stress and imbalance in xenobiotic-metabolizing enzymes. J Med Food. 2007;10: 495-502.
39. Vennila R, Thirunavukkarasub SV and Muthumarya J. *In vivo* studies on anticancer activity of taxol isolated from an endophytic fungus pestalotiopsis pauciseta sacc. VM1. Asian J Pharm Clin Res. 2010; 3(4): 30-34.
40. Koul A, Mukherjee N, Gangar SC. Inhibitory effects of *Azadirachta indica* on DMBA-induced skin carcinogenesis in Balb/c mice. Mol Cell Biochem. 2006; 283: 47-55.
41. Trisha D, Rao AR, Yadava PK. Modulatory effect of Henna leaf (*Lawsonia inermis)* on drug metabolising phase I and phase II enzymes, antioxidant enzymes, lipid peroxidation and chemically induced skin and forestomach papillomagenesis in mice. Mol Cell Biochem. 2003; 245: 11-22.
42. Samy RP, Gopalakrishnakone P, Ignacimuthu S. Anti-tumor promoting potential of luteolin against 7,12-dimethylbenz(*a*)anthracene-induced mammary tumors in rats. Chemico-Biological Interactions. 2006; 164: 1-14.
43. Javanmardi J, Stushnoff C, Locke E, Vivanco JM. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food Chem. 2003; 83: 547-550.
44. Pizzale L, Bortolomeazzi R, Vichi S, Uberegger E, Conte LS, Antioxidant activity of Sage (*Salvia officinalis* & *S fruticosa*) and Oregano (*Origanum* & *O. indercedens*) extracts related to their phenolic compound content. J Sci Food Agric. 2002; 82: 1645-1651.
45. Chung KT, Wong TY, Huang YW, & Lin Y. (1998). Tannins and human health: A review. Crit Rev Food Sci Nutr. 1998; 38: 421-464.
46. Lee KW, Lee HJ, Surh YZ, Lee CY. Vitamin C and cancer chemoprevention: reappraisal1-3. Am J Clin Nutr. 2003;78:1074.
47. Sharma S, Stutzman Id, Kelloff GJ, Steele VE. Screening of potential chemopreventive agents using biochemical markers of carcinogenesis. Cancer Res. 1994:54:5848-55.