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PREVENTIVE POTENTIAL OF *DALBERGIA SISSOO* ROXB. ON DMBA-INDUCED SKIN CARCINOGENESIS IN MICE

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ABSTRACT

The present study was carried out to investigate preventive potential of *Dalbergia sissoo* leaf extract (in Petroleum Ether) against chemical induced skin carcinogenesis in Swiss albino mice. Skin tumor or papilloma was developed by topical application of DMBA on intrascapular region of mice as initiator and croton oil twice weekly for 16 weeks. The animals were divided into four groups: Group I (vehicle treated control); Group II (*Dalbergia sissoo* leaf Petroleum Ether extract (control); group III (carcinogenic treated control) and group IV (*Dalbergia sissoo* leaf petroleum ether extract 200 mg/kg orally for 16 weeks along with DMBA treatment). After the 16th week of treatment; the body weight and tumor morphology were observed and compared with carcinogen treated control as well as vehicle treated control. Two stage protocol was used to evaluate the preventive activity. For initiation single topical application of a carcinogen (7, 12 - Dimethylbenz (a) anthracene (DMBA), is followed by croton oil (promoter) 2 times in a week for 16 weeks. A significantly decline in tumor burden, tumor incidence and cumulative no. of papillomas was detected, along with inhibition of tumor multiplicity in mice treated with *Dalbergia sissoo* leaf extract in comparison to the group treated with DMBA and croton oil.

Keywords: *Dalbergia sissoo*, Petroleum Ether extract, DMBA, tumor morphology, body weight.

Introduction

Cancer is a group of diseases which cause cells in the body to alteration and grow out of control. In both the developed and developing countries, cancer is the second most fatal disease. The worldwide burden of cancer continues to upsurge largely for the reason that of the growth of the world population and aging together with an rising adoption of cancer-causing behaviour, mainly smoking, in developing countries (De Santis *et al.*, 2011; Qian *et al.*, 2011) Amongst all of the human cancers, skin cancer is one of the most common and its incidence is increasing rapidly all over the world. A polycyclic aromatic hydrocarbon, 7,12-Dimethyl benz (a) anthracene (DMBA), is a procarcinogen and thus requires metabolic activation to come to be an ultimate carcinogen. During the metabolic activation of DMBA, the active metabolite, dihydrodiol epoxide, generated which binds to and causes DNA damage. Through the metabolic activation of DMBA higher level reactive oxygen species are also generated. It is widely used in Swiss albino mice as an initiator as well as promoter to induce skin carcinogenesis (Miyata *et al.*, 2001; Nigam *et al.*, 2007; Sharma and Goyal, 2015).

The current treatments of cancer include surgery, conventional medicine, chemotherapy and radiotherapy. Although, the limitations of these therapies are clear as even after end of the standard treatment procedure, there are high rates of metastasis and deterioration (Liang *et al.*, 2010; Hsiao and Liu, 2010).

Began with the folk medicine, the use of plant products as anti-carcinogenic agents has an extensive history and over the years has been assimilated into allopathic and traditional medicine (Sharma *et al.*, 2009). Plants related compounds are also of attention due to their multitargeting character, comparative lack of toxicity, lower cost and easy availability easily (Gupta *et al.*, 2011; Tan *et al.*, 2015; Sayyad *et al.*, 2016).

Dalbergia sissoo Roxb. (Family-Fabaceae) also called 'Shisham' is used since time immemorial for cure of several ailments like dysentery, burning sensations, leucoderma, dyspepsia, and some skin ailments. It is memory enhancer and anti-inflammatory. Its leaves have significant levels of flavonoids which displayed antioxidant activity twice of usually used antioxidants like Selenium and vitamin C (Chetty *et al.*, 2008). It prevents central nervous system damage also (Mukhtar *et al.*, 2010). All the parts of plant are traditionally used in treating different diseases. Antidiabetic, Anti-termite, Analgesic and Antipyretic, Anti-inflammatory, Anthelmintic, Anti-spermatogenic, Antidiarrhoeal, Antinociceptive, Neuroprotective, Molluscicidal, Antioxidant and Osteogenic activities are known. (Bharath *et al.*, 2013; Sultana *et al.*, 2015; Bijauliya *et al.*, 2017).

Materials and Methods

Chemicals: The initiator, "7, 12-dimethylbenz [a] anthracene (DMBA)" and croton oil (promoter) were procured from "Sigma Chemicals Co, St Louis, MO". DMBA was dissolved

at a concentration of 100 µg/100 µL in acetone. Croton oil was as well mixed in acetone to give a solution of 1 per cent dilution.

Plant Extract: Leaves of *D. sissoo* were collected from “University of Rajasthan campus, Jaipur, Rajasthan, India”. The Leaves were authenticated and identified at the Herbarium, “Department of Botany, University of Rajasthan, Jaipur” under the specimen voucher no. (RUBL 211671). The leaves were dried, coarsely powdered and Soxhleted with Petroleum Ether at 55°-60° C for 35 h. Under low temperature and pressure the plant extract was filtered and concentrated to get a dry viscous dark brownish mass. The extract was prepared and suspended in sterile distilled water.

Animals: The present experiment was conducted on 7–8-week old Swiss albino mice and weighing 24 ± 2 g. which were selected from an inbred colony.

Experimental Plan

Swiss albino mice were divided into four groups of 8 mice each.

Group I: Vehicle treated Control: This group of animals was treated topically on the dorsal skin with acetone (100 µl/mouse) and double distilled water (100 µl/mouse/day), orally for 16 weeks.

Group II: *Dalbergia sissoo* leaf extract alone: Mice were treated with Petroleum Ether extract of *D. sissoo* leaf suspended in distilled water at the dose rate of 200 mg/kg, orally, for sixteen weeks.

Group III: Carcinogen Experimented Control (DMBA + Croton Oil): DMBA was applied topically over the shaven area of the skin of these animals with a single dose of 100 µg of DMBA in 100 µl of acetone. After two weeks of DMBA application, croton oil (100 µl of 1% croton oil in acetone) was applied 3 times per week, until the completion of the experiment (i.e. sixteen weeks).

Group IV: DMBA+ *Dalbergia sissoo* leaf extract (200 mg/kg b. wt./day): Test groups—received DMBA individually (25 µg in 0.1 ml Acetone/mouse) topically, twice in a week for 16 weeks with oral administration of graded doses of *Dalbergia sissoo* leaves extract (200mg/kg/body wt.) orally starting one week before the exposure to the carcinogen as DMBA and then continued for 16 weeks with daily administration.

Body weights: Initial body weight and final body weight of every animal were noted.

Morphological study

Tumor incidence

The number of mice carrying at least one tumor was expressed as percent incidence.

Tumor yield

The average number of tumors per mouse was calculated.

Tumor burden

The average numbers of tumors per tumor-bearing mouse was calculated.

Tumor diameters

The diameter of each tumor was calculated at the termination of experiment.

Cumulative number of tumors

The total number of tumors appeared till the termination of the treatment, were recorded.

Inhibition of tumor multiplicity

“Total number of tumors in carcinogen treated control animals – total number of tumors in plant extract treated animals/Total no of tumors in carcinogen treated control group X 100”

Results

Body weight: As presented in Table 1, experimented with the *Dalbergia sissoo* plant affected the numerous phases of skin carcinogenesis in mice. In Group I the animal appeared healthy throughout the experimental period. No adverse effects were noticed in general behaviour, sickness, mortality, food and water consumption habits, pattern of urination and defecation. In addition they did not have any tumor appearance. In Group II the body weight gradually increased in the experimental period, but body weight decreased in the carcinogen treated control animals (Group III), while comparing to normal body weight. The mice in the group IV received *Dalbergia sissoo* treatment before the DMBA application. However, the body weight was noted to decrease with dose of *Dalbergia sissoo* but it was higher than the animals belonging to the carcinogen treated control.

Morphological study: The tumor parameters of both control and experimental animals are shown in Table.1 and 2. Animals of Group I and Group II had no incidence of skin tumor throughout the experimental period. Topical application of DMBA followed by croton oil produced skin tumors in group III. The incidence of skin cancer in DMBA-croton oil treated mice was taken as 100% at the time of the termination of the treatment. However, when *Dalbergia sissoo* was orally administered to mice of Group IV, in addition to the (DMBA), the tumor incidence was found to be 87%, which is significantly lesser.

Discussion

Prevention of tumors by plant products in an emerging, appealing, and innovative approach in experimental oncology, which deals with the inhibition, prevention and suppression of carcinogenesis. To examine to the genetic and biochemical changes, the chemical-induced two stage skin carcinogenesis model of mouse is a predominantly useful model. In the current investigation, to the initiate carcinogenesis, the topical application of DMBA was used for the reason that skin absorption was investigated to be the fastest route of entry for these polycyclic amino hydrocarbons. In to the liver, the metabolic activation of DMBA takes place by phase-one detoxification enzyme cytochrome P450, which converts it into “3, 4-diol-1, 2-epoxide” that covalently binds to DNA and make DNA adducts, at last leading to mutation. Croton oil retains “12-O Tetradecanoylphorbol-13-acetate”, which is utilised for the promotion of skin tumor by the construction of reactive oxygen species and hydroperoxides in keratinocytes (Saha and Hait, 2012).

According to “World Health Organization, 80 per cent of the people living in rural areas depend on natural products as primary healthcare system”. A great deal of pharmaceutical study is being preferred in technologically advanced countries like Germany, USA, France, China and Japan has considerably enhanced quality of the natural products used in the treatment of cancer. By enhancing detoxification functions of the body, some natural products protect the body from cancer. Certain of them decrease the toxic side effects of radiotherapy and chemotherapy.

Various species of *Dalbergia* have been reported to have phenolic compound, flavonoids, tannins, cardiac glycosides, carbohydrates, proteins, terpenoids, isoflavones, stigmaterol and neoflavonoids (Wealth of Indian Raw Materials, 1972; Mohammad and Arun, 2011). Leaf of *D. sissoo* has Isoflavone -O- glycoside and other compounds obtained from *D. sissoo* is a isoflavone, biochanin which is a potent cancer preventive agent. (Dixit *et al.*, 2012; Kharkwal *et al.*, 2012).

Against to the cancer several antioxidants are reported to act as protecting agents (Huang *et al.*, 1994; Kozoumbo *et al.*, 1983; Huang *et al.*, 1997). Antioxidants act as the primary line of defence in contrast to ROS which propose their usefulness in removing the risk of oxidative injury caused during carcinogenesis. Leaf extract of *D. sissoo* is rich in phenolic content. On the basis of the fact that phenolics are known for their capability to trap free radicals ions, it seemed important to evaluate their antioxidant activity (Rijhwani and Bharty, 2016). Since the compounds displaying anti-inflammatory and/or antioxidant activities are believed to be effective anti-tumor agents (Surh, 2002), the investigation suggest that the phytochemical fraction of

Dalbergia sissoo having phenolic compound, flavonoids, tannins, cardiac glycosides, carbohydrates, proteins, terpenoids, isoflavones, norartocarpotin, stigmaterol and neoflavonoids have antioxidant activity and also inhibit the formation of chemically induced mouse skin tumors.

In *Dalbergia sissoo* leaves extract treated mice were found to possess diminished tumor burden, cumulative number of papillomas, tumor yield, tumor incidence, tumor size and tumor weight (group IV). This drop may be due to reasons such as DMBA metabolism inhibition to its active form or delay in the promotion phase of tumorigenesis through down regulation in the production of reactive oxygen species and inhibiting effects on skin tumor promoting-inducing epidermal ornithine decarboxylase activity. For the reason that reactive oxygen species (ROS) have been involved in premature skin aging, DNA damage, carcinogenesis, instigation of signal transduction pathways associated to differentiation, growth and cell death, it is supposed that antioxidants could have potential anti-carcinogenesis activity at several stages of skin carcinogenesis (Rana *et al.*, 2002; Md Roduana *et al.*, 2017).

It can be determined that phytoconstituents present in the Petroleum ether extract of *Dalbergia sissoo* leaf by virtue of their separate or synergistic activity and by activating the antioxidant system induced significant preventive effect, leading to decline of skin tumors in swiss albino mice.

Conclusion

It can be concluded that *D.sissoo* possesses preventive potential against DMBA-induced skin carcinogenesis when given prior to carcinogen administration in mice at the dose rate of 200 mg/kg when given orally during the process.

Table 1: Effect of *D.sissoo* on body weight and tumor diameter in mice having chemical-induced skin carcinogenesis.

Treatment group	Body weight (gm)		Tumor Diameter (mm)	
	Initial	Final	2-5 mm	6-9mm
Group I Control (vehicle)	25.54±0.75	32.55±0.54	-	-
Group II Plant alone	27.72±0.73	32.15±0.43	-	-
Group III DMBA	25.24±0.83	22.30±0.41	28	18
Group IV <i>D. sissoo</i> + DMBA	24.93±0.66	30.77±0.72	21	15

Table 2: Antitumorigenic activity of *D.sissoo*. on DMBA–induced skin carcinogenesis in mice

Treatment group	No. of Tumor	Tumor yield	Tumor burden	TIM (%)	Tumor incidence (%)
Group I Control (vehicle)	-	-	-	-	-
Group II Plant alone	-	-	-	-	-
Group III DMBA	46	5.75	5.75	-	100
Group IV <i>D.sissoo</i> +DMBA	36	4.50	5.14	21.73	87.50

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