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Dalbergia sissoo Leaves Chloroform Extract Prevents DMBA- Induced Skin Carcinoma in Swiss Albino Mice

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Abstract:- *Dalbergia sissoo*, is extensively make use of the treatment of many diseases in different parts of India. In the current investigation, cancer preventive efficacy of *D. sissoo* was evaluated on 7, 12-dimethyl benz (a) anthracene (DMBA) treated skin carcinogenesis in Swiss albino mice. Single topical treatment of DMBA followed 2 weeks later through repeated application of croton oil and continued till the completion of the experiment. Group I (vehicle treated control); group II Carcinogen treated control; Group III *Dalbergia sissoo* leaf chloroform extract control and group IV Leaf chloroform extract of *Dalbergia sissoo* 200, 400 and 600 mg/kg, 7 days before and after that for 16 weeks along with croton oil treatment.

***Keywords*:-** *Dalbergia sissoo Leaves, Chloroform Extract, Carcinogen.*

I. INTRODUCTION

Various parts of *D. sissoo* plant such as bark, leaves, seeds, roots and wood were being used in numerous diseases from ancient time. It is utilized not only in Ayurveda but also in Unani medicines. Earlier studies exhibited that *D. sissoo* have cardiac, antimicrobial, neural, antioxidant, antidiabetic, anti-inflammatory, antiparasitic, dermatological, analgesic, Osteogenic, reproductive, gastrointestinal and many additional impacts.

D. sissoo plant is extensively used in folklore medicine for numerous diseases. Plant bark extract was utilized in sciatica as anti-inflammatory, piles and as blood purifier, in fevers the concentrated extract of heartwood in milk was recommended. The oil was utilized on the outside in the infected ulcers and skin diseases. Plant wood was used as antileprotic, anthelmintic as well as cooling. Arial parts of the plant were used as aphrodisiac, expectorant and spasmolytic. *D. sissoo* leaves extract was used as antioxidant, anti-diabetic, analgesic, antipyretic and anticancergenic. Plant flowers were utilized for skin problems, immunity Booster and as blood purifier (Nadkarni, 1954; Ghani, 1998; Khare, 2007; Ramrakhiyani et al., 2016).

Isoflavones, amyrin, "biochanin-A, muningin, stigmasterol, sissotrin" have been extracted from the aerial parts of *Dalbergia sissoo* Roxb plant (Sarg et al., 1999)

II. MATERIALS AND METHODS

Chemicals: The initiator, "7, 12-dimethylbenz [α] anthracene (DMBA)" and croton oil were procured from "Sigma Chemicals Co, St Louis, MO". In acetone DMBA was dissolved at a concentration of 100 µg/100 µL.

Plant Extract: Leafs of *D. sissoo* were collected from "University of Rajasthan campus, Jaipur, Rajasthan, India". The Leaves were identified and authenticated 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 by chloroform extract at 55˚-60˚ C for 35 h.

Animals: The current experimental study was accompanied on seven- eight week old healthy albino mice and weighing 24 ± 2 gram, chosen from inbreed colony in the laboratory.

Parameters for Study

Cumulative no. of tumors

The total number of tumors seemed till the terminations of the experiment, were noted.

Inhibition of tumor multiplicity

Total tumors in carcinogen treated control mice – total tumors in plant extract treated mice / Total tumors in carcinogen treated control mice X 100

Statistical Analysis

Results were expressed as mean ± S.E. Comparisons between the means of the control and experimental groups were made by one-way analysis of variance (ANOVA) using the SPSS software package for windows.

Experimental Design

Mice were divided into four groups of eight mice each.

Group I: Vehicle treated Control: In this group mice were treated orally with double distilled water (100 µl/ mouse/ day) and topically on the dorsal skin with acetone (100 µl/ mouse).

Group II: Carcinogen treated Control (DMBA + Croton Oil)*:* DMBA was applied topically with a single dose of 100 µg of DMBA in 100 µl of acetone. After 2 weeks of DMBA application, croton oil (100 µl of 1% croton oil in acetone) was applied three times per week, until the end of the experiment.

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Group III: *D. sissoo* leaves extract alone: Animals were treated with *D.sissoo* leaf chloroform extract at the dose level of 200, 400 and 600 milligram/kilogram body weight, orally, for 16 weeks.

Group IV: DMBA+ *Dalbergia sissoo* leaf extract:Test groups–received DMBA and croton oil as in group III. *Dalbergia sissoo* leaves extract (200, 400 and 600 milligram/kilogram) orally was given starting one week before the exposure to the carcinogen and then continuous for 16 weeks.

III. RESULTS

Cumulative Number of Tumors

Mice of group I had no number of skin tumors during the entire experimental period (up to 16 weeks).Group II carcinogen fed group had noticeably higher tumor emergence at different weeks throughout the entire experiment. Cumulative number of tumors in these mice was noted as 51. No occurrence of tumor was seen in this group III when *D. sissoo* was administrated orally at a dose level of 200, 400 and 600 milligram/kilogram body weight/ day in the study period of 16 weeks.

Animals of this group (group iv-a, b, c) when given *D. sissoo* leaves extract (200mg/kg) during the entire experimental period exhibited 31 cumulative numbers of tumors as compared to carcinogen treated control animals.

Mice that were orally treated with *D. sissoo* leaves extract at a dose of 400 mg/kg during the experimental period displayed 20 cumulative number of skin tumors was registered in contrast to the Group-II . The administration of *D.sissoo* leaves extract with the dose of 600 mg/kg during the experimental period exhibited 5 Cumulative number of tumors was noted which were recorded as significantly lower respect to carcinogen treated control animals

While comparing the values obtained in different experimental groups of *D.sissoo* chloroform leaves extract treatment at the dose of 600 mg/kg exhibited the maximum reduction in cumulative numbers of tumors when given throughout the experimental period in contrast to carcinogen experimented group.

Inhibition of Tumor Multiplicity

Group IV

In the absence of *D. sissoo* treatment, inhibition of tumor multiplicity was considered as zero percent for the animals of carcinogen fed control group. The *D. sissoo* administration was documented to increase the inhibition of tumor multiplicity in the dose related way i.e. 19.65, 43.22 and 71.93% in the sub groups IVa, IVb and IVc (Fig. 1).

IV. DISCUSSION

The rising incidence of skin cancer is a result in an increasing public health concern; the present-day chemo-immunotherapy and chemotherapy treatment have shown small clinical advantage with less or no improvement to the overall survival of the patient (Stadler et al., 2015; Swetter et al., 2019). The current investigation reveals the preventive property of *D. sissoo* extract, on the DMBA induced skin carcinoma in mice.

Numerous investigations have revealed that compounds that have anti-inflammatory activity inhibit "12-O- tetradecanoyl phorbol-13-acetate" induced tumor promotion in mice skin while "Aurore and collaborators described that anti-inflammatory steroids extremely inhibits epidermal DNA synthesis and cellular proliferation induced by phorbol ester tumor promoters, a pre-requisite for tumorigenesis" (Aurore et al., 1977) and the anti-inflammatory efficiency of *D. sissoo* which has been described by various researchers (Sidana *et al*., 2012; Kumar and Kumud, 2010;Behera *et al*.,2013;Amrutkar *et al*., 2017) might have played a synergistic function in the inhibition of tumorigenesis as detected in the existing research.

Preventive activity of the plant extracts is may be due to the occurrence of secondary metabolites such as terpenoids, tannins, glycosides, and flavonoids present in the hydromethanolic *D. sissoo* leave extract **(**Wealth of Indian Raw Materials, 1972; Mohammad and Arun, 2011**).** Evidences have also been present which showed that these secondary metabolites possess anticancer activity.

V. CONCLUSION

The present study clearly demonstrates that chloroform leaves extract of *D. sissoo* have preventive activity of skin carcinogen, when compared with the control mice. The presence of secondary metabolites might have provided strengthened this activity.

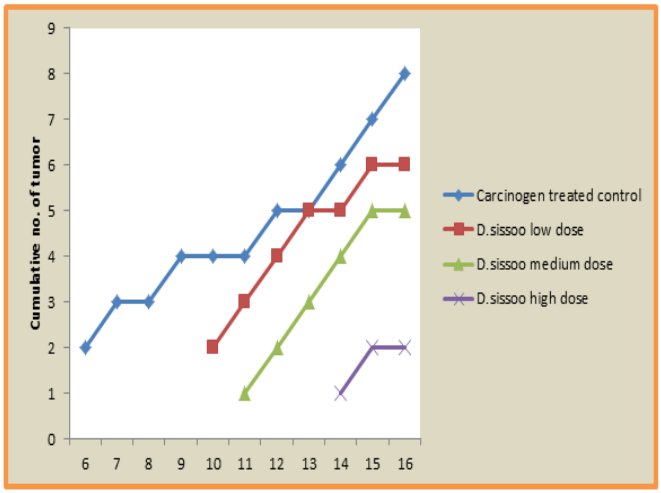

Fig 1: Graph showing variation in cumulative Number of tumors

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Table 1: Variation in tumor inhibition multiplicity in different groups of chloroform extract during chemical induced skin carcinogenesis in mice

Treated Mice Group	Tumor Inhibition multiplicity
I Control	-
II Carcinogenic treated control	-
III a *D. sissoo* treated 200 mg/kg b. wt.	-
III b *D. sissoo* treated 400 mg/kg b. wt.	-
III c *D. sissoo* treated 600 mg/kg b. wt.	-
IV a *D. sissoo* treated 200 mg/kg b. wt. + Carcinogen	19.65
IV b *D. sissoo* treated 400 mg/kg b. wt. + Carcinogen	43.22
IV c *D. sissoo* treated 600 mg/kg b. wt. + Carcinogen	71.93

REFERENCES

[1]. Khare, C. P. (2007) Indian medicinal plants: an illustrated dictionary. Springer-Verlag, New York, USA, 199-201.

[2]. Ramrakhiyani, C., Gaur, V. N. and Athaley, R. (2016) Comparative and therapeutic studies of some medicinal plants of family Fabaceae. IOSR Journal of Pharmacy and Biological Sciences, 11(2): 17-19.

[3]. Nadkarni, K. M. (1954) Indian Materia Medica. 3rd ed. Bombay: Popular Book 1954:432.

[4]. Ghani, A. (1998) Medicinal plants of Bangladesh: chemical constituents and uses. 1st ed. Asiatic Society of Bangladesh, 155-156.

[5]. Sarg, T., Ateva, A. M., Ghani, A. A., Badr, W., Shams, G. (1999) Phytochemical and Pharmacological studies of Dalbergia sissoo growing in Egypt. Pharm Biol., 37:54–62.

[6]. Aurore, V., Thomas, J. S., Michael, W., Weinstein, I. B. (1977) Effects of anti-inflammatory agents on mouse skin tumor promotion, epidermal DNA synthesis, phorbol-ester induced cellular proliferation and production of plasminogen activator. Cancer Res., 37: 1530–1536.

[7]. Stadler, S., Weina, K., Gebhardt, C., Utikal, J. (2015) New therapeutic options for advanced non-resectable malignant melanoma. Adv. Med. Sci., 60, 83–88.

[8]. Swetter, S.M., Tsao, H., Bichakjian, C.K., Curiel-Lewandrowski, C., Elder, D.E., Gershenwald, J.E. et al., (2019) Guidelines of care for the management of primary cutaneous melanoma, J. Am. Acad. Dermatol., 80 208–250.

[9]. Sidana, J. K., Saini, V. and Dahiya, S. (2012) Analgesic and anti-inflammatory activities of *Dalbergia sissoo* leaves extract. International Journal of Natural Product Science, (Spl issue 1): 134.

[10]. Kumar, S. M. and Kumud, U. (2010) Anti-inflammatory Activity of Root of Dalbergia sissoo (Rox.b) in Carrageenan-Induced Paw Edema in Rats. Phcog.Net. vol 2, 427-430.

[11]. Behera, P. C., Verma, S. M., Kumar, P. M., Nalin B. Das, Mishra, P. M. and Sasmita Baliarsingh, S. (2013) Anti-Inflammatory and Anti-Microbial Activity of Chalcone from *Dalbergia Sissoo* Roxb. Leaves. AJPCT1[2]: 186-194.

[12]. Amrutkar, Y., Hajare, S. W., Ingawale, M. V., Bhojane, N. M., Madhuri, Hedau, Ingole, R. S.

[13]. (2017) Anti-inflammatory activity of ethanolic leaf extract of *Dalbergia sissoo* in vitro and in vivo. *Adv Tissue Eng Regen Med Open Access*, 2(3):171–174.

[14]. Wealth of Indian Raw Materials. Publication and information directorate, CSIR, New Delhi, 1972; 2:214-230.

[15]. Mohammad A, Arun K. (2011) Phytochemical investigation and evaluation of antinociceptive activity of ethanolic extract of *Dalbergia sissoo* (Roxb.) bark. J Nat Sci Biol Med., 2:76–9.