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ORIGINAL ARTICLE **OPEN ACCESS**

Alteration in Liver and Skin biochemistry of mice having DMBA induced carcinoma after *Dalbergia sissoo* leaf extract treatment

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ABSTRACT

Effect of D. sissoo leafs extract (200 mg/kg body weight, orally) was treated on chemically induced development of skin carcinogenesis in adult Swiss albino mice. The animals were distributed into four groups: Group I (vehicle treated control); group II Carcinogen treated control; Group III Dalbergia sissoo leaf Petroleum Ether extract control and group IV Dalbergia sissoo leaf petroleum ether extract 200 mg/kg, 7 days before and after that for 16 weeks along with croton oil treatment. After the 16th week of treatment the mice were sacrificed, liver was dissected out, weighed and evaluated for the biochemical contents in it Protein, lipid peroxidation (LPO), glutathione (GSH), superoxide dismutase (SOD) activity and catalase (CAT) activity. Due to DMBA toxicity protein content, GSH level, SOD and CAT activities also diminished in the liver. But there was rise in LPO level in the liver. DMBA act as tumor initiator and induces oxidative stress.

Key words:-Mouse, liver, skin, carcinoma, *Dalbergia sissoo,* protein, lipid peroxidation

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INTRODUCTION

Carcinogenesis is a complex, multistep progression which involves the sequential accumulation of mutations of geneand cellular proliferation that leads to neoplastic transformation and gained the ability to form distant metastasis [1-3]. Two-stage mice skin cancer model was developed to study the sequential and step-wise development of skin tumor in mice [5, 6, 8]. The general protocols include two induction stages of (i) initiation, the application of a sub-carcinogenic dose of carcinogen. Then, the tumor development is (ii) promoted by the repeated treatment of croton oil (tumor-promoting agent) [6, 7]. The involvements of biochemical reactions, free radicals as well as alterations of metabolic action have been highlighted with the help of two-stage skin carcinogenesis model [8].

Studies on plant materials have revealed their health promoting action including cancer prevention. *Dalbergia sissoo* Roxb.also called "shisham" is used sincetime immemorial for treatment of several diseases like sensations, burning, dysentery, leucoderma, dyspepsia, skin ailments, memory enhancing and inflammation. Leaves of the plant have significant amount of flavonoids which has antioxidant activity twice of commonly used antioxidants like vitamin C and Selenium [4, 10]. It also prevents central nervous system damage [1].

D. sissoo is known to possess diverse phytoconstituents including biochanin A, tectorigenin, mesoinisitol, isocaviumin, tectorigenin, dalbergin, dalberginone, tannins, fixed oils and essential oils [20]. Compounds obtained from fresh flowers of *Dalbergia sissoo* like an isoflavone, biochanin are a effective chemotherapeutic cancer preventative agent with a distinct estrogenic activity.

Leaves of *Dalbergiasisssoo* plant contain Isoflavone -O- glycoside, Rhamnose, galactose, and glucuronic acid [25], Pods have Mesoinisited, 7 - O – methyltectorigenin and 4'- rhamnoglycoside. Rural persons in India as well as Nepal use *Dalbergia sissoo* leaves to treat animals suffering from non-specific diarrhoea. Leaf extract has been used to treat heart problems, sore throats, syphilis, gonorrhoea and dysentery [22]. The juice of the leaves is useful for anthelmintic, beneficial for diseases of the nose and the eye. It is used in burning sensation of the body, scabies, scalding urine, digestive disorders and syphilis [23, 24].

MATERIAL AND METHODS

Chemicals: The initiator, "7, 12-dimethylbenz [α] 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 also 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 healthy Swiss albino mice and weighing 24 ± 2 g, selected from inbreed colony in the laboratory.

Parameters for Biochemical Study

Biochemical parameters Protein [26], GSH [27], SOD [28], LPO [30] and Catalase [29] were performed in mice liver and skin. Biochemical alterations were measured in animals of all of the above groups at the time of termination of the experiment.

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 Plan

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

Group I: Vehicle treated Control: Inthis group animals were 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: Carcinogen treated 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 III: *Dalbergia sissoo* leaf extract alone :Mice were treated with Petrolium Ether extract of *D.sissoo*leaf suspended in distilled water at the dose rate of 200 mg/kg body weight, orally, for sixteen weeks.

Group IV: DMBA+ *Dalbergia sissoo* leaf extract (200 mg/kg b. wt./day):Test groups–received DMBA and croton oil as in group III. *Dalbergia sissoo* leaves extract (200mg/kg/body wt.) orally was given starting one week before the exposure to the carcinogen and then continued for 16 weeks.

RESULTS

Total Proteins

Total protein concentration in liver and skin was valued as 109.25±4.54and 84.63±3.89 mg/gm tissue, respectively in the animals of vehicle treated group. The animals belonging to the DMBA croton oil treated group exhibited a significant ($P<0.001$) decrease in the total protein content in liver and skin respectively, in comparison to the vehicle treated control group. There was a slight increase in the protein level of liver and skin of mice, when the animals were administrated with *D. sissoo*. (200 mg/kg b. wt.). *D. sissoo*. administration in the animals of sub group IVresulted in the significant elevation in total protein content as compared to the carcinogen treated control group (group II) .

Lipid peroxidation (LPO)

Lipid peroxidation level was measured as 2.64 ±0.19 n mole/mg in the liver and 3.55±0.28 n mole/mg in skin of Swiss albino mice of(vehicle treated control). *D. sissoo* administration did not bring any noticeable alteration in LPO level. The animals belonging to the DMBA croton oil treated group exhibited a significant ($P<0.001$) increase in the LPO level in liver and skin respectively, in comparison to the vehicle treated control group. Animals of group IV exhibited a significant reduction in the LPO levels in the liver and skin as compared to the animals of the group II.

Glutathione

Reduced glutathione level was observed in animals of vehicle treated control group as 9.25±0.20 and 5.88±0.12 u mole/ gm tissue in liver and skin, Respectively. In comparison to the vehicle treated control group, the animals of DMBA and croton oil treated group exhibited a marked decrease in the glutathione levels in both the liver and skin (Group II). No significant alterations were recorded after *D. sissoo*. alone treatment (200 mg) but levels of GSH were found to increase in liver and skin of the administration of *D. sissoo* in group IV.

Catalase activity

In the animals of vehicle treated control i.e. Group 1, the catalase activity was noted as 47.98±3.02 in liver and 45.28±2.78 U/ mg tissue in skin. The activity of catalase (CAT) in the liver and skin of mice treated with DMBA and croton oil (Group II) exhibited a significant decrease as compared to the vehicle treated control (Group I). In the animals of Group IV in which *D. sissoo*. was administered the catalase activity was found to elevated in the liver and skin when compared with the carcinogen treated control group (group II).

Superoxide Dismutase activity

The SOD levels were noticed as 69.39±2.37 and 56.76±1.88 in liver and skin respectively, in animals belonging to the vehicle treated control group. On application of DMBA and croton oil, the SOD activities in the liver and skin of the animals of Group III showed a significant decrease from the normal value. Mice belonging to the Group IVwas given *D. sissoo*. at the 200 mg/kg dose level had a significant ($P<0.01$) elevation in the SOD activity in the liver and skin All these values were noted to be statistically higher when compared to the respective values of carcinogen treated control.

DISCUSSION

Skin cancer is the most common form of human cancer and its incidence is rising rapidly world-wide. Basal cell carcinoma accounts for 80 percent whereas melanomas and squamous cell carcinoma account for 4% and 16% respectively of all type of skin cancers. It is worth noting that deaths from skin cancer have risen among men in developed countries. Numerous precipitating factors mediate the development of skin cancer, such as chronic inflammation, UV radiation, genetic disorder and chemical inducers [10, 18, 19].

DMBA induced skin cancer is consequently used as antool to test the antioxidant potential of medicinal plants and its constituents. Enzymatic activation of poly aromatic hydrocarbons lead to the generation of active oxygen species such as peroxides and superoxide anion radicals, which induce oxidative stress in the form of lipid peroxidation [11, 12].

Liver, the main metabolic organ, performs an important role in the detoxification procedure and thus analysing its status help to identify the chemopreventive efficacy of the test compound. Chemopreventive agents protect tumor formation through activating multiple biochemical mechanisms including phase-II detoxification enzyme induction and antioxidant defense mechanism. Phase-II detoxification enzyme plays a major role in increasing the polarity and assisting the excretion of xenobiotic agents.

Alteration in the biochemical contents of the skin and liver in carcinogen treated mice and further correction shows the recovery in the plant extract treated group may be due to general inhibition of DNA dependent RNA polymerase. Oxidative stress can induce alteration of chromosomal through oxidative base damage and strand breaks in DNA that participating to mutagenesis [13]. The carcinogenic and mutagenic action of genotoxic substances involves overproduction of DNAattacking reactive oxygen species [14].

In the current investigating significant diminution was observed in total protein, in carcinogen experimented control mice as compared to the normal untreated mice. The hypoproteinemia in cancer may be an expression of cachexia, representing homeostatic imbalance in which the utilization and obliteration of albumin by the tumor cannot be compensated by the organism. In some studies significant positive correlation was also seen among albumin and total thiols, which are effective antioxidants. Increased protein concentration recorded in our study in group IV mice displays that *D. sissoo* exerts prophylactic and ameliorative effects against carcinogens.

D. sissoo is enriched with the phenolic antioxidants, especially vitamin E, which is known to scavenge the peroxy radicals, super oxides and singlet oxygen. The current study depicts a significant ($P<0.05$-0.001) reduction in the LPO level in the skin and liver of the animals treated with *D. sissoo* leaves extract (Group IV)in comparison to carcinogen treated mice (Group II) which indicates that the administration of plant extract showed anti-oxidant activity, which have the capability to break the chain reactions of free radicals.

A significant increase in the reduced glutathione, superoxide dismutase and catalaseactivity in both liver and skin was recorded in the *D. sissoo* leaves extract treated animals (groupIV) when compared to carcinogen treated animals (group II). This antioxidant property of *D. sissoo* may is due to phenolic compounds and due to intrinsic anti-oxidative potential of *D. sissoo.*

As showed in the results, *D. sissoo* plant has significant amounts of phenolics and flavonoids. Flavonoids are a group of natural components are found in vegetables fruits and other parts of the plant. These natural substances are known for their valuable effects on human health. It has been established that flavonoids have anti-oxidative, antitumor and anti-inflammatory activities that were attributed to their capacity to regulate main cellular enzyme functions [15]. Moreover, phenolic substances are large

heterogeneous components of secondary herbal metabolites that have been broadly found in herbs [21]. These natural constituents rich in antioxidants are of crucial value for researchers [17-19].

Table 1: Variation in the Protein, glutathione (GSH), superoxide dismutase (SOD), lipid peroxidation (LPO) and catalase levels in the skin of mice in different treatment groups of petrolium ether during chemical induced skin carcinogenesis

Treatment group	**PROTEIN** (mg/gmtissue)	**GSH** (µ mole/gmtissue)	**SOD** (U/mg tissue)	**LPO** (n mole/mg tissue)	**CATALASE** (U/mg tissue)
Group I Control (vehicle)	84.63±5.62	5.88±0.12	56.76±1.88	3.55±0.28	45.28±2.78
Group II Carcinogen treated control	46.50±3.21^{c}	1.97±0.33 ^{c}	27.46±2.45 ^{c}	11.13±0.54^{c}	26.34±1.07^{c}
Group III Plant alone	86.50±3.77^{ns}	6.74±0.25 ^{ns}	59.46±3.67 ^{ns}	3.14±0.23 ^{ns}	47.36±2.48^{ns}
Group IV *D.sissoo* treated low dose	60.50±4.56^{b}	2.82±0.27^{b}	29.65±1.25^{c}	8.17±0.52^{c}	28.93±3.68^{b}

Levels of significance: ns-non- significant; Values represent mean ± SEM of 8 Animals
a-P<0.05; b- P<0.01; c- P<0.001 when compared with carcinogen treated control

Table 2: Variation in the Protein, glutathione (GSH), superoxide dismutase (SOD), lipid peroxidation (LPO) and catalase levels in the liver of mice in different treatment groups of petrolium ether during chemical induced skin carcinogenesis

Treatment group	**PROTEIN** (mg/gm tissue)	**GSH** (µ mole/gm tissue)	**SOD** (U/mg tissue)	**LPO** (n mole/mg tissue)	**CATALASE** (U/mg tissue)
Group I Control (vehicle)	109.25±4.54	9.25±0.20	69.39±2.37	2.64±0.19	47.98±3.02
Group II Carcinogen treated control	66.38±3.07 ^{c}	4.25±0.53 ^{c}	35.45±3.76 ^{c}	9.29±0.32^{c}	27.59±2.19^{c}
Group III Plant alone	115.50±4.17^{ns}	11.08±0.42^{ns}	72.58±4.68^{ns}	2.32±0.15^{ns}	51.51±1.77^{ns}
Group IV *D.sissoo* treated low dose	79.63±4.59^{b}	5.67±0.28^{b}	44.84±5.11^{b}	6.18±0.62^{c}	31.95±5.20^{b}

Levels of significance: ns-non- significant, Values represent mean ± SEM of 8 Animals
a-P<0.05; b- P<0.01; c- P<0.001 when compared with carcinogen treated control

CONCLUSION

The present study clearly demonstrates thatfeeding of the petroleum ether extract of *D. sissoo* mice reversed the changes in the biochemical parameters to a normal level, when compared with the control mice. The presence of natural antioxidants such as flavanoids and polyphenols might have provided strengthened this activity.

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