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Effect of *Clerodendrum serratum* leaf extract on biochemical and oxidative stress parameters of testis in 7, 12-dimethylbenz[a]anthracene induced skin carcinogenesis in Swiss albino mice

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Abstract
The biochemical contents and antioxidant potential of *Clerodendrum serratum* (Verbenaceae) leaf extract (CSLE) on 7, 12-dimethylbenz[a]anthracene (DMBA) induced skin carcinogenicity in testis of mice was investigated. Group I received distilled water served as control. The skin lesions were induced by twice-weekly topical application of DMBA for 2 weeks on the shaved backs of group II, III, IV and V mice. CSLE was administered to group III, IV and V mice at the dose of 300, 600 and 900 mg/kg b.wt/day, for 4 week before DMBA application, and continued till 45 days. On 46th day the mice were sacrificed, testis were dissected out freed from adherent tissue and weighed to nearest milligram and evaluated the biochemical contents DNA, RNA, protein, glycogen, cholesterol, lactate dehydrogenase (LDH), Succinic dehydrogenase (SDH), acid phosphatase (ACP) and alkaline phosphatase (AKP) activities, oxidative stress parameters, levels of glutathione (GSH), thiobarbaturic acid reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT) and glutathione-s-transferase (GST). DMBA induced skin carcinogenesis decreased body and testis weight, DNA, RNA, protein, glycogen, GSH level, SDH, AKP, SOD, CAT and GST activities. But there was increase in cholesterol content, LDH, ACP activities and TBARS level. DMBA act via generating reactive oxygen species (ROS) as tumor initiator and free radicals inducing oxidative stress. The results revealed that there was a recovery in biochemical contents, dehydrogenases, phosphatases and oxidative stress parameters in testis. Thus, the present study inferred that CSLE administration significantly curtailed tumor development and counteracted all the biochemical effects. Many plant secondary metabolites exhibit potent anticarcinogenic potential and known to exert their effects by quenching reactive oxygen, inhibiting lipid peroxidation.

Keywords: *Clerodendrum serratum*, DMBA, Biochemical parameters, Reactive oxygen species, Mice.

INTRODUCTION

Skin is a barrier or an outer layer of the body that protects humans from heat or cold, chemicals, bacteria, UV and other harmful radiations. Skin, a major environmental interface for the body, is accidentally or occupationally exposed to a number of chemical mutagens and carcinogens [1]. Skin cancer is the most common form of human cancer in which cancer cells are found in the outer layers of the skin and its incidence is increasing rapidly all over the world. Basal cell carcinoma accounts for 80% whereas squamous cell carcinoma and melanomas accounts for 16% and 4% respectively of all skin cancers [2]. Squamous cell carcinoma is the most serious form of cancer than other skin cancers since they can spread into vital organs inside the body [3]. In India, skin cancer accounts for approximately 1-2% of all diagnosed cancers and the annual incidence of skin cancer will increase significantly in future due to its immense population [4].

7, 12-dimethylbenz[a]anthracene (DMBA), the organ specific potent carcinogen, is commonly used to induce skin cancer in Swiss albino mice. DMBA could either be used as an initiator or promoter for inducing skin carcinogenesis. Twice per week for two week topical application of DMBA induced skin carcinogenesis in swiss albino mice [5]. DMBA induced skin cancer is therefore used as an ideal tool to test the antioxidant potential of medicinal plants and its constituents. Enzymatic activation of Poly aromatic hydrocarbons leads to the generation of active oxygen species such as peroxides and superoxide anion radicals, which induce oxidative stress in the form of lipid peroxidation [6,7]. Consequences of the damage initiated by these metabolic by products affect reproductive organ testis by large range of biological reactions, like increases in mutation rate, alteration of cellular membrane composition, structural proteins, metabolic, detoxifying enzymes and cellular signaling proteins [8].

All over the world, studies on plant materials have revealed their health promoting action including cancer prevention. The *Clerodendrum serratum*, Linn (Family: Verbenaceae) commonly known as “Bharngi” in the ayurvedic medicine of Indian system. The genus *Clerodendrum* L. is very widely distributed in tropical and subtropical regions of the world. Plants remain a large chemical library to be explored for new agents. It has been reported that, plant-derived triterpenoids have attracted reasonable attention for their unique antineoplastic activity [9]. The major chemical components reported from the genus *Clerodendrum* are phenolics, steroids, di-terpenes and triterpenes, flavonoids, volatile oils, etc

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[10]. Therefore the present investigation was undertaken to evaluate the effect of *Clerodendrum serratum* leaf extract on biochemical and oxidative stress parameters of testis in 7, 12-dimethylbenz [a] anthracene induced skin carcinogenesis in Swiss albino mice.

MATERIALS AND METHODS

Carcinogen

The carcinogen chemical 7, 12-dimethylbenz[a]anthracene (DMBA) was procured from Sigma Chemicals Co., St. Louis, USA. DMBA is 95% potent carcinogen, with molecular formula $C_{20}H_{16}$ and molecular weight 256.3.

Plant extract preparation

The leaves of *Clerodendrum serratum* were collected from Botanical garden of Karnatak University Dharwad. The plant was authenticated in P. G. Department of Botany, Karnatak University Dharwad. Methanolic leaf extract of *Clerodendrum serratum* was extracted by the Soxhlet apparatus by continuous cycle collection of the extract. The leaves of the plant were washed and dried at room temperature and crushed by the mechanical grinder to fine powder. The powder (500 gm) was then extracted with 2.5 litre of 90% methanol in a Soxhlet apparatus at 65ºC, until the powder became exhausted totally. The resulting extract was filtered, concentrated, and dried in vacuo (yield 8.75% w/w). The extract was stored in a desiccator for administration orally to mice in three increasing graded dose.

Animals

Laboratory bred adult male Swiss albino mice were used in the experiments. Mice aged 90 days old weighing between 25-30 g was used. The study was approved by the Ethical Committee, Dept. of Zoology, Karnatak University, Dharwad, India; CPCSEA (639/02/a/CPCSEA) guidelines were followed for maintanance and use of experimental animals. They were housed in separate polypropylene cages containing sterile paddy husk as bedding material. The mice were provided with standard mice pellet diet "Gold Mohar" (Hindustan Liver Company, Mumbai) and water *ad libitum*. The mice were maintained under normal day/night schedule (12 L: 12 D) at room temperature 25 ± 2°C.

Treatment

The chemical carcinogen, 7, 12-dimethylbenz[a]anthracene (DMBA) induced skin tumorigenesis in male Swiss albino mice [11]. It was applied topically on the dorsal skin surface of the mice, at a dose of 25 µl DMBA in 25 µl acetone (1:1v/v) per mouse twice a week for two weeks to respective groups with a suitable art brush. Methanolic leaf extract of *Clerodendrum serratum* was dissolved in physiological saline and administrated orally in the graded dose of 300, 600 and 900 mg/kg b.wt/day for four weeks before topical application of DMBA on skin to respective groups and continued for two weeks while inducing to respective groups. After 45 days mice were sacrificed and the skin and testis was dissected out and stored in saline. The experiment was designed to determine the preventive effect of methanolic leaf extract of *Clerodendrum serratum* on 7, 12 dimethylbenz[a]anthracene (DMBA) induced skin carcinogenesis on body and testis weight, biochemical contents and oxidative stress parameters of the testis in albino mice.

Biochemical Studies

The biochemical contents such as DNA and RNA carried out as per the method described by [12], protein by [13], glycogen by [14], cholesterol by [15], activities of enzymes such as LDH by [16], SDH by [17], ACP and AKP by [18].

Oxidative stress parameters

The oxidative stress parameters such as GSH level was measured following the method of [19], the product of the reaction between malondialdehyde (MDA) and thiobarbaturic acid reactive substances (TBARS) by [20] were measured by a modified method of Esterbauer and Cheesman, (1990), SOD activity by [21], CAT activity by [22] and GST activity by [23].

Extraction of plasma membrane surface proteins and SDS-PAGE Electrophoresis

The extraction of surface proteins from plasma membrane of mice skin epidermis was carried with 3M KCl according to the procedure of [24]. The skin epidermis was removed at the time of autopsy, minced and washed with 5M phosphate buffered saline (PBS), pH 7.2 by centrifugation at 10,000 rpm for 10 min twice in order to remove the soluble extracts and intracellular components. SDS-PAGE was performed according to [25], to identify the tumor associated proteins with a medium range marker. Silver staining was performed to distinguish the bands as it was not clear with the commasie brilliant blue stain.

Statistical analysis

Statistical significance between the control and experimental data were subjected to analysis of variance (ANOVA) together with Dunnett's test ($P<0.05$).

RESULTS

Body and organ weight

Change in the body and testis weight revealed that there was a significant decrease in the body and testis weight of DMBA treated control mice. However, there was increase in the body and testis weight of mice treated with DMBA along with higher dose 900 mg/kg b.wt/day of plant extract showing its recovery (Table 1).

Biochemical studies

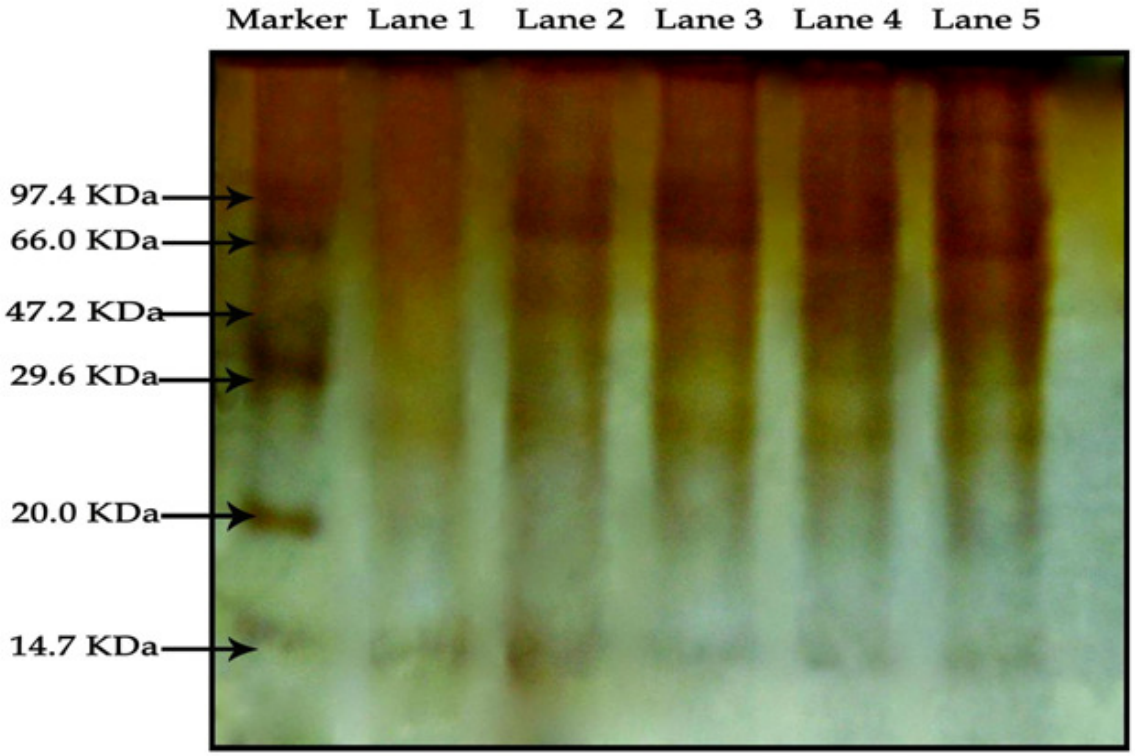
Biochemical contents and the enzyme activities revealed that there was a significant decrease in the level of DNA, RNA, protein, glycogen, SDH and ACP activities in the testis of DMBA treated control mice. However, there was increase in the level of the nucleic acids, protein, glycogen, SDH and ACP activities in the mice treated with DMBA along with higher dose 900 mg/kg b.wt/day of plant extract showing its recovery. But there was a increase in the level of the cholesterol, LDH and AKP activities of DMBA treated control mice and there was a decrease in the level of the cholesterol, LDH and AKP activities in the mice treated with DMBA along with higher doses of plant extract showing its recovery (Table 2 and 3).

Oxidative stress parameters

There was a decrease in the level of GSH, CAT, SOD and GST activity in the testis of DMBA treated control mice. Further, there was an increase in the level of GSH, CAT, SOD and GST activity in the mice treated with DMBA along with higher doses of plant extract showing its recovery. However, there was a increase in the level of TBARS of DMBA treated control mice and there was a decrease in the level of TBARS in the mice treated with DMBA along with higher doses of plant extract showing its recovery (Table 4).

Tumor associated proteins

The results of SDS PAGE of surface proteins in skin epidermis of mice showed the expression of the bands having molecular weights of 27 and 54 kDa indicating the presence of tumor associated proteins in DMBA treated control mice. However, there was expression of tumor associated proteins in DMBA along with plant extract treated mice (Fig. 1).



Marker Lane, Lane 1-Normal control, Lane 2-DMBA control, Lane 3-DMBA+CSLE (300 mg/kg/b.wt), Lane 4- DMBA+CSLE (600 mg/kg/b.wt), Lane 5- DMBA+CSLE (900 mg/kg/b.wt)

Fig 1. SDS PAGE of tumor associated proteins in the skin of the mice on treatment with DMBA and plant extract (CSLE)

Table 1. Effect of *Clerodendrum serratum* leaf extract (CSLE) on body and testis weight of mice on treatment with 7, 12-Dimethylbenz[a]anthracene (DMBA)

Groups	Treatment (mg/kg/d)	Change in body weight (g)	Relative testis weight /100 g body weight (Mean ± S.E) (g)
			Testis
I	Normal Control	2.14±0.32	0.36±0.14
II	DMBA Control (25µl)	-2.43±0.42*	0.21±0.23*
III	DMBA+CSLE (300)	0.62±0.38	0.26±0.15
IV	DMBA+ CSLE (600)	1.12±0.25*	0.32±0.26
V	DMBA+ CSLE (900)	2.52±0.28*	0.40±0.18*

Values are mean± SEM of 10 animals * Significant P ≤ 0.05 vs Control

Table 2. Effect of *Clerodendrum serratum* leaf extract (CSLE) on biochemical contents in the testis of mice on treatment with 7, 12-Dimethylbenz[a]anthracene (DMBA)

Groups	Treatment (mg/kg/d)	Biochemical contents (µg / mg wet weight of tissue)				
		DNA	RNA	Protein	Glycogen	Cholesterol
I	Normal Control	2.68±0.05	4.84±0.03	158.03±5.08	6.98±0.03	8.98±0.12
II	DMBA Control (25µl)	2.05±0.02*	3.34±0.40*	123.23±6.02*	4.53±0.07*	9.84±0.62*
III	DMBA+CSLE (300)	2.12±0.08	3.60±0.67	133.34±6.15	4.86±0.32	9.62±0.45
IV	DMBA+ CSLE (600)	2.28±0.34	4.34±0.83	146.48±7.18*	5.82±0.23*	9.43±0.62
V	DMBA+ CSLE (900)	2.52±0.23*	4.95±0.79*	161.58±6.24*	6.61±0.26*	9.23±0.87*

Values are mean± SEM of 10 animals * Significant $P \leq 0.05$ vs Control

Table 3. Effect of *Clerodendrum serratum* leaf extract (CSLE) on dehydrogenases and phosphatases enzyme activities in the testis of mice on treatment with 7, 12-Dimethylbenz[a]anthracene (DMBA)

Groups	Treatment (mg/kg/d)	Enzyme activity µmoles/ min/ g tissue			
		LDH[a]	SDH[b]	ACP[c]	AKP[d]
I	Normal Control	9.28±0.19	12.42±0.18	18.46±0.18	14.32±0.24
II	DMBA Control (25µl)	11.05±0.27*	11.15±0.05*	16.15±0.38*	16.10±0.84*
III	DMBA+CSLE (300)	10.60±0.45	11.35±0.48	16.52±0.17	15.72±0.45
IV	DMBA+ CSLE (600)	10.04±0.82	11.92±0.63	17.32±0.27*	15.12±0.56
V	DMBA+ CSLE (900)	9.45±0.64*	12.32±0.53*	18.02±0.36*	14.62±0.62*

a µmoles of pyruvate formed/ min/ g tissue b µmoles of formazon formed/ min/ g tissue
c µmoles of inorganic phosphorus formed/min/g tissue d µmoles of p-nitrophenyl formed/min/g tissue
Values are mean± SEM of 10 animals * Significant $P \leq 0.05$ vs Control

Table 4. Effect of *Clerodendrum serratum* leaf extract (CSLE) on oxidative stress parameters in the testis of mice on treatment with 7, 12-Dimethylbenz[a]anthracene (DMBA)

Groups	Treatment (mg/kg/d)	Antioxidant	Oxidative stress byproduct	Oxidative stress enzymes		
		GSH[a]	TBARS[b]	Catalase[c]	SOD[d]	GST[e]
I	Normal Control	9.27±0.08	0.23±0.03	148.60±2.18	45.36±2.74	2.91±0.08
II	DMBA Control (25µl)	6.63±0.09*	0.47±0.01*	126.34±2.84*	32.81±2.68*	1.23±0.06*
III	DMBA+CSLE (300)	7.23±0.07	0.34±0.02*	132.47±2.68	36.32±2.88	1.83±0.07
IV	DMBA+ CSLE (600)	8.10±0.08*	0.26±0.03*	140.53±2.46*	42.38±2.28*	2.20±0.08
V	DMBA+ CSLE (900)	8.95±0.08*	0.22±0.02*	146.40±2.54*	48.26±2.36*	2.46±0.09*

a µmole of glutathione (GSH)/ mg protein b nmoles thiobarbaturic acid (TBARS)/gm protein
c µmole of H_2O_2 d super oxide dismutase (SOD) unit/mg protein
e Glutathione-s-transferase (GST) µmole/min/mg protein
Values are mean± SEM of 10 animals * Significant $P \leq 0.05$ vs Control

DISCUSSION

Free radicals are "any species capable of independent existence that contain one or more unpaired electrons" [26]. Because of their very high chemical reactivity, free radicals are able to induce cellular damage in a variety of ways [27]. The most deleterious effects of free radicals are damage to DNA [28], which is associated with the process of carcinogenesis. This deleterious effect of free radicals can also be reduced by the natural or synthetic antioxidants. Antioxidants can terminate the free radicals chain reaction by

donating hydrogen or electrons to free radicals and converting them to more stable products. The present study shows that the *Clerodendrum serratum* leaf extract contains secondary metabolites which include antioxidants which are implicated in lowering the lipid peroxidation and the risk of cancer.

The decrease in the body weight on treatment with DMBA may be due to suppression towards food and water intake. Similar results are reported on the body weight in the conditions of DMBA induced squamous cell carcinoma of skin [29]. There has been considerable scientific evidence, epidemiologic and experimental, accumulated in the past two decades indicating that modifications in lifestyle, including diet, can have a major effect on the risk for numerous cancers [30]. Further, the increase in the body weight in the DMBA treated along with *Clerodendrum serratum* leaf extract reveal that, cancer prevention involves pharmacological intervention with synthetic or naturally occurring chemicals or substances to prevent or inhibit or reverse the process of carcinogenesis or prevent the development of invasive cancer [31].

There is a decrease in the testis weight in DMBA induced mice and further the increase in weight shows the recovery in the plant extract treated group. Similar results are reported on the testis weight in the conditions of DMBA induced squamous cell carcinoma of skin [32]. As the testis is highly susceptible to damages caused by genetic disorders, environmental or occupational exposure to chemicals or by other means. Quality of sperm production has been adversely affected due to the exposure to UV rays and chemicals, particularly mutagens and carcinogens [33]. The decrease in the testis weight on treatment with DMBA may be due to the release of free radicals which may affect the production of sperms as there is DNA damage in the germ cells. Recovery in the weight is due to the antioxidants of the plant extract which scavenge the free radicals and lowers the lipid peroxidation.

The decrease in the biochemical contents of the testis in DMBA induced mice and further the increase in biochemical contents shows the recovery in the DMBA along with plant extract treated group may be due to the general inhibition of DNA dependent RNA polymerase. Oxidative stress can induce chromosomal aberrations through oxidative base damage and strand breaks in DNA contributing to mutagenesis [34]. The mutagenic and carcinogenic action of genotoxic substances involves overproduction of DNA attacking reactive oxygen species [35]. Reactive oxygen species can react with amino acids and DNA and introduce cross linkages between proteins and nucleic acids, resulting in alterations in replication, transcription and leading to tumor formation [36]. Cholesterol level is increased in DMBA treated control due to inhibition of steroidogenesis in testis, similar reports reported when poly aromatic hydrocarbons and other toxicants were treated [37]. The rise in LDH activity in tissue suggested high turnover of pyruvate to lactate and vice-versa to yield required energy to overcome DMBA induced oxidative stress and reactive oxygen species generation [38]. Effect of carcinogen on carbohydrate metabolism in the tissue is indicated by decrease in SDH activity [39] as this enzyme is related with high metabolic activity such as absorption and secretion. Acid phosphatase (ACP) which hydrolysis the ester linkage of phosphate esters at acidic pH (between 5 to 6) and helps in autolysis of the degenerated cells [40]. Alkaline phosphatases (AKP), which splits phosphorous esters at alkaline pH (10) and mediates membrane transport is associated in protein synthesis [41].

The study reveals that there is a decrease in the level of GSH and increase in the level of TBARS of the testis in DMBA induced mice and further the increase in the level of GSH and decrease in the level of TBARS shows the recovery in the plant extract treated group. It has been suggested that decrease in the level of GSH in the testis of mice induced by DMBA, is a biological antioxidant present in high amounts in the testis, and its presence is a pre-requisite for protection against oxidative damage [42]. The increase in the level of TBARS in the testis of mice induced by DMBA helps to assess the extent of tissue damage in pathological conditions [43]. Lipid peroxidation (LPO) is the most extensively studied manifestation of oxygen activation in biology. LPO is broadly defined as “oxidative deterioration of poly unsaturated fatty acids (PUFA)” which is fatty acids that contain more than two carbon carbon double bonds [44].

The decrease in SOD, CAT and GST activity of the testis in DMBA induced mice and further the increase in SOD, CAT and GST activity shows the recovery in the DMBA along with plant extract treated group. It has been reported that decrease in the SOD, CAT and GST activity in the testis of mice induced by DMBA is because SOD and CAT form a part of the crucial processes involved in cellular antioxidant defence mechanism whereby peroxides and superoxides are inactivated [45]. Similar decline in the level of antioxidant enzymes like SOD, CAT and GST observed in CCl_4 treated mice is a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system resulting in tissue damage (46). Its been reported that reduced activity of CAT in alloxan treated mice results in the accumulation of H_2O_2, which produces deleterious effects (47). GST plays an important role in initiating detoxification by catalyzing the conjugation of GSH to the electrophillic foreign compounds for their elimination from the system, thereby providing cellular protection against a wide variety of xenobiotics [48]. Further, in the present study the SOD, CAT and GST activity in the testis of the mice treated with DMBA along with plant extract is increased may be due to the activation of these enzymes following exposure to the carcinogen DMBA that was found to result in decreased activity of these enzymes. The activation of these enzymes is also accompanied by a reduction in lipid peroxidation, a process known to generate reactive oxygen species that is associated with tissue injury and damage of cellular macromolecules [49]. The effect of plant extract on SOD, CAT and GST activity is due to their antilipidperoxidative and antioxidant functions during papillomagenesis, similar results are observed in several antioxidants of plant materials are experimentally proved and widely used as more effective agents against oxidative stress [50].

The SDS PAGE of tumor associated proteins in the skin of the mice on treatment with DMBA and plant extract (CSLE) reveal that there is a low molecular weight expression of tumor associated proteins in skin epidermis of DMBA treated groups. It is suggested that there have been different conclusions reported by prior workers on the existence of low molecular weight keratins in neoplastic tissues [51]. However, most investigators agree with the absence of high molecular weight keratins in malignancy [52]. This may be due to the fact that the failure of the expression of high molecular weight keratin protein may possibly attribute to either the reduction of their correspondent mRNA or message translation [53]. It was also reported that the indication of tumor associated proteins may be related to the epidermal cell differentiation process [54]. The expression of tumor associated proteins bands even in the DMBA along with plant extract treated mice were observed as the tumor were not completely decreased, is similar to that reported by Osborn and Weber [55] who found that the lower molecular weight keratins

(43 to 58 kDa) were present in all tumor tissue preparations. Further study is required to analyse the proteins from the tumors.

CONCLUSION

The present study conducted in a mouse skin carcinogenesis model clearly reveal that the methanolic leaf extract of *Clerodendrum serratum* major components flavonoids and phenolics can effectively reduce the incidence and multiplicity of skin papilloma, a precancer condition which precedes development of cacinomas. The recovery in the plant extract treated mice groups is due to their antilipidperoxidative and antioxidant functions during papilloma genesis [56,57]. Further this study demonstrates that the functional action of CSLE and its components is mediated by varied pathways, which includes activation of detoxification and prevention of cellular damage, inhibition of cell proliferation and induction of apoptosis. Similar observations are in accordance with a rat colon carcinogenesis model [58]. In view of this study it is suggested CSLE has a potential antioxidants for prevention of diseases involving oxidative damages, including cancer. The secondary metabolites isolation studies are therefore, required to accurately establish the cancer preventive metabolite of CSLE.

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REFERENCES

[1] Raymond, R. 1977. Environment and the Skin. *Environmental Health Perspectives.* 20:27-37.

[2] Rajalingam, K., G. L. Renju., S. Balakrishnan and S. Manoharan. 2008. Effect of *Clerodendron inerme* on Erythrocyte membrane integrity during7, 12-dimethylbenz[a]anthracene induced skin carcinogenesis in Swiss albino mice. *Asian J. Sci Res.* 1: 246-255.

[3] Wagner, R. F. and Casciato, D. A. 2000. Skin Cancers. In: Manual of Clinical Oncology, Casciato, D.A., B.B. Lowitz (Eds.). 4th Edn., Williams and Wilkins, Philadelphia, Pa: *Lippincott,* 336-373.

[4] Ridky, T. W. Nonmelanoma skin cancer.2007. *J. Am. Acad. Dermatol.* 57: 484-501.

[5] Vellaichamy, S., Alias, L. M., Manoharan, L., Balakrishnan, S. and Ramachandran, C. R. 2009. Protective effect of ferulic acid on 7, 12- dimethylbenz[a]anthracene-induced skin carcinogenesis in Swiss albino mice. *Exp. Toxicol. Pathol.* 61:205-14.

[6] Bishayee, A., Oinam, S., Basu, M. and Chatterjee. 2000. Vanadium chemoprevention of 7, 12-dimethylbenz(a)anthracene–induced rat mammary carcinogenesis; probable involvement of representative hepatic phase I and II xenobiotic metabolizing enzymes, Breast. *Cancer Res Treat.* 63(2):133-145.

[7] Batcioglu, K., Karagozler, A. A., Ozturk, I. C., Genc, M., Bay, A., Ozturk, F. and Aydogdu, N. 2005. Comparison of chemopreventive effects of vitamin E plus selenium versus melatonin in 7, 12- dimethylbenz(a)anthracene-induced mouse brain damage. *Cancer Detect Prevent.* 29:54-58.

[8] Marnett, J. Lawrence., James, N. Riggins. and James, D. West. 2003. Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein *J Clin Invest*. 111:583–593.

[9] Seltzer, W. N. and Setzer, M. C. 2003. Plant derived triterpenoids as potential antineoplastic agents. *Mini. Rev Med Chem.* 3:540-556.

[10] Manoharan, S., Singh, R. B. and Balakrishnan, S. 2009. Chemopreventive Mechanisms of Natural Products in Oral, Mammary and Skin Carcinogenesis: An Overview. *The. Open. Nutraceuticals Journal.* 2:52-63.

[11] Yasukawa, K., Yu, S. Y., Yamanouchi, S., Takido, M., Akihisa, T. and Tamura, T. 1995. Some lupene-type triterpenes inhibit tumor promotion by 12-O-tetrade-canoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. *Phytomedicine.* 4:309-13.

[12] Schneider WC. Determination of nucleic acids in tissues by pentose analysis. In: Colowick, S. P., Kaplan , N. D. (eds) Methods in Enzymology, New York Academic Press 1957;p.680-684.

[13] Lowry H, Rosebrough NI, Far AL, Ranall RJ. Protein measurement with folinphenol reagent. *J Biol Chem* 1951;193:265-275.

[14] Carrol NV, Langely RW, Row RH. Glycogen determination in liver and mode by use of anthrone reagent. *J Biol Chem*1956;20:583-593.

[15] Abell LL, Levy BB, Brodie BP, Kendal FE. Simplified method for estimation of total cholesterol in serum and demonstration of its specificity. *J Biol Chem* 1952;195:357-361.

[16] King J. In Practical Clinical Enzymology, Edn. Z. Van, D. Norstrand, Co. London, 1965; p. 83.

[17] Nachlas MM, Margulius SI, Sellirgman AM. Site of electron transfer to tetrazolium salts in the succinoxidase. *J Biol Chem* 1960;235:2739.

[18] Bergemeyer HV, Bernt E In: Methods of enzymatic analysis. (Ed.) H.V. Bergmeyer. Acad. Press Weinheim, NY and London 1963;837.

[19] Ellman G. Tissue sulphydryl groups. *Arch of Biochem Biophy* 1959;32:70-77.

[20] Okhawa H, Ohishi N, Yagi K Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Annal Biochem* 1979;95:351-8.

[21] Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Ind J Biochem Biophys*1984;21:130-132.

[22] Aebi H In: Bergmeyer H.U.: Methods of Enzymatic Analysis. 2nd ed. Vol. 2, Verlag Chemie, Academic Press, Inc. New York and London 1974;673 pp.

[23] Habig WH, Pabst MJ, Jakoby WB. The glutathione S-transferases; the first enzymatic step in mercapturic acid

formation. *Journal of Biological Chemistry* 1974; 249:7130-7139.

[24] Stevens RH, Cole DA, Cheung HF. Identification of a common oncofetal protein in X-ray and chemically induced rat gastrointestinal tumors. *Br J Cancer* 1981;43:817.

[25] Laemmli UK Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680–5.

[26] Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine, Clarendon press, Oxford, England (1989).

[27] Bendich A In Micronutrients and Immune Function. (A Bendich and R.K.Chandra,Eds), New York Acad Sci New York 1990;p.587.

[28] Richter C, Park JW, Ames BN. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc. Natl. Acad. Sc .USA. 1988;85: 6465-7.

[29] Das I, Saha T. Effect of garlic on lipid peroxidation and antioxidation enzymes in DMBA-induced skin carcinoma. *Nutrition* 2009;25:459-71.

[30] Martinez M, Giovanucci E. Diet and the prevention of cancer. Cancer. *Metastasis. Rev.* 1997;16:357-376.

[31] Kelloff GJ. Perspectives on cancer chemoprevention research and drug development. *Adv. Cancer Res* 2000;7:8199-334.

[32] Prakash J, Gupta, SK, Dinda AK. Withania sominifera root extracts prevents DMBA induced squamous cell cacrcinoma in swiss albino mice. *Nutrition and Cancer*. 2002;42:91-97.

[33] Matsumara F, Boush GM, Nisato T. Environmental toxicology of pesticides. Academic Press, New York and London 1972;30-32.

[34] Kussmaul L, Hirst J. The mechanism of superoxide production by NADH:ubiquinone oxidoreductase (complex I) from bovine heart mitochondria. *Proc. Natl. Acad. Sci.* 2006;103:7607-12.

[35] Christou M, Moore CJ, Gould MN, Jefcoate CR. Induction of mammary cytochromes P450: an essential first step in the metabolism of 7,12-dimethylbenz[a]anthracene by rat mammary epithelial cells. *Carcinogenesis* 1987;8:73-80.

[36] Perchellet JP, Perchellet M. Antioxidant and multistage carcinogenesis in mouse skin. *Free. Radical. Biol. Med.*1989;7:377–408.

[37] Shivanandappa T, Krishnakumari MK, Majumdar SK. Inhibition of steriodogenic activity in the adrenal cortex of rats fed benzene hexachloride (hexachlorocyclohexane). *Experentia* 1981; 38: 1251-1253.

[38] Kacker R, Srivastava MK, Raizada RB. Induction of gonadial toxicity to male rats after chronic exposure to mancozeb. *Indus Health1997*;35:104-11.

[39] Preidkalns J, Weber AF. The succinic dehydrogenase and lipid content of follicular and luteal cells of the bovine ovary. *Acta Anat* 1968;71:542-564.

[40] de Duve C, Pressman BC, Gianetto R, Wattiaux R, Appelmans F (1955). *Biochem. J.* 60:604.

[41] Pilo B, Asanani MV, Shah RV. Studies on the wound healing and repair in pigeon liver III. Histochemical studies on acid and alkaline phosphatases during the process. *J. Anm Morphol Physiol* 1972;19:205- 212.

[42] Dhawan D, Balasubramaniam S, Amonkar AJ, Singh N. Chemopreventive effect of 4'-dimethyl epipophyllotoxin on DMBA/TPA-induced mouse skin carcinogenesis. *Carcinogenesis* 1999;20:997-1003.

[43] Dasgupta T, Rao AR, Yadava PK. Chemomodulatory action of curry leaf (Murraya koenigii) extract on hepatic and extrahepatic xenobiotic metabolising enzymes, antioxidant levels, lipid peroxidation, skin and forestomach papillomagenesis. *Nutrition Res* 2003;23:1427- 46.

[44] Halliwell B. How to characterize a biological antioxidant. Free. Radic*, Res. Commun*. 1990;9:1-32.

[45] Vang O, Rasmussen BF, Anderson O. Combined effects of complex mixtures of potentially anticarcinogenic compounds on antioxidant enzymes and carcinogen metabolizing enzymes in the rat. *Cancer Lett* 1997;114:283-6.

[46] Kurjogi, M, M., Sanakal, R. D., Kaliwal, B. B., Antibiotic susceptibility and antioxidant activity of Staphylococcus aureus pigment staphyloxanthin on carbon tetrachloride (ccl_4) induced stress in swiss albino mice. *International Journal of Biotechnology Applications*, 2010; 2 : pp-33-40.

[47] S.S. Dodamani, R.D. Sanakal, and B.B. Kaliwal. 2012. Effect of ethanolic leaf extract of *Nymphaea odorata* on biochemical and oxidative stress parameters of liver and pancreas in alloxan induced diabetic mice. *Res. Opin. Anim. Vet. Sci.* 2(3): 151-157

[48] Szarka CE, Pfeiffer GR, Hum ST. Glutathione-S transferase activity and glutathione-S transferase expression in subjects with risk for colorectal cancer. *Cancer Res.* 1995; 55:2789- 93.

[49] Chung FL, Chen HJC, Nath RG. Lipid peroxidation as a potential endogenous source for the formation of exocyclic DNA adducts. *Carcinogenesis* 1996;17:2105-11.

[50] Prosenjit Saha, Sukta Das. Elimination of Deleterious Effects of Free Radicals in MurineSkin Carcinogenesis by Black Tea Infusion, Theaflavins &Epigallocatechin Gallate. Asian. Pacific. Journal. of Cancer. *Prevention* 2002;3: 225-30.

[51] Loning T, Staouet, MJ, Thivolht J. Keratin polypeptides distribution in normal and diseased human epidermis and oral mucosa. *Virchows Arch Abt A 1980*;388:273-88.

[52] Altmannsberger M, Weber K. Antibodies to intermediate filaments as diagnostic tools: human gastrointestinal carcinomas express keratin. *Lab Invest* 1982;46:520-6.

[53] Toftgard R, Yusp ASH, Roop DR. Keratin gene expression in mouse skin tumors and in mouse skin treated with 12-O-tetradecanoylphorbol-13-acetate. *Cancer Res* 1985;45:5845-50.

[54] Park KK, Chun KS, Lee JM. Lee SS, Surh, YJ. Inhibitory effects of [6]-gingerol, a major pungent principle of ginger, on phorbol esterinduced inflammation, epidermal ornithine decarboxylase activity and skin tumor promotion in ICR mice. *Cancer Lett* 1998;129:139-44.

[55] Osborn M, Weber D. Biology of disease tumor diagnosis by intermediate filament typing: a novel tool for surgical pathology.

Lab Invest 1983;48:372-94.

[56] Renju G L, Manoharan S, Balakrishnan S, Senthil N. Chemopreventive and antilipidperoxidative potential of Clerodendron inerme [L] Gaertn in 7, 12-dimethylbenz [a] anthracene induced skin carcinogenesis in Swiss albino mice. *Pak J Biol Sci* 2007;10:1465-70.

[57] Chinchali, J. F., R. D. Sanakal and B. B. Kaliwal. 2011. Evaluation of anticarcinogenic activity of *Clerodendrum serratum* leaf extract on liver and kidney of 7, 12-dimethylbenz[a]anthracene (DMBA) induced skin carcinogenesis in mice. *Euro. J. Exp. Bio.* 1(4):130-141.

[58] Sengupta A, Ghosh S, Das S. Inhibition of cell proliferation and induction of apoptosis during Azoxymethane induced colon carcinogenesis by Black tea. *Asian. Pacific. J. Cancer Prevention* 2002;3:41-46.