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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

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ABSTRACT

The evaluation of the anticarcinogenic activity of the *Clerodendrum serratum* leaf extract (CSLE) on liver and kidney of 7, 12-dimethylbenz[a]anthracene (DMBA) induced skin carcinogenesis in mice were studied. Group I served as control. The skin lesions were induced by twice-weekly topical application of DMBA for two 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, liver and kidney were dissected out freed from adherent tissue and weighed to nearest milligram and evaluated the effect of administration of CSLE on biochemical and oxidative stress parameters. Our findings showed that there was a recovery in biochemical and oxidative stress parameters in the liver and kidney of the CSLE administered mice. Together, these findings suggest that *Clerodendrum serratum* leaf extract has anticarcinogenic efficacy against skin carcinogenesis.

Key words: Anticarcinogenic activity, *Clerodendrum serratum*, DMBA, Biochemical contents and Oxidative stress.

INTRODUCTION

Living organisms are continuously exposed to both physiological and environmental damaging chemical substances. These chemical substances cause numerous public health problems and disorders in the system physiology which have become prominent issues in recent decades after reports of adverse effects of certain carcinogenic chemicals which induce cancer in respective organs at higher level of exposure to many of these chemicals. Cancer, which is a group of diseases with similar characteristics and not a single disease but rather a general term referring to many kinds of malignant growths that invade adjoining tissue and sometimes spread to distant tissues. Skin cancer is the most common form of human cancer in which cancer cells are found

in the outer layers of the skin. According to the world cancer report, skin cancer contributes approximately 30% of all newly diagnosed cancers in the world and solar ultraviolet radiation is the established cause of 90% of all skin cancers [1]. 7, 12-dimethylbenz(a)anthracene (DMBA) is a potent carcinogenic polycyclic aromatic hydrocarbon (PAH) is a potent cancer inducing agent in animal models. Sources of PAHs are widely distributed in our environment and are implicated in various types of cancer [2]. Enzymatic activation of PAHs 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 [3-4]. Consequences of the damage initiated by these metabolic by products affect a large range of biological reactions, like increases in mutation rate, alteration of cellular membrane composition, structural proteins, metabolic, detoxifying enzymes and cellular signalling proteins [5]. Free radicals play an important role in tumor promotion by direct chemical reaction or alteration of cellular metabolic process and their scavengers represent inhibitors at different stages of carcinogenesis.

In the present study the plant *Clerodendrum serratum*, Linn (Family: Verbenaceae) commonly known as “Bharngi” in the ayurvedic medicine of Indian system which is widely distributed in tropical and subtropical regions of the world is evaluated for the anticarcinogenic activity in Swiss albino mice. Chemoprevention represents a promising strategy that can slow, reverse, or completely halt the process of carcinogenesis [6]. Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species [7]. All over the world, studies on plant materials have revealed their health promoting action including cancer prevention, plant-derived flavonoids and triterpenoids have attracted reasonable attention for their unique antineoplastic activity [8].

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 65°C, 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 (Reg no. 639/02/a/CPCSEA) guidelines were followed for maintenance 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 (12L: 12D) at room temperature $25 \pm 2^\circ\text{C}$.

Treatment:

The chemical carcinogen, 7, 12-dimethylbenz[a]anthracene (DMBA) induced skin tumorigenesis in male Swiss albino mice [9]. 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 administered 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 liver and kidney 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 liver and kidney weight, biochemical contents and oxidative stress parameters of liver and kidney in the albino mice.

Biochemical Studies:

The biochemical contents such as DNA and RNA carried out as per the method described by [10], protein by [11], glycogen by [12], cholesterol by [13], and activities of enzymes such as LDH by [14], SDH by [15], ACP and AKP by method of [16].

Oxidative stress parameters:

The oxidative stress parameters such as GSH level was measured following the method of [17], the product of the reaction between malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) by [18] were measured by a modified method of Esterbauer and Cheesman, (1990), SOD activity by [19], CAT activity by [20] and GST activity [21].

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

Liver and kidney weight:

Liver and kidney weight revealed that there was a significant decrease in DMBA treated control mice. However, there was a significant increase in the liver and kidney weight of mice treated with DMBA along with higher doses 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 liver and kidney of DMBA treated control mice. However, there was a significant increase in the level of the nucleic acids, protein, glycogen, SDH and ACP activities in the mice treated with DMBA along with higher doses of plant extract showing their recovery. But there was a significant increase in the level of the cholesterol, LDH and AKP activities of DMBA treated control mice and there was a significant 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 their recovery (Table 2, 3, 4 and 5).

Oxidative stress parameters:

There was a significant decrease in the level of GSH, CAT, SOD and GST activity in the liver and kidney of DMBA treated control mice. Further, there was a significant 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 their recovery. However, there was a significant increase in the level of TBARS of DMBA treated control mice and there was a significant decrease in the level of TBARS in the mice treated with DMBA along with higher doses of plant extract showing their recovery (Table 6 and 7).

DISCUSSION

Cancer chemoprevention involves pharmacological intervention with synthetic or naturally occurring chemicals or substances to prevent, inhibit or reverse the process of carcinogenesis or to prevent the development of invasive cancer [22]. In the present study, the modulatory influence of *Clerodendrum serratum* leaf extract on biochemical contents and oxidative stress parameters with organ specific carcinogenesis was assessed. The observations revealed that, significant decrease in the liver and kidney weight of the mice is in support of the findings reported earlier [23] in the DMBA induced skin carcinogenesis. These effects can be attributed to plausible biochemical mechanisms including growth arrests at one or more points in the cycle, inhibition of DNA synthesis, and modulation of signal transduction pathways by altered expression of key enzymes such as cyclooxygenases and protein kinases [24]. Further, the liver and kidney weight of the mice treated with DMBA along with plant extract indicates that there was an increase in the liver and kidney weight of the mice in comparison with DMBA treated control mice. Similar results have also reported in the DMBA treated along with different plant extract such as *Boerhaavia diffusa*, *Withania somnifera* root and *Berberis vulgaris* [25-26]. The effect of plant extract on liver and kidney weight is due to their antilipidperoxidative and antioxidant functions during papillomagenesis [27]. This cancer inhibitory action by a variety of human nutrients derived from plants as well as of nonnutritive plant-derived constituents (phytochemicals) has been confirmed in different animal tumor models [28] and has led to an increased emphasis on cancer prevention strategies in which these dietary factors are utilized.

In the present study, the results indicate that the DMBA induced skin carcinogenesis showed a significant decrease in DNA, RNA, protein and glycogen content in the liver and kidney of the mice may be due to more cell proliferation and less apoptosis, the DNA adducts formed is the major factor for decrease in the replication which in turn leads to the decrease in the transcription, which affects the protein and glycogen synthesis. Increased cell proliferation activity accompanied by a decrease in protein and nucleic acids contents in response to DMBA releases of nucleases and proteases affecting RNA, DNA and protein metabolism. RNA damage due to oxidative stress by carcinogen is attributed by adducts in the DNA strands and decrease in RNA level is mainly in concern with DNA level. Similar reports were suggested on decrease in DNA, DNA, RNA, protein and glycogen level on DMBA induced skin carcinogenesis in the liver and kidney of mice [23][29]. Reactive oxygen species (ROS) have been suggested as causative factors in mutagenesis, carcinogenesis and tumor promotion and have been implicated in the etiology and pathophysiology of many human diseases [30].

In the present study, the results indicate that the DMBA induced skin carcinogenesis showed a significant increase in cholesterol content in the liver and kidney of the mice. The earlier reports

suggest that an imbalance in oxidant and antioxidant status plays crucial role in the pathogenesis of several diseases including cancer [31]. Over production of ROS occurring during metabolic activation of DMBA to diol epoxide, can cause oxidative damage to structure and functions of DNA, proteins and lipids [32]. Similar results were reported on increase in cholesterol level in CCl₄ induced liver and kidney damage [33]. Further, the cholesterol content of the mice treated with DMBA along with plant extract is decreased in comparison with DMBA treated control mice may be due to the major metabolic organ liver has crucial role in the detoxication process. Agents that induce the activity of detoxication are considered to have antigenotoxic potential [34]. Detoxication of genotoxic chemicals is influenced by the dietary factors and therefore dietary intervention helps in cancer prevention [35]. Similar reversal results on treatment with natural products containing flavonoids derived from the plant extracts induced by DMBA in rats corresponding to our results both in liver and kidney has been reported by [36]. It is concluded that the chemopreventive potential is due to their antilipidperoxidative and antioxidant functions during papillomagenesis [27].

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

Groups	Treatment (mg/kg/d)	Relative organs weight /100 g body weight (Mean \pm S.E) (g)	
		liver	kidney
I	Normal Control	4.42 \pm 0.23	0.58 \pm 0.10
II	DMBA Control (25 μ l)	3.78 \pm 0.45*	0.36 \pm 0.18*
III	DMBA+CSLE (300)	3.96 \pm 0.68	0.45 \pm 0.05
IV	DMBA+ CSLE (600)	4.22 \pm 0.45*	0.52 \pm 0.27
V	DMBA+ CSLE (900)	4.48 \pm 0.70*	0.63 \pm 0.35*

Values are mean \pm SEM of 10 animals

* Significant $P \leq 0.05$ vs Control

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

Groups	Treatment (mg/kg/d)	Biochemical contents (μ g / mg wet weight of tissue)				
		DNA	RNA	Protein	Glycogen	Cholesterol
I	Normal Control	2.34 \pm 0.32	3.24 \pm 0.03	184.32 \pm 2.66	6.40 \pm 0.80	11.36 \pm 0.32
II	DMBA Control (25 μ l)	1.74 \pm 0.02*	2.68 \pm 0.09*	150.48 \pm 6.32*	4.82 \pm 0.24*	13.32 \pm 0.38*
III	DMBA+CSLE (300)	1.90 \pm 0.38	2.86 \pm 0.13	160.32 \pm 3.56	5.36 \pm 0.86	13.06 \pm 0.07
IV	DMBA+ CSLE (600)	2.10 \pm 0.66*	3.05 \pm 0.62	168.38 \pm 2.34*	5.88 \pm 0.32*	12.45 \pm 0.86
V	DMBA+ CSLE (900)	2.68 \pm 0.56*	3.40 \pm 0.82*	176.28 \pm 3.88*	6.20 \pm 0.36*	11.80 \pm 0.26*

Values are mean \pm SEM of 10 animals

* Significant $P \leq 0.05$ vs Control

Table 3. Effect of *Clerodendrum serratum* leaf extract (CSLE) on biochemical contents in the kidney of mice on treatment with 7, 12-Dimethylbenz[a]anthracene (DMBA)

Groups	Treatment (mg/kg/d)	Biochemical contents ($\mu\text{g} / \text{mg}$ wet weight of tissue)				
		DNA	RNA	Protein	Glycogen	Cholesterol
I	Normal Control	2.26 \pm 0.25	4.48 \pm 0.15	176.23 \pm 0.58	5.83 \pm 0.68	13.29 \pm 0.26
II	DMBA Control (25 μl)	1.64 \pm 0.15*	3.32 \pm 0.11*	132.34 \pm 0.34*	4.12 \pm 0.18*	14.82 \pm 0.35*
III	DMBA+CSLE (300)	1.78 \pm 0.56	3.60 \pm 0.23	148.18 \pm 0.67	4.68 \pm 0.19*	14.52 \pm 0.69
IV	DMBA+ CSLE (600)	1.98 \pm 0.34	4.02 \pm 0.43*	162.46 \pm 0.72*	5.20 \pm 0.23*	14.22 \pm 0.83*
V	DMBA+ CSLE (900)	2.16 \pm 0.28*	4.42 \pm 0.66*	178.68 \pm 0.82*	5.73 \pm 0.48*	13.60 \pm 0.24*

Values are mean \pm SEM of 10 animals

* Significant $P \leq 0.05$ vs Control

Table 4. Effect of *Clerodendrum serratum* leaf extract (CSLE) on dehydrogenases and phosphatases enzyme activities in the liver of mice on treatment with 7, 12-Dimethylbenz[a]anthracene (DMBA)

Groups	Treatment (mg/kg/d)	Enzyme activity $\mu\text{moles} / \text{min} / \text{g}$ tissue			
		LDH ^a	SDH ^b	ACP ^c	AKP ^d
I	Normal Control	13.66 \pm 0.72	14.46 \pm 0.23	15.82 \pm 0.36	15.36 \pm 0.14
II	DMBA Control (25 μl)	16.28 \pm 0.48*	11.85 \pm 0.18*	14.02 \pm 0.87*	17.20 \pm 0.43*
III	DMBA+CSLE (300)	15.66 \pm 0.28	12.52 \pm 0.27	14.62 \pm 0.75	16.56 \pm 0.32
IV	DMBA+ CSLE (600)	14.75 \pm 0.36*	13.52 \pm 0.62*	15.30 \pm 0.63*	15.98 \pm 0.43*
V	DMBA+ CSLE (900)	14.15 \pm 0.46*	14.05 \pm 0.41*	15.76 \pm 0.48*	15.50 \pm 0.34*

a μmoles of pyruvate formed/ min/ g ti

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 \pm SEM of 10 animals

* Significant $P \leq 0.05$ vs Control

Table 5. Effect of *Clerodendrum serratum* leaf extract (CSLE) on dehydrogenases and phosphatases enzyme activities in the kidney of mice on treatment with 7, 12-Dimethylbenz[a]anthracene (DMBA)

Groups	Treatment (mg/kg/d)	Enzyme activity $\mu\text{moles} / \text{min} / \text{g}$ tissue			
		LDH ^a	SDH ^b	ACP ^c	AKP ^d
I	Normal Control	11.63 \pm 0.14	16.54 \pm 0.48	14.86 \pm 0.31	15.32 \pm 0.85
II	DMBA Control (25 μl)	14.32 \pm 0.38*	14.63 \pm 0.18*	12.62 \pm 0.73*	17.65 \pm 0.96*
III	DMBA+CSLE (300)	13.76 \pm 0.23	15.36 \pm 0.28	13.03 \pm 0.98	17.05 \pm 0.15
IV	DMBA+ CSLE (600)	12.88 \pm 0.41*	15.78 \pm 0.68	13.78 \pm 0.15*	16.35 \pm 0.46
V	DMBA+ CSLE (900)	11.90 \pm 0.32*	16.32 \pm 0.58*	14.62 \pm 0.28*	15.68 \pm 0.31*

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 \pm SEM of 10 animals

* Significant $P \leq 0.05$ vs Control

Table 6. Effect of *Clerodendrum serratum* leaf extract (CSLE) on oxidative stress parameters in the liver 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	11.03±0.09	0.21±0.02	170.32±2.29	49.72±2.12	5.23±0.18
II	DMBA Control (25µl)	9.05±0.08*	0.40±0.03*	136.30±5.16*	30.78±2.68*	2.84±0.22*
III	DMBA+CSLE (300)	9.70±0.06	0.36±0.01	145.47±5.27	36.68±2.26	3.26±0.16
IV	DMBA+ CSLE (600)	10.38±0.09*	0.29±0.02*	153.50±3.22*	41.84±2.13*	3.82±0.12
V	DMBA+ CSLE (900)	10.82±0.08*	0.24±0.03*	166.40±6.43*	46.56±2.48*	4.74±0.15*

a µmole of glutathione (GSH)/ mg protein

c µmole of H₂O₂

e Glutathione-s-transferase (GST) µmole/min/mg protein

Values are mean±SEM of 10 animals

b nmoles thiobarbaturic acid (TBARS)/gm protein

d super oxide dismutase (SOD) unit/mg protein

* Significant $P \leq 0.05$ vs Control

Table 7. Effect of *Clerodendrum serratum* leaf extract (CSLE) on oxidative stress parameters in the kidney 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	8.94±0.06	0.28±0.02	166.34±3.33	45.86±2.18	3.36±0.08
II	DMBA Control (25µl)	6.86±0.08*	0.57±0.01*	122.62±5.43*	26.39±2.48*	1.64±0.07*
III	DMBA+CSLE (300)	7.46±0.06	0.43±0.03	134.24±4.81	32.08±2.32	2.42±0.03
IV	DMBA+ CSLE (600)	7.90±0.07*	0.34±0.01*	146.50±2.39*	38.06±2.04*	2.82±0.06*
V	DMBA+ CSLE (900)	8.26±0.06*	0.26±0.02*	159.42±6.48*	42.45±1.45*	3.10±0.04*

a µmole of glutathione (GSH)/ mg protein

c µmole of H₂O₂

e Glutathione-s-transferase (GST) µmole/min/mg protein

Values are mean±SEM of 10 animals

b nmoles thiobarbaturic acid (TBARS)/gm protein

d super oxide dismutase (SOD) unit/mg protein

* Significant $P \leq 0.05$ vs Control

In the present study, the results indicate that the DMBA induced skin carcinogenesis showed a significant increase in LDH and decrease in SDH activity in the liver and kidney of the mice. Similar results were reported as DMBA induced increase in the activity of hepatic and renal LDH may be attributed to the enhanced enzyme synthesis [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]. Sharma [39] has reported that significant decrease in the activity of liver and kidney succinate dehydrogenase suggests that anaerobic metabolism was favoured over aerobic oxidation of glucose through Krebs cycle in order to mitigate the energy crisis for survival. Further, in the present study there was decrease in LDH and increase in SDH activity in the liver and kidney of the mice treated with DMBA along with plant extract is decreased in comparison with DMBA treated control mice. Several studies have shown the flavonoids to act as scavengers of superoxide anions, singlet oxygen, hydroxyl radicals, and lipid peroxy radicals [40]. The chemopreventive activity of flavonoids triterpenoids is dependent on their structural features. Similar reports in accordance with the flavonoids of the *Clerodendrum serratum* leaf extract justify that plant has chemopreventive potential to the DMBA induced carcinogenesis [41].

In the present study, the results indicate that the DMBA induced skin carcinogenesis showed a significant decrease in ACP and increase in AKP activity in the liver and kidney of the mice. Decrease in ACP activity may be taken as index of hepatic parenchymal damage and hepatocytic necrosis [42]. Increase in AKP activity reflects alterations in protein synthesis and uncoupling of oxidative phosphorylation [43]. The increase in AKP by stressors probably indicates an altered transport of phosphate [44] and an inhibitory effect on the cell growth and proliferation [45]. Further, in the present study there was increase in ACP activity and decrease in AKP activity in the liver and kidney of the mice treated with DMBA along with plant extract in comparison with DMBA treated control mice, may be due to the plant extract treatment might have significantly reduced lipid peroxidation in mice exposed to DMBA by their antilipidperoxidative and antioxidant functions during papillomagenesis [41]. A chemopreventive agent that decreases cell proliferation markedly reduces the susceptibility to cancer [46]. Similar results have been reported that treatment with natural products containing flavonoids derived from the plant extracts induced by DMBA in rats corresponding to our results both in liver and kidney has reported by Manoharan *et al.*, [29].

In the present study, the results indicate that the DMBA induced skin carcinogenesis showed a significant decrease in GSH and increase in TBARS level in the liver and kidney of the mice may be due the significantly elevated lipid peroxidation level in liver and kidney in turn produced ROS, which caused oxidative stress in these organs [47]. GSH alters the profile of lipoxygenase and cyclooxygenases [48-49] which are involved in tumorigenesis. Excessive generation of reactive oxygen species, as evidenced by increased formation of lipid peroxidation by products (TBARS), has been reported in DMBA-induced genotoxicity [50]. Decreased activities of enzymatic antioxidants and decline in non-enzymatic antioxidant level were well documented in skin cancer [23]. Further, in the present study the GSH level in the liver and kidney of the mice treated with DMBA along with plant extract is increased and TBARS level is decreased in comparison with DMBA treated control mice may be due to the scavengers of oxygen radicals which have shown to inhibit the cancer causation in animals and in human trials [51]. *Clerodendrum serratum* leaf extract effectively elevated the GSH level reduced TBARS levels in liver and kidney in a dose independent manner. Similar results are observed in several antioxidants of plant material are experimentally proved and widely used as more effective agents against oxidative stress [29].

In the present study, the results indicate that the DMBA induced skin carcinogenesis showed a significant decrease in CAT, SOD and GST activity in the liver and kidney of the mice. The low levels of antioxidant enzymes CAT, SOD and GST in DMBA treated mice show poor antioxidant status. Oberley, and Oberley, [52] reported decreased SOD and CAT activity in papillomas and squamous cell carcinoma leading to a pro-oxidant state of cells, facilitating tumorigenesis. The increase in antioxidant enzymes by plant extract reflects that it inhibits the process of oxidative stress induced carcinogenesis. Several reports suggest that GSH is a more efficient antioxidant agent than SOD or CAT [53-54]. Increased activity of CAT, SOD and GST in the liver and kidney tissue of mice treated with plant extract suggests certain role for reactive free oxygen radical in DMBA induced skin carcinogenesis [55-56]. The experimental observations therefore suggest that protective role of *Clerodendrum serratum* leaf extract against carcinogenic exposure by their action on the physiological detoxification processes.

Peroxisomes are involved in a number of important cellular metabolic processes as well as detoxication of H₂O₂ [57-58]. More than 90% of oxygen consumed by the mitochondria is converted to water (H₂O) and the rest to super oxide radical (O₂) whereas the oxygen consumed by peroxisomes is converted to H₂O₂ and only a little amount is converted to O₂ [59]. These

antioxidant enzymes provide protection to cell against ROS i.e. O_2 and H_2O_2 by detoxifying them at the site where they are produced. SOD detected in a large number of tissues and organs, is also thought to protect cells from damage by superoxide radicals [60]. Superoxide radical $O_2^{\cdot-}$ is the product of biological oxygen reduction as a result of electron transfer oxidase system. SOD catalyzes the one electron reduction of oxygen to superoxide radical. This $O_2^{\cdot-}$ is harmful to the respiring cells. SOD minimizes the toxicity resulting from the mechanism against oxygen formation of free radicals [61].

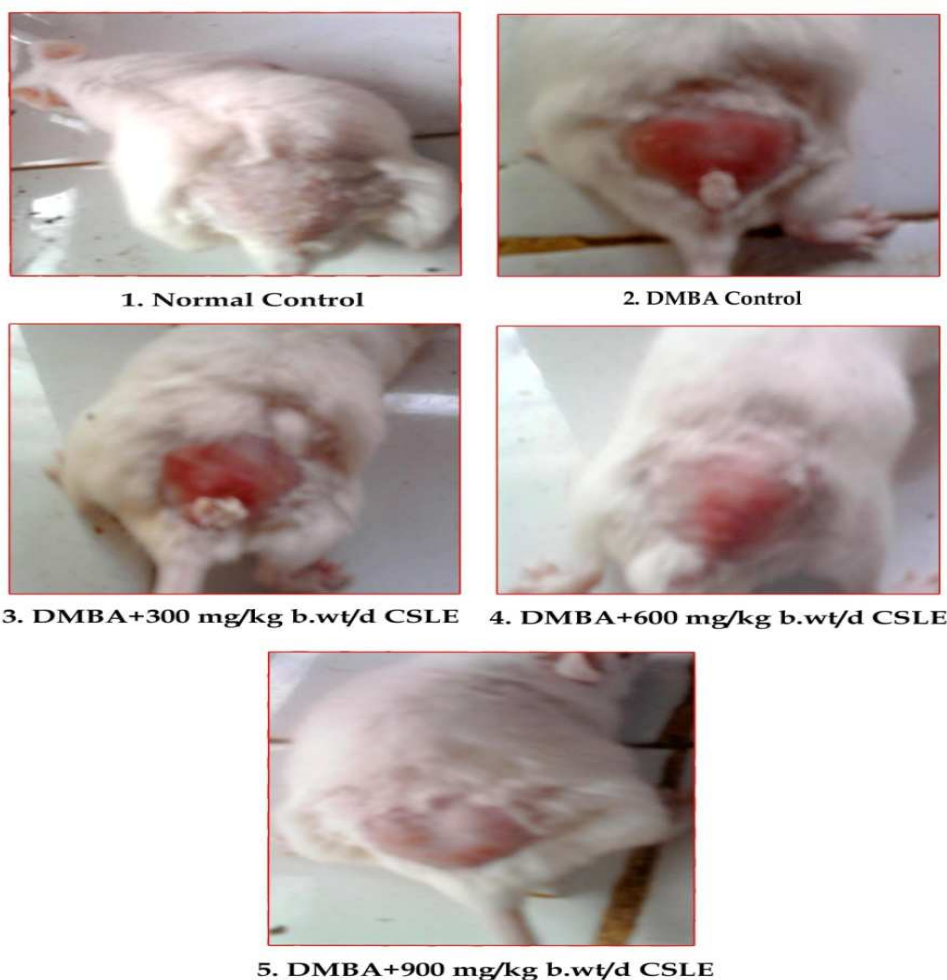


Fig. 1 Effect of *Clerodendrum serratum* leaf extract (CSLE) on DMBA induced papillomas in mouse skin. Pictures were taken at the end of the experiment and are representative for each group. Treatments were as indicated in the figure.

GST is thought to play a physiological role in initiating the detoxication of many alkylating agents [62] and environmental chemicals including mutagens and carcinogens [63]. Their main function is the conjugation of GSH to a variety of electrophilic compounds. GST reduced the covalent binding of epoxides of carcinogens with DNA and other macromolecules and this reduction in DNA binding was found to be effective in decreasing carcinogenesis caused by the carcinogens. Further, in the present study the CAT, SOD and GST activity in the kidney of the mice treated with DMBA along with plant extract is increased. The increased activity of CAT, SOD and GST in liver tissue of the mice treated with DMBA along with plant extract and decreased activity of these enzymes in the liver tissue of the mice treated with DMBA suggested that plant extract could influence host detoxification processes. Increased activity of CAT, SOD

and GST in the liver tissue of mice treated with plant extract suggests certain role for reactive free oxygen radical in DMBA induced skin carcinogenesis [55-56]. In the present study it has been shown that the activation of three detoxification enzymes studied viz, CAT, SOD and GST was accompanied by significant reduction in lipid peroxidation and inhibition of Dimethylbenz(a)anthracene (DMBA) induced papillomas may be due to anticarcinogenic activity of *Clerodendrum serratum* by their action on the physiological detoxification processes. It is therefore, implied that agents that can reduce generation of free radicals in vivo may be considered to have the potential for chemoprevention of cancer. The present study shows that plant extract treatment may significantly reduce the apoptosis, cell proliferation and lipid peroxidation in mice induced by DMBA due to the antiproliferative, antilipidperoxidative and antioxidant functions during skin papillomagenesis [23].

1. The skin of the normal control mouse showing clear skin.
2. The skin of the DMBA control mouse treated with 25 μ l:25 μ l (v/v) DMBA in the acetone for two weeks twice weekly respectively was showing dark skin papillomas.
3. The skin of the DMBA control mouse treated with 25 μ l:25 μ l (v/v) DMBA in the acetone and plant extract 300 mg/kg b.wt/day for 45 days respectively showing reduced skin papillomas compared to DMBA control.
4. The skin of the DMBA control mouse treated with 25 μ l:25 μ l (v/v) DMBA in the acetone and plant extract 600 mg/kg b.wt/day for 45 days respectively showing more reduced skin papillomas compared to DMBA control and 300 mg plant extract treated mouse.
5. The skin of the DMBA control mouse treated with 25 μ l:25 μ l (v/v) DMBA in the acetone and plant extract 900 mg/kg b.wt/day for 45 days respectively showing less skin papillomas compared to DMBA control and 600 mg plant extract treated mouse.

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