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**Effect of *Clerodendron inerme* on Erythrocyte  
Membrane Integrity During 7,12-dimethylbenz(a)anthracene  
Induced Skin Carcinogenesis in Swiss Albino Mice**

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**Abstract:** Aim was to investigate the modifying effects of ethanolic extract of *Clerodendron inerme* leaves on membrane integrity by measuring the levels of plasma and erythrocyte membrane glycoconjugates and red blood cell osmotic fragility during 7,12-dimethylbenz(a)anthracene (DMBA) induced skin carcinogenesis in Swiss albino mice. The skin squamous cell carcinoma was induced in the shaved back of mice, by painting with DMBA (25 µg/0.1 mL acetone) twice weekly for 8 weeks. We have observed 100% tumor formation in the fifteenth week of experimental period. The status of glycoconjugates in plasma and erythrocyte membrane and red blood cell osmotic fragility was assayed by using specific colorimetric methods. The levels of glycoconjugates were increased in plasma whereas decreased in erythrocyte membrane of DMBA treated animals as compared to control animals. Red blood cells from tumor bearing animals were more fragile than those from control animals. Oral administration of ethanolic leaf extract of *Clerodendron inerme* (CILEE) 300 mg kg<sup>-1</sup> b.wt. significantly prevented the tumor formation as well as restored the status of glycoconjugates and red blood cell osmotic fragility in DMBA treated animals. The present study thus demonstrates the protective effect of CILEE on red blood cell membrane integrity during DMBA induced mouse skin carcinogenesis.

**Key words:** DMBA, skin cancer, glycoconjugates, sialic acid, osmotic fragility,  
*Clerodendron inerme*

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## INTRODUCTION

Cancer is the most arrogant, independent, uncontrolled, highly destructive and proliferative, tissue invasive, hardly inevitable, renegade, apparently immortal population of body's own cells, with the potentiality to metastasize and pollute the system with a fatal terminal (Ranjit, 2004). Agents that cause genetic damage and induce neoplastic transformation of cells include chemical carcinogen, radiant energy and oncogenic microbes, chiefly viruses. 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 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. Epidemiological figures show a wide range of skin cancer incidence between 40 and over 700 or 5 and 250, respectively per 1,00,000 populations per year depending on the country or area of the report (Dummer *et al.*, 2001; Perkins *et al.*, 2005). Basal cell carcinoma accounts for 80 % whereas squamous cell carcinoma and melanomas account for

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16 and 4%, respectively of all skin cancers. Squamous cell carcinoma is the most serious form of cancer than other skin cancers since they can spread into vital organs inside the body. The incidence of squamous cell carcinoma is increasing rapidly worldwide and accounts for 16% of all skin cancers (Wagner and Casciato, 2000).

Chemical carcinogenesis in skin as well as in other organs is a multi-step process comprising initiation, promotion and progression stages that require both initiating and promoting substances for the development of cancer. Polycyclic aromatic hydrocarbons such as 7,12-dimethylbenz(a)anthracene have been shown to transform cells under both *in vivo* and *in vitro* conditions. DMBA on metabolic activation produces diol epoxide (ultimate carcinogen) which mediates carcinogenic process by inducing chronic inflammation, over production of Reactive Oxygen Species (ROS) and oxidative DNA damage (Tennant, 1997). DMBA induced mouse skin carcinogenesis model is commonly employed to study the chemopreventive potential of medicinal plants and its active components.

Measurements of biomarkers in plasma or serum are valuable in the assessment of tumor aggressiveness and also helpful in the selection of treatment strategies (Manoharan *et al.*, 2004). Cell surface glycoconjugates play an important role in cell differentiation, intercellular recognition, tumorigenesis and as receptors for many hormones and viruses. They influence cell growth and cell to cell interaction and therefore of importance in the development of cancer (Zhao *et al.*, 2006). Profound studies on altered characteristics of malignant cells have shown that altered blood cell surface is the hall mark of cancer (Aranganathan *et al.*, 2005; Suresh *et al.*, 2007). Sialic acids, a family of 9 carbon sugars, are widely distributed in nature as non-reducing termini of glycoprotein and glycolipids (Sebzda *et al.*, 2006). Evaluation of total sialic acid and lipid bound sialic acid status could be very helpful for the diagnosis of patients with cancer and to monitoring their progression and response to treatment (Sebzda *et al.*, 2006; Manoharan *et al.*, 2004). Fucose, one of the essential sugars for optimal function of cell-cell communication, plays a significant role in slowing the growth of cancer cells (Rao *et al.*, 1998).

The integrity of the red blood cells may be determined by measuring the changes in erythrocyte osmotic fragility. Osmotic fragility, the sensitivity to change in osmotic pressure characteristic of red blood cells, has been found to be altered in various pathological conditions including cancer. Measurement of osmotic fragility of erythrocytes has been applied to the diagnosis of hemolytic diseases, studies of membrane permeability and alteration leading to destruction of erythrocytes. The red blood cells become more fragile if there is an imbalance in oxidant and antioxidant status in the body (Abou-Seif *et al.*, 2000; Kolanjiappan *et al.*, 2002).

A large number of Indian medicinal plants have been shown to have chemopreventive potential in experimental carcinogenesis including skin cancer (Manoharan *et al.*, 2006; Renju *et al.*, 2007). *Clerodendron ineme* is commonly known as Seaside Clerodendron in English, Sankupi in Hindi and Peechangu in Tamil. Different parts of *Clerodendron ineme* plant products are considered beneficial for the treatment of rheumatism, skin disease, venereal infections beriberi and tumors (Kiritikar and Basu, 1975). Preliminary phytochemical studies on *Clerodendron ineme* (L.) Gaertn revealed the presence of flavones, diterpenes, phenyl propanoid, glycosides, glucosides, verbascoside, clerosterol, isoverbscoside, leucosceptoside A, 5-hydroxy-7-4' dimethoxy flavone, leucosceptoside A, salvigenin, acacetin, apigenin etc., (Rehman *et al.*, 1997). Previous studies from our laboratory have shown the chemopreventive and antilipidperoxidative potential of *Clerodendron ineme* (L.) Gaertn in 7,12-dimethylbenz(a)anthracene induced skin carcinogenesis in Swiss albino mice (Renju *et al.*, 2007). The present study demonstrates the modifying effects of *Clerodendron ineme* on red blood cell membrane integrity by examining the status of glycoconjugates and red blood cell osmotic fragility during 7,12-dimethylbenz(a)anthracene induced skin carcinogenesis in Swiss albino mice.

## MATERIALS AND METHODS

### Chemicals

The carcinogen, 7,12-dimethylbenz(a)anthracene (DMBA) was purchased from Sigma Aldrich Chemical Pvt Ltd., Bangalore, India. All other required chemicals used were of analytical grade.

### Animals

Male Swiss Albino mice 4-6 weeks old, weighing 15-20 g were purchased from National Institute of Nutrition, Hyderabad, India and maintained in the Central Animal House, Rajah Muthaiah Medical College and Hospital, Annamalai University. The animals were housed in groups of four or five in polypropylene cages and provided standard pellet diet and water *ad libitum* and maintained under controlled conditions of temperature and humidity, with a 12 h light/dark cycle as per the principles and guidelines of the ethical committee for animal care of Annamalai University in accordance with Indian National Law on animal care and use.

### Plant Material

*Clerodendron inerme* (L.) Gaertn was collected in and around Chidambaram and Cuddalore, Tamil Nadu, India. Dr. R. Panneer Selvam, Botanist, Department of Botany, Annamalai University verified the identity of the plant and a voucher specimen was also deposited in the Department of Botany, Annamalai University.

### Ethanollic Extract of *Clerodendron inerme* Leaves

The ethanollic extract of *Clerodendron inerme* leaves was prepared according to the method of Hossain *et al.* (1992). Five hundred gram of fresh leaves of *Clerodendron inerme* leaves were dried, powdered and soaked in 1500 mL of 95% ethanol over night. After filtration, the residue obtained was again resuspended in equal volume of 95% ethanol for 48 h and filtered again. The above two filtrates were mixed and the solvents was evaporated in a rotovapour at 40-50°C, under reduced pressure. A 14% semisolid light greenish yellow material obtained was stored at 0-4°C until used. A known volume of the residual extract is suspended in distilled water and was orally administered to the animals during the experimental period.

### Experimental Design

The experimental design was approved (Proposal No. 315; dated 15-02-2006) by the Annamalai University animal ethical committee (Reg. No: 160/1999/CPCSEA), Annamalai University, Annamalainagar. The present study was conducted during 2005-2006 in the Department of Biochemistry and Biotechnology, Annamalai University, Annamalainagar, Tamilnadu, India.

A total number of 40 male Swiss albino mice were divided into four groups of 10 each. Skin carcinogenesis was developed in Swiss albino mice according to the method of Azuine and Bhide (1992). Depilatory cream was applied to remove hair from the back of each mouse and the mice were left untreated for two days. Mice having no hair growth after two days were selected for the present study.

The depilated back of group I mice was painted with acetone alone (0.1 mL<sup>-1</sup> mouse) twice weekly for 8 weeks (vehicle treated control). The depilated back of group II and III mice were painted with DMBA (25 µg in 0.1 mL acetone/mouse) twice weekly for 8 weeks. Group II mice received no other treatments. Group III mice were orally administered with ethanollic extract of *Clerodendron inerme* leaves (300 mg kg<sup>-1</sup> b.wt. in 1 mL distilled water, three times per week) starting 1 week before the exposure to the carcinogen and continued throughout the experimental period. Group IV animals received CILEE alone (300 mg kg<sup>-1</sup> b.wt. in 1 mL distilled water) three times per week throughout the experimental period. At the end of experimental period all the animals were sacrificed by cervical dislocation.

### **CILEE Dose Fixation**

Previous studies from our laboratory have identified the pharmacological dose ( $300 \text{ mg kg}^{-1} \text{ b.wt.}$ ) of CILEE for experimental cancer studies (Manoharan *et al.*, 2006; Renju *et al.*, 2007).

### **Toxicity Studies**

The toxicity studies for the administered dose of CILEE were evaluated by histological studies and estimating the activities of serum aspartate transaminase (AST) and alanine transaminase (ALT) (Mohun and Cook, 1957) and  $\gamma$ -glutamyl transferase (GGT) (Fiala *et al.*, 1972). Measurement of these enzymes activities help to assess the extent of tissue toxicity and damage.

### **Histopathological Studies**

For histopathological examination, skin tissues were fixed in 10% formalin and embedded with paraffin, 2-3  $\mu\text{m}$  sections were cut in a rotary microtome and stained with hematoxylin and eosin.

### **Biochemical Analysis**

After plasma separation, the erythrocyte membrane was prepared by the method of Dodge *et al.* (1968) modified by Quist (1980). The precipitate obtained after treating the plasma with 95% ethanol was used for the estimation of protein bound hexose and hexosamine. Similarly, the precipitate obtained after treating the erythrocyte membranes with 1% phosphotungstic acid followed by 5% TCA was used for the estimation of protein bound hexose and hexosamine. The protein bound hexose, hexosamine, total sialic acid and fucose in plasma and erythrocyte membrane were estimated by the methods of Niebes (1972), Wagner (1979), Warren (1959) and Dische and Shettles (1948), respectively. Plasma lipid bound sialic acid level was determined by the method of Katopodis and Stock (1980). The levels of thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) in erythrocyte membrane were measured by the methods of Donnan (1950) and Beutler and Kelley (1963), respectively. Osmotic fragility was determined by the method of Parpart *et al.* (1946) and mean corpuscular fragility was calculated by recording the saline concentration, which would have resulted in 50% hemolysis.

### **Statistical Analysis**

The data are expressed as mean $\pm$ SD. Statistical comparisons were performed by One way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) and Student's t-test. The results were considered significant if the p-values were less than 0.05.

## **RESULTS**

The activities of serum AST, ALT and GGT in control animals ( $60.4\pm 4.8$ ,  $27.5\pm 1.9$  and  $1.47\pm 0.08$ , respectively) and in control animals treated with CILEE alone ( $61.8\pm 5.1$ ,  $28.1\pm 2.1$  and  $1.53\pm 0.09$ , respectively) showed no significant difference between them.

The levels of total hemoglobin and red blood cell count were significantly decreased ( $p < 0.001$ ) where as the mean corpuscular fragility was increased in tumor bearing animals as compared to control animals. Oral administration of CILEE at a dose of  $300 \text{ mg kg}^{-1} \text{ b.wt.}$  to DMBA painted animals restored the status of above said parameters ( $p < 0.001$ ). Mice treated with CILEE alone showed no significant differences in the levels of total hemoglobin, red blood cell count and mean corpuscular fragility as compared to control animals (Table 1).

A significant increase in TBARS ( $p < 0.001$ ) and decrease in GSH ( $p < 0.001$ ) were observed in tumor bearing animals as compared to control animals. Oral administration of CILEE at a dose of  $300 \text{ mg kg}^{-1} \text{ b.wt.}$  to DMBA painted animals restored the status of TBARS and reduced glutathione ( $p < 0.001$ ). Mice treated with CILEE alone showed no significant differences in TBARS and GSH levels as compared to control animals (Table 2).

Table 1: Levels of total hemoglobin, red blood cell count and mean corpuscular fragility in control and experimental animals in each group (n = 10)

Parameters	Total hemoglobin (g dL <sup>-1</sup> )	Red blood cell count (million cells mm <sup>-3</sup> )	Mean corpuscular fragility <sup>Δ</sup>
Control	11.5±0.59 <sup>a</sup>	3.90±0.27 <sup>a</sup>	0.41±0.03 <sup>a</sup>
DMBA	7.8±0.73 <sup>b*</sup>	2.80±0.32 <sup>b*</sup>	0.56±0.05 <sup>b*</sup>
DMBA + CILEE	10.4±0.92 <sup>ab#</sup>	3.50±0.34 <sup>ab#</sup>	0.46±0.04 <sup>ab#</sup>
CILEE alone	11.7±0.72 <sup>a</sup>	3.82±0.22 <sup>a</sup>	0.42±0.03 <sup>a</sup>

Values are expressed as mean±SD, (n = 10). Values not sharing a common superscript significantly different at p<0.05 (DMRT); \*: Significantly different from control animals p<0.001 (Student's t-test); #: Significantly different from control animals p<0.05 (Student's t-test); #: Significantly different from DMBA alone painted animals p<0.001 (Student's t-test); CILEE-*Clerodendron inerme* ethanolic leaf extract; Δ: Concentration of NaCl solution (g%) at 50% hemolysis

Table 2: Levels of TBARS and reduced glutathione in erythrocyte membranes of control and experimental animals in each group (n = 10)

Parameters	TBARS (nmol mg <sup>-1</sup> protein)	GSH (mg dL <sup>-1</sup> )
Control	0.27±0.03 <sup>a</sup>	36.5±2.4 <sup>a</sup>
DMBA	0.87±0.14 <sup>b*</sup>	23.6±3.1 <sup>b*</sup>
DMBA + CILEE	0.32±0.04 <sup>ab#</sup>	31.8±4.2 <sup>ab#</sup>
CILEE alone	0.26±0.03 <sup>a</sup>	37.1±3.1 <sup>a</sup>

Values are expressed as mean±SD, (n = 10). Values not sharing a common superscript significantly differ at p<0.05 (DMRT); \*: Significantly different from control animals p<0.001 (Student's t-test); #: Significantly different from control animals p<0.05 (Student's t-test); #: Significantly different from DMBA alone painted animals p<0.001 (Student's t-test); CILEE-*Clerodendron inerme* ethanolic leaf extract

Table 3: Protein bound hexose, hexosamine, total sialic acid, lipid bound sialic acid and fucose in plasma of control and experimental animals in each group

Parameters	Protein bound hexose (mg dL <sup>-1</sup> )	Protein bound hexosamine (mg dL <sup>-1</sup> )	Total sialic acid (mg dL <sup>-1</sup> )	Lipid bound sialic acid (mg dL <sup>-1</sup> )	Fucose (mg dL <sup>-1</sup> )
Control	75.7±5.4 <sup>a</sup>	65.1±4.6 <sup>a</sup>	35.4±2.8 <sup>a</sup>	8.7±0.6 <sup>a</sup>	5.9±0.32 <sup>a</sup>
DMBA	114.3±12.9 <sup>b*</sup>	92.6±8.9 <sup>b*</sup>	62.5±7.3 <sup>b*</sup>	20.4±2.3 <sup>b*</sup>	11.4±0.9 <sup>b*</sup>
DMBA+CILEE	83.8±8.7 <sup>ab#</sup>	71.4±6.9 <sup>ab#</sup>	39.7±5.3 <sup>ab#</sup>	9.6±1.1 <sup>ab#</sup>	6.4±0.61 <sup>ab#</sup>
CILEE alone	74.7±6.2 <sup>a</sup>	63.9±5.1 <sup>a</sup>	35.1±3.2 <sup>a</sup>	8.5±0.7 <sup>a</sup>	5.7±0.43 <sup>a</sup>

Values are expressed as mean±SD, (n = 10). Values not sharing a common superscript significantly differ at p<0.05 (DMRT); \*: Significantly different from control animals p<0.001 (Student's t-test); #: Significantly different from control animals p<0.05 (Student's t-test); #: Significantly different from DMBA alone painted animals p<0.001 (Student's t-test); CILEE-*Clerodendron inerme* ethanolic leaf extract

Table 4: Protein bound hexose, hexosamine and total sialic acid levels in erythrocyte membranes of control and experimental animals in each group (n = 10)

Parameters	Protein bound hexose (μg mg <sup>-1</sup> protein)	Protein bound hexosamine (μg mg <sup>-1</sup> protein)	Total sialic acid (μg mg <sup>-1</sup> protein)
Control	112.5±7.9 <sup>a</sup>	69.8±4.9 <sup>a</sup>	30.1±1.8 <sup>a</sup>
DMBA	71.3±8.3 <sup>b*</sup>	52.4±6.1 <sup>b*</sup>	19.4±2.2 <sup>b*</sup>
DMBA + CILEE	103.5±8.8 <sup>ab#</sup>	64.7±5.4 <sup>ab#</sup>	27.9±2.8 <sup>ab#</sup>
CILEE alone	113.1±8.7 <sup>a</sup>	70.1±5.6 <sup>a</sup>	30.4±2.1 <sup>a</sup>

Values are expressed as mean±SD, (n = 10). Values not sharing a common superscript significantly differ at p<0.05 (DMRT); \*: Significantly different from control animals p<0.001 (Student's t-test); #: Significantly different from control animals p<0.05 (Student's t-test); #: Significantly different from DMBA alone painted animals p<0.001 (Student's t-test); CILEE-*Clerodendron inerme* ethanolic leaf extract

The levels of these glycoconjugates were significantly increased (p<0.001) in plasma of tumor bearing animals as compared to control animals. Oral administration of CILEE at a dose of 300 mg kg<sup>-1</sup> b.wt. to DMBA painted animals restored the status of glycoconjugates (p<0.001). Mice treated with CILEE alone showed no significant difference in glycoconjugates levels as compared to control animals (Table 3).

The levels of these glycoconjugates were significantly decreased (p<0.001) in erythrocyte membranes of tumor bearing animals as compared to control animals. Oral administration of CILEE at a dose of 300 mg kg<sup>-1</sup> b.wt. to DMBA painted animals restored the status of glycoconjugates (p<0.001). Mice treated with CILEE alone showed no significant differences in glycoconjugates levels as compared to control animals (Table 4).

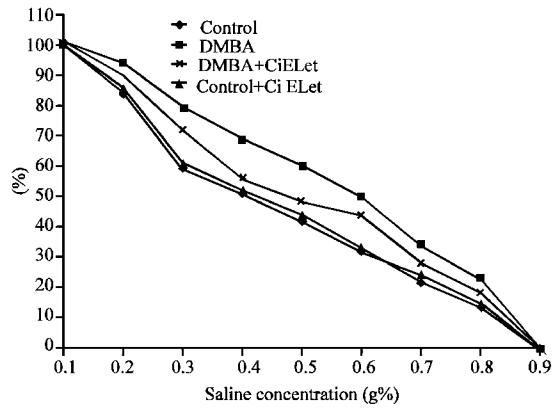
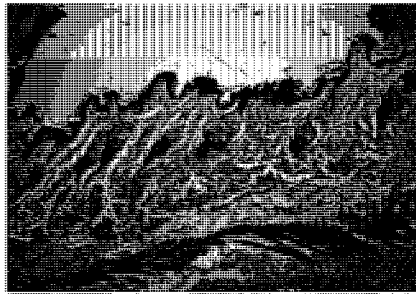
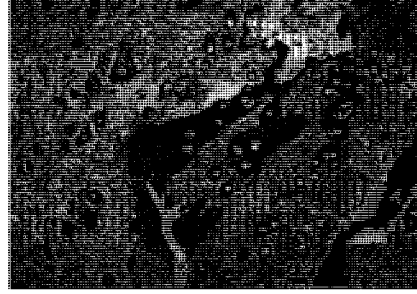


Fig. 1: Osmotic fragility curve of control and experimental animals in each group. The degree of haemolysis was calculated by comparing with 0.1% NaCl solution which represented 100% lysis



Microphotographs showed normal mouse skin and subcutaneous tissues (H and E magnification 40X)



Microphotographs of DMBA alone treated mouse skin showing well differentiated squamous cell carcinoma (H and E magnification 40X)



Microphotographs of DMBA alone treated mouse skin and showing well differentiated squamous cell carcinoma with keratin pearls (H and E magnification 40X)



Microphotographs of DMBA + CILEE treated mouse skin showed normal skin tissues (H and E magnification 40X)

Fig. 2: Histopathological features of control and experimental animals in each group

The fragility curve of tumor bearing animals was shifted to the right for the control animals. The mean corpuscular fragility was also significantly higher in tumor bearing animals as compared to control. Treatment of tumor bearing animals with CILEE at a dose of 300 mg kg<sup>-1</sup> b.wt. shifted the curve to the left of cancer animals. Mean Corpuscular Fragility (MCF) values did not differ significantly in animals treated with CILEE alone as compared to control animals (Fig. 1).

The skin tissues from DMBA treated mice (Group II) revealed severe keratosis, hyperplasia, dysplasia and well differentiated squamous cell carcinoma. A mild to moderate preneoplastic lesions (hyperplasia (+) keratosis (++) and dysplasia (+) were noticed in group III animals (DMBA+CILEE). We did not observe any histological changes in the skin tissues of mice treated with CILEE alone (Fig. 2).

## DISCUSSION

The simplicity, availability and ease of isolation make erythrocyte membrane as an excellent model for membrane studies. The goal of the present study was to investigate the protective effect of *Clerodendron inerme* on erythrocyte membrane integrity in DMBA induced skin carcinogenesis. The membrane integrity was assessed by measuring the status of RBC membrane glycoconjugates and red blood cell osmotic fragility in experimental animals.

Control animals treated with acetone alone (vehicle) showed well defined skin and the presence of subcutaneous tissues. DMBA alone treated animals showed 100% tumor incidence and the tumor was histopathologically diagnosed as well differentiated squamous cell carcinoma. The tumor cells have pleiomorphic, hyperchromatic nuclei with epithelial pearl formation. Oral administration of CILEE completely prevented the formation of well differentiated squamous cell carcinoma. We have however observed precancerous lesions such as hyperplasia and dysplasia. Present results thus indicate that CILEE has suppressing effect on cell proliferation.

Oral administration of CILEE alone at a dose of 300 mg kg<sup>-1</sup> b.wt. did not produce any histological changes in liver, kidney and skin of control animals. We have also assessed the toxic effect of CILEE by estimating the activities of AST, ALT and GGT in serum. No significant difference in the activities of AST, ALT and GGT in control animals treated with CILEE alone. Our results thus suggest that the administered dose has no toxic effect to the host tissues.

Erythrocytes of tumor bearing mice were more fragile than those from control mice. Osmotic fragility has been found to be altered in various pathological conditions including cancers (Abou-Seif *et al.*, 2000; Kolanjiappan *et al.*, 2002). Increased osmotic fragility in cancer animals can be correlated to elevated lipid peroxidation in erythrocytes. Lipid peroxidation has been implicated in the alterations of membrane structure and functions. Increased lipid peroxidation has been reported to cause an increase in osmotic fragility and decrease in cell fluidity (Manoharan *et al.*, 1996; Kolanjiappan *et al.*, 2002).

Several studies have reported that reduced glutathione plays a central role in cellular defense against reactive oxygen species (McLellan and Wolf, 1999). Decline in red blood cell reduced glutathione in cancer mice is partially responsible for the increased osmotic fragility of erythrocytes. The tripeptide glutathione plays an important role in protecting the erythrocytes from free radical-induced lipid peroxidation. Hence, we feel that the decrease in red blood cell glutathione due to increased lipid peroxidation may in turn leads to changes in red blood cell osmotic fragility. Oral administration of CILEE protected the changes in red blood cell fragility in DMBA alone painted animals. This is probably due to antilipidperoxidative and antioxidant potential of CILEE. In the present study, we also observed that CILEE significantly restored the levels of total Hemoglobin and red blood count in DMBA painted animals. This suggests that CILEE protected the alterations in red blood cells during skin carcinogenesis.



In the present study, an increase in plasma glycoconjugates and decrease in erythrocyte membrane glycoconjugates levels were noticed in tumor bearing animals as compared to control animals. Analysis of glycoconjugates, the vital components of cell surface, has been the field of intensive investigation due to the fact that they play a crucial role during neoplastic transformation (Dabelsteen, 1996; Suresh *et al.*, 2007). Altered glycosylation of glycoconjugates is one of the important molecular changes that accompany malignant transformation. Dabelsteen (1996) have reported that epithelial cell surface carbohydrates were lost during experimental carcinogenesis. The decrease in erythrocyte membrane glycoproteins can therefore be related to increased membrane degradation during malignancy.

Increased concentration of serum glycoproteins have been reported in human and experimental carcinogenesis (Manoharan *et al.*, 2004; Suresh *et al.*, 2007). Macbeth and Bekesi (1964) have reported that the presence of neoplastic cells in the body stimulate the synthesis of glycoproteins in the liver, which subsequently enter into circulation. Increased levels of plasma glycoproteins in tumor bearing mice are either due to spontaneous release of glycoproteins from tumor cells or due to increased hepatic synthesis with subsequent shedding into plasma.

Sialic acids, group of acylated neuraminic acid, impart a net negative charge to cell membrane and has important role in cell-cell and cell-matrix interactions. Sialic acid concentration on the surface of malignant cell can be used to correlate directly with its ability to metastasize (Sebzda *et al.*, 2006). Elevated levels of total sialic acid and lipid bound sialic acid were reported in several cancers (Manoharan *et al.*, 2004; Suresh *et al.*, 2007). Elevated plasma sialic acid concentration is probably either due to shedding or secretion of sialic acid from tumor cell surfaces or due to membrane degradation (Macbeth and Bekesi, 1964; Manoharan *et al.*, 2004). The observed increase in plasma lipid bound sialic acid is probably due to shedding of aberrant sialic acid rich glycolipids into circulation.

A positive association between serum fucose level and cancer progression has been reported (Manoharan *et al.*, 2004). The observed increase in plasma total sialic acid and fucose in tumor bearing mice could be either due to increased turnover of malignant cells or shedding from tumor tissues and membranes into circulation. In the present study, oral administration of *Clerodendron inerme* ethanolic leaf extract restored the status of plasma and erythrocyte membrane glycoconjugates in DMBA painted mice. Our results thus suggest that *Clerodendron inerme* protected the levels of plasma and erythrocyte membrane glycoconjugates by maintaining the structural integrity and stability of the erythrocyte membrane during DMBA induced skin carcinogenesis.

Increased membrane lipid peroxidation, insufficient antioxidant potential and loss of membrane glycoconjugates caused structural and functional abnormalities in the erythrocytes of skin tumor bearing mice. Oral administration of CILEE restored these abnormalities during DMBA induced skin carcinogenesis. The protective effect of CILEE on membrane integrity is probably due to the presence of one or more bioactive constituents such as flavonoids and isoflavones and their synergistic interactions. The present study thus demonstrates the protective effect of CILEE on red blood cell membrane integrity during DMBA induced skin carcinogenesis.

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