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Treating the 7,12-dimethylbenz(a)anthracene (DMBA) induced buccal pouch carcinoma in Syrian hamster, *Mesocricetus auratus* (L) with ethanolic extractives of leaves of mulberry, *Morus alba* (L)

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ABSTRACT

The leaf extract of mulberry, *Morus alba* (L) in ethanol was used to treat the 7,12-dimethylbenz-anthracene (DMBA) - induced carcinoma in the buccal pouch of Syrian hamster, *Mesocricetus auratus* (L). The 0.5 percent DMBA in liquid paraffin was used for painting the buccal pouch. The DMBA treatment was carried thrice a week for 14 weeks. This was resulted into squamous cell carcinoma. The parameters analyzed include: The tumor incidence, volume and burden. It was followed by oral treatment with ethanolic extractives of mulberry leaves (TpEt) at a dosage of 300 mg/kg, body weight, to DMBA (on alternate days for 14 weeks) - painted animals. Administration of ethanolic extractive of mulberry leaves was found preventing significantly the incidence, volume and burden of the buccal pouch carcinoma. The ethanolic extractive of mulberry leaves exhibited significant effect, especially, the antilipidperoxidation and antioxidative enhancement in DMBA individuals of Syrian hamsters. The results are demonstrating the potency of ethanolic extractive of mulberry leaves for chemoprevention and significant antilipidperoxidative influence in 7,12-dimethylbenz-anthracene (DMBA)- induced carcinoma in the buccal pouch of Syrian hamster, *Mesocricetus auratus* (L). The activation of caspase through release of cytochrome C through disruption of mitochondrial membrane potential may be the sequence of action of the active principles of mulberry leaf extractives. With it's bioactive compounds, mulberry, *Morus alba* (L) may open a

new avenue in the cancer prevention and treatment. Taken together, the present attempt provide experimental evidence that leaves of mulberry may have chemopreventive effects on cancerous growth. Mulberry, *Morus alba* (L) may provide a therapeutic option for controlling the growth of cancer cells.

1. INTRODUCTION

Most of the plants are with chemical compounds that are analogous with animal hormones and therefore they are accredited as "Healthy and Wealthy" (Vitthalrao Khyade and Vivekanand Khyade, 2013 and 2013). The plants, through their biocompounds serve to orchestrate the progression of cytological health. The mulberry, *Morus alba* (L) is with many more medicinally important constituents. Moracin is the compound isolated from mulberry fruits. This compound have been reported to cure the skin cancer in mice (Vitthalrao Khyade, *et al*, 2013). The mulberry leaf extravtive was found excellent to cure the diabetes in the experimental mice (Vitthalrao Khyade, 2014).

The carcinoma of oral squamous cells ranks the fifth common malignancy worldwide. It occur most commonly in human males. The western countries accounts three to four percent for this oral carcinoma. The highest incidences of most of the cancers are recorded in Indian population, which accounts for about 30 – 40 percent. According to Moore, et al (2000), the cancers of the oral cavity are frequently associated with chewing of betel quid containing tobacco. The smoking and consumption of alcohol are the other reasons for cancers.

For the purpose to study precancerous and cancerous lesions of human oral squamous cell carcinoma, the best suitable model is the 7,12-dimethylbenz(a)anthracene (DMBA)-induced hamster buccal pouch carcinogenesis. This is because of morphological and histological similarity to human tumors. Further, the 7,12-dimethylbenz(a)anthracene (DMBA)-induced hamster buccal pouch carcinogenesis is expressing many biochemical and molecular markers that are expressed in human being (Boring, *et al*, 1994). The most significant implications of pathogenesis of oral (and others too) carcinoma include: lipid peroxidation and a potent reactive oxygen species (ROS)- mediated chain reaction.

The mutagenesis and carcinogenesis are contributions related to overproduction of reactive oxygen species within tissues can damage DNA and possibly contribute. For the purpose to defend carcinogenesis, however the organisms have an array of potent adaptive antioxidant defense mechanisms. This array of adaptive spectrum include the enzymatic antioxidants: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and non-enzymatic antioxidants: reduced glutathione (GSH), vitamin C and vitamin E within he cells, to combat the deleterious influences of reactive oxygen species- mediated oxidative damages (Ray and Husain, 2002).

There are many more natural or synthetic chemicals that concerned with cancer chemoprevention. This is supposed to be a new promising strategy for prevention, inhibition or reversal of carcinogenesis, which are induced by specific natural or / and synthetic chemicals (Hans Peter, *et al* , 2000).

The medicinal plants are the best source of preventing and treating various pathological aspects including carcinogenesis. Craig (1999) reported many medicinal plants and their contents for prevention of multistage carcinogenesis.

The phytoochemical contents of mulberry, *Morus alba* (L) serve as wholesome food for the progression of insect live cycles, like silkworm, *Bombyx mori* (L). There are reports on use of mulberry leaves for feeding the live stock (cattle, goat, …etc.). In the areas of dry seasons, restriction of availability of ground vegetation is a common problem. In such areas, mulberry leaves are cut for food for the livestock. The bioochemical constituents of mulberry include: moracin – M; Steppogenin – 4' – o – beta – D – glucoside and mulberroside are the novel biocompounds of mulberry, which found to produce hyperglycemic effects (Zhang, *et al*, 2009). Use of fruits of mulberry for human consumption seems to be a general method in Japan, China and India. The fruits of mulberry are also used to treat the premature grey hairs; to fortify the blood and to treat the constipation and diabetes. The most significant constituents of mulberry fruits is the Moracin. It is extracted and isolated from the mature fruits of mulberry, *Morus alba* (L).

The extractives of leaves of mulberry, *Morus alba* (L) in ethanol was reported to have antihyperglycemic, antioxidant and antiglycation effects in chronic diabetic rats (Naowaboot, *et al*, 2009 and Vitthalrao B. Khyade, 2014). The efforts conducted to assess the protective influence of Moracin, the major constituent of fruits of mulberry, *Morus alba* (L) on tumor promotion in 7,12-dimethylbenz (alpha) anthracene (DMBA) – initiated and 12 – O – tetradecanoylphorbol 13 – acetate (TPA) – promoted skin tumerigenesis in mice was found most significant effects (Vitthalrao B. Khyade, *et al* , 2013).

The expression of tumor necrosis factor (TNF) – alpha protein and the level of 4-hydroxynoneal (4HNF) in the normal epidermis were significantly reduced in moracin treated mice (Ujjwala Lonkar and Vitthalrao B. Khyade, 2013). Moracin treatment furthermore significantly suppressed the elevation in 4 – HNE level and elevated expression of c – fos; c – myc and cyclooxygenase – 2 (COX – 2) in epidermal tumerigenesis induced by double application of TPA (Babita Sakdeo and Vitthalrao, 2013).

To the best of present knowledge, the scientific report on the chemopreventive efficacy and antilipidperoxidative effects of leaves of mulberry, *Morus alna* (L) in DMBA – induced, hamster buccal pouch carcinogenesis are nowhere. This situation made to plan the present attempt to evaluate the effectiveness of ethanol extractives of leaf of mulberry, *Morus alba* (L) for treating the carcinogenic process in DMBA- induced hamster buccal pouch carcinoma in Syrian hamster, *Mesocricetus auratus* (L).

2. MATERIAL AND METHODS

The chemical 7,12-dimethylbenz(a)anthracene (DMBA) was obtained from Sigma-Aldrich Chemical Pvt. Ltd., Bangalore, India through local dealer. Remaining all other chemicals used were of analytical grade available in the "Dr. APIS" Laboratory. The National Institute of Nutrition, Hyderbad, India availed the male, golden Syrian hamsters, *Mesocricetus auratus* (L) 8-10 weeks old, weighing 80 to120 grams. They were maintained in the domestic cages, made up of polypropylene in the "Dr. APIS" Laboratory. The male, golden Syrian hamsters, *Mesocricetus auratus* (L) were housed in groups of four or five in polypropylene cages.

They were provided standard diet in the form of pellet. Provision of drinking water was made through the use of plastic bottles. The hamsters in the laboratory were maintained under controlled conditions of temperature and humidity, with a 12 h light/dark cycle through standard methods prescribed (Thirunavukkarasu, C., *et al*, 2001).

The fresh leaves of mulberry, *Morus alba* (L) were collected from the mulberry garden at the sheti (Malegaon) farm of Agriculture Development Trust, Baramati (India). The leaves washed through running tap water and allowed for drying in shade. Shade dried leaves of mulberry were powdered through the use of domestic mixture. 500 g of dried finely powdered mulberry leaves were kept in 1500 ml of 95% ethanol for 24 hours. After 24 hours, the content was filtered. This filtrate was labeled as, "A". The residue thus obtained was again immersed in equal volume of 95% ethanol for 48 hours. The content was filtered. This filtrate was labeled as, "B". The two filtrates, "A" and "B" were mixed. The mixture was allowed for the solvent evaporation through the use of rotovapour at 40-50 °C, under the reduced pressure. A dark semisolid material (13%) was obtained. This was the ethanolic extractives of leaves of mulberry, *Morus alba* (L) (TpEt).

This dark semisolid material was stored at -4 °C, until use. For further use, in experimental studies, a known volume of the extract was suspended in distilled water. It was used for oral administered to the experimental animals, the male, golden Syrian hamsters, *Mesocricetus auratus* (L). The "gastric intubation using force-feeding needle" method was used for feeding the ethanolic extractives of leaves of mulberry, *Morus alba* (L) to the experimental animals. The committee of institutional animal ethics availed the approval for the design of the study attempt. The male, golden Syrian hamsters, *Mesocricetus auratus* (L) were randomly divided into four groups. Each group was with ten individuals. The first group (I) was used as untreated control. The groups II and III were used for treating (painting) with 0.5% DMBA.

The DMBA was used in liquid paraffin. The treatments (painting with 0.5% DMBA) was carried for three times for a week. This treatment was carried for 14 weeks on the left buccal pouches of the male, golden Syrian hamsters, *Mesocricetus auratus* (L). Group II received no other treatment (Except, 0.5% DMBA). The Group III was treating with mulberry leaf extractives (300 mg/kg, b.w.) orally. The mulberry leaf extract treatment was started 1 week before the exposure to the carcinogen (0.5% DMBA). And this was continued on days alternate to DMBA painting, until the animals were used for bioassays. Group IV received oral TpEt, only throughout the experimental period. The experimentation was carried for 14 weeks. For the bioassay purpose, all the animals were sacrificed through cervical dislocation.

The blood and buccal mucosa were used for Biochemical assays. The buccal mucosal tissues were fixed in ten percent formalin for histopathological studies. The formalin fixed tissues were embedded in paraffin and blocks were prepared. The section of 2-3 µm sections were cut in a rotary microtome and processed for histochemical analysis. The membrane of erythrocytes was prepared by the method described by Dodge *et al* (1968) and Quist (1980). The bioassay of thiobarbituricacid reactive substances (TBARS) was carried through the use of plasma, erythrocytes and buccal mucosa. The methods of Yagi (1978) Donnan (1950) and Ohkawa *et al* (1979) respectively, utilized for the bioassay of TABRS of Plasma, erythrocytes and buccal mucosa.

The method described by Beutler and Kelley (1963) was utilized for the bioassay of reduced glutathione (GSH). The biochemical measurements of Vitamin C and E were carried out through the methods of Omaye *et al* (1979) and Desai (1984) respectively.

The methods of Kakkar, et al (1984); Sinha (1972) and Rotruck, et al (1973) were for the bioassays of the enzymatic antioxidant activities; SOD; CAT and GPx respectively. For the purpose to obtain consistency in the results, the whole experimentation was repeated for three times.

The data was collected and subjected for analysis through standard statistical methods. data was expressed as mean with standard deviation and subjected for statistical analysis.

3. RESULTS, DISCUSSION AND COLNLUSIONS

The results of present study has been categorized in the parameters, which include: Incidence, volume and burden of tumor; Histopathological changes; TBARS and antioxidant status of Plasma; Erythrocytes; and Buccal mucosa.

A. Incidence, volume and burden of tumor: The influence of ethanol extractives of leaves of mulberry, *Morus alba* (L) (TpEt) on the parameters like incidence of tumor, volume of tumor and the burden of tumor in the DMBA- induced hamster buccal pouch carcinoma are presented in Table - 1. In all the individuals of Group II, DMBA - painted hamsters, the tumor formation appeared. There was 100% tumor formation in this group. The 472 mm^3 was the mean tumor volume was measured. The tumor burden correspond to 2029 mm^3. Treating the cancerous hamsters (Group III) with ethanol extractives of leaves of mulberry, *Morus alba* (L) (TpEt) at the rate of 300 mg/kg, b.w. was found significantly preventing the incidence tumor formation, volume of tumor and burden of tumor. The hamsters of the DMBA untreated and the hamsters treated with DMBA followed by oral treatment with ethanol extractives of leaves of mulberry, *Morus alba* (L) (Group IV) were found without the tumor formation.

B. Histopathological Changes: The histopathological features of control and experimental animals in each group are summarized in Table – 2. The hamsters painted with DMBA alone (Group III) (DMBA + TpEt) were found with myriad of histopathological changes, which include: severe keratosis, hyperplasia, dysplasia and squamous cell carcinoma of the epithelium. The hamsters of Group III were found with a mild to moderate preneoplastic lesions [hyperplasia (++), keratosis (+) and dysplasia (+)].

C. Plasma TBARS and antioxidant status: The Table – 3 explain the status of TBARS and antioxidant in plasma and erythrocytes of the control and experimental groups. In comparison with the control group, the hamster individuals of Group II (DMBA alone) were exhibiting increased concentration of TABRS. There was significant decrease in the levels of nonenzymatic antioxidants (GSH, Vitamin C and Vitamin E) and activities of enzymatic antioxidants (SOD, CAT and GPx). The oral administration of ethanol extractives of leaves of mulberry, *Morus alba* (L) (TpEt) was thus found significantly decrease in the levels of TBARS and improvement in the antioxidants status in DMBA- painted hamsters. The ethanol extractives of leaves of mulberry, *Morus alba* (L) (TpEt) alone- treated hamsters exhibited no significant difference in TBARS and the status of antioxidative activity.

D. Erythrocyte TBARS and antioxidant status: The Table – 4 depict the status of TBARS and antioxidant in plasma and erythrocytes of the control and experimental groups. The concentration of TBARS was increased, whereas the levels of nonenzymatic antioxidants (GSH, Vitamin C and Vitamin E) and activities of enzymatic antioxidants (SOD, CAT and GPx), were significantly decreased in group II (DMBA alone), as compared to control animals. Oral administration of TpEt significantly decreased the levels of TBARS and improved the antioxidants status in DMBA - painted hamsters. TpEt alone - treated hamsters showed no significant difference in TBARS and antioxidants status, in comparison with the control group.

E. Buccal Mucosa TBARS and antioxidant status: The changes in TBARS and antioxidant properties of buccal mucosa are summarized in Table – 5. Decrease in TBARS concentration and alterations in the antioxidant status (Vitamin E, GSH and GPx were increased; SOD and CAT were decreased), were noticed in cancer animals (Group II) as compared to control (Group I). However oral administration of TpEt (Group III), reverted the concentration of TBARS and antioxidants to near normal range in DMBA - painted animals. Hamsters treated with TpEt alone (Group IV) showed no significant difference in TBARS and antioxidants status, as compared to control animals.

The developing countries are with the report of incidence and mortality rates of oral cancer. The highest rate of death through cancer are reported every year from developing countries, particularly from India. The cancer of oral pouch is one among the few human cancers. The oral cancer deserve the vast potential for prevention.

The high content of polyunsaturated fatty acids is a major target of reactive oxygen species is cell membrane. The damage of cellular DNA, membrane structure and inhibition of functions of several enzymes and alterations in the immune system are brought through reactive oxygen species (ROS) mediated lipid peroxidation (Thirunavukkarasu, *et al*, 2001). Enormous production of free radicals in the system is the distinguishing feature of cancer cell. It seems to have a close relationship between free radical activity and neoplastic transformation (McCord, 2000).

The enzymatic and non-enzymatic antioxidants play a vital role in scavenging reactive oxygen species and help to protect the cells from damage (oxidative). The primary defense antioxidants are Vitamin E, Vitamin C and reduced glutathione. The Vitamin E; Vitamin C and reduced glutathione provide protection against several reactive oxygen species. They prevent the development of cancer and other oxidative stress- mediated dysfunctions (Ray and Hussain, 2002).

Enhanced lipid peroxidation causes the tissue damage. From such damage, that is to say the tissue damage through enhanced lipid peroxidation, the protection is availed by SOD, CAT and GPx. The cancerous tissue is with elevated lipid peroxidation and poor antioxidant systems. Absence of antioxidant defense exert the elevated lipid peroxidation in the red blood corpuscles (erythrocytes) (Sabitha and Shyamaladevi, 1999).

There is altered activities of enzymatic antioxidants during the carcinogenesis or after formation of tumor. The poor antioxidant defense mechanism, therefore leads to the elevated lipid peroxidation in the circulation of cancer animals. Most possibly, the elevated lipid peroxidation or sequestration through tumor tissue for their rapid growth are due to lowered non - enzymatic antioxidants (Vitamin C, Vitamin E and GSH) in the circulation of body fluid

like blood. In comparison with normal, healthy tissue, there is diminished lipid peroxidation and disturbed antioxidants status (increase in GSH and GPx whereas decrease in SOD and CAT) in the tumor tissues.

According to Subpriya, *et al* (2002), the cancer cells exhibit decreased susceptibility to lipid peroxidation, compared to normal tissues.

There exert an inverse relationship between the lipid peroxidation process and the rate of cell proliferation. The glutathione is a biologically important tripeptide.

It is essential for maintaining cell integrity, through its reducing properties and participation in the cell metabolism. The glutathione peroxidase and its co-substrate glutathione, are with regulatory effects on proliferation of cell. The elevated glutathione and GPx activity, have been demonstrated in several tumor tissues, including oral cancer. This lead to conclude that, the diminished peroxidation of lipid, combined with the enhanced glutathione-dependent antioxidant capacity of oral carcinogenesis, facilitates the cell - proliferation.

This may be offering selective growth advantage in tumor cells in comparison with their surrounding normal cells. Our results of present attempt lend credibility for the above observations (Subapriya *et al,* 2002 and Vitthalrao B. Khyade, *et al*, 2013).

Decrease in SOD and catalase activities described in tumors, is regarded as markers of malignant transformation. Lowered activities of SOD and CAT were reported in several cancers, including skin tumerigenesis and oral cavity cancer (Sabita and Shymaladevi, 1999; Subapriya *et al,* 2002 and Babita Sakdeo and Vitthalrao Khyade, 2013). The results of present attempt thus, indicate, that ethanol extractives of leaves of mulberry, *Morus alba* (L) (TpEt) possess significant chemopreventive potential against DMBA - induced buccal pouch carcinoma.

Furthermore, ethanol extractives of leaves of mulberry, *Morus alba* (L) (TpEt) is responsible for significant reduction in the levels of TBARS and enhancement in the status of antioxidants in the circulation of DMBA - painted hamsters. There is elevation of TBARS level and improvement in the antioxidant defense system in the buccal mucosa of DMBA-painted hamsters, through treatment with ethanol extractives of leaves of mulberry, *Morus alba* (L) (TpEt). The chemopreventive features of plant anticarcinogens are either due to antilipidperoxidative action, modulating carcinogen detoxification or through improvement in the antioxidant defense system (Johnson, 1997 and Ujjwala Lonkar and Vitthalrao Khyade, 2013).

Most of the plants are with chemical compounds that are analogous with animal hormones, which accredited them for medicinal plants (Vitthalrao Khyade and Vivekanand Khyade, 2013 and 2013). The plants of medicinal importance and their contents, possibly exerting the chemopreventive effect, through scavenging reactive oxygen species (ROS) and improving the antioxidant defense systems of individuals.

The present attempt reveals, that the chemopreventive effect of ethanol extractives of leaves of mulberry, *Morus alba* (L) (TpEt) 7,12-dimethylbenz(a)anthracene (DMBA) induced buccal pouch carcinoma in Syrian hamster, *Mesocricetus auratus* (L) is possibly due to its antilipidperoxidative and antioxidant properties. Taken together, the present attempt provide experimental evidence that leaves of mulberry may have chemopreventive effects on cancerous growth. Mulberry, *Morus alba* (L) may provide a therapeutic option for controlling the growth of cancer cells.

Table 1. Influence of treatment with leaf extract of mulberry, *Morus alba* (L) on oral squamous cell carcinoma in 0.5 percent DMBA painted Syrian hamster, *Mesocricetus auratus* (L).

Groups (Alone) Parameters	Control	0.5 % DMBA	o.5 % DMBA + Mulberry Extract (300 mg / Kg)	Mulberry Extract (300 mg / Kg)
Tumor Incidence	00.00	100 %	20 %	00.00
Total No. of Tumors	00.00	43 %	4.00 %	00.00
Tumor volume (cubic mm)	00.00	472.41 ($\pm$ 57.33)	97.96 ($\pm$ 13.07)	00.00
Tumor Burden (cubic mm)	00.00	2029.61($\pm$ 178.60)	194.92 ($\pm$ 38.18)	00.00

Each value is the mean of three replications. Figures in parenthesis with ($\pm$) signs are the standard deviations. Tumor volume was measured through the use of formula: V = (4 divided by 3) x Pi x (D1 divided by 2) x (D2 divided by 2) x (D3 divided by 2), where D1; D2 and D3 are the three diameters of tumor. Tumor burden was calculated by multiplying tumor volume and number of tumors per animal. Mulberry leaf extract was administered orally (300 mg / kg body weight of expt. Animal) one week before BMBA painting and continued on alternate days to DMBA paintings until sacrifice. DMBA (0.5 % in liquid paraffin) was painted on the left buccal pouch thrice a week for 14 weeks.

Table 2. Histopathological changes in oral cheek mucosa of mulberry leaf extract treated, 0.5 percent DMBA painted Syrian hamster, *Mesocricetus auratus* (L).

Groups (Alone) Parameters	Control	0.5 % DMBA	0.5 % DMBA + Mulberry Extract (300 mg / Kg)	Mulberry Extract (300 mg / Kg)
Keratosis	Absent	Severe	Moderate	Absent
Hyperplasia	Absent	Severe	Mild	Absent
Dysplasia	Absent	Severe	Mild	Absent
Squamous Cell Carcinoma	Undifferentiated	Moderately Differentiated	No	Undifferentiated

Number of individual Experimental animals (n) in each group was ten (n = 10). Mulberry leaf extract was administered one week before and continued on alternate days to DMBA painting until sacrifice. 0.5 % DMBA in liquid paraffin was painted on the left buccal pouches thrice a week for total 14 weeks.

Table 3. Plasma TBARS and antioxidant status in mulberry leaf extract treated, 0.5 percent DMBA painted Syrian hamster, *Mesocricetus auratus* (L).

Parameters Groups	TBARS (nmoles / ml)	GSH (mg/dl)	Vitamin C (mg/dl)	Vitamin E (mg/dl)	SOD (U*/ml)	CAT (U**/ml)	GPx (U***/ml)
Control	2.97 ($\pm$ 0.25)	28.88 ($\pm$ 2.4)	1.48($\pm$ 0.12)	1.21($\pm$ 0.12)	2.87($\pm$ 0.12)	0.43 ($\pm$ 0.12)	133.8 ($\pm$ 0.1)
DMBA	4.64($\pm$ 0.41)	19.71($\pm$ 1.31)	0.86($\pm$ 0.08)	0.70 ($\pm$ 2.4)	1.76 ($\pm$0.07)	0.27 ($\pm$ 2.4)	98.3($\pm$ 8.6)
DMBA+Mul.	3.41($\pm$ 0.36)	25.65($\pm$ 2.2)	1.25($\pm$ 0.11)	1.08($\pm$ 0.10)	2.58($\pm$ 0.20)	0.38($\pm$ 0.03)	126.4 ($\pm$11.8)
Mul. alone	2.84 ($\pm$ 0.16)	30.21($\pm$ 2.8)	1.50($\pm$ 0.12)	1.28($\pm$ 0.17)	2.96($\pm$ 0.23)	0.45($\pm$ 0.04)	138.2($\pm$12.3)
One – Way F	63.431	39.239	70.251	58.215	60.312	56.575	22.246
ANOVA df	3,36	3,36	3,36	3,36	3,36	3,36	3,36
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05 ---

Each value is the mean of three replications. Figures in parenthesis with sign are the standard deviations. * indicate the amount of enzyme required to inhibit fifty percent NBT reaction. ** indicate micromoles of hydrogenperoxide utilized per second. *** indicate micromoles of glutathione utilized per minute. 0.5 % DMBA in liquid paraffin was painted on the left buccal pouches thrice a week for total 14 weeks.

Table 4. Erythrocytes TBARS and antioxidant status in mulberry leaf extract treated, 0.5 percent DMBA painted Syrian hamster, *Mesocricetus auratus* (L).

Parameters Groups	Erythrocyte TBARS (pmoles / mg Hb)	Erythrocyte TBARS (n moles / mg protein)	Vitamin E (microgram / mg protein)	Erythrocyte GSH (mg / dl)	Erythrocyte Lysate SOD (U*/mg Hb)	CAT (U**/mg Hb)	GPx (U***/mg Hb)
Control	1.91 ($\pm$ 0.18)	0.34 ($\pm$ 0.03)	2.32($\pm$ 0.12)	42.35($\pm$ 3.81)	2.17($\pm$ 0.22)	1.24 ($\pm$ 0.13)	14.63 ($\pm$ 0.11)
DMBA	2.65($\pm$ 0.22)	1.2($\pm$ 0.11)	1.48($\pm$ 0.09)	29.67 ($\pm$ 2.6)	1.46 ($\pm$ 0.17)	0.82 ($\pm$ 0.08)	7.58($\pm$ 1.63)
DMBA+Mul.	2.16($\pm$ 0.19)	00.92($\pm$ 0.06)	2.12($\pm$ 0.20)	38.21($\pm$ 3.51)	1.92($\pm$ 0.19)	1.12($\pm$ 0.09)	11.32 ($\pm$ 1.08)
Mul. alone	1.82 ($\pm$ 0.16)	00.51($\pm$ 0.04)	2.40($\pm$ 0.19)	44.56($\pm$ 3.27)	2.28($\pm$ 0.23)	1.31($\pm$ 0.14)	15.74 ($\pm$ 1.26)
One – Way F	29.207	388.58	60.27	35.942	37.141	41.394	111.79
ANOVA df	3,36	3,36	3,36	3,36	3,36	3,36	3,36
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Each value is the mean of three replications. Figures in parenthesis with sign are the standard deviations. * indicate the amount of enzyme required to inhibit fifty percent NBT reaction. ** indicate micromoles of hydrogenperoxide utilized per second. *** indicate micromoles of glutathione utilized per minute. 0.5 % DMBA in liquid paraffin was painted on the left buccal pouches thrice a week for total 14 weeks.

Table 5. Buccal Mucosa TBARS and antioxidant status in mulberry leaf extract treated, 0.5 percent DMBA painted Syrian hamster, *Mesocricetus auratus* (L).

Parameters Group	TBARS (nmoles / 100gm Protein)	GSH (mg/ 100. g tissue)	Vitamin E (mg/ 100 g tissue)	SOD (U*/gm Protein)	CAT (U**/gm Protein)	GPx (U***/gm Protein)
Control	76.431 ($\pm$ 5.45)	8.54 ($\pm$ 3.73)	1.94 ($\pm$ 0.17)	5.43 ($\pm$ 1.36)	38.2 ($\pm$ 2.9)	6.36 ($\pm$ 1.59)
DMBA	51.5 ($\pm$ 3.62)	12.02 ($\pm$ 3.96)	2.85 ($\pm$ 0.19)	3.81 ($\pm$ 0.68)	24.4 ($\pm$ 1.18)	9.37 ($\pm$ 1.78)
DMBA+Mul.	69.2 ($\pm$ 5.23)	9.32 ($\pm$ 1.74)	2.28 ($\pm$ 0.27)	4.86 ($\pm$ 1.38)	33.93 ($\pm$ 2.13)	7.04 ($\pm$ 1.49)
Mul. alone	77.821 ($\pm$ 6.29)	8.48 ($\pm$ 1.85)	1.87 ($\pm$ 0.19)	5.62 ($\pm$ 1.43)	39.51 ($\pm$ 4.219)	6.38 ($\pm$ 1.61)
One – Way ANOVA F	48.467	36.858	45.992	43.533	80.143	63.868
df	3,36	3,36	3,36	3,36	3,36	3,36
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Each value is the mean of three replications. Figures in parenthesis with sign are the standard deviations. * indicate the amount of enzyme required to inhibit fifty percent NBT reaction. ** indicate micromoles of hydrogenperoxide utilized per second. *** indicate micromoles of glutathione utilized per minute. 0.5 % DMBA in liquid paraffin was painted on the left buccal pouches thrice a week for total 14 weeks.

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