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Research Article

Chemopreventive efficacy of berberine in 7,12-dimethylbenz[a]anthracene (DMBA) induced skin carcinogenesis in Swiss albino mice

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ABSTRACT

Chemoprevention is a novel approach to study the anti-initiating and anti-tumor promoting efficacy of medicinal plants and its active principles. Chemopreventive agents exert their anticancer potential through their anti-lipid peroxidative and antioxidant function. The present study investigated the chemopreventive potential of berberine, an isoquinoline alkaloid, in 7,12-dimethylbenz[a]anthracene induced skin carcinogenesis. The chemopreventive potential of berberine was assessed by measuring the tumor incidence and by analyzing the status of lipid peroxidation by products, antioxidants and phase II detoxification enzymes during DMBA-induced skin carcinogenesis. Repeated topical applications of DMBA (2 times per week for 8 weeks) induced skin carcinogenesis in Swiss albino mice. DMBA alone treated animals showed 100% tumor incidence and the tumor was histopathologically confirmed as well differentiated squamous cell carcinoma. The status of lipid peroxidation by products, antioxidants and detoxification enzymes activities were marked by altered in mice treated with DMBA alone. Oral administration of berberine completely prevented the formation of well differentiated squamous cell carcinoma as well as restored the status of biochemical parameters in DMBA treated animals. The present study thus demonstrated the chemopreventive potential of berberine in DMBA-induced skin carcinogenesis. Although the exact mechanism of action of chemopreventive potential of berberine is unclear, its anti-lipid peroxidative, antioxidant and modulating effect on detoxification cascade could play a possible role.

Keywords: Berberine; Skin Cancer; Antioxidant; Lipid peroxidation.

INTRODUCTION

Skin is a shield that protects people from heat or cold, chemicals, UV-radiation and bacteria. Skin cancer is one of the most common of all human cancers and its incidence is increasing rapidly all over the world. Skin cancer contributes approximately 30% of all newly diagnosed cancer in the world and solar ultraviolet radiation is an established cause of approximately 90% of all skin cancers (Armstrong & Kricker, 2001). Almost all skin cancers start as a small, low risk lesions but can grow and become high risk lesions if left untreated. People with fair or sun sensitive skin, with red hair and many freckles are at risk of developing skin carcinogenesis. Worldwide, the highest rates of skin cancer are reported in South Africa and Australia, these are the areas that receive high amount of UV radiation (Gloster & Neal, 2006: Staples, et al., 2006). Skin cancer accounts for around 80% of all new cancers diagnosed each year in Australia (Staples, et al., 2006). Skin cancer is also the most common form of cancer in the United States (Miller & Weinstock, 1994). In India, skin cancer accounts for 1-2% of all cancers (Deo, et al., 2005). The chances of complete cure of skin cancer are excellent if this form of cancer is detected before it has spread to surrounding tissues.

7,12-dimethylbenz(a)anthracene (DMBA), the site and organ specific carcinogen, is commonly employed to induce skin cancer in Swiss albino mice. DMBA could either be used as an initiator or promoter for inducing skin carcinogenesis. Repeated topical applications of DMBA (2 times per week for 8 weeks) induced skin carcinogenesis in Swiss albino mice (Vellaichamy, et al., 2009). DMBA induced skin cancer is therefore used as an ideal tool to test the chemopreventive efficacy of medicinal plants and its constituents.

Free radical is an atom or group of atoms that have one or more unpaired electrons. Free radical mediated oxidative stress can cause structural and functional abnormalities in the cells, making them weak and defenseless (Raha & Robinson, 2000). Over production reactive oxygen species has been implicated in the etiology of several disorders including cardiovascular diseases, diabetes, cancer and Alzheimers diseases (Selek, et al., 2008). Mammalian cells possess elaborate enzymatic (Superoxide dismutases (SOD), Catalase (CAT)

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and Glutathione peroxidase (GPx)) and non-enzymatic (Reduced Glutathione (GSH)) antioxidant defense mechanisms to detoxify radicals. Antioxidants protect cellular components by acting as radical scavenger, hydrogen donors, electron donors, peroxide decomposer, singlet oxygen quencher and metal-chelating agents (Fang, et al., 2002). Superoxide dismutases are enzymes that catalyze the conversion of two superoxides into hydrogen peroxide which is substantially less toxic than superoxide. Catalase is found in peroxisomes in eukaryotic cells. It degrades hydrogen peroxide to water and oxygen, and hence finishes the detoxification reaction started by SOD (Escobar, et al., 1996). Glutathione peroxidase detoxifies hydrogen peroxide using reduced glutathione as co-substrate. Reduced glutathione, the most important intracellular antioxidant protect cells against damage by reactive oxygen species (Circu, et al., 2009). Though skin posses elaborate and sophisticated antioxidant defense mechanism, increased ROS load can promote skin cancer and premature ageing of skin. Altered status of lipid peroxidation and antioxidants was well documented in skin carcinogenesis (Ishii, 2007).

Chemoprevention is a novel approach to study the anti-initiating and anti-tumor promoting efficacy of medicinal plants and its active principles. Chemopreventive agents exert their anticancer potential through their anti-lipid peroxidative and antioxidant function (Naithani, et al., 2008). Profound studies demonstrated the chemopreventive potential of natural products and synthetic entities in DMBA-induced skin carcinogenesis (Renju, et al., 2007: Alias, et al., 2009). Berberine is an isoquinoline alkaloid isolated from the roots and bark of several herbs including *Berberis vulgaris, Coptis chinensis, Hydrastis Canadensis* and *Berberis aquifolium*. It has also been used historically as a dye, due to its yellow color. Berberine exerted diverse pharmacological effects, which include antimicrobial activity, cardioprotective effect, anti-inflammatory effect, hypoglycemic effect and destruction of cancerous cells (Tang, et al., 2009: Schiller, 1995: Ivanovska & Phillipov, 1996). Berberine showed anti-hyperglycemic and anti-hyperlipidemic effects in experimental models (Anis, et al., 2001: Hwang, et al., 2006). Berberine also suppressed the growth of wide variety of tumor cells including breast cancer, leukemia and melanoma (Iizuka, et al., 2000). To the best of our knowledge we found no scientific literature on chemopreventive potential of berberine in DMBA-induced skin carcinogenesis. The present study thus investigated the same effect in DMBA induced skin carcinogenesis.

MATERIALS AND METHODS

Chemicals

The carcinogen, 7,12-dimethylbenz(a)anthracene (DMBA), berberine and other biochemicals such as reduced glutathione, reduced nicotinamide adenine dinucleotide, 1,1',3,3'-tetramethoxypropane, were obtained from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India. All other chemicals and solvents used were of analar grade.

Animals

Male, Swiss Albino mice 4-6 weeks old, weighing 15-20g 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 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.

The local institutional animal ethics committee (Register number 160/1999/ CPCSEA), Annamalai University, Annamalai Nagar, India, approved the experimental design (Proposal No. 620: dated. 25-05-2009). The animals were maintained 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.

EXPERIMENTAL DESIGN

A total number of 24 male Swiss albino mice were divided into four groups of 6 each. Skin carcinogenesis was developed in Swiss albino mice according to the method of Azuine and Bhide (Azuine & 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 experimental study.

The depilated back of group I mice was painted with acetone (0.1 ml/mouse) twice weekly for 8 weeks (vehicle treated control). The depilated back of groups 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 treatment. Group III mice were orally administered with berberine (75 mg/kg body wt) by gastric gavage starting 1 week before the exposure to the carcinogen and continued for 25 weeks (3 times/week on alternate days) thereafter. Group IV animals were orally administered with berberine alone by gastric gavage throughout the experimental period. At the end of experimental period all the animals were sacrificed by cervical dislocation.

Preparation of Tissue Homogenate

Tissue samples from animals were washed with ice cold saline and dried between folds of filter paper, weighed and homogenized using appropriate buffer [appropriate buffer of concerned parameter (TBARS– 0.025 M Tris-HCl buffer, pH 7.5; GSH and GPx – 0.4 M phosphate buffer, pH – 7.0; SOD – 0.025 M sodium pyrophosphate buffer, pH 8.3; CAT – 0.01 M phosphate buffer, pH 7.0)] in an all glass homogenizer with teflon pestle. The homogenate was centrifuged

at 1000g for 5 minutes and the supernatant was then used for the biochemical estimations.

Histopathology

For histopathological studies, tumor tissues and normal skin tissues were fixed in 10 % formalin and were routinely processed and paraffin embedded, 2-3µm sections were cut in a rotary microtome and were stained with hematoxylin and eosin.

Biochemical Estimations

Biochemical estimations were carried out in blood and tissues of control and experimental animals in each group. Lipid peroxidation was estimated as evidenced by the formation of thiobarbituric acid reactive substances (TBARS). TBARS in plasma were assayed by the method of Yagi (Yagi, 1987). TBARS in erythrocyte membrane was estimated by the method of Donnan (Donnan, 1950). Lipid peroxidation (TBARS) in tissues was estimated by the method of Ohkawa *et al* (Ohkawa, et al., 1979). Superoxide dismutase, catalase and glutathione peroxidase activities were determined in erythrocytes and skin tissues by the method of Kakkar *et al* (Kakkar, et al., 1984), Sinha (Sinha, 1972) and Rotruck *et al* (Rotruck, et al., 1973) respectively. The reduced glutathione level in erythrocytes, liver and skin tissues was determined by the method of Beutler and Kelley (Beutler & Kelley, 1963). The activity of GST in liver was assayed by the method of Habig *et al* (Habig, et al., 1974). Glutathione reductase activity in liver was assayed by the method of Carlberg and Mannervik (Carlberg & Mannervik, 1985).

Statistical analysis

Values are expressed as mean ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT). The values were considered statistically significant, if p value was less than 0.05.

RESULTS

The body and liver weight of control and experimental animals in each group are shown in table 1. The body and liver weight were significantly decreased in DMBA treated animals as compared to control animals. Oral administration of berberine three times per week for 25 weeks significantly increased the body and liver weight in DMBA treated animals. Oral administration of berberine alone to mice (Group IV) showed no significant difference in body and liver weight as compared to control animals (Group I).

Table 1: Body and liver weight of control and experimental animals in each group

Groups	Body wt. (g)		Liver wt. (g)
	Initial	Final	
Control (Vehicle Treated)	22.39 ± 0.41^a	28.09 ± 0.42^a	1.19 ± 0.09^a
DMBA alone	22.18 ± 0.38^a	19.36 ± 0.46^b	0.71 ± 0.08^b
DMBA + berberine	22.24 ± 0.35^a	24.28 ± 0.53^c	1.08 ± 0.07^c
Berberine alone	22.35 ± 0.43^a	28.29 ± 0.57^a	1.21 ± 0.08^a

Values are expressed as mean ±SD (n=6).

Values that are not sharing common superscript in the same column differ significantly at p<0.05.

Table 2: Effect of berberine on tumor incidence, tumor volume and tumor burden in DMBA treated mice

Groups	Tumor incidence	Total number of tumors	Tumor volume (mm^3)	Tumor burden (mm^3)
DMBA alone	100% (6/6)	19 / (6)	661.6 ± 59.92^a	2095.1 ± 189.6^a
DMBA + berberine	0	0	0^b	0^b

Values are expressed as mean±SD (n=6). Tumor volume was measured using the formula $v = \frac{4}{3}\pi\left[\frac{D_1}{2}\right]\left[\frac{D_2}{2}\right]\left[\frac{D_3}{2}\right]$ where D_1, D_2 and D_3 are the three diameters (mm) of the tumors.

Tumor burden was calculated by multiplying tumor volume and the number of tumors / animal. Number in parenthesis indicated total number of animals bearing tumors.

Values that are not sharing a common superscript in the same column differ significantly at p<0.05.

The tumor incidence, tumor volume and burden of DMBA alone and DMBA+berberine treated animals are shown in table 2. In DMBA painted mice (group II), 100% tumor formation with mean tumor volume ($661.6mm^3$) and tumor burden ($2095.1\ mm^3$) was observed. The gross appearance of skin tumors in DMBA alone and DMBA+berberine treated mice is depicted in figure 1a and 1b respectively. Oral administration of berberine completely prevented tumor incidence in DMBA painted mice.

The histopathological evaluation in skin tissues of control and experimental animals in each group is shown in figure 2 (a-d). Skin tissues from vehicle

treated control mice (2a) and berberine alone treated mice (2d) exhibited well defined subcutaneous tissue and intact epithelial layer. We observed severe hyperplasia, hyperkeratosis, dysplasia and well-differentiated squamous cell carcinoma in all DMBA alone painted mice (2b). Although, we noticed hyperplasia and dysplasia (2c) in DMBA+berberine treated mice, the squamous cell carcinoma was not developed in any animal.

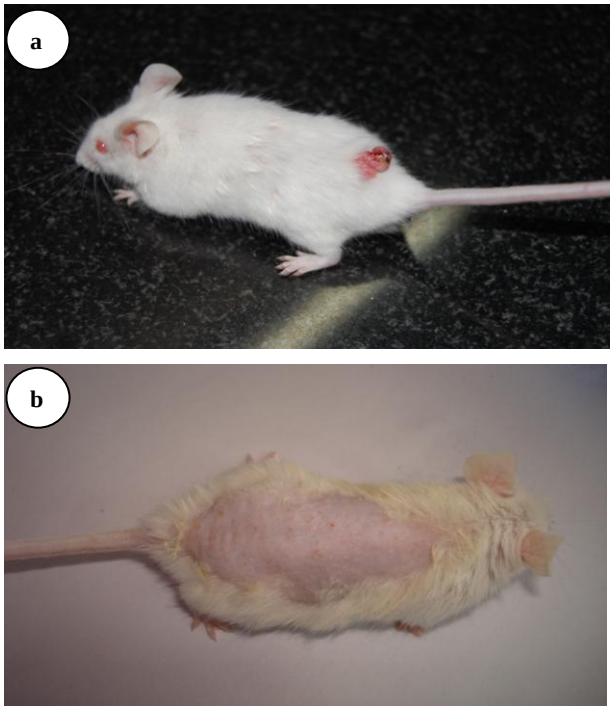


Figure 1: The gross appearance of skin tumors in DMBA alone and DMBA + berberine treated mice. – DMBA alone treated; (b) – DMBA + berberine treated

The levels of TBARS in plasma, erythrocyte membrane and skin tissues of control and experimental animals in each group are shown in figure 3. The levels of TBARS were significantly increased in plasma, erythrocyte membranes and skin tissues of tumor bearing animals (group II) as compared to control animals. Oral administration of berberine at a dose of 75mg/kg bw three times per week for 25 weeks to DMBA painted animals significantly reduced the levels of TBARS. Control mice treated with berberine alone (group IV) showed no significant difference in plasma, erythrocyte membrane and skin tissue TBARS as compared to control mice (group I).

The activities of enzymatic antioxidants (SOD, CAT, GPx) and non-enzymatic antioxidant (GSH) level in erythrocytes and skin tissues of control and experimental animals in each group are shown in figures 4 and 5. The activities of SOD, CAT, GPx and GSH level were significantly decreased in erythrocytes and skin tissues of tumor bearing animals (group II) as compared to control animals. Oral administration of berberine to DMBA painted animals significantly increased the activities of enzymatic antioxidants and non-enzymatic antioxidants levels. Control mice treated with berberine alone (group IV) showed no significant difference in erythrocytes and skin tissue enzymatic antioxidants and non-enzymatic antioxidant status as compared to control mice (group I).

The status of phase II detoxication agents (GST, GR and GSH) in the liver of control and experimental animals in each group are shown in figure 6. The status of GSH, GST, and GR were significantly decreased in the liver of tumor bearing animals (group II) as compared to control animals. Oral administration of berberine to DMBA painted animals significantly improved the status of phase II detoxification agents. Control mice treated with berberine alone (group IV) showed no significant difference in the activities of phase II detoxification enzymes and reduced glutathione level as compared to control mice (group I).

DISCUSSION

Skin carcinogenesis, the most common of all cancers, has been increasing in recent years all over the world. Skin is the most common site of malignancy and represents 55% of all human cancers with tremendous impact on health and morbidity. DMBA-induced mouse skin carcinogenesis is commonly used to test chemopreventive efficacy of medicinal plants and their constituents. In recent years, profound interest has been focused in the identification of non toxic natural products that are capable of reducing the tumorigenicity of the environmental carcinogen. In the present study, we assessed the chemopreventive potential of berberine by monitoring the percentage of tumor bearing animals, tumor volume and burden as well as by analysing the status of detoxification enzymes, lipid peroxidation and antioxidants in DMBA painted animals.

In the present study, DMBA alone treated animals showed 100% tumor incidence and the tumor was histopathologically confirmed as well differentiated squamous cell carcinoma. The tumor cells have pleiomorphic hyperchromatic nuclei with epithelial pearl formation. Oral administration of berberine completely prevented the formation of well differentiated squamous cell carcinoma in DMBA treated animals. We have however observed precancerous lesions such as hyperplasia and dysplasia in berberine treated DMBA painted animals. Our results thus suggest that berberine suppressed abnormal skin cell proliferation occurring during DMBA-induced skin carcinogenesis.

Liver, the major metabolic organ, performs an important role in the detoxification process and thus analyzing the status of detoxification agents help to identify the chemopreventive efficacy of the test compound. Skin cancer chemopreventive agents protected tumor formation through activating multiple biochemical mechanisms including phase-II detoxification enzyme induction and antioxidant defense mechanism (Keeney, et al., 2009: Meijerman, et al., 2008). Phase-II detoxification enzyme plays a major role in increasing the polarity and assisting the excretion of xenobiotic agents.

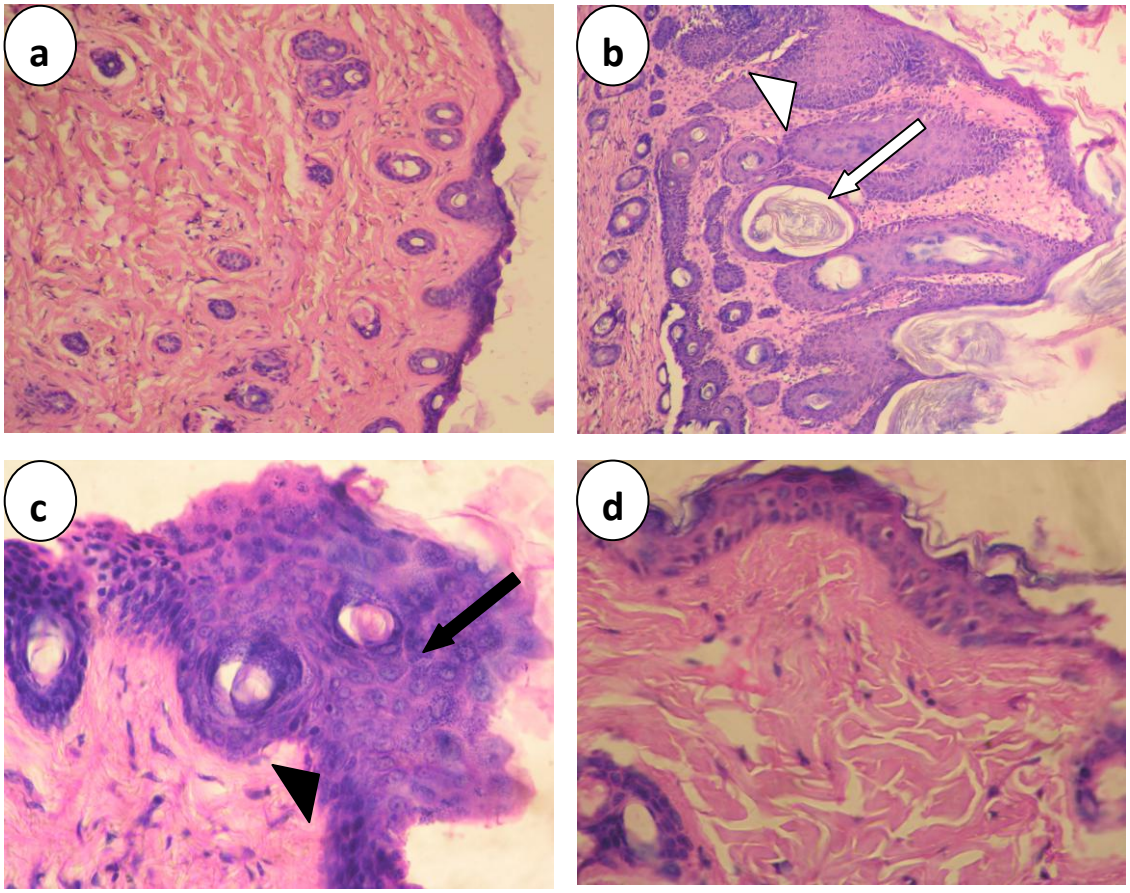
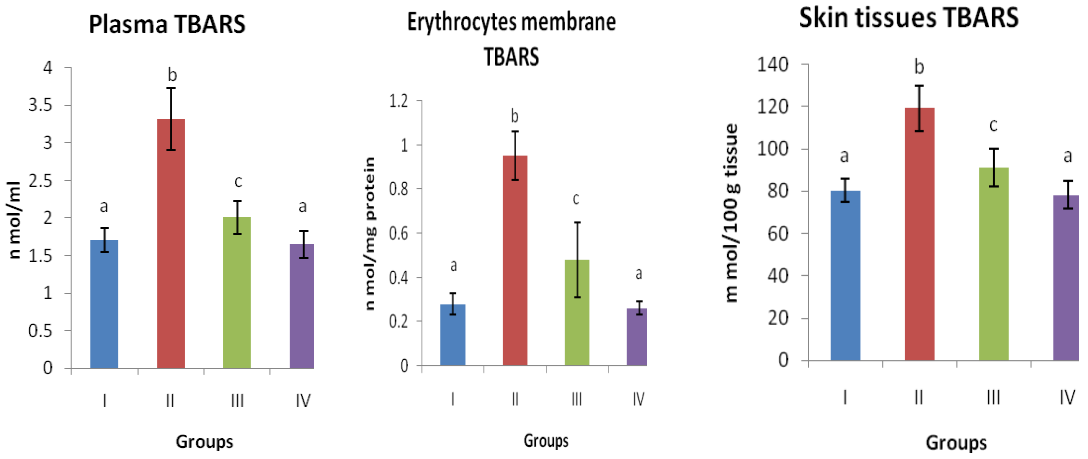
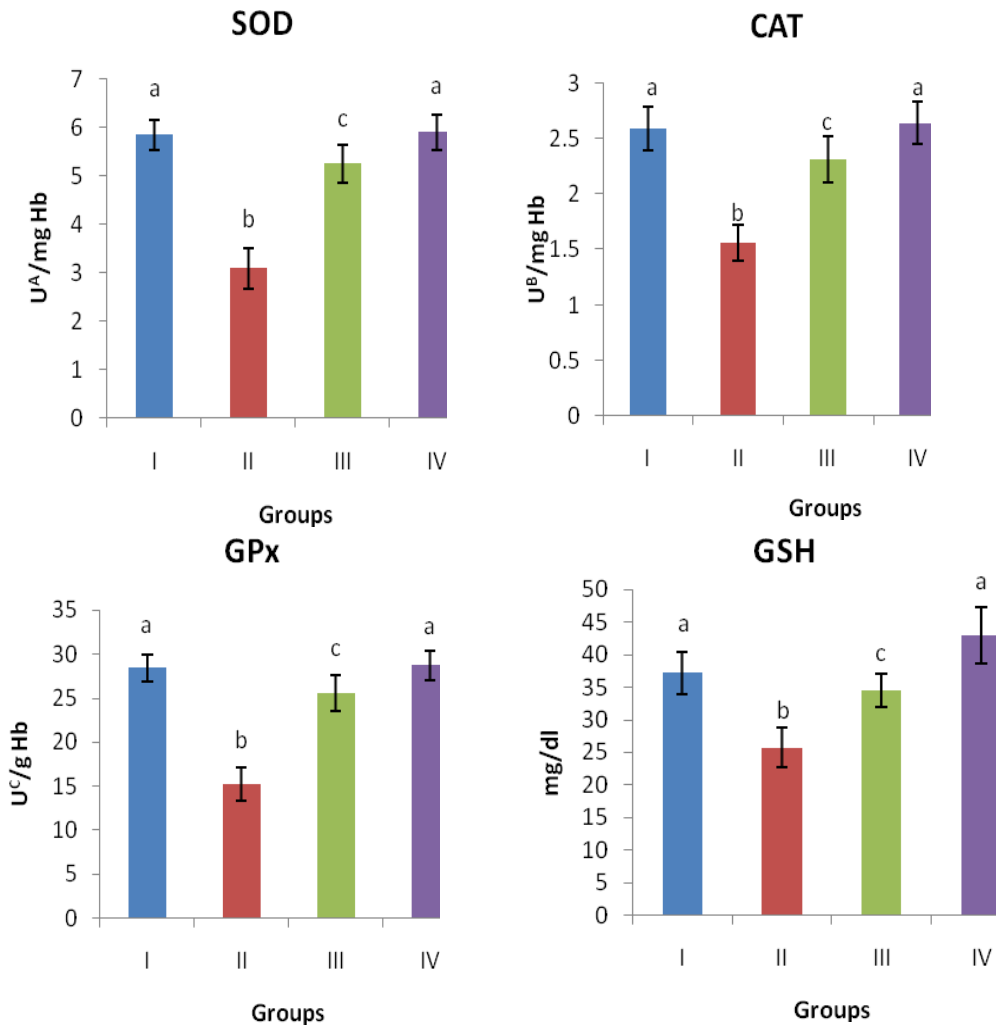


Figure 2: The histological evaluation in skin tissues of control and experimental animals in each group. (a) & (d) - Microphotographs of skin tissues from control and berberine alone treated animals respectively, showing well-defined subcutaneous tissues and intact epithelial layer; (b) - Microphotograph of skin tissues from DMBA alone painted animals showing well-differentiated squamous cell carcinoma with dysplastic epithelium (arrow head) and keratin pearls (arrow); (c) - Microphotograph of skin tissues from DMBA + berberine treated animals showing hyperplastic (block arrow) and dysplastic (block arrow head) epithelium.



I-Control (Vehicle Treated), II- DMBA alone, III- DMBA + berberine, IV- Berberine alone.

Figure 3: Effect of berberine on TBARS status in plasma, erythrocyte membrane and skin tissues of control and experimental animals in each group. Values are expressed as mean ± SD (n=6). Values that are not sharing a common superscript differ significantly at p<0.05.

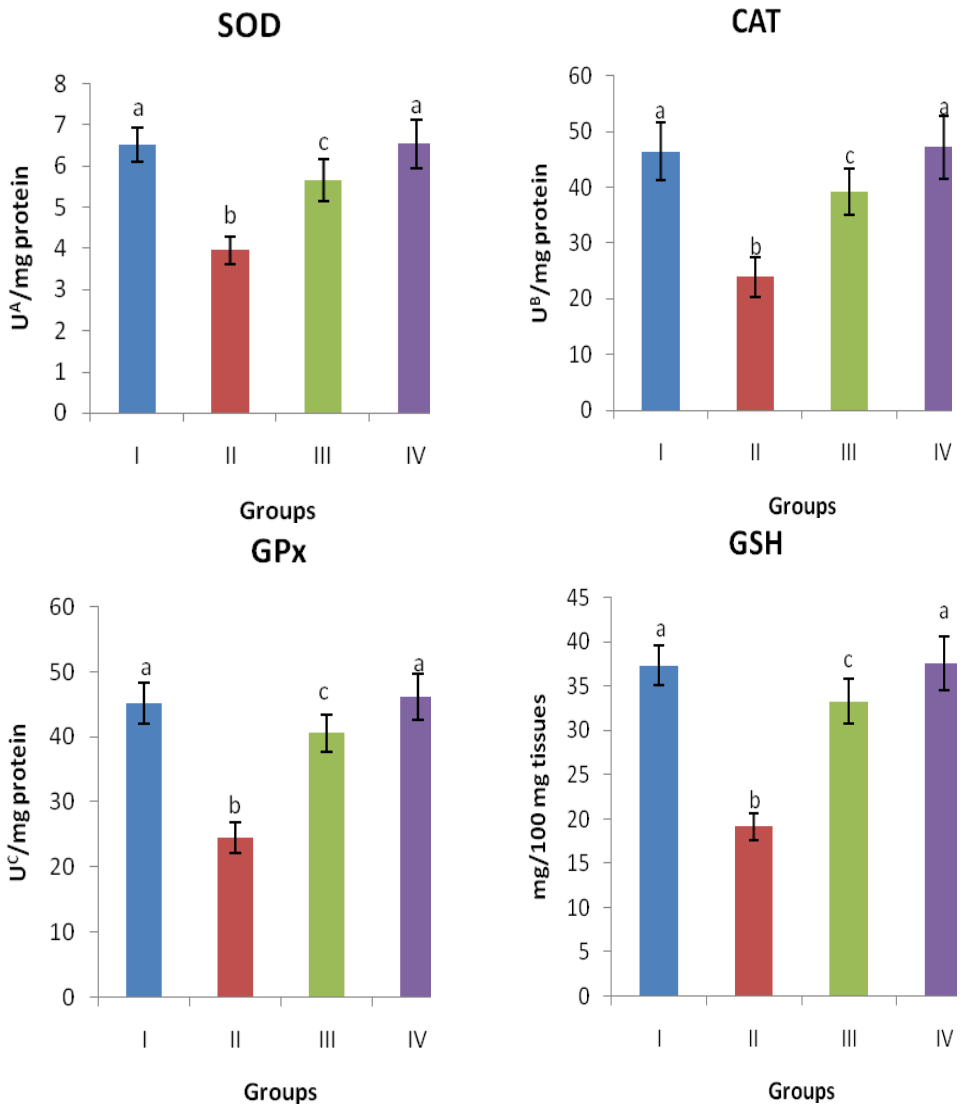


I-Control (Vehicle Treated), II- DMBA alone, III- DMBA + berberine, IV- Berberine alone

Figure 4: Effect of berberine on the status of enzymatic and non-enzymatic antioxidants in erythrocytes of control and experimental animals in each group. Values are expressed as mean ±SD (n=6). Values that are not sharing a common superscript differ significantly at p<0.05. A- The amount of enzyme required to inhibit 50% NBT reduction. B- Micromoles of H2O2 utilized/ Sec. C- Micromoles of glutathione utilized/ min.

They detoxify carcinogens either by destroying their reactive centres or by conjugating them with endogenous ligand, facilitating their excretion. A large number of experimental studies reported that chemopreventive agents convert DNA damaging entities through the induction of detoxication agents such as glutathione-s-transferase (Choi, et al., 2003). The activities of glutathione-s-transferase and glutathione reductase and reduced glutathione content were decreased in DMBA painted mice as compared to control mice. Oral administration of berberine increased the activities of phase-II detoxification agents in DMBA painted mice, which indicates that berberine stimulated the activities of phase-II detoxification enzymes to facilitate the excretion of the active metabolite of DMBA, dihydrodiol epoxide.

Over production of reactive oxygen species in the cell cause DNA damage and thereby contributing to carcinogenesis. Enzymatic and non-enzymatic antioxidants form the first line of defense against ROS mediated lipid peroxidation. Measurement of plasma TBARS helps to assess the extent of tissue damage in pathological conditions (Dasgupta, et al., 2003). Enhanced plasma TBARS in DMBA alone painted animals could be due to over production and diffusion from the tumor tissues and others damaged host tissues with subsequent leakage into plasma. Repeated topical applications of DMBA on the skin could account for increased TBARS in the skin tumor tissues. Decreased activities of enzymatic antioxidants and decline in non-enzymatic antioxidant level were well documented in skin cancer (Das & Saha, 2009). Decreased level of reduced glutathione in plasma is probably due to its utilization by tumor tissue or to combat the deleterious effects of lipid peroxidation. Lowered activities of enzymatic antioxidants in tumor tissue are probably due to the exhaustion of these enzymes to scavenge the excessively generated lipid peroxidation by-products during DMBA-induced skin carcinogenesis.



I-Control (Vehicle Treated), II- DMBA alone, III- DMBA + berberine, IV- Berberine alone.

Figure 5: Effect of berberine on the status of enzymatic and non-enzymatic antioxidants in skin tissue of control and experimental animals in each group. Values are expressed as mean ±SD (n=6). Values that are not sharing a common superscript differ significantly at p<0.05. A- The amount of enzyme required to inhibit 50% NBT reduction. B- Micromoles of H_2O_2 utilized/ Sec. C- Micromoles of glutathione utilized/ min.

Oral administration of berberine at a dose of 75mg/kg bw to DMBA painted mice significantly improved the status of lipid peroxidation and antioxidants. Our results suggests that berberine has a potent anti-lipid peroxidative and antioxidant function during DMBA-induced skin carcinogenesis. The present study thus demonstrated the chemopreventive potential of berberine in DMBA-induced skin carcinogenesis. Although the exact mechanism of action of chemopreventive potential of berberine is unclear, its anti-lipid-peroxidative, antioxidant and modulating effect on detoxification cascade could play a possible role.

REFERENCE

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

Anis, K.V., Rajeshkumar, N.V., Kuttan, R. Inhibition of chemical carcinogenesis by berberine in rats and mice. J Pharm Pharmacol, 53, 2001 pp. 763–68.

Armstrong, B.K., Kricker, A. The epidemiology of UV induced skin cancer. J Photochem Photobiol B, 63, 2001 pp. 8-18.

Azuine, M.A., Bhide, S.V. Chemopreventive effect of turmeric against stomach and skin tumors induced by chemical carcinogens in Swiss mice. Nutr Cancer, 17, 1992 pp. 77-83.

Beutler, E., Kelley, B.M. The effect of sodium nitrate on red cell GSH. Experientia, 19, 1963 pp. 96-97.

Carlberg, I., Mannervik, B. Glutathione reductase. Methods enzymol, 113, 1985 pp. 484-90.

Choi, S.C., Yun, K.J., Kim, T.H., Kim, H.J., Park, S.G., Oh, G.J., Chae, S.C., Nah, Y.H., Kim, J.J., Chung, H.T. Prognostic potential of glutathione S-transferase M1 and T1 null genotypes for gastric cancer progression. Cancer Lett, 195, 2003 pp. 169-75.

Circu, M.L., Moyer, M.P., Harrison, L., Aw, T.Y. Contribution of glutathione status to oxidant induced mitochondrial DNA damage in colonic epithelial cells. Free Radic Biol Med, 47, 2009 pp. 1190-98.

Das, I., Saha, T. Effect of garlic on lipid peroxidation and antioxidation enzymes in DMBA-induced skin carcinoma. Nutrition, 25, 2009 pp. 459-71.

Dasgupta, T., Rao, A.R., Yadava, P.K. 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, 23, 2003 pp. 1427- 46.

Deo, S.V., Hazarika, S., Shukla, N.K., Kumar, S., Kar, M., Samaiya, A. Surgical management of skin cancers: experience from a regional cancer centre in North India. Indian J Cancer, 42, 2005 pp. 145-50.

Donnan, S.K. The thiobarbituric acid test applied to tissues from rats treated in various ways. J Biol Chem, 182, 1950 pp. 415-19.

Escobar, J.A., Rubio, M.A., Lissi, E.A. SOD and catalase inactivation by singlet oxygen and peroxyl radicals. Free Radic Biol Med, 20, 1996 pp. 285-90.

Fang, Y.Z., Yang, S., Wu, G. Free radicals, antioxidants, and nutrition. Nutrition, 18, 2002 pp. 872-79.

Gloster, H.M., Neal, K. Skin cancer in skin of Color. J Am Acad Dermatol, 55, 2006 pp.741-60.

Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem, 249, 1974 pp. 7130-39.

Hwang, J.M., Kuo, H.C., Tseng, T.H., Liu, J.Y., Chu, C.Y. Berberine induces apoptosis through a mitochondria/caspases pathway in human hepatoma cells. Arch Toxicol, 80, 2006 pp. 62–73.

Iizuka, N., Miyamoto, K., Okita, K., Tangoku, A., Hayashi, H., Yosino, S., Abe, T., Morioka, T., Hazama, S., Oka, M. Inhibitory effect of Coptidis Rhizoma and berberine on the proliferation of human esophageal cancer cell lines. Cancer Lett, 148, 2000 pp. 19–25.

Ishii, N. Role of oxidative stress from mitochondria on aging and cancer. Cornea, 26, 2007 pp. 3–9.

Ivanovska, N., Phillipov, S. Study on the anti-inflammatory action of Berberis vulgaris root extract, alkaloid fractions and pure alkaloids. Int J Immunopharmacol, 18, 1996 pp. 553–61.

Kakkar, P., Das, B., Viswanathan, P.N. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys, 21, 1984 pp. 130-32.

Keeney, S., McKenna, H., Fleming, P., McIlfatrick, S. Attitudes, knowledge and behaviours with regard to skin cancer: a literature review. Eur J Oncol Nurs, 13, 2009 pp. 29-35.

Meijerman, I., Beijnen, J.H., Schellens, J.H. Combined action and regulation of phase II enzymes and multidrug resistance proteins in multidrug resistance in cancer. Cancer Treat Rev, 34, 2008 pp. 505-20.

Miller, D.L., Weinstock, M.A. Nonmelanoma skin cancer in the United States: incidence. J Am Acad Dermatol, 30, 1994 pp. 774-78.

Naithani, R., Huma, L., Moriarty, R.M., McCormick, D.L., Mehta, R.G. Comprehensive review of cancer chemopreventive agents evaluated in experimental chemoprevention models and clinical trials. Curr Med Chem, 15, 2008 pp. 1044–71.

Ohkawa, H., Ohishi, N., Yagi, K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95, 1979 pp. 351-58.

Raha, S., Robinson, B.H. Mitochondria, oxygen free radicals, disease and ageing. Trends Biochem Sci, 25, 2000 pp. 502-08.

Renju, G.L., Manoharan, S., Balakrishnan, S., Senthil, N. Chemopreventive and antilipid peroxidative potential of Cleodendron inereme (L) Gaertn in 7,12-dimethylbenz(a)anthracene induced skin carcinogenesis in Swiss albino mice. Pak J Biol Sci, 10, 2007 pp. 1465-70.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., Hockstra, W.G. Selenium: biochemical role as a component of glutathione peroxidase. Science, 179, 1973 pp. 588-90.

Schiller, L.R. Review article: anti-diarrhoeal pharmacology and therapeutics, Aliment Pharmacol Ther, 9, 1995 pp. 87–106.

Selek, S., Herken, H., Bulut, M., Ceylan, M.F., Celik, H., Savas, H.A., Erel, O. Oxidative imbalance in obsessive compulsive disorder patients: a total evaluation of oxidant–antioxidant status. Prog Neuro-psychopharmacol Biol Psychiatry, 32, 2008 pp. 487-91.

Sinha, A.K. Colorimetric assay of catalase. Anal Biochem, 47, 1972 pp. 389-94.

Staples, M.P., Elwood, M., Burton, R.C., Williams, J.L., Marks, R., Giles, G.G. Non-melanoma skin cancer in Australia: the 2002 national survey and trends since 1985. Med J Aust, 184, 2006 pp. 6–10.

Tang, J., Feng, Y., Tsao, S., Wang, N., Curtain, R., Wang, Y. Berberine and Coptidis rhizoma as novel antineoplastic agents: a review of traditional use and

biomedical investigations. J Ethnopharmacol, 126, 2009 pp. 5-17.

Vellaichamy, L., Balakrishnan, S., Panjamurthy, K., Manoharan, S., Linsa, M.A. Chemopreventive potential of piperine in 7,12-dimethylbenz[a]anthracene-induced skin carcinogenesis in Swiss albino mice. Environ Toxicol Pharmacol, 28, 2009 pp. 11-18.

Yagi, K. Lipid peroxides and human diseases. Chem Phys Lipids, 45, 1987 pp. 337-51.