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Research Article

PROTECTIVE EFFECT OF HYDROALCOHOLIC EXTRACT OF BOERHAAVIA DIFFUSA AGAINST DMBA INDUCED BREAST CANCER IN MICE

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A B S T R A C T

Breast cancer is the leading cause of morbidity and mortality worldwide and the most commonly diagnosed cancer among women. Reactive oxygen species (ROS) directly or indirectly involves in multistage process of carcinogenesis. Anticancer activity of Hydroalcoholic extract of Boerhaavia diffusa on 7, 12 dimethylbenz(*a*)anthracene (DMBA) induced breast cancer was investigated in female albino mice. Thirty mice were divided into five groups: Normal control, DMBA, DMBA+standard and DMBA with low & high dose of HAEBD. G_2 served as the tumor control,was treated with a single dose of 7.5 mg of DMBA dissolved in sun flower oil (0.5 ml) given subcutaneously once a week for four consecutive weeks whereas G3 & G4 received 200mg/kg & 400mg/kg of hydroalcoholic extract of *Boerhaavia diffusa* orally daily for a period of 45 days. Derived parameters such as Body weight, Tumour Volume, Percentage reduction of tumour volume were determined. Tumour volume was measured weekly. After the experimental period of 45 days, oxidative stress parameters were assessed in homogenates of breast of both control and experimental groups. Changes in the levels of lipid peroxidation and antioxidants system was evaluated in addition to tumour development. A significant increase in lipid peroxidation levels were observed in the tested samples of cancer induced rats while the activities of enzymic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) were decreased in cancer-bearing animals when compared to control animals. Decrease in body weight & increase in tumour volume was observed in the DMBA group whereas body weight reduces & significant reduction in tumour volume were observed after treatment with HAEBD groups. Oral administration of HAEBD remarkably reduced the lipid peroxidation activity and increased the antioxidants level in drug treated animals and decreased the tumour volume significantly ($P < 0.05$). Histopathological examination revealed the formation of tumor and angiogenesis in DMBA-induced rats and these abnormal changes were ameliorated by treatment with 400mg /kg of HAEBD. This result suggests that HAEBD shows anticancer activity and play a protective role against DMBA induced breast cancer.

INTRODUCTION

Breast cancer is an uncontrolled growth of breast cells originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules as a result of mutations in the genes responsible for regulating the growth of cells and keeping them healthy. One of the most serious problems in oncology is breast cancer which is the leading cause of morbidity and mortality worldwide and the most commonly diagnosed cancer among women.[1] Reactive oxygen species (ROS) are involved in a variety of important pathophysiological conditions including mutagenesis and carcinogenesis.[2,3]

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Oxidative stress has the potential to cause cellular DNA damage, lipid peroxidation, and membrane disruption.[4] Human body is equipped with various antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione (GSH), ascorbic acid (vitamin C), a-tocopherol (vitamin E), and so on, which can counteract the deleterious action of ROS and protect from cellular and molecular damage.[5]

DMBA commonly employed as a chemical model for inducing experimental carcinogenesis.Dimethyl Benz (a) anthracene (DMBA), a member of the polycyclic aromatic hydrocarbons a PAH, and also a procarcinogen needs metabolic activation to become an ultimate carcinogen.[6] Being a well-known cytotoxic, carcinogenic, mutagenic, and immunosuppressive agent, it is often used as a tumor initiator in animal models.[7,8] Being an indirect carcinogen, DMBA requires metabolic

activation by cytochrome P450 enzymes to acquire carcinogenicity results in the formation of oxidative free radicals.[9] Breast cancer and cancer related diseases have been treated using surgery, chemotherapy, and radiation therapy, or a combination of these. Despite these therapeutic options, cancer remains associated with high mortality. Bioactive compounds from plant origin have the potential to subside the biochemical imbalances induced by various toxins associated with free radicals. They provide protection without causing any side effects and therefore, development of drugs from plant products is desired. For that account natural antioxidants from plant sources have been viewed as promising therapeutic drugs.[10] Boerhaavia diffusa one of the famous medicinal plant belonging to the family Nyctaginacea known to have medicinal properties. It has a long history of uses by indigenous and tribal people and in Ayurvedic or natural herbal medicines.[11] Phytochemical investigation reveals that the plant contains a large number of compounds such as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoprotein's.[12] The plant widely used in numerous disorder like nephritic syndrome,[13] asthma, jaundice, drosical swelling, worm infections, liver disorder, heart disorder, urinary tract infections, cough, cold and inflammation.[14,15] It's also shown to have laxative, diuretic, antiurethritis, anticonvulsant, antifibrinolytic, antinematodal and antibacterial properties.[16-20] Therefore the object of present study is to investigate the anticancer activity of Hydroalcoholic extract of *Boerhaavia diffusa* against DMBA induced breast cancer in experimental rats.

MATERIALS AND METHODS

Collection and authentification of Plant

Whole plant of Boerhaavia diffusa was collected from local traders, Tamilnadu. The plant material was taxonomically identified and authenticated by Dr.stephan, American college, madurai and the voucher specimens (KMCP/GN/BD/0290) were retained in the Institute for future reference. The whole plant of Boerhaavia diffusa linn were dried in a shade, milled into coarse powder by a mechanical grinder and stored in closed vessel for further use.

Plant crude extract

About 500gm of air dried fine powder of Boerhaavia diffusa were soaked in the extractor and macerated for 30 hrs with petroleum ether. There it is reflexed successfully with chloroform, after that it is extracted with alcohol and water by continuous hot percolation method using soxhlet apparatus for 40hrs separately. Hydro alcoholic extracted was filtered and concentrated in vacuum using rotary flask evaporator under reduced pressure. Then the extract of *Boerhaavia diffusa* concentrated to brownish residue stored in air tight container

Technique for inducing tumour

Currently available mouse models for human breast cancer can be categorized into three main groups: (a) xenograft models (b) chemically induced, virally induced or ionizing radiationinduced models; and (c) genetically engineered mice (GEM) such as transgenics and knockouts [21] DMBA induced Breast cancer model.

Animals

Female mice weighing (25g) were procured from animal experimental laboratory and used throughout the study. The animals were housed in well ventilated large spacious polypropylene cages and had 12±1 h light and dark cycle throughout the experimental period. The animals received a balanced diet of commercially available pellet rat feed and water *ad libitum*. As per the standard practice the mice were quarantined for 15 days before the commencement of the experiment.[22,23] The Guidelines for Breeding and Experiments on Animals, 1998 defined by the Ministry of Social Justice and Empowerment of India were followed and the protocol was approved by the Institutional Animal Ethics Committee (IAEC/KMCP/123/2014-2015).

Experimental design

30 female albino mice weighing 25 gms were segregated into 5 groups, each group comprising of 6 animals each. Group 1 served as the normal control. Group 2 served as the tumor control, was treated with a single dose of 7.5 mg of DMBA dissolved in sun flower oil (0.5 ml) given subcutaneously.[24,25] Group 1 and 2 receives normal diet and Water. Group 3 served as the positive control, was treated with injection Vinblastine at 0.5 mg/kg body weight, Intra peritoneally.[26] Group 4 served as the treatment control, which was treated with Hydro alcoholic extract of Boerhaavia *diffusa* (200 mg/kg body weight) orally. Group 5 served as treatment control which was treated with Hydro alcoholic extract of Boerhaavia *diffusa* (400 mg/kg body weight) orally.[27]

The experimental mice were regularly monitored for food and water consumption, the apparent signs of toxicity, weight loss, or mortality. Forty-five days after DMBA administration, all the experimental animals were sacrificed. At the end of the experimental period, all the mice were alive, starved overnight anesthetized with diethyl ether and sacrificed by euthanasia method. The breast tumor was surgically dissected out, tumor volumes (mm in diameter) of both cancer controls, as well as the experimental groups were measured, and total body weight (g) also recorded.

Breast were dissected out, cut into small pieces with a heavy-duty blade and washed with ice-cold 0.9% NaCl solution.

Group 1- Normal control	Received vehicle
Group 2 - Tumor control	Received subcutaneous injection of DMBA at 7.5mg/kg b.w. once a week for four consecutive weeks [33,34]
Group 3 - Standard	Received subcutaneous injection of DMBA at 7.5mg/kg b.w. once a week for four consecutive weeks and injection vinblastin at 0.5 mg/kg body weight intra peritoneally once a week for 45 days[35]
Group 4 Treatment control	Received subcutaneous injection of DMBA at 7.5mg/kg b.w. once a week for four consecutive weeks and 200mg/kg b.w. hydroalcoholic extract of *Boerhaaviadiffusa* orally daily for 45 days.
Group 5 Treatment control	Received subcutaneous injection of DMBA at 7.5mg/kg b.w. once a week for four consecutive weeks and 400mg/kg b.w. hydroalcoholic extract of *Boerhaaviadiffusa* orally daily for 45 days.

Chemicals

DMBA were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All the other Chemicals used in this study were of analytical grade available commercially.

One hundred milligrams of the tissues were homogenized in 0.1M Tris–HCl buffer (pH 7.4). The homogenate was used for estimation of biochemical parameters.[28] such as SOD radical scavenging activity, Inhibition of LPx formation, GPx activity,

CAT activity. Derived parameters such as Body weight, Tumour Volume, Percentage reduction of tumour volume[29] were also determined. Tumour volume was measured weekly. Tumor volume was calculated using the formula (cc) = 4/3 πr^3. On 45th day, mice were sacrificed and tumors were removed from the animals and weighed. The tumor incidence and its percentage reduction were also recorded at the termination of the experiment.[30, 31]

Evaluation of Biochemical Parameters

The following biochemical measurements were carried out in the rat breast tissues. Effects of HABD on activities of superoxide dismutase, catalase, glutathione peroxidase and lipid peroxide were estimated in the breasts of treated, as well as untreated, rats.

Assay of Super oxide dismutase radical scavenging activity

SOD activity was assessed by the Nitroblue tetrazolium(NBT) reduction method. Approximately, a known concentration of tissue supernatant was added to a reaction mixture containing 0.1m MEDTA, 0.12mM riboflavin and 0.6M phosphate buffer(pH 7.8) in a final volume of 3 ml. The optical density was measured at 560 nm.[32, 33]

Assay of of lipid Peroxidase activity:

Induction by Fe3+/ascorbate system: the reaction mixture containing rat Breast homogenate(0.1 ml, 50%, w/v) in Tris–HCl (30 mM), ferrous ammonium sulfate (0.16 mM), ascorbic acid (0.06 mM) and the reaction mixture was incubated for 1 h at 37 ◦C, and the resulting thiobarbituric reacting substances (TBARS) were measured.[34,35] Briefly, a 0.4 ml aliquot of the reaction mixture was treated with sodium dodecyl sulfate(0.2 ml, 8%), thiobarbituric acid (1.5 ml, 0.8%) and acetic acid (1.5 ml, pH 3.5), made up to a total volume of 4ml by adding distilled water, and then kept in a waterbath at 95 ◦C for 1 h. After cooling, 1ml of distilled water and 5ml of *n*-butanol/pyridine (15:1, v/v) were added. The organic layer was separated after shaking and centrifugation. LPx activity was measured in terms of thiobarbituric acid formation and the color intensity measured spectrophotometrically at 530 nm.[36]

Assay of Glutathione peroxidase activity

The activity of glutathione peroxidase (GPx) was assayed by the method of Rotruck *et al*.[37] The reaction mixture containing 0.2 ml of EDTA (0.8 mM, pH 7.0), 0.4 ml of phosphate buffer (10 mM), and 0.2 ml of tissue homogenate was incubated with 0.1 M of H2O2 and 0.2 ml of glutathione for 10 min. Oxidation of glutathione by the enzyme was measured spectrophotometrically at 420 nm. The activity of GPx was expressed as lmol glutathione oxidized/min/mg protein.

Assay of Catalase activity

Breast catalase activities were determined spectrophotometrically. The reaction mixture (2 ml) contained 1.95 ml of 10mM H2O2 in 60 Mm phosphate buffer (pH 7.0). The reaction was initiated by adding 0.5 ml supernatant to it, and the absorbance was taken for 3 min at 240 nm. Phosphate buffer (60 mM, pH 7.0) was used as a reference. The extinction coefficient of 0.04mM−1 cm−1 was used to determine the specific activity of CAT. The data is expressed as mol H2O2 consumed/ (min (mg protein)).[38]

Histopathological Examination

Mammary tissues were fixed in 10% buffered formalin, embedded in paraffin using a conventional automated system. Tissue fragments were fixed in formalin and 5µm section was obtained from the paraffin block and stained with haematoxylin and eosin for histologic examination. Breast tissue pathology and histologic type were evaluated by application of the same pathologic criteria used for the classification of human tumours. Serial paraffin sections of each tissue image were captured by light microscopy.

Statistical analysis

Statistical comparisons between control and treatment mean values of two parameters were analyzed using the Student's *t*-test. Multiple comparisons were done using ANOVA. The differences were statistically significant at $P < 0.01$; $P < 0.05$.

RESULTS

Anti-tumor activity

Table 1 illustrates the body weight, protection percentage& tumour volume in DMBA induced Breast cancer in mice. The body weight was significantly decreased in tumor-induced animals versus control (P\0.01). Tumor promotional stage is a reversible stage in the multistage carcinogenesis; therefore, it is the most suitable stage for the anti-carcinogenic agent to prevent, reverse, or slow down the process of carcinogenesis. Animals in the breast cancer control Group 2 attaineda promotional stage tumor after 45 days. At the end of the experiment in non-treated mice, DMBA-induced breast tumors increased to the maximum in terms of tumor incidence (100%), tumor multiplicity, compared to the normal control rats. A Significant reduction in Tumor volume was observed in **G_3** achieved (37.85%) of tumor reduction after 45 days treatment wheras **G_4**HAEBD (200 mg/kg, orally) treated animals achieved a significant decrease (24%) in the mammary tumor size with change in the total body weight of the animals **G2**. However, HAEBD at a dose of 400mg/kg treated group achieved 31.70% breast tumor reduction after 45 days as shown in **Table No 1**.

Table 1 Effect of HAEBD on Anti-Tumour activity in experimental Breast tissue cancer in Mice

Group	Body Weight(mg)	Tumour Volume(mm)	Reduction of Tumour Percentage (%)
G1	30.5±1.40	-	-
G2	48.0±1.65^{**a}	46.78 ± 1.88^{**a}	-
G3	36.0±1.44^{**b}	9.07 ±1.54^{**b}	37.84^{**b}
G4	40.5±1.50^{**b}	35.54±0.86^{**b}	24.02^{**b}
G5	38.0±1.48^{**b}	31.95±0.94^{**b}	31.70^{**b}

Note: G1 – Normal Control, G2 – Toxic Control, G3 – Positive control, G4 – Treatment (HAEBD 200mg/kg), G5 – Treatment (HAEBD 400mg/kg)

Values are expressed as Mean ± SEM.Values are found out by using one way ANOVA followed by NewmannKeul's multiple range tests.

*a − values are significantly different from Normal control at p< 0.001.

*b – values are significantly different from Toxic control (G2) at p< 0.001.

Antioxidant activity

Free radical scavengers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and lipid

peroxide (LPx) levels were recorded (**Tables 2**). Results in **Table 2** showed a significant reduction in the activities of antioxidant enzymes like SOD, CAT, GPX in DMBA induced Breast cancer in mice whereas the values were significantly elevated after treatment with HAEBD (200mg/kg & 400mg/kg). Mice treated with standard drug vinblastine **G_3** showed similar changes. The GPx level was equally increased in vinblastin and both doses of Hydroalcoholic extract of *Boerhaavia diffusa* therapy treated groups versus the cancer control rats. The level of LPx rises in DMBA induced breast cancer animals which is much influenced by the chemical carcinogen in the control animals, whereas significant reduction was observed in the mice treated with vinblastin and both doses of Hydroalcoholic extract of *Boerhaavia diffusa.*

Table 2 Effect of HAEBD on enzymatic antioxidants in the breast tissue of control and experimental animals

GROUP	SOD (units/mg protein)	CAT (µmol H_2O_2 consumed/ [min(mg protein)])	GPx (µg GSH utilized/[min(mg protein)])	LPO(n mol MDA found/[min/(mg protein)])
G1	3.75±0.08	45.85±0.47	3.83±0.10	0.75±0.02
G2	1.53±0.07^{**a}	14.51±1.08^{**a}	2.09±0.03^{**a}	2.16±0.07^{**a}
G3	3.20±0.06^{**b}	37.91±0.78^{**b}	3.55±0.07^{**b}	1.11±0.04^{**b}
G4	2.80±0.07^{**b}	27.71±0.65^{**b}	3.06±0.04^{**b}	1.46±0.04^{**b}
G5	3.14±0.06^{**b}	34.05±0.72^{**b}	3.22±0.02^{**b}	1.35±0.02^{**b}

Note: G1 – Normal Control, G2 – Toxic Control, G3 – Positive control, G4 – Treatment (HAEBD 200mg/kg), G5 – Treatment (HAEBD 400mg/kg)

Values are expressed as Mean ± SEM.Values are found out by using one way ANOVA followed by NewmannKeul's multiple range tests.

*a – values are significantly different from Normal control at p< 0.001.

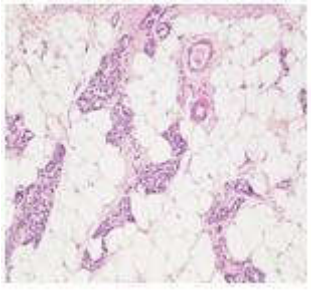
desmoplastic reaction. *In vivo*, the treated groups with vinblastin and both doses of Hydroalcoholic extract of *Boerhaavia diffusa* showed tubules formation & reduce intra-tumor vascularization (G2,G3 & G4). Treatment with hydroalcoholic extract of *Boerhaavia diffusa* showed reduced proliferation and replacement of normal ductular and alveolar structure of mammary tissue.

DISCUSSION

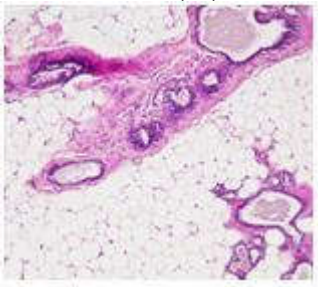
Breast cancer is a complex and multifactorial disease. Since the rat mammary gland shows a high susceptibility to developing neoplasms which closely mimic human breast cancer, they have been selected in comparison to other animal models[39] Treatment of female mice with DMBA induces the development of hyperplastic lesions like alveolar/ductal hyperplasia, benign tumors such as adenoma, malignant neoplasms like adenocarcinomas similar to those seen in humans.[40-42] However, there was a severe body weight loss observed in the toxic control group, versus the control rats. Tumour volume was also increased in toxic group. In the present investigation, vinblastin and both doses of Hydroalcoholic extract of *Boerhaavia diffusa* treatment reduced the breast tumor by an average of 37, 24 and 32 %. As a result, the body weight had also slightly increased, the tumor volume decreased, and the percentage of tumor inhibition was statistically significant ($P < 0.05$).

Toxic manifestation of DMBA is associated with its oxidative metabolism leading to the formation of reactive metabolites (epoxides and quinines) capable of generating free radicals. Metabolism of DMBA by the mixed function oxidases system

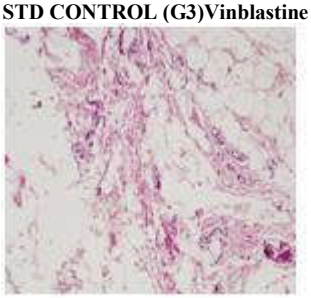
NORMAL CONTROL(G1)



DMBA INDUCED (G2) Toxic



STD CONTROL (G3)Vinblastine



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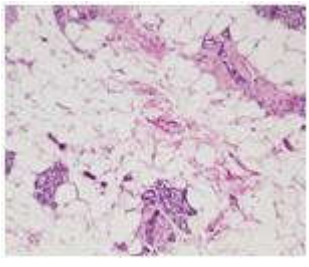
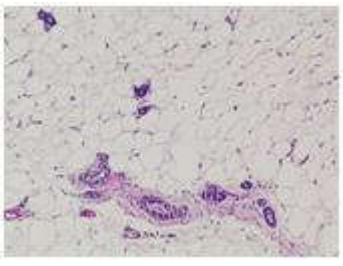
TREATMENT HAEBD(200mg/kg (G4)



TREATMENT HAEBD(G5)(400mg/kg)



Histopathological examination

Histopathology revealed that in G2, the vast majority of the lesions that developed in the mice mammary glands were mostly carcinomas exhibited an identical nuclear pattern with predominant epithelial cell & fibrous tissue surrounding the mammary ducts. It also exhibit a mixed structural pattern with invasion of neighbouring tissues and intense stromal

often results in the formation of oxy radicals ''O2 •-, H2O2, and •OH,'' which bind covalently to nucleophilic sites on cellular macromolecules there by eliciting cancerous responses.[43] The generation of ROS and the peroxidation of membrane lipids are well associated with the initiation of carcinogenesis affecting the normal biochemical process, which further leads to the reduction of body weight.[44] Taking

lead from this, ROS and intracellular Ca2+ levels were analyzed, and an increase in both ROS and Ca2+ generation was observed in DMBA treated animals in comparison to control animals. This excessive ROS signal can cause Ca2+ overload, mitochondrial depolarization, and DNA damage leading to apoptotic cell death[45] which was also observed in this investigation. Increasing evidence indicates that intracellular Ca2+ modulates ROS homeostasis that might influence redox balance and vice versa which, in turn, would determine cancer progression.[46,47]

Oxidative damage in a cell or tissue occurs when the concentration of reactive oxygen species (O2, H2O2 and OH) generated exceeds the antioxidant capability of the cells.[48] Free radical scavenging enzymes like SOD and catalase protect the biological system from oxidative stress. The present study revealed that SOD activity was decreased in the cancer-bearing animal, which may be due to altered antioxidant status caused by carcinogenesis. SOD acts as an anti-carcinogen inhibitor during initiation and promotion/transformation stages of carcinogenesis. SOD is a major intracellular enzyme, which protects against oxygen free radicals by catalyzing the dismutation of superoxide free radical and anions to H2O2 and oxygen. Decrease in CAT activity was measured in patients with breast cancer and benign breast disease conditions.[48] Catalase is an peroxisomalhaem protein which is present in most cells and which catalyses the decomposition of H2O2 to water and oxygen. SOD accelerates the conversion of superoxide radical (O2-) to hydrogen peroxide while CAT catalyses the removal of hydrogen peroxide formed during the reaction catalysed by SOD. This is in accordance with our results, which indicate that decreased CAT in cancer-bearing animals may be due to the utilization of antioxidant enzymes in the removal of H2O2 released Thus, SOD and CAT act as mutually supportive antioxidative enzymes, which provide protective defence against reactive oxygen species. [49]

GPx is an important defense enzyme against oxidative damage and this in turn requires glutathione as a cofactor. Decreased GPx activity was also observed in cancerous conditions.[50] Glutathione is one of the essential compounds for maintaining cell integrity because of its reducing properties and participation in the cell metabolism. GPx catalyses the oxidation of GSH to GSSG at the expense of H2O2.[51] GPx is an equally important antioxidant, which reacts with H2O2, thus preventing intracellular damage caused by the same. [52-54]

The present findings include elevated ROS production with use of the chemical mutagen (DMBA), and the decreased level of antioxidants in breast cancer-bearing animals indicate oxidative stress, which may be the cause of lipid peroxidation-induced DNA damage, mutation and elevated level of LPO also play an important role for higher pathology of breast cancer in animals. It is regarded as one of the basic mechanism of cellular damage caused by free radicals. Increased lipid peroxidation alters membrane fluidity and membrane potential and there by leading to loss of cellular function and cell death.[55] Malondialdehyde is the major end product of LPO and readily reacts with DNA to form DNA-MDA adduct. However, the potential reduction of lipid peroxides was recorded in the vinblastin and both doses of Hydroalcoholic extract of *Boerhaavia diffusa* treated group and it was near to the normal in the control group. The biochemical alterations observed in cancer bearing animals in the present study may be due to the induction of LPO and reduction of antioxidant level following carcinogen administration. However, administration of vinblastin and Hydroalcoholic extract of *Boerhaavia diffusa* significantly reversed the alteration to near normal level in cancer-bearing animals. It did not promote tumor growth and metastasis by the incidence of lesser production of ROS formation due to the antioxidant enzymes such as SOD, CAT and GPx that can directly counter the oxidant attack and may protect cells against LPO and DNA damage.

From the results it can be inferred that Hydroalcoholic extract of *Boerhaavia diffusa* positively modulated the antioxidant activity by quenching and detoxifying the free radicals induced by DMBA. Histopathopathological report also support the studies The attenuation of DMBA induced oxidative stress by the plant extract could be attributed to the antioxidants activity of flavonoids, terpenoids, phenolic compounds, alkaloids such as punarnovine, punarnoviside, boerhavin, boerhavic acid ursolic acid present in the *B.diffusa* plant, which is known to quench the free radicals by maintaining antioxidants levels.[56-59]

References

1. Gonenc A, Ozkan Y, Torun M, Simsek B. Plasma malondialdehyde (MDA) levels in breast and lung cancer patients. *J Clin Pharm Ther*. 2001; 26:141-4
2. Aruoma OI (1994) Nutrition and health aspects of free radicals and antioxidants. *Food Chemistry and Toxicology* 32(7): 671-685.
3. Cerutti PA (1985) Prooxidant states and tumor promotion. Science 227(4685): 375–381.
4. Gilbert DL, Colton CA (1999) Reactive Oxygen Species in Biological Systems: An Interdisciplinary Approach. New York, NY: Kluwer Academic/Plenum Publishers.
5. Jagetia GC, Rao SK (2006) Evaluation of the antineoplastic activity of guduchi (Tinospora cordifolia) in Ehrlich ascites carcinoma bearing mice. *Biol Pharm Bull* 29:460-466.
6. Manoharan S, Vasantha Selvan M. Chemopreventive potential of geraniol in 7, 12-dimethylbenz (a) anthracene (DMBA) induced skin carcinogenesis in Swiss albino mice. *J Environ Biol.* 2012; 33:255-60.
7. Miyata M, Furukawa M, Takahashi K, Gonzales FJ, Yamazoe Y (2001) Mechanism of 7,12-dimethylbenz (a) anthracene-induced immunotoxicity: role of metabolic activation at the target organ. *Jpn J Pharmacol* 86:302-309
8. Wijnhoven SW, Kool HJ, Mullenders LHF, Slater R, Van Zeeland AA, Vrieling H (2001) DMBA-induced toxic and mutagenic response vary dramatically between NER-deficient Xpa, Xpc and Csb mice. *Carcinogenesis* 22:1099-1106
9. Conney AH (1982) Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons, Clowes GHA memorial lecture. *Cancer Res* 4:4875-4917
10. Vijayavel K, Anbuselvam C, and Balasubramanian MP (2006) Free radical scavenging activity of the marine mangrove Rhizophora apiculata bark extract with reference to naphthalene induced mitochondrial dysfunction. *Chemico-Biological Interactions* 163(1-2): 170-175.
11. Dhar, M.L., Dhar, M.M., Dhawan, B.N., Mehrotra, B.N., and Ray, C. 1968. Screening of Indian plants for

biological activity: Part I. *Indian Journal of Experimental Biology* 6:232-247.
12. Surange S.R., Pendse G.S. *J. Res. Indian Med*. 1972; 7: 1.
13. Singh RH and Udupa KN Studies on the Indian indigenous drug punarnava (Boerhaavia diffusa). Part IV: Preliminary controlled clinical trial in nephritic syndrome. *Journal of Research in Indian Medicine* 7: (1972), *28-33*
14. Lad, V... The complete Book of Ayurvedic Home Remedies, Three Rivers Press: New York. 1999.
15. Guhabakshi, D.N, Sensarma, P., and Pal, D.C., A lexicon of Medicinal Plants of India, *Vol. I*, *Naya Prakash, Calcutta*, India. 1999.
16. Chopra RN and Nayar S. Glossary of Indian Medicinal Plants. Publications New Delhi: C.S.I.R.1956.
17. Adesina SK. Anticonvulsant properties of the roots of Boerhaavia diffusa. *Quarterly Journal of Crude Drug Research*.1979; 17: 84-86.
18. Olukoya DK, Tdika N, and Odugbemi T. Antibacterial activity of some medicinal plants from Nigeria. *Journal of Ethnopharmacology.* 1993; 39: 69-72.
19. Vijayalakshmi K, Misra SD and Prasad SK. Nematicidal properties of some indigenous plant materials against second stage juveniles of Meloidogyne incognita (Kofoid and White) Chitwood. *Indian J. of Entomology*. 1979;41(4):326-331.
20. Jain GG and Khanna NM. Punarnavoside: A new antifibrinolytic agent from Boerhaavia diffusa Linn. *Indian Journal of Chemistry*. 1989; 28(B): 163-166.
21. Marklund, S., Marklund, G., Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem*, 1974,47: 469-474.
22. Benakanakere, I., Besch-Williford, C., Carroll, C.,E., Hyder, S.,M., Synthetic progestins differentially promote or prevent 7,12-DMBA induced mammary tumors in Sprague-Dawley rats. *Cancer Prev Res*, 2010, 3:1157-67.
23. Radhakrishnan Padmavathi, Palaniyandi Senthilnathan, Dechen Chodon, Dhanapal Sakthisekaran, Therapeutic effect of paclitaxel and propolis on lipid peroxidation and antioxidant system in 7,12 dimethyl benz(a)anthracene-induced breast cancer in female Sprague Dawley rats, *Life Sciences*, 2006,78: 2820 - 2825.
24. Sinha, A., K., Colorimetric assay of catalase. *Anal. Biochem*, 1972, 47:389-394.
25. Rotruck, J., T., Pope, A., L., Ganther, H.,E., Swanson, A.,B., Hafeman, D.,G., Hoekstra, W.,G., Selenium: biochemical role as a component of glutathione peroxidase. *Science*, 1973, 179: 588-590
26. Ray.G., Batra,S., Shukla,N., K., Deo,S., Raina,V., Ashok,S., Husain, S.,A., Lipid peroxidation, free radical production and antioxidant status in breast cancer, *Breast Cancer Res. Treat*, 2000, 59 163-170.
27. G.Nalini[a*], N.Chidambaranathan[a], R.Meera[b],R.Thaila.[a] Protective effect of hydro alcoholic extract of *Clitoria ternatea* against dimethyl nitrosamine induced liver cirrhosis in rats. *The Journal of Physiology and Health. Photon* 110 (2014) 247-254.
28. Lowe, S., W., Lin, A.,W., Apoptosis in cancer. *Carcinogenesis,* 2000; 21: 485-95.
29. Balasenthil, S., Nagini, S., Inhibition of 7,12-dimethylbenz[a]anthracene- induced hamster buccal pouch carcinogenesis by Sallylcysteine. *Oral Oncology*, 2000; 36:382–6.
30. 30) Fisher, B., Costantino, J., P., Wickerham, D., L., Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst*, 1998, 90, 1371-1388.
31. Constantinou, A.,I., Rossi, H., Lantvit, D., Soy protein isolate prevents chemically induced rat mammary tumors. *Pharm Biol*, 2002, 40, 24-34.
32. Constantinou, A.,I., Husband,A., Phenoxodiol (2H-1-benzopyran-7- 1,3-(4-hydroxyphenyl), a novel isoflavone derivative, inhibits DNA topoisomerase II by stabilizing the cleavable complete. *Anticancer Res*, 2002, 22, 2581-2585.
33. Bayoumy, K., Evaluation of chemopreventative agents against breast cancer and proposed strategies for future clinical intervention trials. *Carcinogenesis*, 1994, 15: 2395- 2420.
34. Apurba Sarker Apu., Mahmuda Sultana Liza., Jamaluddin, A.T.M., Amran Howlader., Repon Kumar Saha. Phytochemical screening and *invitro* bioactivities of the extracts of aerial part of *Boerhaavia diffusa* Linn. *Asian Pacific Journal of Tropical Biomedicine*. 2012, 2(9):673-678.
35. Mahesh, A.R., Harish Kumar., Ranganath, M.K., and Raviraj Anand Devkar. Detail Study on *Boerrhaavia diffusa* Plant for its Medicinal Important a Review. *Research Journal of Pharmaceutical science.*,2012, 1(1): 28-36
36. Katoh, R., Zhou, H., Wang, P.,Y., Promoting effects of milk on the development of 7, 12-dimethylbenz (a) anthracene (DMBA)-induced mammary tumors in rats. *Acta Histochem Cytochem,* 2007, 40 (2):61-7.
37. Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82:70-77.
38. Hall, P.,A., Levison, D.,A., Woods, A.,L., Yu, C.,C., Kellock, D.,B., Watkins, J.,A., Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. *J Pathol*, 1990; 162(4): 285-94.
39. Flohe,L., Otting,F., Superoxide dismutase assays. *Meth. Enzymol,*1984,105: 93.
40. Fischer SM, Conti CJ, Locniskar M, Belury MA, Maldve RE, Lee ML, Leyton J, Slaga TJ, Bechtel DH (1992) The Effect of dietary fat on the rapid development of mammary tumors induced by 7, 12-dimethylbenz(a)anthracene in SENCAR mice. Cancer Res 52:662-666
41. Qing WG, Conti CJ, LaBate M, Johnston D, Slaga TJ, MacLeod MC (1997) Induction of mammary cancer and lymphoma by multiple, low oral doses of 7, 12-dimethylbenz[a]anthracene in sencar mice. Carcinogenesis 18(3):553-559
42. Cardiv RD, Wellings SR (1999) The comparative pathology of human and mouse mammary glands. *J Mammary Gland Biol Neoplasia* 4:105-122
43. Giri U, Sharma SD, Abdulla M, Athar M (1995) Evidence that in situ generated reactive oxygen species act as a potent stage I tumor promoter in mouse skin. *Biochem Biophys Res Commun* 209(2):698-705.

44. Davis L, Kuttan G (2001) Effect of Withania somnifera on DMBA induced carcinogenesis. *J Ethnopharmacol* 75:165–168.
45. Yan Y, Wei CL, Zhang WR, Cheng HP, Liu J. Cross-talk between calcium and reactive oxygen species signaling. *Acta Pharmacol Sin*. 2006;27(7):821-6.
46. Joseph P, Muchnok TK, Klishis ML, Roberts JR, Antonini JM, Whong WZ, *et al*. Cadmium-induced cell transformation and tumorigenesis are associated with transcriptional activation of c-fos, c-jun, and c-myc proto-oncogenes: role of cellular calcium and reactive oxygen species. *Toxicol Sci*. 2001;61:295-303.
47. Kesba HH, El-Belagi HS. Biochemical changes grape rootstocksresulted from humic acid treatments in relation to nematode infection. *Asian Pac J Trop Biomed* 2012; 2(4):287-93.
48. 48) Gonenc A, Erten D, Aslan S, Akyncy M, Sximsxek B, and Torun M (2006) Lipid peroxidation and antioxidant status in blood and tissue of malignant breast tumour and benign breast disease. *Cell Biology International* 30(4): 376-380.
49. Weydert CJ, Waugh TA, Ritchie JM, Iyer KS, Smith JL, Li L, Spitz DR, Oberley LW (2006) Overexpression of manganese or copper-zinc superoxide dismutase inhibits breast cancer growth. *Free Radic Biol Med* 41:226-237
50. Bewick M, CoutieW, Tudhope GR (1987) Superoxide dismutase, glutathione peroxidase and catalase in the red cells of patients with malignant lymphoma. *Br J Haematol* 65:347-350. doi: 10.1111/j.1365-2141.1987.tb06866.x
51. Cerutti P, Ghosh R, Oya Y, Amstad P (1994) The role of cellular antioxidant defence in oxidant carcinogenesis. *Environ Health Perspect* 102(10):123-129. doi:10.2307/3432228
52. Gupta M, Mazumder UK, Kumar RS, Sivakumar T, and Vamsi ML (2004) Antitumour activity and antioxidant status of Caesalpina Bonducella against Ehrlich ascites carcinoma in swiss albino mice. *Journal of Pharmacological Sciences* 94(2): 177-184.
53. Weydert CJ, Waugh TA, Ritchie JM, Iyer KS, Smith JL, Li L, *et al*. (2006) Over expression of manganese or copper-zinc superoxide dismutase inhibits breast cancer growth. *Free Radical Biology and Medicine* 41(2):226-37.
54. Harbone,J.,B., Phytochemical methods, *Champion and Hall publications, London*, 1984, 2nd edition:84-196.
55. Kolanjiappan K, Manoharan S, Kayalvizhi M, Measurement of erythrocyte lipids, lipid peroxidation antioxidants and osmotic fragility in cervical cancer patients, Clinica Chimica ACTA, 326: 143-149, (2002).
56. Edeoga, H.,O., Okwu,D., E., Mbaebie, B., O ., Phytochemical constituents of some Nigerian medicinal plants. *Afr.J. Biotechnol*, 2005, 4:685-688.
57. Okwu,D.,E., Phytochemicals, minerals content of two Nigeria plants. *Int.J. Mol. Med. Adv.Science.,* 2005,1:375-381.
58. Kokate,C.,K., Purohit,A.,P., Gokhale,S.,B., Pharmacognosy. *Nirali Prakasan, Pune, India*, 2005, 38:537-538.
59. Misra, A.,N.,Thiwari, H.,P., Constituents of roots of Boerhaaviadiffusa. *Phytochemistry,* 1971, 10:3318-3319.

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