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DOSE DEPENDENT CHEMOPREVENTIVE EFFICACY OF NEROLIDOL ON CHEMICAL INDUCED MAMMARY CARCINOGENESIS IN RATS

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ABSTRACT: Chemoprevention has evolved as a promising strategy by dietary constituents and emerged as a novel approach to control cancer. Numerous plant-based phytochemicals are predictable to exert their anti-carcinogenic effects. The present study was aimed to investigate the chemopreventive effect of nerolidol against 7, 12-dimethylbenz (a) anthracene (DMBA)-induced mammary carcinogenesis in rats.Mammary carcinomas were induced by a single dose of DMBA (25 mg/rat) injected near the mammary gland. The rats were orally administered with different doses of nerolidol 100, 200 and 400 mg/kg bw andby monitoring tumor incidence, volume, burden and histopathological changes as well as by analyzing the status of biochemical markers in liver and mammary tissues. Administration of nerolidol at a dose of 400 mg/kg bw shows more pronounced effect in inhibition of tumor incidence and restored status of biochemical markers such as, lipid peroxidation, enzymatic and non-enzymatic antioxidants, phase I and phase II detoxification enzymes. Histopathological results also evidence the protection of cells in the mammary and liver tissues in dose dependent manner.The result suggests that nerolidol has chemopreventive potential for DMBA -induced mammary carcinogenesis in female rats.

KEYWORDS: Mammary carcinogenesis, DMBA, Biochemical markers, Nerolidol, Chemoprevention.

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1.INTRODUCTION

Globally, breast cancer is the principal cause of cancer-related death in women, accounts for about 3.27 lakh every year. Approximately 12.4 percent of women diagnosed with breast cancer during

their lifetime. The incidence of breast cancer is associated with tuning of fine balance between the rate of cell proliferation and apoptotic cell death [22]. The most common types of breast cancer are ductal breast cancer (arise from milk ducts) and lobular breast cancer (arise from milk lobules), which can be either in situ or invasive to normal tissue. The signs are a painless lump and thickened tissue in the breast, nipple discharge or a change in the nipple, dimpling or puckering of the skin of the breast and change in breast size or shape.7, 12-dimethylbenz (a) anthracene (DMBA), a potent environmental carcinogen is normally used to study the chemopreventive potential of natural products and synthetic agents in rat models [24]. DMBA-induced mammary carcinogenesis in rat model is similar to human breast cancer both biochemical and molecular aspects. DMBA is a procarcinogen, which is metabolically activated by phase I detoxification enzymes [13] to produce reactive metabolite, dihydrodiol epoxide, which binds to DNA to form DNA adduct and also generate free radicals to cause oxidative stress [42]. Metabolic detoxification of DMBA occurs mainly in the liver and the reactions are catalyzed by phase I detoxification agents (Cytochrome P450 and cytochrome b5) and phase II detoxification enzymes (Glutathione-S-transferase (GST), and Glutathione reductase (GR) respectively. The phase I and phase II detoxification enzymes are significantly altered during mammary carcinogenesis [40].Carcinogenesis is attributed with oxidative stress, a condition occur due to over production of reactive oxygen species (ROS) which affects DNA via oxidative damage through the formation of 8-oxoguanosine. Lipid peroxidation (LPO), a free radical mediated chain reaction causes oxidative deterioration of polyunsaturated fatty acids in the cellmembrane. LPO by-products (thiobarbituricacid reactive substances (TBARS), and lipid hydro peroxides (LOOH) causes extensive membrane damage and disorganization of cell structure and function. Oxidative stress alters the antioxidant defense system such as reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidise (GPx). The dietary antioxidants play a crucial role in the prevention of cancer, inflammation, neurodegenerative, and cardiovascular diseases. Recently, researchers have focused on phytochemicals as antioxidants or free radical, scavengers; inhibit the production of free radicals before the initial stage of neoplastic transformation. Plant essential oils have been used for centuries in various indications including cancer. Chemoprevention has been evolved as a promising strategy by dietary constituents and emerged as a novel approach to control cancer. Numerous plant-based phytochemicals have anti-carcinogenic effects by inhibiting cell proliferation and induced apoptosis. Medicinal plants serve as an important property for the exploration of therapeutically helpful compounds. The use of plant-derived antioxidants as chemoprevention is likely to be acceptable in clinical trials.A number of essential oils and sesquiterpenes, their main components, have been found to inhibit proliferation, induce apoptosis, suppress angiogenesis, retard metastasis and enhance chemotherapy both in vitro and in vivo [5]. Nerolidol is a sesquiterpene present in the essential oils of many plants and flowers, such as neroli, ginger, jasmine, lavender and tea tree [34].

Nerolidol is used as a fragrance additive in cosmetics (e.g., shampoos and perfumes), non-cosmetics (e.g., detergents) and as a flavoring agent. They exhibit diverse biological activity, including hepatoprotective [20], anticonvulsive [2] and antitumor actions. Hence, the present study was investigated the dose dependent chemopreventive potential of nerolidol against DMBA induced mammary carcinogenesis in Sprague-Dawley rats by analyzing the biochemical and histopathological changes. Figure 1 shows the biochemical structure of Nerolidol.

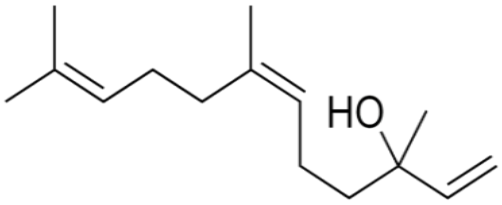

Figure.1 Chemical structure of nerolidol

2. MATERIALS AND METHODS

Chemicals

Nerolidol, DMBA, TBA, GSH and H_2O_2 were purchased from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India. Reagent kits for TC, TG and HDL-C were purchased from Agappe Diagnostics, Ernakulam, India. All the other chemicals used were of analytical grade.

Animal model

Six to seven weeks old female Sprague-Dawley rats (weighed 120–130 g), were purchased from Biogen, Bangalore, India. Animals were maintained in the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram, Tamilnadu, India. After obtaining proper approval from the Institutional Animal Ethics Committee for the Control and Supervision of Experimental Animal (CPCSEA approval no: 1148) guidelines. The animals were maintained under controlled conditions of temperature (24±2˚C), humidity (50±10%) and 12 h light/dark cycle. Feed and water provided *ad libitum*.

Induction of mammary carcinogenesis

Mammary carcinogenesis was induced in Sprague-Dawley rat using a single subcutaneous injection of 25 mg of DMBA in 1 ml emulsion of sunflower oil (0.75 mL) and physiological saline (0.25 ml) to each rat[12].

Selection of relavent dose

It has been found to possess strong anti-tumor activity by inhibiting the intestinal carcinogenesis induced by azoxymethane (15 mg/kg bw) administered twice per week for a duration of three weeks in male F344 rats [45] Beneficial effects of nerolidol (100 and 200mg/kg bw) on thioacetamide-induced damage of the reproductive system in male rats [21]and nerolidol prevented TAA induced testicular damage in rats. We decided to do dose dependent study of nerolidol with the concentration

of 100, 200 and 400 mg/kg bw to determine the effective dose for further study.

Experimental design

For the dose fixation study, total numbers of 48 animals were randomly divided into six groups and each group contains 8 animals. The group I animals were served as untreated control and provided food and water ad libitum throughout the experimental period. Groups II–V animals had a single subcutaneous injection of DMBA (25 mg/rat) near mammary gland, at the end of the first week. Group II animals received no other treatment. Group III–V animals were administered nerolidol at different doses (100, 200 and 400 mg/ kg bw) by intubation once in a day by starting one week before the exposure of the carcinogen and were continued till the end of the experimental period. Group VI animals were received oral administration of nerolidol alone throughout the experimental period. At the end of 16th week, rats were fasted overnight and sacrificed by cervical decapitation. Blood samples were collected in heparinized tubes and the separated plasma was used for the biochemical analysis. Liver and mammary tissues were excised immediately from the rats and stored in ice-cold containers. Tissues were homogenized with suitable buffer, centrifuged at 3000 g and the supernatant were used for biochemical estimations on the same day of sacrifice. Liver and mammary tissues also preserved in 10% formalin and stored at -80˚ C for histopathological studies.

Biochemical analysis

Excised liver and mammary tissues were rinsed in ice-cold saline.A known amount of the tissue were homogenized in 0.1 MTris-HCl buffer (pH 7.4), at 4˚C, in a Potter-Elvehjem homogenizerwith a Teflon pestle at 600 rpm for 3 min. The homogenate wascentrifuged at 3000g for 10 min at 4˚C. The supernatant was collected as tissue homogenate, which was used to assay various biochemical parameters. Liver and mammary tissue microsomes were isolated by the method [19].The microsomal protein content was estimated by the method [29]. The concentration of plasma TBARS was estimated by the method [46]. The concentration of mammary tissue TBARS was estimated by the method [31]. The concentration of plasma and mammary tissue LOOH were estimated by the method [23]. SOD activity in plasma and mammary tissue were determined by the method [25]. CAT activity in plasma and mammary tissue were determined by the method [41]. GPX activity in plasma and mammary tissue were determined by the method [37]. GSH in plasma and mammary tissues were determined by the method [6]. Cyt p450 and Cyt-b5 levels in liver and mammary tissue microsomes were estimated by the method [32]. GST activities in liver and mammary tissues were assayed by the method [18]. GR activities in liver and mammary tissues were assayed by the method [10].

Lipid profile

Lipid extraction from plasma and mammary tissue were done by the method [15]. Totalcholesterol (TC) in plasma and mammary tissues were estimated by using the kit method [48]. Triglyceride (TG) in plasma and mammary tissue were measured by the method [16]. Free fatty acid (FFA) in

plasma and mammary tissue were estimated by the method [14]. Phospholipid (PL) in plasma and mammary tissues were determined by the method [47]. High density lipoprotein (HDL) cholesterol (C) in plasma was estimated by the method [9] and the cholesterol content was determined by the method [48]. Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL-C) levels were evaluated by the method [17] LDLC = TC - (HDL-C + VLDL-C), VLDL-C = TG/5.

Histopathological analysis

For histopathological analysis, the liver and mammary tissues were sliced and immersed in 10% formalin solution for fixation, dehydrated with graded ethanol solutions from 50% to 100%, and then embedded in paraffin. Sections of 3-5 mm in thickness were cut and stained with hematoxylin and eosin and the slides were observed using microscope.

Statistical analysis

Statistical analysis was performed using SPSS 16 (SPSS, Inc., Chicago). The data are expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) comparison method was used to correlate the difference between the variables. Data are considered statistically significant if p values are less than 0.05.

3. RESULTS AND DISCUSSION

Cancer chemoprevention is a process by which natural or biological agents prevent the development of cancer Bandari et al. [7]. In the present study, we observed 100% tumor formation in DMBA injected rats and histopathologically confirmed a well differentiated mammary cell carcinoma. Nerolidol treatment to DMBA injected animals showed a significant reduction in tumour incidence, volume and burden in dose dependent manner.Sachdanandam et al [35]. reported that the phytochemical formulation Shemamruthaa possesses strong anticancer effects through its role in modulating body weight, tumor volume and marker enzymes in DMBA induced mammary carcinoma in rats. In the way our drug also phytochemical compound of nerolidol possess anti tumor and anticarcinogenic effect.

Effect of nerolidol on body weight changes, tumor incidence, total number of tumors, tumor volume and tumor burdenin control and experimental animals

Table.1 shows the body weight,tumor incidence, total number of tumors, tumor volume and tumor burden of control and experimental animals. Initially, there were no significant changes in the body weight of control and experimental animals. Finally, we observed the body weight of DMBA alone treated animals decreased significantly ($p<0.05$) when compared with the control group. Nerolidol treatment significantly ($p<0.05$) increase in the body weight of DMBA treated animals. Nerolidol alone treated animals show no significant changes when compared with control animals. In DMBA alone treated group, the tumor incidence was 100% and the mean tumor volume and burden was found to be 20.45 mm and 163.62 mm respectively. Upon the treatment with nerolidol (Group III-V), the tumor burden, tumor volume was found to decrease significantly (10.63, 4.23 and 0 mm and

85.05, 33.84 and 0 mm) in dose dependently when compared to DMBA alone treated group.

Table 1: Effect of nerolidol on Body weight changes, tumor incidence, total number of tumor, tumor volume and tumor burden in control and experimental animals

Groups	Initial body weight (g)	Final body weight (g)	Total number of tumors (n)	Tumor incidence (%)	Tumor volume (mm^3/rat)	Tumor burden (mm/rat)
Control	125.22±9.53	153.72±11.70[a]	0/8	0	0	0
DMBA	129.66±9.92	103.45±7.92[b]	(8)/8	100	20.45±1.55[a]	163.62±12.45[a]
DMBA+nerolidol(100mg)	130.52±9.93	128.12±9.45[c]	(3)/8	45	10.63±0.81[b]	85.05±6.47[b]
DMBA+nerolidol(200mg)	131.51±10.06	150.92±11.55[a]	(2)/8	25	04.23±0.32[c]	33.84±2.58[c]
DMBA+nerolidol(400mg)	132.32±10.07	151,45±11.53[a]	(0)/8	0	0	0
Nerolidol (400mg)	128.00±9.47	155.64±11.85[a]	(0)/8	0	0	0

Tumor volume was measured using the formula V = 4/3π (D1/2) (D2/2) (D3/2), where D1, D2 and D3 are the three diameters (in mm) of the tumor; Tumor burden was calculated by multiplying tumor volume and the number of tumors per animal.

() indicates total number of rats bearing tumors.

Values are expressed as mean ±SD for eight animals in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

The induction of phase I detoxification enzyme, such as cyt p450 and cyt b5 systems are the potential cancer risk factor due to the activation of procarcinogens to ultimate carcinogen. Therefore, the inhibition of phase I enzyme activities by nerolidol is an importance aspects for the chemopreventive mechanism of DMBA induced carcinogenesis. Phase II enzymes perform conjugation reactions which helps to convert the bio transformed intermediates of DMBA from phase I into less toxic, watersoluble substances which are easily excreted from the body [4].Kumar et al**. [27]** Reported that the anticarcinogenic activity of dietary phytochemicals is mediated through the induction of hepatic GST and GR during the DMBA induced carcinogenesis. Increased activities of phase II detoxification enzymes in rats treated with nerolidol to the detoxification cascade is stimulated to metabolize as well as detoxify the carcinogenic DMBA. The results indicate that the bioactive compounds of nerolidol alter both phase I and II metabolism of xenobiotics and it could act as an ideal chemopreventive drug in breast cancer. In this present study, we noticed an elevated levels of CYP450, Cyt-b5 and decreased levels of GST and GR in DMBA alone treated animals. Mammary tumor bearing rats showed elevated levels of phase I enzymes and reduced activities of phase II enzymes. However, nerolidol treated animals significantly altered these enzyme levels.

Effect of nerolidol on lipid peroxidation by products in plasma and mammary tissues of control and experimental animals

Table.2 shows the levels of lipid peroxidation by-products (TBARS and LOOH) in plasma and mammary tissues of control and experimental animals. A significant ($p<0.05$) increase in TBARS and LOOH plasma and mammary tissue in DMBA alone treated animals when compared with control animals. However, the levels were decreased significantly ($p<0.05$) in animals treated with nerolidol in dose dependent manner. Nerolidol alone treated animals did not show any significant variations when compared to control rats.

Table 2: Effect of nerolidol on lipid peroxidation in plasma and mammary tissues of control and experimental animals

Samples	Plasma		Mammary tissue	
Groups	TBARS (mM/dL)	LOOH (mM/dL)	TBARS (mM/100 g wet tissue)	LOOH (mM/100 g wet tissue)
Control	1.63±0.12^{a}	1.59±0.12^{a}	1.42±0.10^{a}	0.79±0.05^{a}
DMBA	3.66±0.27^{b}	3.16±0.23^{b}	2.59±0.19^{b}	1.83±0.13^{b}
DMBA+ nerolidol (100mg)	2.48±0.18^{c}	2.38±0.17^{c}	2.02±0.15^{c}	1.47±0.11^{c}
DMBA+ nerolidol (200mg)	1.79±0.13^{a}	1.74±0.13^{a}	1.56±0.11^{a}	1.07±0.08^{d}
DMBA+ nerolidol (400mg)	1.76±0.13^{a}	1.71±0.12^{a}	1.52±0.11^{a}	1.03±0.07^{a}
Neroidol (400mg)	1.70±0.12^{a}	1.62±0.12^{a}	1.45±0.11^{a}	0.86±0.06^{a}

Values are expressed as mean ±SD for eight animals in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

Effect of nerolidol on antioxidant status in plasma and mammary tissues of control and experimental animals

Table.3 represents the status of enzymatic and non-enzymatic antioxidant in plasma and mammary tissues of control and experimental animals. DMBA alone treated animals showed a significantly ($p<0.05$) decreased in the status of SOD, CAT, GPx and GSH, when compared with control animals. Oral administration of nerolidol,the status of SOD, CAT. GPx and GSH at the dose dependent manner significantly increased in plasma andsignificantly decreased near to normal levels in mammary tissue. However, nerolidol alone treated animals did not show any significant changes.

Table 3: Effect of nerolidol on antioxidant status in plasma and mammary tissues of control and experimental animals

Samples	Plasma				Mammary tissue			
Groups	SOD (U^*/mL)	CAT ($U^\#$/mL)	GPx ($U^@$/mL)	GSH (mg/dL)	SOD (U^*/mg of protein)	CAT ($U^\#$/mg of protein)	GPx ($U^@$/mg of protein)	GSH (mg/100 g of wet tissue)
Control	3.50±0.26^a	2.31±0.17^a	83.20±6.33^a	26.51±2.02^a	14.37±1.09^a	43.28±3.29^a	13.51±1.03^a	14.00±1.06^a
DMBA	1.73±0.13^b	1.26±0.09^b	51.21±3.92^b	12.27±0.93^b	08.66±0.66^b	26.10±1.99^b	06.15±0.46^b	06.57±0.50^b
DMBA+nerolidol(100mg)	2.35±0.17^c	1.70±0.12^c	61.45±4.68^c	18.67±1.42^c	10.29±0.78^c	28.88±2.19^b	09.36±0.71^c	09.23±0.70^c
DMBA+nerolidol(200mg)	3.16±0.23^d	2.23±0.17^a	78.85±6.03^a	24.02±1.83^a	13.51±1.03^a	41.89±3.20^a	11.64±0.89^a	12.83±0.98^a
DMBA+nerolidol(400mg)	3.19±0.24^d	2.27±0.17^a	78.89±6.00^a	24.14±1.83^a	13.62±1.03^a	41.83±3.18^a	11.71±0.89^a	12.87±0.98^a
Nerolidol (400mg)	3.46±0.26^a	2.30±0.17^a	82.07±6.24^a	26.19±1.99^a	14.21±1.08^a	42.68±3.24^a	11.34±0.86^a	13.73±1.04^a

U*: amount of enzyme to inhibit 50% NBT reduction/min.

U#: µmol of H_2O_2 consumed/min.

U@: µg of GSH consumed/min.

Values are expressed as mean ±SD for eight animals in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

Effect of nerolidol on lipid profile and lipoprotein in plasma and lipid profile in mammary tissue of control and experimental animals

Table 4 shows the levels of lipid profile (TC, TG, PL, and FFA) and lipoprotein (HDL, LDL, VLDL) in plasma and mammary tissue of control and experimental animals. The levels of TC, TG, PL, FFA, LDL and VLDL were significantly ($p<0.05$) increased and HDL was significantly ($p<0.05$) decreased in plasma. The levels of PL and FFA are decreased in mammary tissue of DMBA alone treated animals when compared with control animals. Nerolidol treatment with different doses (100mg, 200mg and 400mg/kg bw) significantly ($p<0.05$) decreased the levels of TC, TG, PL, FFA, LDL and VLDL. However, HDL was significantly ($p<0.05$) increased in plasma, where as the level of PL and FFA are increased in mammary tissue, when compared with the group-II animals. Nerolidol alone treated animals have shown no significant difference, when compared to control animals.ROS are excessively generated during the metabolic activation of DMBA into its active metabolite dihydrodiol epoxide [36]. Lipidperoxidation (LPO) by-products are generated as an indication of oxidative stress. Increased levels of LPO by-products in plasma confirmed oxidative stress in tumor-bearing animals. Decreased activities of plasma and mammary enzymatic antioxidants are probably due to exhaustion of these enzymes due to scavenge excessively produced free radicals in the system and the total body weight also decreased. Because, LPO plays an

important role in the initiation of tumor development and changes in the energy metabolism in DMBA treated animals. Oral administration of nerolidol restored the status of plasma LPO by-products, body weight and antioxidants status to near-normal range, which indicates that nerolidol, has potent free radical scavenging property during DMBA-induced mammary carcinogenesis.Previous study shows that the augmented level of TBARS intumor cells is associated with overproduction of free radical andserves as an index to assess the extent of tissue damage [30]. In thispresent study, we observed increased levels of TBARS and LOOH inDMBA-induced animals. Lakshmi and Subramanian [28], reportedthat mammary tumor induced rats were found in increased levelof TBARS. On the other hand nerolidol also significantlyaltered the lipid peroxidation status indicating its anti-lipid peroxidativeproperty.

Table 4: Effect of nerolidol on lipid profile, lipo protein in plasma and lipid profiles in mammary tissues of control and experimental animals

Samples	**Plasma**						
Groups	TC (mg/dl)	TG (mg/dl)	PL (mg/dl)	FFA (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	94.52±7.19^{a}	68.12±5.18^{a}	94.33±7.18^{a}	10.38±0.78^{a}	26.54±2.02^{a}	22.45±1.71^{a}	14.52±1.10^{a}
DMBA	131.26±10.04^{b}	120.14±9.19^{b}	143.81±11.01^{b}	17.15±1.31^{b}	12.16±0.92^{b}	83.16±6.36^{b}	25.03±1.91^{b}
DMBA+nerolidol(100mg)	108.09±8.23^{c}	102.89±7.83^{c}	126.02±9.59^{c}	14.74±1.12^{c}	16.76±1.27^{c}	58.63±4.46^{d}	21.60±1.64^{c}
DMBA+nerolidol(200mg)	81.79±6.26^{a}	75.61±5.78^{a}	98.00±7.50^{a}	10.66±0.81^{a}	24.60±1.88^{a}	30.10±2.30^{c}	16.19±1.24^{a}
DMBA+nerolidol(400mg)	81.64±6.21^{a}	75.04±5.71^{a}	97.86±7.45^{a}	10.62±0.81^{a}	24.65±1.87^{a}	30.06±2.28^{c}	16.03±1.21^{a}
Nerolidol (400mg)	75.28±5.72^{a}	69.05±5.25^{a}	94.89±7.22^{a}	10.47±0.79^{a}	26.23±1.99^{a}	23.34±1.77^{a}	14.81±1.12^{a}

Mammary tissue			
TC (mg/g)	TG (mg/g)	PL (mg/g)	FFA (mg/g)
05.06±0.38^{a}	05.16±0.38^{a}	15.27±1.16^{a}	11.10±0.84^{a}
13.11±1.00^{b}	10.58±0.81^{b}	08.02±0.61^{b}	05.94±0.45^{b}
10.44±0.79^{c}	07.24±0.55^{c}	12.66±0.96^{c}	08.43±0.64^{c}
05.52±0.42^{a}	04.70±0.36^{a}	14.74±1.13^{a}	10.68±0.81^{a}
05.48±0.41^{a}	04.66±0.35^{a}	14.79±1.12^{a}	10.74±0.81^{a}
05.08±0.38^{a}	04.20±0.32^{a}	15.17±1.15^{a}	10.96±0.83^{a}

Values are expressed as mean ±SD for eight animals in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

Enzymatic antioxidant systems consist of SOD, CAT and GPx are act as the principal defence against oxidative damage during the carcinogenesis. Samy et al. [38]have documented that SOD acts as an effective enzymatic antioxidant during chemical induced carcinogenesis. CAT catalyzes the decomposition of hydrogen peroxide to improve the status of antioxidant system, which provides a protective effect against reactive oxygen species. The decreased level of enzymatic antioxidants

levels in DMBA treated animals indicating oxidative stress, which might be the cause of LPx leads to subsequent damage of DNA. Bose et al.[8]studied regular consumption of lycopene (rich in tomato, spinach, etc.) significantly induced the antioxidant enzymes like SOD, GSH, GPx, and glutathione reductase (GR) and reduced form of glutathione (GSH); moreover this bioactive compound also reduced the levels of lipid peroxide malondialdehyde (MDA), LDL oxidation, and ROS mediated DNA damage. In the way our drug also phytochemical compound of nerolidol significantly induced the antioxidant enzymes. GSH plays an essential role in shielding the cells from oxidative stress and be the first line of defense system against free radical damage. GSH is the main intracellular antioxidant and its central role in xenobiotic or eicosanoid metabolism, and also it is maintaining the cell integrity because of its reducing properties and participation in the cell metabolism. During the detoxifying metabolism of DMBA, GSH in conjunction with GST to detoxifies reactive intermediate species of DMBA and thereby enhancing resistance against oxidative stress. The reduction of GSH level has been observed in cancerous animals in response to DMBA mediated oxidative stress in experimental animals. The same lines of observation were documented in DMBA induced mammary carcinogenesis. Nerolidol treatment significantly improves the non□enzymatic antioxidant status.Abdul Lateef et al.[1].Well documented with the level of reduced glutathione, the activities of glutathione dependent enzymes and antioxidant enzymes were also enhanced to significant levels in the pretreatment with farnesol in oxidative stress in prostate of Wistar rats. Farnesol is one of analogue for nerolidol. So, nerolidol also augmented to the levels of detoxification enzymes in DMBA induced animals.

Effect of nerolidol on phase I and phase II detoxification agents in liver and mammary tissues of control and experimental animals

Table.5 shows the level of phase I (cyt p450, cyt b5) and phase II (GST, GR) detoxification agents in liver and mammary tissues of the control and experimental animals. The level of cyt p450 and cyt b5 were significantly ($p<0.05$) increased and GST, GR activities were significantly decreased in DMBA treated animals when compared with control animals. Oral administration of nerolidol at different doses (100mg, 200mg and 400mg/kg bw) significantly ($p<0.05$) decreased level of phase I agents andincreased activities phase II enzymes in liver and mammary tissues of the (group III-IV) animals are compared with group II animals. However, nerolidol alone treated animals of have no significant difference when compared to control animals.

Table 5: Effect of nerolidol on detoxification agents in liver and mammary tissues of control and experimental animals

Tissues	Liver tissue				Mammary tissue			
Groups	Cyt p450	Cyt b5	GST	GR	Cyt p450	Cyt b5	GST	GR
Control	0.74±0.05[a]	0.44±0.03[a]	1.34±0.10[a]	25.09±1.91[a]	0.86±0.06[a]	0.52±0.04[a]	1.13±0.08[a]	3.47±0.26[a]
DMBA	1.43±0.10[b]	0.76±0.05[b]	0.73±0.05[b]	12.42±0.95[b]	1.34±0.10[b]	0.84±0.06[b]	0.61±0.04[b]	1.27±0.09[b]
DMBA+nerolidol(100mg)	1.14±0.08[c]	0.67±0.04[c]	1.03±0.07[c]	17.44±1.33[c]	1.18±0.08[c]	0.65±0.04[c]	0.85±0.06[c]	2.08±0.16[c]
DMBA+nerolidol(200mg)	0.79±0.05[a]	0.48±0.03[a]	1.23±0.09[a]	25.01±1.91[a]	1.02±0.07[d]	0.57±0.04[a]	1.05±0.07[a]	3.24±0.24[a]
DMBA+nerolidol(400mg)	0.77±0.05[a]	0.47±0.03[a]	1.25±0.09[a]	25.12±1.91[a]	1.00±0.07[d]	0.56±0.04[a]	1.08±0.08[a]	3.28±0.25[a]
Nerolidol (400mg)	0.75±0.05[a]	0.45±0.03[a]	1.30±0.09[a]	25.00±1.90[a]	0.88±0.06[a]	0.53±0.04[a]	1.11±0.08[a]	3.45±0.26[a]

Cyt p450 – nmol/mg of microsomal protein.

Cyt b5 – nmol/mg of microsomal protein.

GST – µmol of CDNB-GSH conjugate formed/mg of microsomal protein/min.

GR – µmol of NADPH oxidized/mg of microsomal protein/min.

Values are expressed as mean ± SD for eight animals in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

Serum lipids and lipoproteins are associated with breast cancer [26].Cell proliferations of breast tissue have been linked with changes in serum TC and TG levels in breast cancer. In our study, we observed increased levels of TC and TG in DMBA-induced experimental rats. The increased level of cholesterol deposited in breast epithelial tissues which cause the production of free radicals due to oxidative stress and hypercholesterolemia. Similar results were observed in plasma. The decreased PL concentration may also be due to the decreased FFA level in mammary tissue [44].It has been suggested that HDL-cholesterol prevents both enzymatic and non-enzymatic generation of O_2 , H_2O_2 and OH^- [11]**.** Moreover, the effective dose of nerolidol treated with DMBA induced mammary carcinogenic rats are control to the serum lipids and lipoproteins level. Arroyo-Acevedo et al [3]. Reported that the possible protective effect of *Piper aduncum*capsule on DMBA (dimethylbenz[α]anthracene)-induced breast cancer in rats was assessed lipid-lowering effect.P. *auduncum*capsule has been hypolipidemic effect and antigenotoxic properties. Then the report is compared to the above the results prove that nerolidol also anti-lipedemic activity against DMBA-induced mammary carcinogenesis.

Histopathological changes in mammary tissue

Fig.2. shows the histopathological analysis of mammary tissues of control and experimental animals. The group I (A) controls and group VI (F) Nerolidol alone treated animals' exhibit normal architecture of mammary tissues. In contrast, group II (B) DMBA-induced animals showed infiltrating malignant tumor. The group III (C) nerolidol (100 mg/kg bw) treated animals showed fibrosis and mild tumor infiltration. The group IV (D) nerolidol (200 mg/kg bw) treated animals

showed almost normal architecture of mammary tissue with increased area of fibrosis. The group V (E) nerolidol (400 mg/kg bw) treated animals showed extensive areas of fibrosis.

Histopathological analysis of mammary tissues

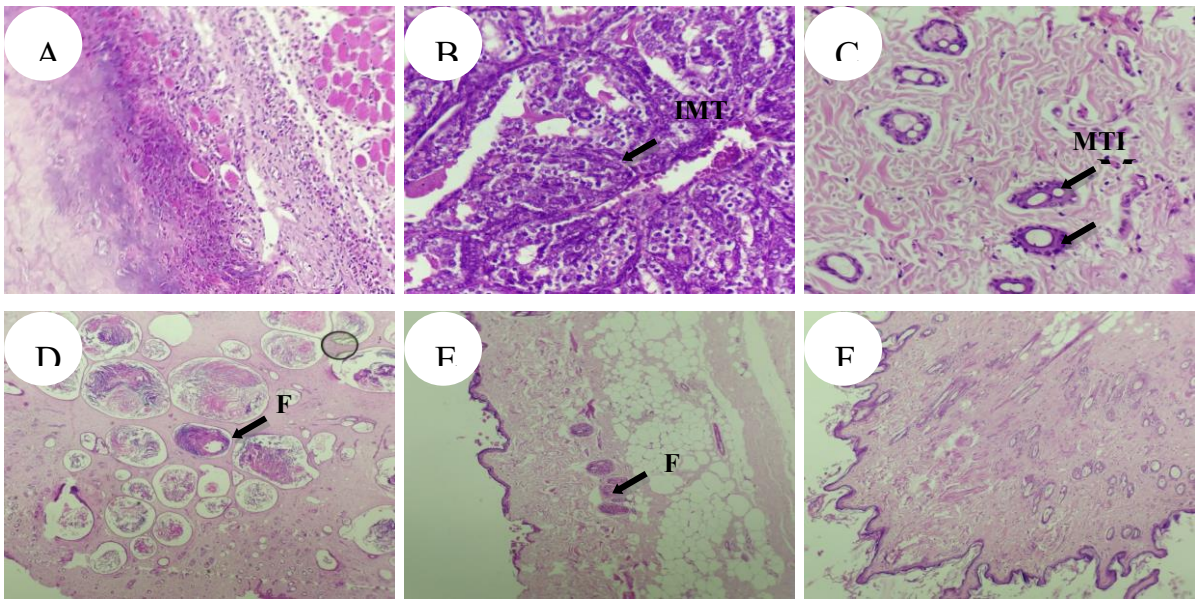

Figure 2: (A–F) Shows the histopathological analysis of mammary tissues of control and experimental animals (hematoxylin and eosin staining). The group I (A) control and group VI (F) Nerolidol alone treated animals showed normal architecture of mammary tissues. Group II (B) DMBA-induced cancer bearing animals showed infiltrating malignant tumor. The group III (C) Nerolidol (100 mg/kg bw) treated animals showed fibrosis and mild tumor infiltration. The group IV (D) Nerolidol (200 mg/kg bw) treated animals showed almost normal architecture of mammary tissue with increased area of fibrosis. The group V (E) Nerolidol (400 mg/kg bw)treated animals showed extensive areas of fibrosis. Arrows indicate (IMT) infiltrating malignant tumor, (MTI) mild tumor infiltration and (F) fibrosis.

Histopathological examination of mammary tissue of cancer bearing animals showed carcinomas exhibited infiltrating malignant tumor. In contrast, nerolidol treated rats revealed no sign of cellular proliferation and necrosis. The more pronounced effective dose dependently in nerolidol treated animals by the normal architecture of mammary tissue with increased area of fibrosis [39].Therefore, nerolidol has the potential to be a safe and effective in cancer treatment and also proves the anti-neoplastic activity of nerolidol.

Histopathological changes of liver tissue

Fig.2. shows thehistopathological analysis of liver tissues of control and experimental animals. The group I (A) control and group VI (F) nerolidol alone treated animals showed normal architecture of hepatocytes. In contrast, group II (B) DMBA-induced cancer bearing animals showed dilated sinusoids along with loss of architecture. The group III (C) nerolidol (100 mg/kg bw) treated animals showed vacuolar degeneration of hepatocytes. The group IV (D) nerolidol (200 mg/kg bw) treated

animals showed almost normal architecture of hepatocytes with dilated sinusoids and mild necrosis. The group V (E) nerolidol (400 mg/kg bw) treated animals showed markedly dilated sinusoids.

Histopathological analysis of liver tissues

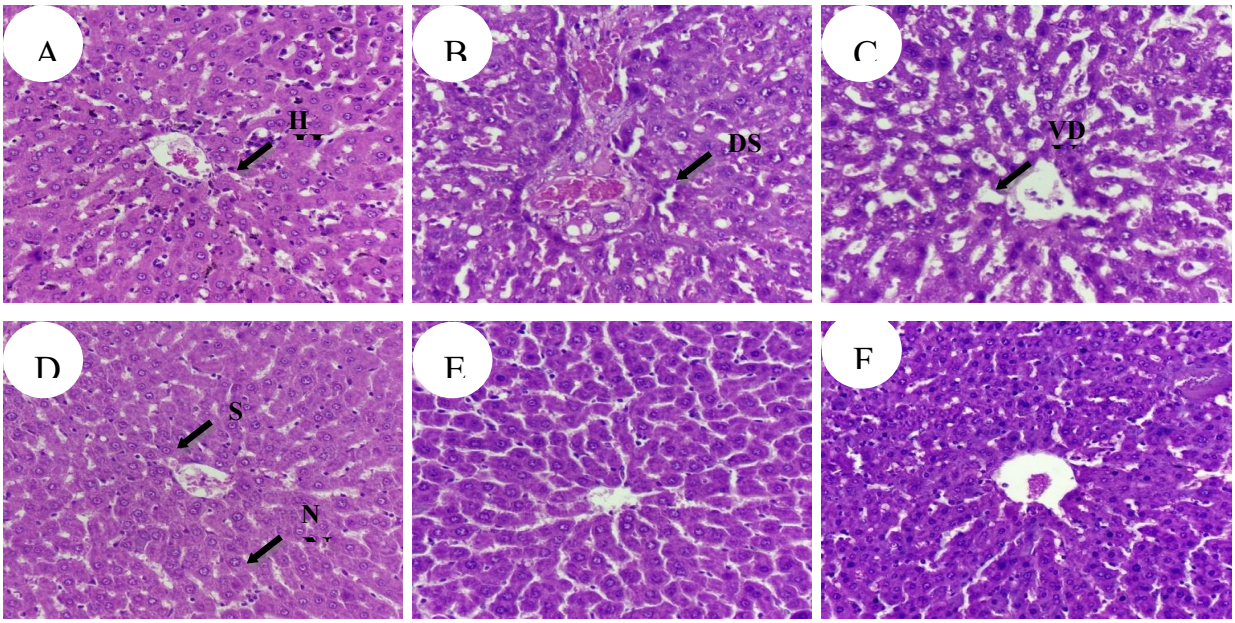

Figure 3: (A–F) Shows the histopathological analysis of liver tissues of control and experimental animals (hematoxylin and eosin staining). The group I (A) control and group VI (F) Nerolidol alone treated animals showed normal architecture of hepatocytes. Group II (B) DMBA-induced cancer bearing animals showed dilated sinusoids along with loss of architecture. The group III (C) Nerolidol (100 mg/kg bw) treated animals showed vacuolar degeneration of hepatocytes. The group IV (D) Nerolidol (200 mg/kg bw) treated animals showed almost normal architecture of hepatocytes with dilated sinusoids and mild necrosis. The group V (E) Nerolidol (400 mg/kg bw) treated animals showed markedly dilated sinusoids. Arrows indicate (H) hepatocytes, (DS) dilated sinusoids, (VD) vacuolar degeneration, (S) sinusoids and (N) necrosis.

Histopathological examination of liver tissue of cancer bearing animals showed with loss of architecture. These changes might be due to the free radical generation by the carcinogen. In the contrary, nerolidol treated animals, resolved these changes might be the free radical scavenging antioxidant properties of this compound, the more pronounced effective dose dependently in nerolidol treated animals by the normal architecture of hepatocytes with dilated sinusoids and mild necrosis. Therefore, it suggests that the nerolidol has the potential to protect liver from carcinogen.

4. CONCLUSION

Based on the present findings, it may be concludes that the administration of nerolidol effectively inhibits DMBA induced mammary cancer. Nerolidol relies on its anti-lipid peroxidative and antioxidant function as well as modulatory effects on phase I and II detoxification enzymes to excrete the carcinogenic metabolites. Biochemical findings are supported by histopathological studies of liver and mammary tissues. However, further study is required at the molecular level to

elucidate the putative mechanism of nerolidol in DMBA-induced mammary carcinogenesis.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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