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Histopathological study of the protective effect of vitamin E against DMBA induced mammary cancer in rats

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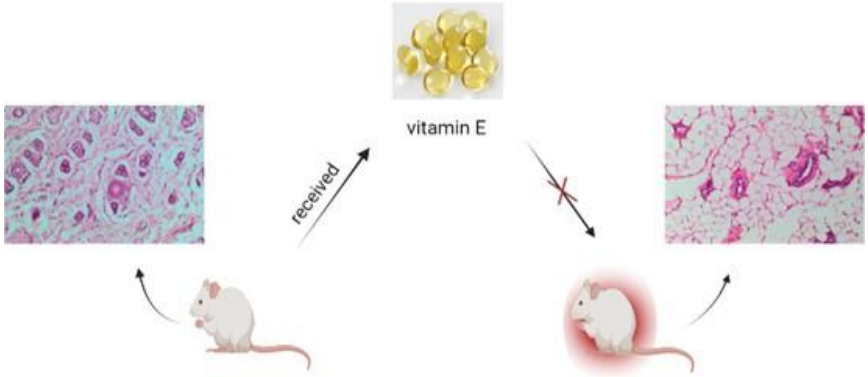
Graphical Abstract



Abstract

In our study, estimation of the preventive role of vitamin E against DMBA induced mammary cancer in rats was achieved. Forty albino rats were divided into four equal groups. The first group was normal group; second group was administered Vit E (100mg/Kg B.W/d) by gastric tube; cancer was induced in third group by DMBA (50 mg/kg B.W); fourth group administered DMBA + Vit E. Our results revealed that cancer induced by DMBA significantly reduced the body weights and CAT activity of rats, increased MDA levels & altered the histopathological picture. Meanwhile vitamin E ameliorated these alterations. Our study revealed that vitamin E supplementation provided antioxidant properties & antitumor defense with strong preventive activity against DMBA-induced carcinogenesis.

Keywords: Breast cancer, Histopathology, DMBA, Vitamin E

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1. Introduction

Breast cancer (BC) is a common and frequently fatal malignant tumor, one of the most diagnosed invasive cancers in women worldwide and the second ranking cause of cancer death in the Eastern Mediterranean Region [1, 2]. Over 1.7 million women are diagnosed with breast cancer annually worldwide. In America, it is estimated that 30% of all new cancer cases (276,480) among women are breast cancer in 2020 [3].

According to WHO Global Cancer Country Profiles, BC is the most prevalent type among Egyptian women. It represents 16.4% of total cancer cases (32.4% in women and 2.2% in men) in 2020 [4]. Also, it has particularly cleared familial, societal, and economic consequences since it can transmit to other organs such as lung, bone, brain and liver which mainly explained its incurability [5].

Polycyclic aromatic hydrocarbons are potent carcinogens. Among these, 7,12-dimethylbenz[a]anthracene (DMBA) is the most widely used chemical carcinogens for the induction of mammary tumor in female rats as tumors appeared in these animals closely similar to those of breast cancer in human [6].

BC prevention remains challenging globally. Early diagnosis of breast cancer is one of the best approaches to prevent this disease. It also can lead to a good prognosis and a high survival rate [7, 8].

Supplementation with Vit E is shown to have strong chemopreventive effect against carcinogenesis [9]. Vitamin E is a lipid soluble supplement that characterized by a potent antioxidant effect on various chronic conditions as it inhibits free radicals from damaging DNA, cell membranes & protein [10]. Anticancer effects of vit E resulted from its antioxidant, antiproliferative, anti-inflammatory, immune modulatory mechanisms and anti-angiogenic properties where it is able to promote the production of antibodies and enhance the immunity of both animals & humans [10, 11]. This study was conducted to estimate the effect of vitamin E on the chemically hazardous damage of DMBA on breast tissues of rats and prove the ability of Vit E to reduce and prevent cancer formation.

2. Materials and Methods

2.1. Chemicals

7,12-dimethylbenz[a]anthracene (DMBA) & corn oil were purchased from Sigma Chemical Company, USA. Vitamin E was purchased from (Pharco-Egypt). Malondialdehyde & Catalase kits were obtained from Biovision (Cairo, Egypt). Other used chemicals were of analytical degree.

2.2. Animals

Forty female albino rats were 30 days old; which weighing 130 ± 10g. Animals were supplied by Pharco pharmaceutical Company, Egypt. They were kept in metallic cages (3/cage) in the ani- mal house of Faculty of Veterinary Medicine, Suez Canal University. All animals were subjected to natural day-light rhythm at relative humidity of 60 ± 5% and temperature of 25°C (±1°C) with free access to food and water. Rats were housed and cared in accordance to the ethical guidelines of Faculty of Veterinary Medicine, Suez Canal University.

2.3. Experimental design

After 5 days of acclimatization, rats were randomly divided into four equal groups (ten for each). The first group administered corn oil (0.2 mL/rat) by gastric tube, fed on basal diet according to Kilany et al. [12] and served as control. The second group administered Vit E (100mg/Kg B.W/d) by gastric tube dissolved in 4 mL corn oil as reported by Abdo et al. [13].The third group administered corn oil (0.2 mL/rat) by gastric tube and subcutaneously injection with a single dose of 50 mg/kg BW of DMBA to induce mammary tumor in rats of ~55 days of age as reported by Nguedia et al. [14]. The fourth group administered Vit E 21 days before DMBA injection & continued for 6 months.

2.4. Evaluation of body weight

Rats' initial body weights were recorded before starting the experiment. Then rats were weighed weekly till the end of the experimental period.

2.5. Estimation of oxidative stress biomarkers

Malondialdehyde (MDA) & Catalase (CAT) levels were detected in sera according to Nguedia et al. [14].

2.6. Examination of histopathology

At the end of the experimental period (six months), animals were anesthetized and sacrificed by decapitation. Then mammary tumors were removed and fixed in 10% formalin for histopathology examination according to Bancroft and Gamble [15].

2.7. Statistical analysis

The present results were expressed as mean± SE (n = 10) & analyzed using statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc., Chicago) for windows. One way ANOVA followed by Duncan test were used for analysis. $P<0.05$ was considered significant.

3. Results

3.1. Evaluation of body weight

Table 1 shows the body weight of control and DMBA treated rats. Rats' initial body weights demonstrated that there were no significant changes between normal control, Vit E control, DMBA treated rats and (DMBA + Vit E) treated rats. Finally, we observed significant ($P<0.05$) reduction in the final body weight & body weight gain of DMBA treated rats when compared to control & Vit E treated rats. Meanwhile, treatment with Vit E inhibited the progressive reduction caused by DMBA injection.

3.2. Estimation of oxidative stress biomarkers

In DMBA control group MDA level was increased significantly ($P<0.001$) (**Figure 1**) while CAT activity was significantly decreased (**Figure 2**) compared to normal control groups. Meanwhile, in (DMBA + Vit E) treated rats MDA level was significantly decreased & CAT activity was significantly increased compared to cancer group. In parallel lines, there were no significant difference observed between normal control and Vit E control rats.

3.3. Examination of histopathology

Mammary sections of control & Vit E con- trol groups showed breast tissues with normal epithelial cells of uniform architecture and histology, with complete absence of any lesion along with absence of abnormal cell proliferations or hyperplasia. While in DMBA group breast sections showed degeneration, abnormal cancer cell proliferations, necrosis and hemorrhage around the neoplastic tissue. Treatment with Vit E (DMBA + Vit E) decreased necrotic areas & neoplastic tissues, reduced inflammation & cellular proliferations therefore improving cellular architecture (**Figure 3**).

4. Discussion

In this study, data expressing body weight parameter of rats declared that there was a significant decrease in the final body weights & weight gain of rats in cancer control group (DMBA) when compared to those of normal control group. This decrement might be attributable to the mutations resulted from DMBA injection. Since DMBA changed the standard biochemical parameters of the animal body, altered antioxidants enzymes activities, increased reactive oxygen species (ROS) & lipid peroxidation which resulted in damaging the cellular structure and membrane of organelles including: DNA, lipids & proteins as represented by Rojas-Armas et al. [16]. This result agreed with Kinoshita et al. & Rojas-Armas et al. [16, 17].

While the final body weights & weight gain of rats treated with (DMBA + Vit E) were elevated and appeared to be near non treated control groups when compared to the data of DMBA control group. The result attributed to Vit E treatment that inhibited the body weight loss as it weakens ROS signals required for adipose tissue expansion due to its antioxidant activity. This result came in accordance with Abo-Elmaaty [18] who confirmed Vit E (100 mg/kg BW) preventive role against cisplatin-inducing acute kidney damage because of its ability to return body weights of rats to normal control values.

Table 1: Body weight changes in control and (DMBA +Vit E) treated animals.

Groups	Control	Vit E control	DMBA	Vit E + DMBA
Initial body weight	133.00 ± 7.00[a]	132.67 ± 7.79[a]	132.00 ± 4.36[a]	134.76 ± 7.31[a]
Final body weight	234.33 ± 6.69[a]	237.00 ± 7.75[a]	166.67 ± 10.33[b]	213.67 ± 13.64[a]
Body weight gain	101.33 ± 11.79[a]	104.33 ± 6.64[a]	34.67 ± 10.74[b]	79.75 ± 9.46[a]

Data are represented as mean± S.E (n = 10), Vit E (Vitamin E) and 7,12dimethylbenz[a] anthracene (DMBA), Means in the same row with different superscript letters are significantly different at (P<0.05).

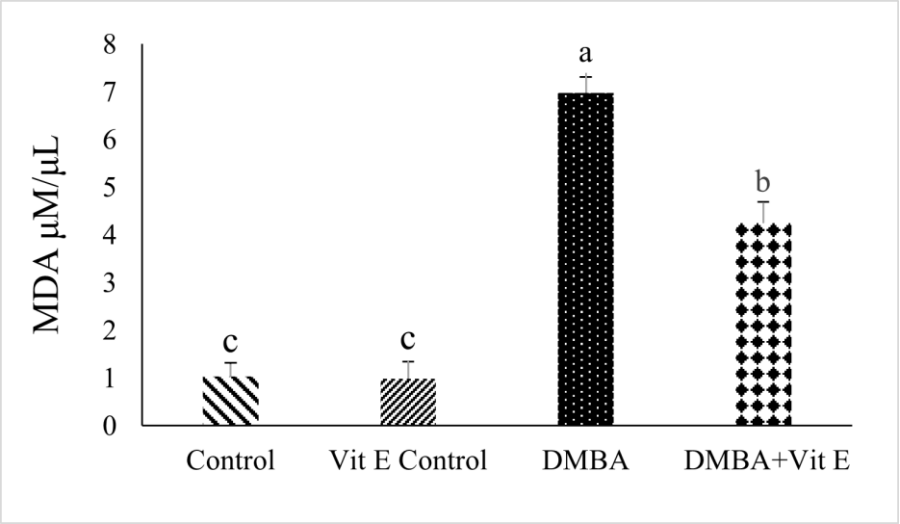

Figure 1: Effect of vitamin E on serum tumor marker MDA in normal and induced breast cancer female albino rats. Data represents the mean values±E from 10 rat/ group. Means with different superscript letters are significantly different at(P<0.001).

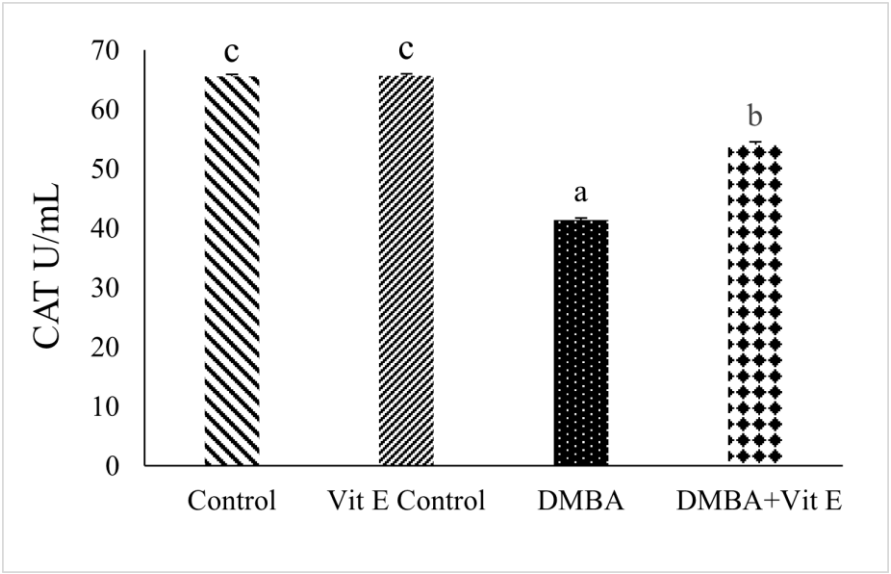

Figure 2: Effect of vitamin E on serum tumor marker CAT in normal and induced breast cancer female albino rats. Data represents the mean value ±S. E from 10 rat/ group. Means with different superscript letters are significantly different at(P<0.001).

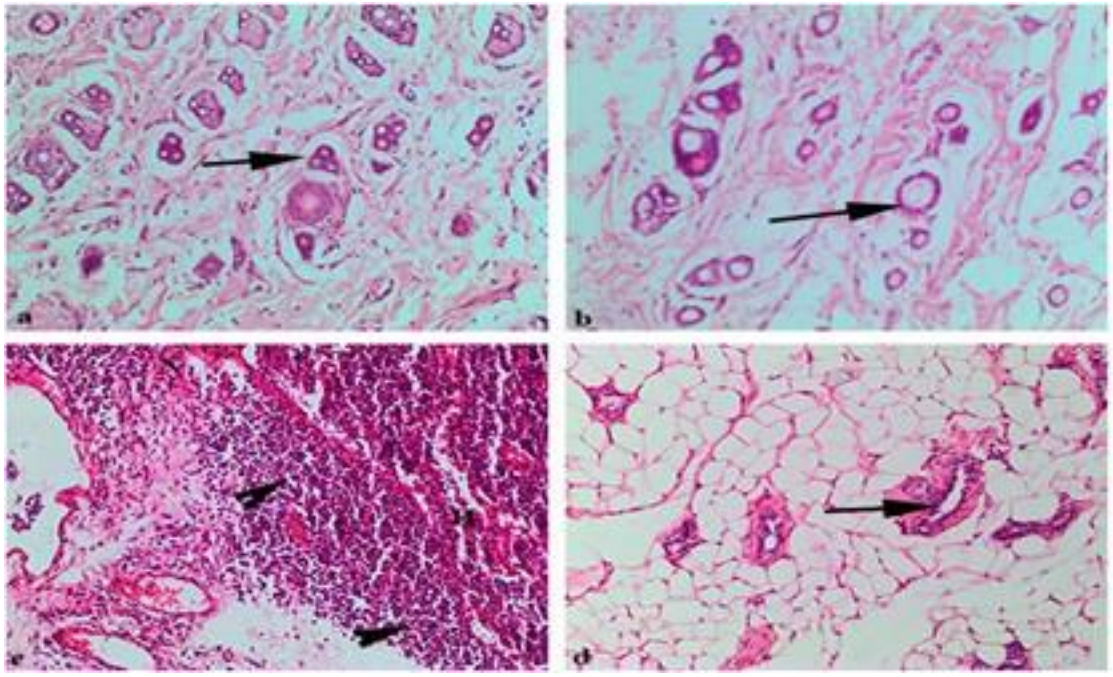

Figure 3: Histopathological profiles (H&E, X 200) of breast tissues from various groups. (a) & (b) Mammary sections of normal control group& Vit E control group, showing normal breast architecture with normal small glands and ducts (arrow)respectively. (c) Mammary sections of DMBA control group, showing infiltration of mammary tissue with neoplastic cells, necrosis(N) and hemorrhage (H). (d) Mammary sections of Vit E treated group, showing obvious reduction of inflammation, neoplastic tissues & cellular proliferations so improving cellular architecture.

The pathogenesis of various diseases is influenced by oxidative stress. MDA and CAT were measured in serum to see if Vit E could prevent breast cancer in albino rats caused by DMBA. Our study revealed that DMBA elevated MDA levels and decreased antioxidant activity (CAT) when compared to control and Vit E treated groups. This alteration is due to the increased formation of ROS, which caused damage to a variety of biomolecules and had a variety of molecular and cellular effects, including cytotoxicity and mutagenicity, which can lead to cancer onset and progression. This result came in accordance with Nguedia et al. [14].

Meanwhile, Administration of Vit E with DMBA significantly improved MDA levels & CAT activity compared to DMBA treated rats. As Vit E restored antioxidant functions. This improvement was due to the antioxidant, anti-inflammatory and immune modulatory mechanisms of Vit E. This result agreed with Abo-Elmaaty [18].

Our findings revealed that DMBA injection in rats caused substantial alterations in the breast tissues of cancer control rats. In the third group (DMBA) breast sections showed degeneration, abnormal cancer cell proliferations necrosis and hemorrhage around the neoplastic tissue. As DMBA alters the normal process by which mammary gland differentiated. This result agreed with Nguedia et al., Rojas-Armas et al. &Mehraban et al. [14, 16, 19].

Mammary tissues of Vit E treatment group (DMBA + Vit E) revealed higher reduction in necrotic areas & neoplastic tissues. Also, it decreased inflammation & cellular proliferations therefore improving cellular architecture in contrast with DMBA group. This result might be attributed to stopping the action of estrogen. This result came in accordance with Nadia et al. [20].

5. Conclusion

From our study we concluded that administration of vitamin E is very important for protection

of different body tissue, especially breasttissue, against cancer or even inflammation. As it characterized by antioxidant properties and antitumor defense.

Abbreviations

Analysis of variance: ANOVA; Breast cancer: BC; Body weight: B.W; Catalase: CAT; 7,12dimethylbenz[a]anthracene: DMBA; Day: d; Gram: gm; Hematoxylin and eosin: H&E; kilogram: kg; Malondialdehyde: MDA; Milliliter: mL; Probability: P; reactive oxygen species: ROS; Standard error of the mean: SE; Statistical Package for Social Sciences: SPSS; vitamin E: Vit E.

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