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# EVALUATION OF THE EFFECT OF AQUADEKS SUPPLEMENT VERSUS SINGLE VITAMINS ON OXIDATIVE STRESS

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## ABSTRACT

Breast cancer is a very important epidemiologic problem and the most common cause of death in women. Oxidative stress play an important role in this disease. Antioxidants such as A, C, E, K, D vitamins have drawn the attention of many scientists for reducing oxidative stress. AquADEKs is one of the antioxidant supplements. The purpose of this study was to investigate the effect this supplement in reducing oxidative stress in breast cancer.

In this applied research, 38 BALB-C mice were divided into 4 groups. In the sera of the mice, the levels of catalase, superoxide dismutase, and total antioxidant capacity were measured by the Elisa method. Data analysis was performed using ANOVA and Graphpad.

The catalase level significantly increased in mice that received vitamin supplements and were implanted with cancer cells (P = 0.049) this relationship was not significant with vitamin D. Superoxide  dismutase levels significantly increased in the mice that received vitamin supplements and were implanted with cancer cells (P = 0.024) but this relationship was not significant with vitamin D. The total antioxidant capacity increased significantly in mice that received vitamin supplements and were implanted with breast cancer cells (P < 0.001) and

this relationship was significant with vitamin D.

The use of AquADEKs as supplement had a significant correlation with the levels of catalase, superoxide dismutase, and total antioxidant capacity. Antioxidant markers can be improved with the use of AquADEKs if this supplement is imported and available in market.

## INTRODUCTION

Breast cancer is a very important epidemiologic problem worldwide and the most common cause of death in women. This disease develops due to malignancy in breast duct cells and lobules [1,2]. Different genetic and environmental factors play a role in the development of breast cancer. According to the National Health Institute of USA, 1 in every 8 women will develop breast cancer at some time during her life [3]. According to different studies in this regard, the age of the development of breast cancer is about 5 years lower in Iran with 10,000 new cases annually. This disease is the 5th cause of cancer death among Iranian women, and comprises the highest percentage of mortality after gastric cancer and leukemias [4].

In general, oxidative stress and lipid peroxidation have a prominent role in carcinogenic compounds. With an increase in free radicals resulting from oxidative stress, the chance of breast cancer increases, especially in individuals receiving chemotherapy [5,6]. Oxidants are formed in response to both endogenous and exogenous processes and are capable of destructing important cellular macromolecules and DNA. Antioxidants neutralize the negative effects of free radicals in different ways; in fact, an imbalance between oxidation-antioxidants can damage the ductal and lobular cells of the breast, leading to an increase in the risk of breast cancer [7-9]. Antioxidants including vitamin A and its provitamins, vitamin C, E, K, D, selenium, zinc, etc. have drawn the attention of scientists to cope with oxidative stress in many diseases like diabetes, cystic fibrosis, cardiovascular diseases, and different cancers including breast cancer [10-12]. Previous studies did not evaluate the effect of the daily use of antioxidants as multivitamin supplements; they investigated the use of single vitamins like A/C/K/E/D3 and provitamin A and reported potential effects [13]. AquADEKs is an antioxidant supplement that is available in three forms of tablet, capsule, and oral drops. It is a combination of antioxidant vitamins and minerals like zinc and selenium. This supplement is capable of elevating antioxidant indexes in the serum and erythrocytes after 2-3 months

which in turn decreases free radicals resulting from oxidative stress and strengthens the body's resistance against cancers, cardiovascular problems, diabetes, and cystic fibrosis in children [14]. Since this supplement is a comprehensive source of antioxidant vitamins and minerals, which play a major role in balancing the levels of total plasma antioxidants and malondialdehyde, hydrogen peroxidase, glutathione (GSH), superoxide dismutase, catalase, etc., it was a valuable source for our study to make a relative comparison with single vitamins used in previous studies. A study in 2011 evaluated the effect of the use of this multivitamin in children suffering from cystic fibrosis and reported a balance in the antioxidants levels and improved pulmonary function [15,16]. Since very few studies have evaluated the effect of the daily administration of these antioxidants for the prevention of and fighting against breast cancer in Iran, we found it necessary to conduct a study to investigate the important effects of these antioxidant supplements on the reduction of oxidative stress in breast cancer. To the best of our knowledge, no study has been performed to assess the effect of the daily use of AquADEKs and other complements on the prevention or treatment of breast cancer. The aim of this study was to investigate the levels of antioxidant indexes in female mice with breast cancer that

received this multivitamin supplement and to compare them with mice that received A/D3/ E single vitamins at defined doses daily.

## MATERIALS AND METHODS

In this applied research, 38 BALB-C mice weighing 18-20gr were used as animal models. The mice were provided from Pasteur Institute, Karaj, Iran. The animals were kept at $23 \pm 2°C$ in light cycles of 12:12 hours. The mice were fed condensed rodent food (Dam Pars Co., Tehran, Iran). The mice underwent research after 1 week. The mice were divided into 4 main groups. In the first group, 16 mice were subdivided into four groups.

–       Four mice received AquADEKs for 1 month. AquADEKs was purchased from AmericaRX Co. Each vial of AquADEKs contained 60ml of the supplement; 400µL of the solution was dissolved in 10cc distilled water and 100µL of the solution was fed to each mouse through gavage every day.

–       Four mice received vitamin A for 1 month (Osveh Pharmaceutical Company, Iran); 300µL of vitamin A was dissolved in 10mL olive oil and each mouse received 100µL through gavage every day. Two mice died during the research.

–       Four mice received vitamin E for 1 month (Osveh Pharmaceutical Company, Iran); 25µL of vitamin E was dissolved in

10mL water and each mouse received 100µL through gavage every day. One mouse died during the research.

– Four mice received vitamin D3 for 1 month (Osveh Pharmaceutical Company, Iran); 300µL of vitamin D3 was dissolved in 10mL olive oil and each mouse received 100µL through gavage every day. Two mice died during the research.

After one month, all mice in the four subgroups were implanted with breast cancer cells through the cell implant method [17]. In this method, one BALB-C mouse with breast cancer was selected and after confirmation of the tumor and the spontaneous model of cancer, the tumor was excised in a sterile fashion and placed in sterile physiological solution. The tumor was cut into 3mm$^3$ pieces in the solution. After anesthetizing the mice with intraperitoneal injection of ketamine (10mg/kg) and xylene (5mg/kg), the prepared pieces were transplanted subcutaneously in the left flank of the mice and the site of surgery was sutured using special clips [18]. After 3 weeks, the tumor could be visualized and was palpable and the mice gained 2 gr. After visualizing the tumor, the administration of the vitamins and supplement continued for one month. Then, blood samples were taken via cardiac puncture and their sera were separated and stored at -70ºC. In total, 5 mice died in this group and 11 mice entered the next stage.

In the second groups, the mice were subdivided into 4 groups as group 1. After the animals spent 1 week in the animal shelter with no administration of vitamin supplement, the mice were injected with 4T1breast cancer cells [19].

At this time, A, E, and AquADEKs supplements started for the breast cancer-afflicted mice. After 2 months, blood samples were collected through cardiac puncture and their sera were separated and stored at -70ºC.

In the third group, 11 mice received vitamin supplements for 2 months; then, after 2 months, blood samples were collected through cardiac puncture and their sera were separated and stored at -70ºC.

The level of catalase was measured using an ELISA kit (Cat. No. CKE 90105) purchased from HANGZHOU EAST BIOPHARM Company.

The kit uses a double – antibody sandwich enzyme – linked immunosorbent assay (ELISA) to assay the level of Human Catalase.

Catalase was added to the monoclonal antibody enzyme well which was pre-coated with monoclonal antibody and incubation was performed.

Then Catalase antibodies labeled with biotin was added and combined with streptavidin-horseradish peroxidase (streptavidin-HRP) to form immune

complex, then carry out incubation and washing again to remove the uncombined enzyme. Then Chromogen solution A, B were add, the color of the liquid changes into the blue, and at the effect of acid, color finally becomes yellow.

The level of superoxide dismutase was measured using a kit (Cat. No. BYEK1111) purchased from Biospes Company.

This kit was based on standard sandwich enzyme-linked immune-sorbent assay technology.

The purified anti-superoxide dismutase (anti-SOD) antibody was pre-coated onto 96-well plates. And the HRP conjugated anti-SOD antibody was used as detection antibodies. The standards, test samples and HRP conjugates detection antibody were added to the wells subsequently, mixed and incubated, then, unbound conjugates were washed away with wash buffer. Tetramethyl-benzidine (TMB) substrates (A & B) were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow is proportional to the SOD amount of sample captured in plate. Read the O.D. absorbance at 450 nm in a microplate reader, and then the concentration of SOD can be calculated.

Total antioxidant capacity (TAOC) was measured using a kit (Cat. No. DM P-4100) purchased from LDN Company (Labor Diagnostika Nord GmBH & Co. KG).

The determination of the total antioxidative capacity is based on the reaction of peroxides with peroxidase followed by a colour reaction of the chromogenic substrate tetramethyl benzidine. Its blue colour turns to yellow after addition of the stop solution and can be measured photometrically at 450 nm.

One way analysis of variance and Tukey's test were used for statistical analysis. P values less than 0.05 were considered significant.

## RESULTS

The levels of catalase and SOD, and TAOC were evaluated in the four groups of mice.

In the first group, 16 mice were divided into 4 subgroups:

– In the first subgroup, the mice were implanted with breast cancer cells after receiving AquADEKs for 1 month

– In the second subgroup, the mice were implanted with breast cancer cells after receiving vitamin D for 1 month

– In the third subgroup, the mice were implanted with breast cancer cells after receiving vitamin E for 1 month

– In the fourth subgroup, the mice were implanted with breast cancer cells after receiving vitamin A for 1 month

In the second group, the mice were divided into similar subgroups but received vitamin

supplements after developing breast cancer through the injection of breast cancer cell.

The mice in the third group received vitamin supplements but were not injected or implanted with breast cancer cells.

The mice in the fourth group, as the control group, received no supplement and were not implanted or injected with breast cancer cells.

Statistical analysis showed a significant relationship between TAOC and vitamin administration for 1 month before developing the disease for preventive purposes ($P < 0.05$) while the relationship was not significant in the second group when the mice received vitamin after developing cancer. It is obvious that the relationship between TAOC and vitamin administration ($P < 0.05$) (Table 1).

The relationship between catalase activity and vitamin administration for 1 month before developing the disease for prevention was significant ($P < 0.05$). Moreover, the relationship between catalase activity and vitamin use in the second group in which the mice received vitamin after developing breast cancer was also significant ($P < 0.05$) (Table 2).

The relationship between SOD activity and vitamin use for 1 month before developing the disease for preventive purposes (first group) was significant ($P < 0.05$). The relationship between SOD activity and vitamin use was not significant in the remaining three groups (Table 3).

The results showed that TAOC significantly correlated with the use of AquADEKs and vitamin D3 and E among all 4 groups ($P < 0.05$) (Table 4). Catalase activity also significantly correlated with the use of AquADEKs and vitamin E in all 4 groups ($P < 0.05$) but the relationship was not significant for vitamin A and D3 (Table 5).

This study showed that SOD activity had a significant correlation with the use of AquADEKs and vitamin D3 and E in all four groups ($P < 0.05$) while the relationship was not significant for vitamin A (Table 6).

**Table 1: Total antioxidant capacity in the four groups of mice with receiving 4 vitamins (A, E, D3, and AquADEKs) (mmol/L)**

| Group | Mean | Std. Deviation | Mean square | F | Sig |
|---|---|---|---|---|---|
| Group 1 | 0.6636 | 0.4272 | 0.557 | 42.899 | P < 0.001 |
| Group 2 | 1.5 | 0.503 | 0.072 | 0.217 | P = 0.882 |
| Group 3 | 1.009 | 0.8859 | 2.520 | 60.820 | P < 0.001 |
| Group 4 | 0.3182 | 0.0751 | 0.002 | 0.297 | P = 0.827 |

The difference is significant according to the statistical analysis ($P < 0.05$)

**Table 2: The level of catalase activity in the four groups of mice with receiving 4 vitamins (A, E, D3, and AquADEKs) (KU/L)**

| Group | Mean | Std. Deviation | Mean square | F | Sig |
|---|---|---|---|---|---|
| Group 1 | 92.3889 | 13.3657 | 638.891 | 25.32 | P = 0.001 |
| Group 2 | 76.4556 | 34.7947 | 3651.949 | 9.201 | P = 0.015 |
| Group 3 | 70.7222 | 17.65098 | 120.247 | 0.320 | P = 0.738 |
| Group 4 | 80.5333 | 3.2035 | 6.775 | 0.593 | P = 0.582 |

The difference is significant according to the statistical analysis ($P < 0.05$)

**Table 3: The level of SOD activity in the four groups of mice with receiving 4 vitamins (A, E, D3, and AquADEKs) (U/ml)**

| Group | Mean | Std. Deviation | Mean square | F | Sig |
|---|---|---|---|---|---|
| Group 1 | 4.4182 | 0.5913 | 0.861 | 6.591 | P = 0.019 |
| Group 2 | 5.4091 | 1.2193 | 2.871 | 3.213 | P = 0.092 |
| Group 3 | 52.455 | 0.7594 | 1.031 | 2.699 | P = 0.126 |
| Group 4 | 4.2273 | 0.4076 | 0.095 | 0.482 | P = 0.705 |

The difference is significant according to the statistical analysis (P < 0.05)

**Table 4: Total antioxidant capacity in the four groups of mice according to the vitamins (A, E, D3, and AquADEKs) (mmol/L)**

| Groups | Mean | Std. Deviation | Mean square | F | Sig |
|---|---|---|---|---|---|
| AQ | 0.6750 | 0.5272 | 1.302 | 58.943 | P < 0.001 |
| $D_3$ | 1.15 | 0.8053 | 1.4300 | 22.880 | P = 0.006 |
| E | 0.6167 | 0.4764 | 0.721 | 17.307 | P = 0.001 |
| A | 1.3750 | 0.8795 | 1.168 | 2.447 | P = 0.204 |

The difference is significant according to the statistical analysis (P < 0.05)

**Table 5: The level of catalase activity in the four groups of mice according to the vitamins (A, E, D3, and AquADEKs) (KU/L)**

| Groups | Mean | Std. Deviation | Mean square | F | Sig |
|---|---|---|---|---|---|
| AQ | 76.9438 | 21.1951 | 1049.937 | 3.511 | P = 0.049 |
| A | --- | --- | --- | --- | --- |
| $D_3$ | 70.050 | 19.7183 | 520.623 | 1.796 | P = 0.287 |
| E | 90.7833 | 19.4557 | 1352.963 | 103.194 | P < 0.001 |

The difference is significant according to the statistical analysis (P < 0.05)

**Table 6: The level of SOD activity in the four groups of mice according to the vitamins (A, E, D3, and AquADEKs) (U/ml)**

| Groups | Mean | Std. Deviation | Mean square | F | Sig |
|---|---|---|---|---|---|
| AQ | 1937.5 | 1.0981 | 3.186 | 4.480 | P = 0.025 |
| $D_3$ | 4.8625 | 0.8165 | 1.458 | 19.120 | P = 0.008 |
| E | 4.5750 | 0.56749 | 0.990 | 13.810 | P = 0.002 |
| A | 4.4250 | 0.9794 | 1.635 | 3.613 | P = 0.123 |

The difference is significant according to the statistical analysis (P < 0.05)

## DISCUSSION

Breast cancer is the most common cancer in women in developing countries and its mortality and incidence is increasing in developing countries [20]. The increased mortality in developing countries can be due to diverse factors such as population growth, age, lifestyle changes, and immigration (urbanization) [21]. Twenty-year forecasts for the increased prevalence and mortality of breast cancer in developing and developed parts of the world shows that the WHO should provide more support for research on primary prevention of cancer and development of effective strategies like factors related to nutrition [22].

In the past decades, low levels of vitamin D were regarded as a potential risk factor for different malignancies [23,24] including colorectal [25] and breast cancer [26]. The level of 25-hydroxy vitamin D is considered the best biomarker because of its association with nutrition and sunlight [27]. Studies have shown that 1,25 dihydroxy vitamin D, the active form of vitamin D, can inhibit the proliferation of cancer cells and angiogenesis and induce apoptosis [28].

Maalmi et al reported that vitamin D levels were associated with the survival of the patients with breast cancer. Increased

vitamin D concentrations significantly correlated with longer survival of the patients [29]. Shao et al also showed that vitamin D deficiency was a risk factor for breast cancer [30]. The available information on the levels of vitamin D does not confirm the relationship between this metabolite and breast cancer [31].

Our investigation on vitamin D was performed on 4 mice in each group of which 2 died during the research. The results showed that vitamin D administration to the mouse, as a laboratory model, resulted in a significant increase in SOD (P = 0.008) (Table 6) and TAOC (P < 0.006) (Table 4) while it had no significant correlation with catalase (Table 5).

Vitamin E, or tocopherol, is known as lipid oxidation inhibitors [32]. Its antioxidant properties are due to the phenolic hydrogens in the chromanol ring that are donated to lipid free radicals [33].

α-tocopherol is a better antioxidant than other tocopherols and has a higher capacity than γ-tocopherol and δ-tocopherol [34]. The biological effects of α-tocopherol have been under investigation for a long time but the current concept regarding its role in the inhibition of breast carcinogenesis is not yet complete [35].

Many studies have investigated the relationship between vitamin E and cancers. Eleven studies suggest that vitamin E decreases the risk of cancer [36-46] while some studies have failed to find a relationship between the occurrence of breast cancer and vitamin E [47-53].

In our study, the levels of catalase (P < 0.001) (Table 5) and SOD (P = 0.002) showed a significant relationship with vitamin E. Moreover, TAOC was also significantly associated with vitamin E (P = 0.001) (Table 4). Studies have shown that the use of carotenoids and the level of retinol have no relationship with the risk of breast cancer [54-56]. We also found no significant relationship between vitamin A and the level of SOD and TAOC.

Studies show that oxidative stress plays a role in the pathophysiology of all cancers. Although Sener et al reported no significant difference in lipid hydroperoxide levels between patients with breast cancer and controls, the available information shows that a lower TAOC, i.e. oxidative stress, can be associated with breast cancer [57].

According to our study, TAOC increased significantly when vitamin supplements started before developing breast cancer for prevention (Table 1). The increase was also observed following the administration of AquADEKs and vitamin E, separately (Table 4).

Moreover, we noted that the catalase activity increased significantly when vitamins were administered before the development of breast cancer (Table 2). The increase was also detected following

the administration of AquADEKs and Vitamin E, separately (Table 5).

The activity of SOD increased significantly when vitamin supplements started before developing breast cancer for prevention (Table 3). The increase was also observed following the administration of AquADEKs, vitamin D3, and vitamin E, separately (Table 6). TAOC and SOD activity had no significant correlation with vitamin A administration. Our study showed that the preventive use of AquADEKs could increase TAOC and catalase and SOD activity. However, the administration of vitamin E as an endogenous antioxidant has the same effect.

## CONCLUSION

The administration of vitamin E and D may have a significant relationship with oxidative stress markers and their increase and also with TAOC. After producing AquADEKs in Iran or importing this supplement, it can be used to markedly increase antioxidant markers in the body before developing cancer to achieve desirable preventive or therapeutic results.

## ACKNOWLEDGEMENTS

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