Original Article

Pathological Effects of High Dose of Acrylamide on The Mammary Glands of Female Albino Rats and The Possible Protective Role of Vitamin E (Histological and Immunohistochemical Study)

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ABSTRACT

Introduction: Acrylamide (ACR) was discovered in foods in 2002, mainly in starchy foods such as potato chips and bread that had been heated higher than 120 °C . It was not found in food that had been boiled or in non heated food. Vitamin E is a fat-soluble vitamin, its main role is to act as an antioxidant. It also enhances immune function and Vitamin E has the ability to protect cells from free radical damage

Aim of the Work: To study the possible protective role of Vitamin E in Acrylamide induced mammary gland damage in female albino rats through histological and immunohistochemical studies.

Materials and Methods: Forty-five female virgin albino rats weighing 180-250g were used in the study and were subdivided into three groups: Control group (given saline); Acrylamide group (given 10 mg/kg of acrylamide) by oral gavage for 3weeks; Acrylamide + Vitamin E group (given the same dose of acrylamide + vitamin E orally at a dose of 100 mg/kg) for 3 weeks. Animals of the three groups were sacrificed and mammary glands were excised for H&E, Masson Trichrome and Immunohistochemicalstudies.

Results: Rats received acrylamide showed altered general attitude, generalized malaise with diminished activity up to complete hind limb paralysis these results ameliorated with Vit E administration. ACR-treated mammary glands showed pathological changes in the form of dilated alveolar ducts with pizzar arranged epithelial cells and dark pyknotic nuclei, hypertrophied alveoi with increased collagen deposition fibers found mainly around the ducts and alveoli, there wereincreased in the CASPS-3 expression and expression of the pancytokeratins. VitEadministration appeared to decreaseallthese resultsas seen with histological and Immunohistochemicalstudies.

Conclusions: Addition of vitamin E supplements can produce less damage of mammary glands treated with high dose of Acrylamide.

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Key Words: Acrylamide; mammary; vitamin E.

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INTRODUCTION

The anatomy and histology of the mammary gland reflects its function to synthesize and secrete milk to the newborn offspring, and as the parenchyma undergoes a majority of growth and development postpubertally, it is a good field to be performed in researches in the juvenile or adult. Mammary gland also is a target of viral, chemical, and physical carcinogens and being a good model for neoplastic development[1].

The Swedish National Food Administration (SNFA) said that prolonged heating of starchy foods can create large amounts of Acrylamide[2]. Acrylamide does not present in raw foods and formed during the heating process above 120°C or[3]. Acrylamide was found to be harmful of the male and female genital systems in rodents and humans[4]. It was indicated that such effects of ACRis due to oxidative stress, hence occurred infertility[5].

To antagonize oxidative stress, antioxidants and plant phenolics are used as the chemo protective agents against oxidative stress-dependent diseases; VitE (Anti-infertility Vitamin)is a powerful antioxidant, capable of protecting the cells and tissues againstoxidative stress-induced damage through increasing the antioxidant capacity by antagonizing free radicals[6].

Vit E also has a beneficial effects on the general health due to its anti-inflammatory, anti-thrombotic, and anti-hypertensive effects[7].

AIM OF THE WORK

Study toxic effects of Acrylamide on the mammary gland of female albino rats andthe Potential protective effects of Vitamin E through histological and immunohistochemicalstudies.

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MATERIALS AND METHODS

Materials

Acrylamide was purchased from El Gomhorya Company, Egypt as white powder and was dissolved in distilled water in a concentration of100mg/10ml and given according to the weight of the animal.

Vitamin E was purchased as soft gelatin capsules from pharco pharmaceuticals Company, Egypt. Each capsule contained 400 mg of vitamin E, each capsule was dissolved in 4 ml olive oil.

Animals

Forty-five adult virgin female rats weighed 180-250 gm were used in the present study. All animals were housed under the same conditions and allowed food and water.

Ethical approval

The experiment was performed according to the "Guide for the Care and Use of Laboratory Animals" (Institutes of laboratory Animal Research)[8] and in accordance with the guidelinesof the Sohag University Animal Ethics and approved by Research Ethics Committee considering care and use of laboratory animals.

Rats were randomly divided into three equal groups.

- **Controlgroup:** received saline orally for 3 weeks.
- **Acrylamide-treated group:** were given ACR by oral gavage at a dosageof10 mg/kg/d,dissolved in distilled water, for 3 weeks[9]
- **ACR + Vit. E treated group:** were given ACR as previous group and vitamin E at a dose of 100 mg/kg body weight by oral gavagefor 3weeks[10].

At the end of the experiment the animals were sacrificed by cervical dislocation, mammary glands were dissected. Using hemostatic forceps, gently peel the abdominal and thoracic skin to the side of the rat, exposing the mammary glands attached to the underside of the skin. (mammary glands present along the mammary line which extend from the thoracic to the ingunal region). Using forceps, gently lift the inguinal mammary gland (most developed gland) and cut between the gland and the skin with small sharp scissors. Making sure no pieces of skin or muscle remain on the gland[11]. (Figure 1)

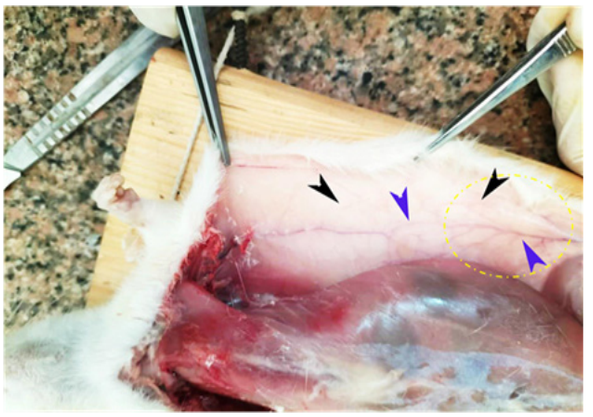


Fig. 1: Picture of the mammary gland of control female rat showing normal appearance and texture of the mammary line (black arrow heads) with normal vasculature of the gland along the milk line (blue arrow heads) , yellow ring referred to the inguinal mammary gland

The gland then subjected to the usual histological studies. Specimens were fixedin 10% formalin, processed and embedded in paraffin. Serial sections (5 microns) were prepared and subjectedtoHematoxylin and eosinandMassonTrichrome stains to detect histological changes and collagen fibres distribution[12].

Immunohistochemistry study

Mammary glands were prepared to detect immunohistochemical reactivity using a mouse monoclonal antibody (Lab Vision, USA) in appreciate dilution for CASPS-3 (detecting apoptosis)[13] and immunoreactivity for Pancytokeratins (PCKS) (detection of dysplasia)[14].

Morphometric methods

Detection of the mean Body weight before and at the end of the experiment and the density of collagen fibers in the three groups using massontrichrome stained sections (magnification X 100) , 5 fields per animal for 5 animals in the 3 groups using image J program (version:1.5.0)

Statistical analysis

Results were expressed as mean value ± Standard deviation. The data were statistically analyzed (SPSS: 16) using one-wayANOVA and post-hoc test when ANOVA was significant $P \leq 0.05$[15].

RESULTS

General observations

Control Group

Generally, rats receiving saline showed normal attitude, features and activity, dissection for organs extraction revealed normal gross anatomical appearance and vasculatureofthe mammary gland.

ACR treated Group

In this group, rats showed altered attitude, features and activity in the form of irritation followed by obvious malaise from the day following receiving the treatments, rats also showed diminished activity up to complete hind limb paralysis and ruffling of the fur with yellowish orange discoloration of the teeth with perioral redness(Figure 2).

Combined ACR +VIT E Group:Rats showed some sort of normal attitude, some weakness of the hind limb and some ruffling of the fur with yellowish orange discoloration of the teeth. (Figure 3).

Hematoxylin and eosin stain

Control Group (Figures 4,5)

The mammary gland showed scattered ducts embedded in abundant adipose tissue stroma with no capsule ,the ducts had a lumen lined with cubical epithelial cells surrounded by flat basal myoepithelial cells, all were surrounded by connective tissue sheets with Scattered few undeveloped alveoli were also seen within the section

ACR treated Group (Figures 6,7)

The mammary gland showed increased and dilated alveolar ducts with dark picknotic nuclei and detached epithelium in the lumen with thickened connective tissue sheaths, dilated and congested blood vessels, with disturbed fatty hyper cellular eosinophilicstroma with pleiomorphicfibroblasts, and hypertrophied alveoli lined by epithelial cells with dark piknotic nuclei,.

Combined ACR +VIT E Group (Figures 8,9)

The mammary gland showed moderately dilated ducts lined by arranged epithelial cells with dark piknotic nuclei with some ducts lumen containing detached dead cells,some surrounded by moderately thick connective tissue sheaths , mildly cellular eosinophilicstroma containing pleiomorphic fibroblasts with some ducts showed localized multilayered lining with dark nuclei

Masson Trichrome stain (MTS)

Control Group (Figure 10)

The mammary gland showed normal picture of green collagen fibers distributed around the ducts and around the blood vessels, with minimal distribution around the alveoli and in the stroma.

ACR treated Group (Figure 11)

The mammary gland showed increased collagen fibers deposition pronounced around the dilated ducts, dilated blood vessels and around the alveoli with massive generalized collagen fibers deposition in the stroma when compared to the control ones.

Combined ACR +VIT E Group (Figure 12)

The mammary gland showed a histological picture similar to that of the control groupin the form ofprominent collagen fibers appearing green in color surrounding the ducts, around blood vessels Also, there are collagen fibers within the stroma.

Immunohistochemical studies

(CASPS3)

Control Group (Figure 13)

The mammary gland showing positivity of ductal epithelium cells in the form of granular diffuse yellow brown cytoplasmic staining and with negative reaction of the connective tissue sheaths and of stroma cells in the form of blue coloration due to heamatoxilin contrast.

ACR treated Group (Figure 14):

The mammary gland showed strong granular brown positive immunostaining of the cytoplasm in epithelium of the widely dilated ducts and the hypertrophied alveoli with immunopositive secretions in the lumens of the ducts and the alveoli, positive immunostaining of the connective tissue sheaths surrounding. stromaalsoshowed positive immunostained many cells.

Combined ACR +VIT E Group (Figure 15):

The mammary gland showed brown positive immune-staining of the cytoplasm in the epithelium of the moderately dilated ducts with negative staining of the surrounding connective tissue , scattered positive cells in thealvoli, stroma showed scattered immunoposive cells in the.

Pancytokeratines (PCKs)

Control Group (Figure 16)

The mammary gland showing positivite reaction of ductal epithelial cells in the form of brown cytoplasmic staining with negative reaction of the connective tissue sheaths and of stroma cells in the form of blue colouration due to heamatoxiline contrast.

ACR treated Group (Figures 17,18)

The mammary gland showed intense brown positive immunostaining of the cytoplasm in epithelium of the widely dilated ducts and the hypertrophied alveoli with immune positivepizzare arranged cells around the ducts, positive immunostaining of the connective tissue sheaths surrounding, also stroma showed multiple positive immunostained cells.

Combined ACR +VIT E Group (Figure 19)

The mammary gland showed positive immunostaining of the cytoplasm in the epithelium of the moderately dilated

ducts with negative staining of the surrounding connective tissue and stroma.

Morphometric and statistical results

Rat weight (Table 1, Histogram 1)

The mean rat weight in control (235.7±25) showed very highly significant increase in comparison with group 2 (ACR trated-161.2±17),Comparison between group 1 and group 3(ACR + vit E treated-231.5±19) showed non -significant difference, Comparison between group 2 and group 3 showed very highly significant difference.

Collagen density (Table 1, Histogram 1)

The mean collagen density(in pixel) in control (82.7±9) showed very highly significant decrease in comparison with group 2 (ACR trated-98.4±20), Comparison between group 1 and group 3(ACR + vit E treated-68±18) showed non -significant difference, Comparison between group 2 and group 3 showed very highly significant difference.

Fig. 2: a picture of female rats received ACR showing discoloration of the teeth(1) and paralysis of the hind limb (2).

Fig. 3: a picture of female rat received ACR+ vit E; showing discoloration of the teeth(A)and weakness of the hind limb(B)

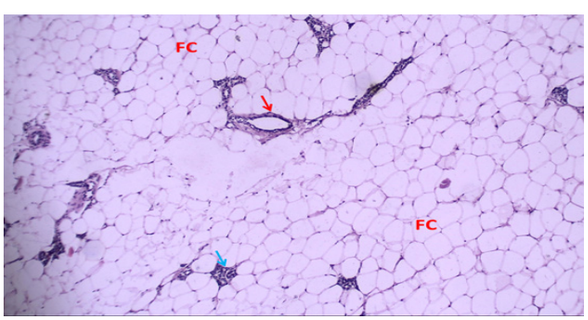


Fig. 4: a Photomicrograph of H&E - stained section of control adult female rat mammary gland (group1),appeared as duct (Red arrows) in abundant fat cells (FC), scattered few under developed alveoli (blue arrow) (H & E X100)

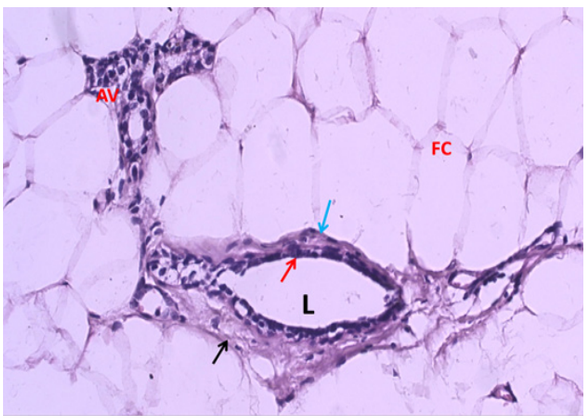
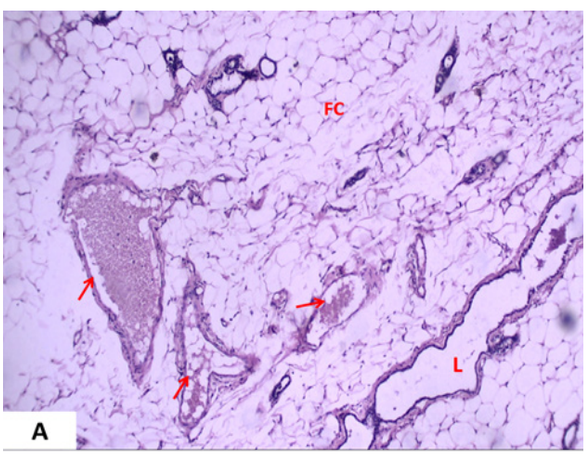


Fig. 5: a magnification of the previous section showing alveolar ductal lumen (L) lined by inner cubical cells (red arrow) and flat outer myoepithelial cells (blue arrows) surrounded by connective tissue sheet (black arrow) and with under developed multiple alveoli (AV), all embedded in abundant adipose tissue (FC) (H & E X400).



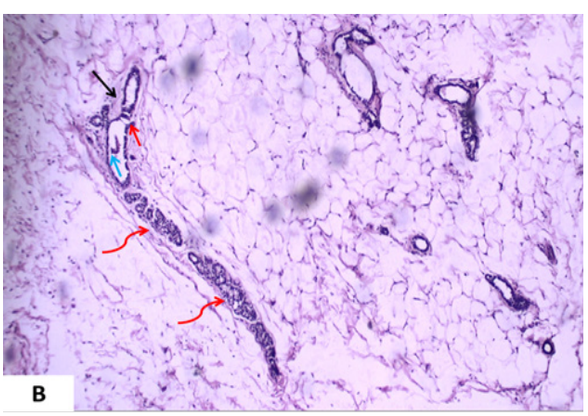


Fig. 6: (A) a Photomicrograph of H&E - stained section of ACR treated mammary gland (group2), showing multiple dilated congested blood vessels (Red arrows) with disturbed adipose tissue (FC) stroma with dilated duct lumens (L). (B) a Photomicrograph of H&E – stained section of ACR treated mammary gland (group2) showing the dilated duct lined by disarranged epithelial cells (red arrow) with detached epithelium in the lumen(blue arrow), surrounded by thick connective tissue sheaths (black arrow) with hypertrophied alveoli(irregular arrows) (H & E X100).

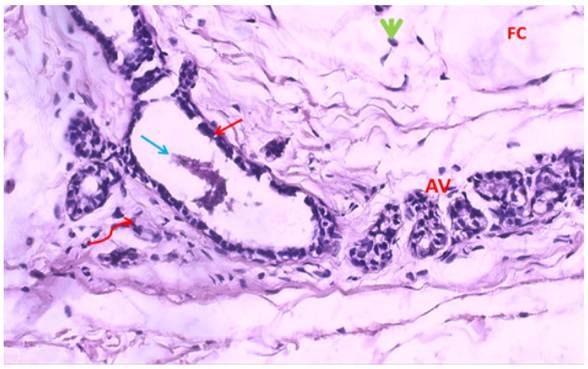


Fig. 7: a magnification of the previous section showing dilated duct lumen containing detached epithelium (blue arrow), lined by disarranged epithelial cells (red arrow) surrounded by thick connective tissue sheaths (irregular arrow), with hypertrophied alveoli (AV) lined by epithelial cells with dark piknotic nuclei, dense eosinophilic stroma(FC) containing pleiomorphic fibroblasts (green head arrow).(H & E X400).

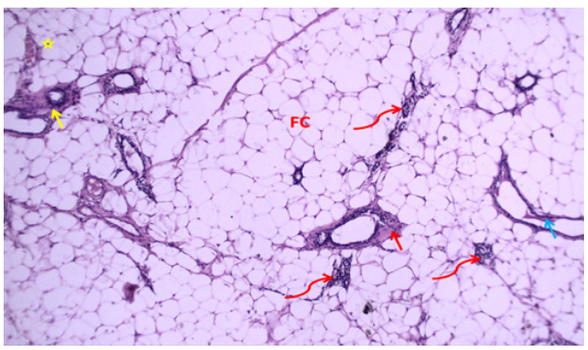


Fig. 8: Photomicrograph of H&E - stained section of ACR+ VIT E treated mammary gland (group3), showing moderately dilated duct lumen, some ducts containing detached epithelium (blue arrow) lined by arranged epithelial cells , some surrounded by moderately thick connective tissue sheaths (red arrow), with scattered under developed alveoli (irregular arrows), eosinophilic stroma(FC),some ducts showed localized multilayered lining (yellow arrow) with dilated blood vessel also seen in the section(star) . (H & E X100)

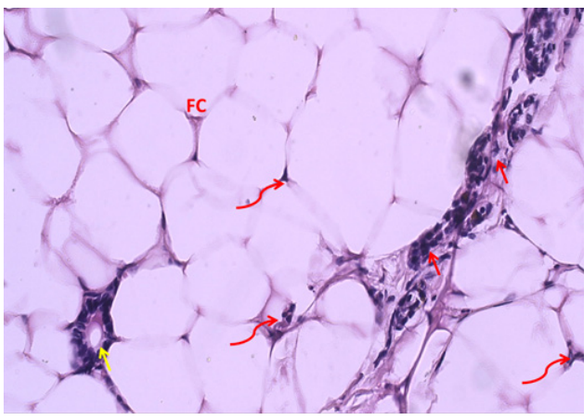


Fig. 9: a magnification of the previous section showing duct lined by arranged epithelial cells with dark piknotic nuclei (yellow arrow), eosinophilic stroma (FC)with scattered pleomorphic fibroblasts (irregular arrows),also multiple alveoli lined by dark piknotic nuclei can be seen (red arrows)(H & E X400).

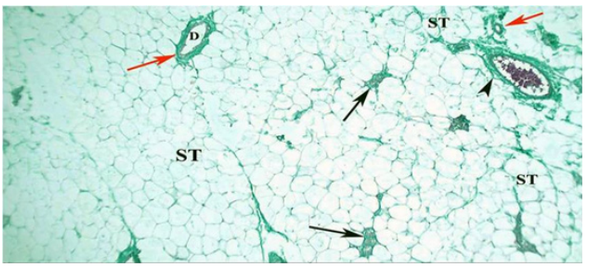


Fig. 10: A Photomicrograph of MTS- stained section of a control adult rat (group1) mammary gland showing normal appearance of collagen fibers appearing green in color surrounding the ducts (D)(red arrows) ,around blood vessels (head black arrow) and around alveoli (black arrows).with minimal collagen fibers within the stroma(ST)(MTS, ×100).

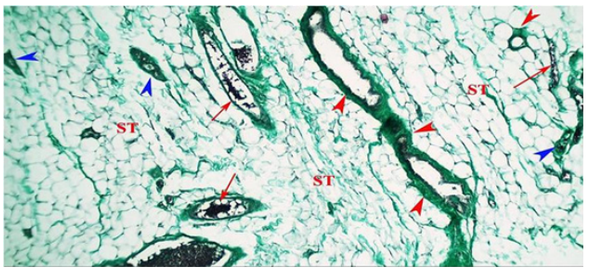


Fig. 11: A Photomicrograph of MTS- stained section of a ACR-treated adult rat (group2) mammary gland showing massive collagen fibers appearing green in color surrounding the dilated ducts (red head arrows) ,around the dilated blood vessels (red arrows) and around the hypertrophied alveoli (blue head arrows).Also, massive collagen fibers distribution within the stroma(ST)(MTS, ×100)

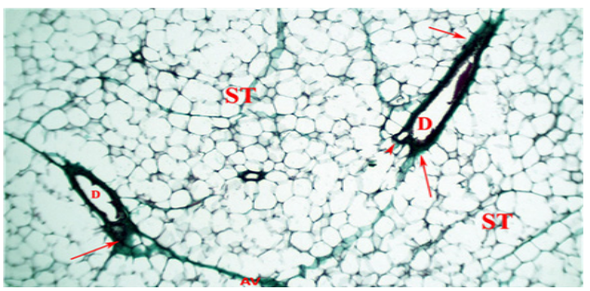


Fig. 12: A Photomicrograph of MTS- stained section of a ACR + VIT E -treated adult rat (group3) mammary gland showing prominent collagen fibers appearing green in color surrounding the ducts (D)(red arrows) with normal picture around blood vessels (head red arrow) , alveoli(AV) and within the stroma (ST) (MTS, ×100).

Fig. 13: Immuno-histochemical localization of CASP3 in control mammary gland showing positivitereactionof the ductal epithelium (red arrows) and with negative reaction of the connective tissue sheaths (blue head arrows) and of stromal fatty cells (FC) (Magnification, ×400).

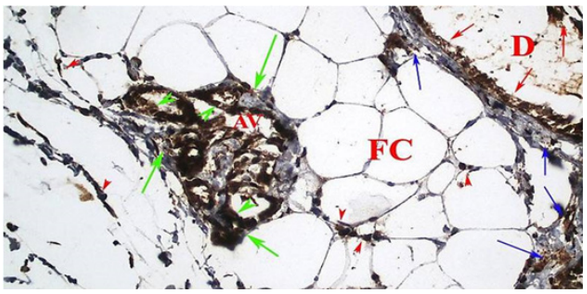


Fig. 14: Immunohistochemical localization of CASP3 in ACR-treated mammary gland showing intense positivity in epithelial cells(red arrows) and secretions in the lumens of the dilated ducts (D), with immunopositivity in its surrounding connective tissue (blue arrows),immunopositivity in epithelial cells(green head arrow) of the hypertrophiedalveoli (AV) and its surrounding connective tissue (green arrows) many immunopositive cells(red head arrows) of stroma cells (FC), (Magnification, ×400).

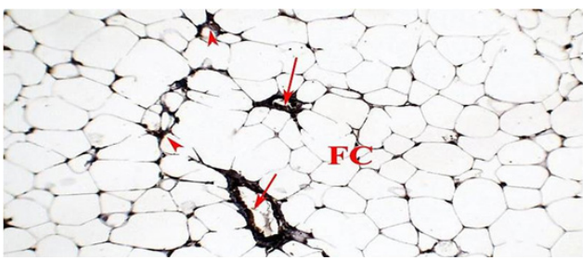


Fig. 15: Immunohistochemical localization of CASP3 in ACR+ VIT E treated mammary gland showing immunopositive epithelial cells of the ducts (red arrows), scattered immunopositive cellsin the alvoli(red arrow heads) with negative reaction of stromal fatty cells (FC).(Magnification, ×400).

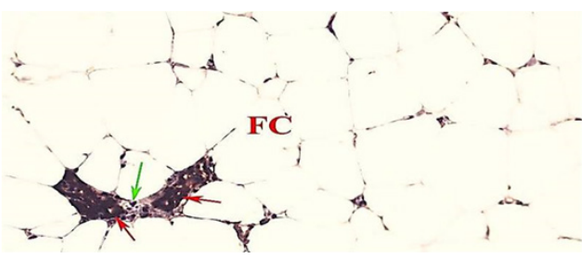


Fig. 16: Immunohistochemical localization of PCKs in control mammary gland showing positivite reaction of the ductal epithethelial cells (red arrows) and negative reaction of the surrounding connective tissue sheaths (green arrows) and of stroma cells (FC) (Magnification, ×400)

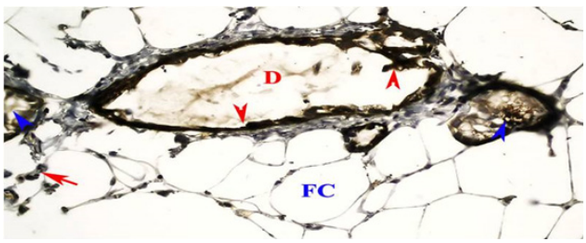


Fig. 17: Immunohistochemical localization of PCKs in ACR-treated mammary glands dilated ducts (D) showing intense positivity in their epithelial cells (red arrow heads) with ,immune positivepizzar arranged cellsaround the ducts (blue arrow heads) with many immunopositive cells (red arrow) in the stroma(FC) . (Magnification, ×400).

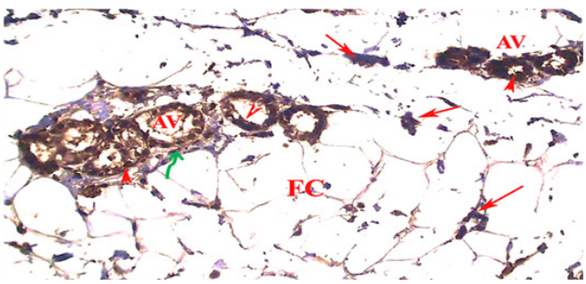


Fig. 18: Immunohistochemical localization of PCKs in ACR-treated mammary glands showing immunopositive reaction (red arrow heads) in the epithelial cells the and surrounding CT(green irregular arrow) of the hypertrophied alveoli (AV) with many immunopositive cells (red arrows) in the stroma(FC) . (Magnification, ×400)

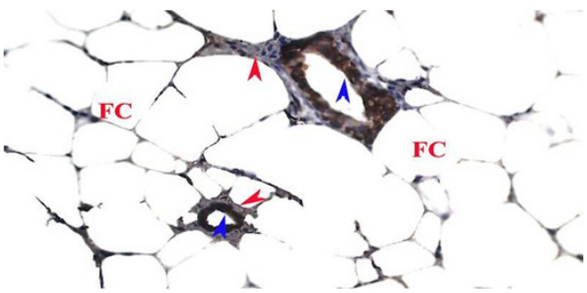
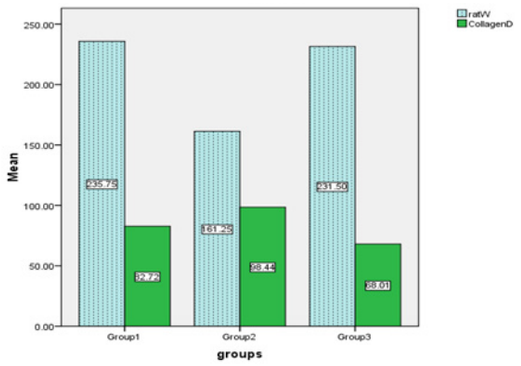


Fig. 19: Immunohistochemical localization of PCKs in ACR + vit E treated mammary gland showing intense positivity in epithelial cells (blue head arrows) of the ducts and immunonegative connective tissue sheaths (red arrow heads) ,immunonegative of stroma(FC), (Magnification, ×400).

Table1: Mean rat weight and collagen density in control and treated groups

	Group1	Group2	Group3	ANOVA	P1(1,2)	P2(1,3)	P3(2,3)
Rat Weight/gm	235.7±25	161.2±17	231.5±19	.000	.000***	.8NS	.000***
Collagen Density	82.7±9	98.4±20	68±18	.000	.005***	.8NS	.000***

P1: Differences between group 1 and 2
P2: Differences between group 1 and 3
P3: Differences between group 2 and 3
***: Very highly significant NS: Non significant



Histogram 1: Mean rat weight and collagen denity in control and treated groups

DISCUSSION

In recent years, it is clear that harmful effects resulted from free radical processes occurring in living system, so it is important to increase the effect of naturally occurring antioxidants in diseases prevention in experimental studies and their possible roles in prevention of humans diseases[16].

In this study, animals received ACR showed deteriorating general condition with irritability and paralysis of the hind limb, these results are in agreement with previous studies[17,18] who found that Acrylamide is an important neurotoxin which has harmful effects on both central and peripheral nervous system, and its administration can resulted in ataxia and weakness of the skeletal muscles[19].

Acrylamide caused a significant reduction in body weights with decrease of daily food and water intake. Similar results have been found by previous studies[20,21] who reported that the reduction in the weight may be due to metabolic disorder causing energy metabolism pathways which interfere with acrylamide.

Histological results showed ACR mammary damage in the form of ductal dilatation with detached epithelium , thickened connective tissue sheaths and dilated congested blood vessel with hyper cellular eosinophilicstroma and The hypertrophied alveoli , the same was found by previous researches[16,22] which showed that Acrylamide treated rats showed increasing alveoli with hypertrophic and hyperplastic epithelium, an increase in the proteinaceous secretory fluid in the alveolar lumen with Vacuolization in many alveolar cells.

The dilated blood with separation of the endothelial lining after ACR administration.Vascular dilatation revealed in ACR treated rats could be explained by activation of inflammatory cells with subsequent elevation of production of nitric oxide considered contributing factor for vascular dilatation[23].

In the present study ACR-treated rats mammary gland showed increased collagen deposition specially around the dilated ducts,dilated blood vessels and around the alveoli with abnormal collagen fibers deposition in the stroma when compared to the control ones.

Cytokines resulting from inflammatory infiltration can stimulate the production of prostaglandins E2 and collagenase and thus are believed to be involved in tissue damage with subsequent fibrosis[24].

Collagen synthesis is enhanced by Reactive Oxygen Species (ROS) which promote fibrosis as they induce proliferation of stellate cells. Activated stellate cells leaded to the deposition of collagen type I all after ACR administration[25].

Immunohistochemical sections for CASPS-3 localization in ACR-treated mammary gland showed increased positivity in the cytoplasm of epithelium of the ducts and the alveoli with immunopositive secretions within the lumens, positive immunostaining of the connective tissue sheaths surrounding, stroma showed positive immunostained many cells.

These results were similar to results of previous investigators who reported that Acrylamide induce CASP-3 over expression andon the apoptosis in testis cells of mice[26].

The present study Immunohistochemical sections for PCKs localization in ACR-treated mammary gland showed positivity in the epithelium of the dilated ducts and the hypertrophied alveoli with immunopositivepizzare arranged cells present newly arround the ducts, positive immunostaining of the connective tissue sheaths surrounding, stroma showed positive immunostained cells.

Previous study[27] found that after acrylamide administration rats showed multiple dysplastic lesions, considered to be premalignant in the form of areas of dysplastic alveoli and squamous metaplasia, with areas of inflammation in the stroma and the epithelium.

Maronpot *et al*.,[28] reported ACR-related neoplastic changes in acrylamide-treated rats by the end of two years administration included mammary gland fibroadenomas, also Maier *et al*.,[22] stated that Fibromas showed a significant increased tumor incidence in acrylamide treated females groupthis is because of its genotoxicity related to glycidamide formation and oxidative stress..

On the other hand previous study[29], reported that breast tissues in acrylamide treated group showed no signs of malignancy but only hyperplasia was found.

Multiple researches studed the relationship between breast cancer and dietary acrylamide found little evidence of increased breast cancer risk[30,31]. Of both, onlyOlesen *et al*.[31] reported a significant relationship between breast cancer risk and dietary acrylamide.

In the present study, the histological and immunohistochemical and statical results of rats treated with acrylamide and Vit E showed some improvement in comparison with a group of rats received ACR only, This improvement include less number of dilated ducts with less thick connective tissue sheaths,stroma appear similar to the control one,In addition. Masson stain and

immunohistochemical studies were improved after vitamin E administration to a degree similar to the normal pattern .

These results are in agreement with previous results[32,33] who mentioned that supplementation with Vit E produced organ -protection in the form of preserved normal architecture also they stated that the level of apoptosis was markedly decreased and there was minimum collagen fibers in stroma and around blood vessels. Preserved normal histological architecture structure in ACR and VE-treated subgroups is attributed to VE natural anti-inflammatory and antioxidant properties which ameliorate the oxidative damage by trapping of reactive oxygen and nitrogen species, and also by down-regulating of inflammatory markers as CRP and IL-6 .

In contrast with these results, Rahangadale, *et al*.[34] concluded that VIT E doesn't alleviate the ACR induced toxicity during active exposure.

CONCLUSION

Acrylamide causes hyperplasia and fibrosis in mammary gland, Addition of vitamin E cause restoration of the normal picture of the ducts and stroma of mammary gland.

RECOMMENDATIONS

Limitation of the use of acrylamide containing food is necessary specially for young females.

Further researches is recommended to found the perfect protective agents against acrylamide.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

الآثار المرضية لجرعة عالية من مادة الأكريلاميد على الغدد الثديية لإناث الجرذان البيضاء والدور الوقائى المحتمل لفيتامين E (دراسة نسيجية وكيميائية مناعية)

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المقدمة: تم اكتشاف مادة الأكريلاميد في الأطعمة في عام ٢٠٠٢ ، وخاصة في الأطعمة النشوية مثل رقائق البطاطس والخبز التي تم تسخينها أعلى من ١٢٠ درجة مئوية. لا يوجد في الطعام المغلي أو غير المسخن.
فيتامين (هـ) هو فيتامين قابل للذوبان في الدهون ، ودوره الرئيسي هو العمل كمضاد للأكسدة. كما أنه يعزز وظيفة المناعة ولفيتامين هـ القدرة على حماية الخلايا من أضرار الجذور الحرة
هدف العمل: دراسة الدور الوقائي المحتمل لفيتامين E في تلف الغدة الثديية الناجم عن مادة الأكريلاميد في إناث الجرذان البيضاء من خلال الدراسات النسيجية والكيميائية المناعية.
المواد والطرق: تم استخدام ٤٥ جرذًا عذراء ألبينو وزنها ١٨٠-٢٥٠ جرامًا في الدراسة وتم تقسيمها إلى ثلاث مجموعات: المجموعة الضابطة (أعطيت محلولًا ملحيًا). مجموعة الأكريلاميد (اعطيت ١٠ مجم / كجم من مادة الأكريلاميد) عن طريق الفم لمدة ٣ أسابيع ؛ مجموعة الأكريلاميد + فيتامين هـ (تُعطى نفس جرعة الأكريلاميد + فيتامين هـ عن طريق الفم بجرعة ١٠٠ مجم / كجم) لمدة ٣ أسابيع ، وتم استئصال حيوانات المجموعات الثلاث التي تم التضحية بها والغدد الثديية من أجل دراسات الهيماتوكسلين والايوسين والماسون ودراسات الكيمياء المناعية.
النتائج: أظهرت الفئران التي تلقت مادة الأكريلاميد تغيرًا في الشكل العام ، وتوعكًا عامًا مع انخفاض النشاط حتى اكتمال شلل الأطراف الخلفية ، وقد تحسنت هذه النتائج باستخدام فيتامين هـ. أظهرت الغدد الثديية المعالجة بـالأكريلاميد تغيرات مرضية في شكل اتساع فى الحويصلات مع عدم انتظام فى ترتيب الخلايا
مع زيادة ترسيب الياف الكولاجين الموجودة بشكل رئيسي حول القنوات والحويصلات الهوائية ، كان هناك زيادة في تعبير CASPS-٣ والتعبير عن pancytokeratins ويبدو أن تناول الفيتامين يقلل جميع النتائج كما شوهد في الدراسات النسيجية والكيماوية المناعية.
الخلاصة: يمكن أن تؤدي إضافة مكملات فيتامين (هـ) إلى تلف أقل للغدد الثديية المعالجة بجرعة عالية من مادة الأكريلاميد.