Original Article

Effect of Amygdalin (Vitamin B17) on Induced Mammary Tumor in young Virgin Female Albino Rats: Histological and Morphometric Study

Shaimaa M. Motawea[1]; Samia A. Youssef[1]; Ghada A. Abdel-Aleem[2]; Maysa F.M. Salem[1] and Magdy S. Mostafa[1]

[1]Department of Anatomy and Embryology, [2]Department of Medical Biochemistry, Faculty of Medicine, Tanta University, Egypt

ABSTRACT

Introduction: Breast cancer is one of the common causes of mortality among women worldwide. Metastasis from breast cancer may occur and commonly affects bone, lung, liver and the central nervous system. Amygdalin (vitamin B17) is a plant compound found in the pits of many fruits e.g. peach and apricot. Intestinal bacteria produce betaglucosidases enzymes which break down amygdalin to benzaldehyde, glucose and cyanide or hydrocyanic acid (HCN).The balance between pro-apoptotic proteins such as B-cell lymphoma protein 2 -associated X (Bax) protein and anti-apoptotic protein e,g, B-cell lymphoma protein 2 (Bcl-2) can play a critical role in control of carcinogenesis.

Aim of the Work: This work aimed to study the effect of amygdalin (vitamin B17) on experimentally induced mammary tumor in virgin young female albino rats.

Materials and Methods: Fifty virgin young female albino rats were used and divided into: control group: included 30 rats and experimental groups: included 20 rats and subdivided to: Subgroup IIa: 10 rats. Each received 80 mg / kg (12 mg) 7,12 Dimethylbenzanthracane to induce breast tumor. Subgroup IIb: as subgroup IIa then received 5mg/kg amygdalin.

Results: The H&E stained sections of subgroup IIa showed hyperplasia of the lining epithelial cells of lactiferous ducts. Subgroup IIb showed lactiferous ducts with well-organized layers of epithelial cells and prominent nuclei. Other ducts showed pyknotic nuclei and vacuolations. Transmission electron microscope of subgroup IIa showed large nuclei with prominent nucleoli of the epithelial cells lining lactiferous ducts, increased euchromatin mass and nuclear pockets. Subgroup IIb showed half-moon-shaped nucleus of the lining epithelial cells of lactiferous ducts. Sections from subgroup IIa stained with Bcl-2 immunostain showed increased cytoplasmic Bcl-2 immunoreactivity than control group and positive cytoplasmic Bax immunoreactivity. Subgroup IIb revealed less cytoplasmic Bcl-2 while increased cytoplasmic Bax immunoreactivity than DMBA-administered subgroup.

Conclusion: Amygdalin which is a natural compound had proven its cytotoxic effect on breast cancer cells mainly by increasing pro-apoptotic Bax protein.

Received: 31 January 2022, **Accepted:** 18 Feruary 2022

Key Words: Amygdalin, bax, Bcl-2, breast cancer, histological and morphometric study.

Corresponding Author: Shaimaa Mamdouh Motawea, MD, Department of Anatomy and Embryology, Faculty of Medicine, Tanta University, Egypt, **Tel.**: +20 10 0120 9892, **E-mail:** shaimaa.metawee@med.tanta.edu.eg

ISSN: 1110-0559, Vol. 46, No. 2

INTRODUCTION

Breast cancer is one of the common causes of mortality among women worldwide[1]. Cancer is the second leading cause of death after myocardial infarction[2]. It is a pathological condition which involves uncontrolled division and abnormal growth of cells. These abnormal cells have the potential to spread to other parts of the body[3]. Metastasis from breast cancer may occur and commonly affects bone, lung, liver and the central nervous system[4]. The risk for the development of female breast cancer is usually related to age, family history, reproductive factors, environmental and genetic factors[5]. The balance between pro-apoptotic proteins such as B-cell lymphoma protein 2 -associated X (Bax) protein and anti-apoptotic protein e,g, B-cell lymphoma protein 2 (Bcl-2) can play a critical role in control of carcinogenesis[6]. Chemotherapy which is the most common used cancer therapy is associated with numerous severe side effects ranging from mild to severe toxicity. All organs of the body can be affected including the heart, lungs and brain. In addition, the chronic effects of chemotherapy include drug resistance and infertility[7]. Amygdalin is a plant compound found in the pits of many fruits e.g. peach and apricot. Intestinal bacteria produce betaglucosidases enzymes which break down amygdalin to benzaldehyde, glucose and cyanide or hydrocyanic acid (HCN)[8].

Personal non-commercial use only. EJH copyright © 2023. All rights served *DOI: 10.21608/ejh.2022.119301.1637*

MATERIALS AND METHODS

Drugs

a. 7, 12-dimethylbenzanthracene (DMBA)

7,12-dimethylbenzanthracene was obtained from Cornell lab-chemistry company, Cairo, Egypt. It was in the form of yellow crystalline powder. Each rat received 12 mg (80 mg / kg) DMBA diluted in sesame oil (0.5 ml) single dose orally by orogastric tube[9].

b. Amygdalin

Amygdalin was obtained from Cornell lab-chemistry company, Cairo, Egypt. It was in the form of white crystalline powder. Each rat received 0.75 mg (5mg/kg) diluted in 1 ml saline 0.9% once per week for 3 successive weeks by intraperitoneal injection[10].

Experimental animals

In this study fifty virgin young female albino rats aged 6-7 weeks and of average body weight 150 grams were used. The animals were housed in clean separated well ventilated cages (not more than five rats per cage). The tops of cages were made of steel wire and bedded with wood shavings. Animals were kept under similar environmental condition and fed the same laboratory diet. Care and treatment of animals as well as the experimental procedures were provided as per the ethics regulation at Tanta University. The animals were divided into two groups: group I (control) and group II (experimental):

Group I (Control): (30 rats) and was equally subdivided into two subgroups:

- Subgroup Ia (negative control): 10 rats received no treatment.
- Subgroup Ib (vehicle control): 10 rats received sesame oil 0.5 ml single dose orally by orogastric tube.
- Subgroup Ic (vehicle control): 10 rats received saline 0.9% 1 ml once per week for 3 weeks by intraperitoneal injection.

Group II (experimental group): (20 rats) and divided to the following subgroups:

IIa (10 rats): Each rat received 80 mg / kg 7,12-dimethylbenzanthracene (DMBA) diluted in sesame oil (0.5 ml) single dose orally by orogastric tube[9].

IIb (10 rats): Each rat received the same dose of DMBA as subgroup IIa then after 10 weeks (as DMBA takes approximately 10 weeks to induce tumors in female albino rats)[9] received amygdalin 5 mg/kg by intraperitoneal injection once per week for 3 successive weeks[10].

At the end of experiment, all animals were anaesthetized with ether then sacrificed at the optimum time and specimens of mammary glands were taken and examined. Finally sacrificed rats were safely collected in a special package according to safety and health precaution measures to be incinerated later.

Histological and immunohistochemical study

The procedures were done at Faculty of Medicine, Tanta University.

a. Light microscopic study

Specimens were fixed in 10% neutral buffered formalin for 24 hours[11], then subjected to the following stains to study the normal histological structure of rat's mammary glands and the pathological changes among experimental groups according to[12,13].

i. Hematoxylin and eosin (H&E) stain

ii. Bcl-2 and Bax immunostains

b. Transmission Electron Microscopic (TEM) study

Sections prepared for TEM and were examined and photographed using (JEOL-JEM-100 SX electron microscope, Japan) at the electron microscopic unit, Faculty of Medicine, Tanta University.

Morphometric study

1. Counting and determining the mean number of epithelial cells that expressed Bcl-2. The cytoplasm of cells that expressed Bcl-2 protein appeared brown in color, while those did not express the protein appeared purple or blue. Cells were counted in 10 non-overlapping different fields at magnification 400/slide in each group using image J software program. Based on the data obtained, the percentage analysis (%) of the Bcl-2 immunohistochemical expression was calculated by calculating the mean number of cells that expressed Bcl-2 divided by the mean number of total cells multiplied by 100%.
2. Counting and determining the mean number of epithelial cells that expressed Bax. The cytoplasm of cells expressed Bax protein appeared brown in color, while those did not express the protein appeared purple or blue and the total cell number were counted. Cells were counted in 10 non-overlapping different fields at magnification 400/slide in each group using image J software program. Based on the data obtained, the percentage analysis (%) of the Bax immunohistochemical expression was calculated by calculating the mean number of cells that expressed Bax divided by the mean number of total cells multiplied by 100%. The formula used:

% cell expression = number of cell expressed/number of total cell X 100%[14]

3. Estimation of the mean Bax/Bcl-2 ratio: was calculated using the following formula:

The percentage of positive expression of Bax immunostain is divided by the percentage of positive expression of Bcl-2 immunostain[15]

Statistical Analysis

All data were collected and analyzed using Statistical Package for the Social Sciences (SPSS) 27.0 software.

Data were compared by using mean and standard deviation of number of Bcl-2 and Bax immune-stained epithelial cells in all groups. The t-test (independent) was performed for each group against control group using Statistical Package for the Social Sciences (SPSS) software. A difference among the means of a *p value* ≤ 0.05 was considered statistically significant and a *p value* ≤ 0.001 was considered statistically highly significant[16].

RESULTS

Light microscopic examination of the mammary glands

a. Hematoxylin & Eosin (H&E)

H&E stained-sections of rat's mammary glands of the control group showed lactiferous ducts. Each was composed of well-organized one layer of epithelial cells. These epithelial cells were cuboidal to columnar cells with prominent nuclei. They were surrounded by myoepithelial cells and blood vessels appeared near the ducts. The lumens of ducts were patent and the connective tissue consisted mainly of fat cells well connected to each other (Figure 1A). Subgroup IIa (received DMBA): showed hyperplasia of the lining epithelial cells of lactiferous ducts (Figure 1B). The wall was composed of more than two layers of epithelial cells. Some lining epithelial cells showed cellular pleomorphism (Figure 1C). Some lactiferous ducts revealed some discontinuity of their lining epithelium with detachment of some epithelial cells to the lumen (Figure 1D). Fat cells in the connective tissue appeared irregular with loose connections in between and excessive collagen fibers were seen (Figure 1E). Subgroup IIb (received amygdalin): showed marked improvement of hyperplasia of ductal epithelial cells and tumor cells growth in which most of lactiferous ducts appeared with well-organized layers of epithelial cells with prominent nuclei and eosinophilic necrotic materials present in their lumens (Figure 1F). Another sections of this group showed many lining epithelial cells with pyknotic nuclei (Figure 1G).

b. Toluidine blue-stained sections

Toluidine blue stained-sections of the rat's mammary glands of the control group showed lactiferous ducts composed of one well-organized layer of columnar epithelial cells with prominent nuclei and obvious nucleoli. The lining epithelial cells were surrounded by myoepithelial cells (Figure 2A). Toluidine blue-stained sections of the rat's mammary glands of (subgroup IIa) showed hyperplasia of the lining epithelial cells of most of lactiferous ducts obstructing their lumen (Figure 2B). Toluidine blue-stained sections of rat's mammary glands of subgroup IIb demonstrated lactiferous ducts composed of one layer of epithelial cells with prominent nuclei. The lining epithelial cells were surrounded by myoepithelial cells. Numerous epithelial cells appeared with pyknotic nuclei. Vacuolations were obvious and necrotic material was present in the lumen (Figure 2C).

c. Bcl-2 and Bax immunohistochemistry

Light microscopic examination of sections in mammary glands of control group stained with Bcl-2 immunostain showed positive cytoplasmic Bcl-2 immunoreactivity which appeared as cytoplasmic brown coloration (Figure 3A). While this group showed negative Bax immunoreactivity (Figure 3B). Subgroup IIa (received DMBA): demonstrated marked increase in cytoplasmic Bcl2 immunoreactivity (appeared as cytoplasmic brown coloration) than control group (Figure 3C). This subgroup also showed some positive cytoplasmic Bax immunoreactivity (cytoplasmic brown coloration) (Figure 3D). Subgroup IIb (received DMBA then amygdalin): showed less cytoplasmic Bcl2 immunoreactivity than DMBA-administered subgroup (Figure 3E), while it showed increased cytoplasmic Bax immunoreactivity than DMBA-administered subgroup (Figure 3F).

Transmission Electron Microscopic results

The ultrathin sections of the control group illustrated epithelial cells with large centrally located nuclei with intact and regular nuclear membranes, prominent nucleoli and well distributed euchromatin and heterochromatin material. The cells cytoplasm contained rough endoplasmic reticulum and spherical mitochondria with apparent cristae pattern (Figure 4A).

Subgroup IIa: showed epithelial cells with irregular-shaped nuclei with prominent two nucleoli, chromatin clumping and nuclear pockets (Figure 4B). Another cells showed increase of euchromatin mass and markedly dilated rER (Figure 4C). Subgroup IIb; showed epithelial cells with half-moon-shaped nucleus with peripheral nuclear chromatin condensation and vacuolations were present in the cytoplasm (Figure 4D).

Morphometric and statistical results

1- The mean percentage of Bcl-2

Control group (group I) was 20.27% ± 5.48. In DMBA-administered subgroup (subgroup IIa) the mean percentage of Bcl-2 expression was 50.71% ± 9.99 which was highly significantly increased compared to control group (*p2 value* 0.00**). In subgroup IIb (after receiving amygdalin) the mean percentage of Bcl-2 expression was 31.38% ± 5.94 (*p1 value* 0.001**) which was highly significantly lower than DMBA-administered subgroup and still significant compared to control group (p2 value 0.003*) (Table 1, Figure 6A: blue columns).

The mean percentage of Bax expression in all groups

The mean percentage of Bax expression in control group (group I) was 5.72% ± 1.35. After receiving DMBA

(subgroup IIa), the mean percentage of Bax expression was 22.74% ± 5.83 with *p2 value* 0.00** which was highly significantly increased compared to control group. In subgroup IIb (after receiving amygdalin), the mean expression of Bax was 57.32 % ± 8.62 which was highly significantly increased compared to DMBA-administered subgroup and control group (p1 and p2 values 0.00**) (Table 2, Figure 6A: red columns).

The mean Bax/Bcl-2 ratio in all groups

In control group, the mean Bax/Bcl-2 ratio was 0.304 ± 0.09. In subgroup IIa, the mean Bax/Bcl-2 ratio was 0.453 ± 0.141 which was significantly increased than control group (*p2 value* 0.03*). In subgroup IIb, the mean Bax/Bcl-2 ratio (1.85 ± 0.401) was highly significantly increased compared to DMBA-administered subgroup and control group (p1 and p2 values 0.00**) (Table 3, Figure 6B).

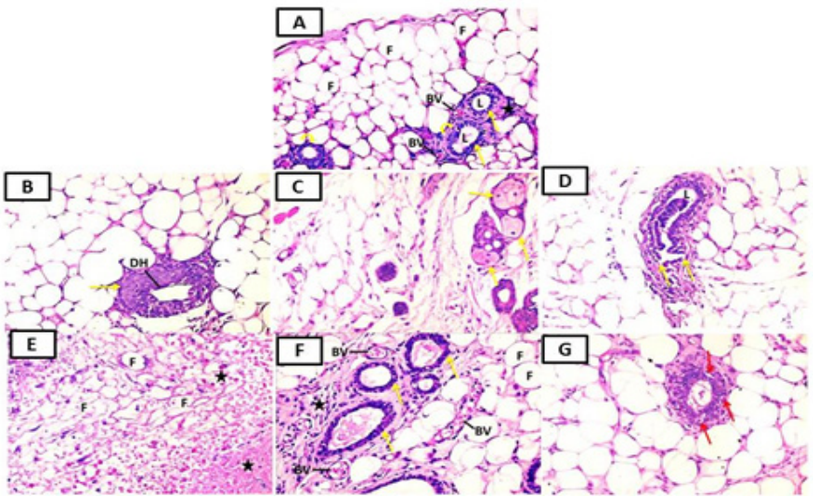

Fig. 1: (H & E × 400): Fig. 1 (A): A photomicrograph of a section in mammary gland of a control young virgin female albino rat demonstrates lactiferous ducts with patent lumen (L). Each duct is composed of one well-organized layer of cuboidal to columnar epithelial cells with prominent nuclei (yellow arrows) surrounded by myoepithelial cells (curved arrows) and blood vessels appear near the ducts (BV). The connective tissue is mainly consisting of fat cells well connected to each other (F). Fig. 1(B): A photomicrograph of a section in mammary gland of a young female albino rat received DMBA demonstrates a lactiferous duct (yellow arrow). Its wall shows ductal epithelial cells hyperplasia (DH). It consists of more than two layers of epithelial cells. Fig. 1(C): Another photomicrograph of a section in mammary gland of a young female albino rat received DMBA shows cellular pleomorphism in the lining epithelium of a lactiferous duct (yellow arrows). Fig. 1(D): A photomicrograph of a section in mammary gland of a young female albino rat received DMBA reveals some discontinuity of the lining epithelium of a lactiferous duct (yellow arrows) with detachment of some epithelial cells to the lumen (L). Fig. 1(E): A photomicrograph of a section in mammary gland of a young female albino received DMBA reveals irregular shapes of fat cells with loose connections in-between (F) and dense collagen fibers (stars) scattered in some areas. Fig. 1(F): A photomicrograph of a section in mammary gland of a young female albino rat received DMBA then amygdalin shows lactiferous ducts. Each is composed of one well-organized layer of cuboidal to columnar epithelial cells with prominent nuclei (yellow arrows). The connective tissue contains fat cells well connected to each other (F). Some blood vessels appear normal but other blood vessel is slightly congested (BV). Fig. 1(G): Another photomicrograph of a section in mammary gland of a young female albino rat received DMBA then amygdalin shows a lactiferous duct with numerous epithelial cells appear with pyknotic nuclei (red arrows).

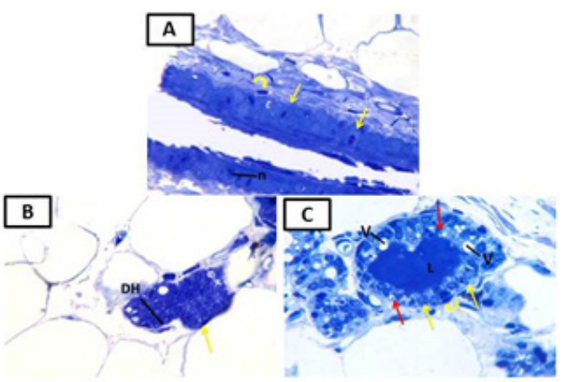

Fig. 2: (Toluidine blue x1000): Fig.2 (A): A semithin section in mammary gland of a control young female albino rat shows a lactiferous duct composed of one well-organized layer of columnar epithelial cells with prominent nuclei (yellow arrows) and obvious nucleolus (n). The lining epithelial cells are surrounded by myoepithelial cells (curved arrow). Fig.2 (B): A semithin section in mammary gland of a young female albino received DMBA shows a lactiferous duct (yellow arrow) with ductal epithelial cells hyperplasia (DH) obstructing its lumen. Fig.2 (C): A semithin section in mammary gland of a young female albino received DMBA then amygdalin demonstrates a lactiferous duct composed of one layer of epithelial cells. Some of the cells show prominent nuclei (yellow arrows). Others show pyknotic nuclei (red arrows) and vacuolations (V). There is necrotic material present in the lumen (L). Myoepithelial cells (curved arrow) are seen surrounding the lining epithelium

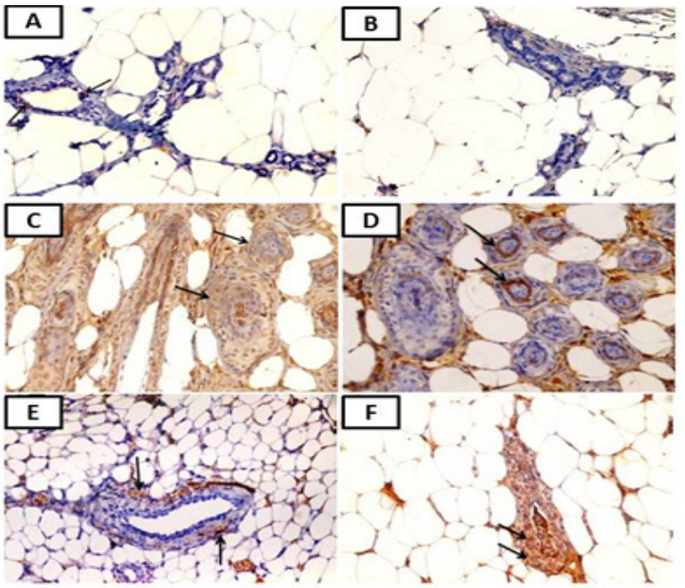

Fig. 3: Mammary gland (Immunostaining Bcl-2.and Bax X 400): Fig. 3(A): A photomicrograph of a section in mammary gland of a control young female albino rat reveals positive cytoplasmic Bcl-2 immunoreactivity which appears brownish in color (black arrows). Fig. 3(B): A photomicrograph of a section in mammary gland of: a control young female albino rat shows negative Bax immunoreactivity. Fig. 3(C): a young female albino rat received DMBA demonstrates markedly increased cytoplasmic Bcl-2 immunoreactivity which appears brownish in color (black arrows) than the control group. Fig.3(D): a young female albino rat received DMBA shows positive cytoplasmic Bax immunoreactivity which appears brownish in color (black arrows). Fig.3(E): a young female albino rat received DMBA then amygdalin shows less cytoplasmic Bcl-2 immunoreactivity (black arrows) than DMBA-administered group. Fig. 3(F): a young female albino rat received DMBA then amygdalin shows increased cytoplasmic Bax immunoreactivity (black arrows) than DMBA-administered subgroup.

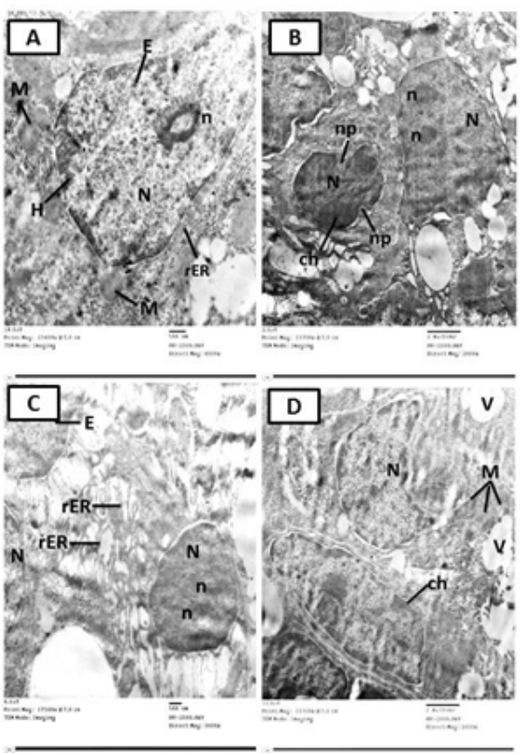

Fig. 4: Mammary gland (TEM): An electron micrograph of an ultrathin section in mammary gland of: Fig. 4(A) a control young female albino rat illustrates large centrally located nucleus (N) with intact and regular nuclear membrane and a prominent nucleolus (n). Well distributed euchromatin (E) and heterochromatin (H) material with rough endoplasmic reticulum (rER) and spherical mitochondria (M) are seen in the cell cytoplasm (TEM X 4000). Fig.4(B) a young female albino rat received DMBA shows irregular-shaped nuclei (N) with prominent two nucleoli (n). Chromatin clumping (ch) and nuclear pockets (np) are also seen (TEM X 2000). Fig.4(C) of a young female albino rat received DMBA shows regular shaped nuclei with prominent nucleoli (n), increased euchromatin mass (E) and markedly dilated rER (rER) are obvious in the cytoplasm (TEM X 3000). Fig.4(D) a young female albino rat received DMBA then received amygdalin reveals half-moon-shaped nucleus (N) with peripheral nuclear chromatin condensation (ch) and vacuolations are seen in the cytoplasm (V) (TEM X 2000)

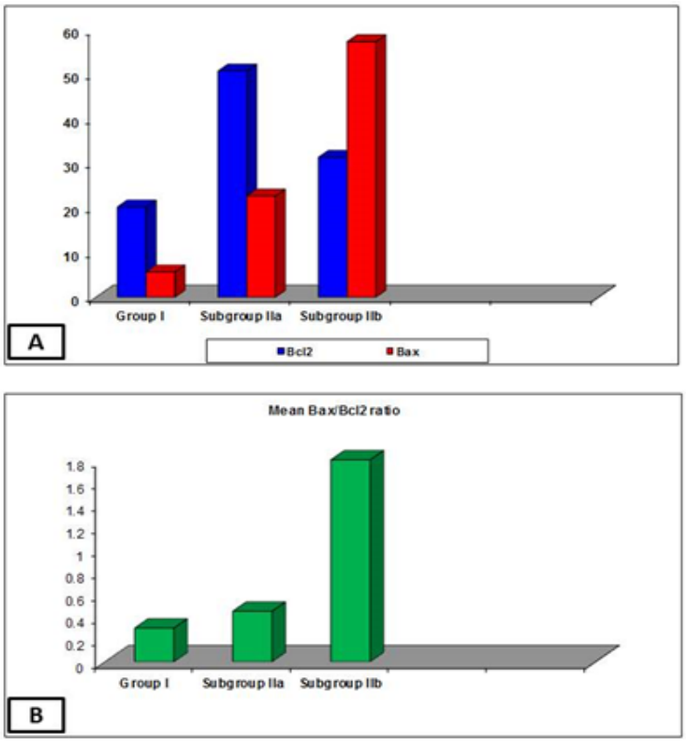

Fig. 5: Bar Graph (A): Illustrates the mean values of Bcl-2 (blue columns) and Bax (red columns) expression in the different groups. Bar Graph (B): Illustrates the mean values of Bax/Bcl-2 ratio in the different groups.

Table 1: Mean percentage of Bcl-2 expression in the different groups

Mean Bcl-2 expression (%)	Group I	Group II	
	Control group	Subgroup IIa	Subgroup IIb
Range	14.28-30.3	41.1-71.7	22- 27.3
Mean	20.27	50.71	31.38
Standard deviation (SD)	5.48	9.99	5.94
P1 value			0.001**
P2 value		0.000**	0.003*

Table 2: Mean percentage of Bax expression in the different groups

Mean Bax expression (%)	Group I	Group II	
	Control group	Subgroup IIa	Subgroup IIb
Range	4-8	13.3-32.7	42.2-70
Mean	5.72	22.74	57.32
Standard deviation (SD)	1.35	5.83	8.62
P1 value		0.000**	0.000**
P2 value			0.000**

Table 3: Mean Bax/Bcl-2 ratio in the different groups

Mean Bax/Bcl2 ratio	Group I	Group II	
	Control group	Subgroup IIa	Subgroup IIb
Range	0.17-0.5	0.28-0.76	1.2-2.5
Mean	0.304	0.453	1.85
Standard deviation (SD)	0.09	0.141	0.401
P1 value			0.000**
P2 value		0.03*	0.000**

P1: Subgroups IIb compared to subgroup IIa(DMBA) subgroup. P2: subgroup IIb compared to group I (control).
P value > 0.05 → Non significant difference.
P value ≤ 0.05* → Significant difference.
P value ≤ 0.001** → High significant difference.

DISCUSSION

In the present work, light microscopic examination of sections in mammary glands after administration of (DMBA) to virgin young female albino rats (subgroup IIa) revealed hyperplasia of the lining epithelial cells of lactiferous ducts. Some discontinuity of the cells occurred with detachment of these cells to the lumens of ducts. Fat cells in the connective tissue appeared irregular with loose connections in between. By transmission electron microscopy, mammary glands showed epithelial cells of lactiferous ducts with irregular shaped nuclei and prominent nucleoli. Chromatin clumping and nuclear pockets were seen. Also there was increase in nuclear euchromatin mass and extensively swollen rough endoplasmic reticulum appeared in the cell cytoplasm.

Bishayee *et al*.[17] stated that there is a specific time for (DMBA) exposure. They reported that administration of DMBA to young age (45-55 days) virgin female rats induced mammary tumor. It was evident that rats at this young age have high frequency of terminal end buds which were more sensitive to DMBA in initiating mammary tumors[18].

Karimi *et al*.[19] also announced that administration of DMBA to young rodents enhanced the breast cancer induction because their mammary glands are still undifferentiated and have high rate of cell proliferation. They reported that DMBA is a polycyclic aromatic hydrocarbons (PAH) has both carcinogenic and immunosuppressive effects. It acts by producing various metabolic free radicals leading to oxidative stress due to disturbance in cellular oxidant-antioxidant balance

The present study is in agreement with Soliman & Elfeky[20] who proved that administration of DMBA 20 mg/kg dissolved in corn oil orally to virgin female rats, aged 7 weeks old caused loss of normal architecture of mammary ducts with hyperplasia (adenocarcinoma) of its lining epithelium and hemorrhage in the blood vessels. This study also coincided with Bishayee *et al*.[17] who reported that 50 mg/kg body weight DMBA (dissolved in corn oil) administered by oral gavage to rats aged about 8 weeks showed extensive epithelial proliferation, resulting in a fused glandular pattern, nuclear pleomorphism, characterized by nuclear enlargement, prominent nucleoli and clumping of chromatids. Moreover, epithelial cells demonstrated gross variation in nuclear size and irregular chromatin. Sections stained with Bcl-2 immunostain in mammary glands of this group (DMBA-administered subgroup IIa) showed increased cytoplasmic Bcl-2 immunoreactivity which was highly significantly increased compared to control group. It also showed positive cytoplasmic Bax immunoreactivity (22.74% ± 5.83), (the mean percentage of Bax stained cells in control group was 5.72% i.e. negative Bax-immunostained) and the Bax/Bcl-2 ratio in this group was 0.453.

Tumors were considered as Bax negative if all or most of the cancer cells were unstained or (there is <10% of positive stained cells) and described as Bax positive if more than 10% of cells are positive immunostained[21]

These results are in agreement with Bishayee *et al*.[22] who found that the frequency of Bcl-2-immunopositive cells was increased in tumors from oral administration of 50 mg/kg DMBA to 8 weeks old virgin rats. This is also in agreement with Mulyati *et al*.[14] who reported that the percentage of positive stained Bcl-2 cells after DMBA administration to rats was 52.83 ± 3.61 which was significantly higher than control group (20.62 ± 10.09).

Overexpression of Bcl-2 in malignancies was found to be associated with drug resistance, while a high Bax level was considered a good prognostic indicator[23]. On the other hand, the decreased Bcl-2 (antiapoptotic protein) expression was found to stimulate the release of cytochrome c from mitochondria to the cytoplasm and subsequently caused apoptosis by activation of caspases[24,25].

The Bax/Bcl-2 ratio is suggested to be more important than either Bcl-2 or Bax alone in determining apoptosis[23]. This ratio is a critical determinant of the apoptosis pathway and initiation of apoptosis[26]. With higher Bax/Bcl-2 index, a significant higher rate of complete remission, a longer overall survival and disease-free survival were observed, while lower Bax/Bcl-2 ratio was found to be associated with poorer prognosis[23]. Also Kubatka *et al*.[27] stated that the increasing Bax/Bcl-2 ratio caused enhanced caspase-3activity and subsequently induces apoptosis in tumor cells. A patient whose tumor has more Bcl-2 or less Bax (a Bax: Bcl-2 ratio < 1) may have a poorer response to therapy and prognosis than a patient whose tumor has more Bax expression or decreased Bcl-2 (a Bax: Bcl-2 ratio ≥ 1)[15]. In the present study, light microscopic examination of sections in mammary glands after administration of 7,12-dimethylbenzanthracene (DMBA) followed by amygdalin injection (subgroup IIb) showed marked improvement of epithelial cells hyperplasia evident by appearance of numerous lactiferous ducts composed of well-organized layers of epithelial cells with prominent nuclei and others with pyknotic nuclei and cytoplasmic vacuolations. Eosinophilic necrotic materials were present in the lumens of milk ducts. The connective tissue showed fat cells well connected to each other. The ultrathin sections in mammary glands of this group showed many epithelial cells with irregular-shaped and half-moon-shaped nuclei with peripheral nuclear chromatin condensation and some vacuolations were present in the cytoplasm.

Apoptosis is a highly regulated type of cell death that leads to removal of cells that are defective and regulated to die. Apoptosis is characterized by morphological changes, mainly evident in the nucleus, where the chromatin compacts and condenses and takes a crescent or half-moon shape before being fragmented to nuclear hyperchromatic apoptotic bodies[28].

Amygdalin is a cyanogenic component in bitter almond. The enzymatic hydrolysis of amygdalin yields benzaldehyde and hydrocyanic acid. Prunasin is the

precursor of amygdalin. Prunasin may be degraded to hydrogen cyanide, glucose, and benzaldehyde[29]. So it has been a subject of doubt whether amygdalin is a toxic chemical or a therapeutic drug when used at the correctly administered dose. Several studies were conducted to evaluate amygdalin toxicity mainly when administered orally. However, rhodanese which is an enzyme present in the mitochondria of many organisms is able to convert the high cyanide content resulting from amygdalin into a harmless thiocyanate[30].

Several studies had shown that amygdalin inhibited the proliferation of human colon cancer cell and could also reduce the incidence of prostate cancer, lung cancer, colon cancer and rectal cancer[31,32,33].

In the current work, sections from (subgroup IIb) stained with Bcl-2 immunostain showed decreased cytoplasmic Bcl-2 immunoreactivity than DMBA-administered subgroup while it showed increased cytoplasmic Bax immunoreactivity than DMBA-administered subgroup and the Bax: Bcl-2 ratio of this group was 1.85. This coincided with several studies which supported that amygdalin can induce apoptotic cell death of various types of cancer cells such as leukemia, prostate cancer and liver cancer They had also postulated that amyg¬dalin administration increased expression of Bax, while decreased expression of Bcl-2[31,32,34,35].

The antitumor effects of amygdalin are mainly through affecting cell cycle, inducing apoptosis, producing a cytotoxic effect, and regulating the body's immune function[36]. Lee & Moon[37] measured the levels of expression of apoptosis-related proteins in breast cancer cells, which treated with various concentrations of amygdalin, and their results showed that amygdalin caused increase in the expression of pro-apoptotic protein Bax and decreased the expression of antiapoptotic Bcl-2. However, the clinical application of amygdalin may be associated with toxic effects due to cyanide (one of its metabolites)[38].

Clinically, cyanide toxicity is manifested by Cherry-red color of skin, nausea, vomiting, dyspnea, confusion, seizures and lactic acidosis[39]. Therefore, caution is seriously required, although it is reported that amygdalin is metabolized in non-toxic dose in normal cells[40].

CONCLUSION

From the present study it could be concluded that Amygdalin which is a natural compound, had proven its cytotoxic effect on cancer cells mainly by increasing the pro-apoptotic Bax protein and also by decreasing anti-apoptotic Bcl-2 protein.

RECOMMENDATIONS

- Amygdalin has an evident anti-cancer effect. Further studies are needed using higher doses or longer duration of amygdalin to evaluate its toxicity before reporting its usage safely in human.
- Studies needed to evaluate the use of safe doses of amygdalin as adjuvant to traditional cancer therapy.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Azamjah, N.; Soltan-Zadeh, Y. and Zayeri, F. (2019): Global Trend of Breast Cancer Mortality Rate: A 25-Year Study. Asian Pacific Journal of Cancer Prevention, 20(7): 2015–2020.
2. Khan, M.A.; Chen, H.; Tania, M. and Zhang, D. (2011): Anticancer activities of nigella sativa (black cumin). African Journal of Traditional, Complementary and Alternative Medicine, 8(5): 226–232.
3. Ramesh, R.G.V. (2017): Types of Cancer and Surgery in Rats (Rattus norvegicus). Research & reviews. Journal of Veterinary Sciences, 3(1):4-9.
4. Jin, X. and Mu, P. (2015): Targeting breast cancer metastasis. Breast Cancer (Auckl), 9(1): 23–34.
5. Shah, R.; Rosso, K. and Nathanson, S.D. (2014): Pathogenesis, prevention, diagnosis and treatment of breast cancer. World Journal of Clinical Oncology, 5(3): 283–298.
6. Hardwick , J.M. and Soane, L. (2013): Multiple Functions of BCL-2 Family Proteins. Cold Spring Harbor Perspectives in Biology, 5(2): a008722.
7. Schirrmacher, V. (2019): From chemotherapy to biological therapy: A review of novel concepts to reduce the side effects of systemic cancer treatment (Review). International Journal of Oncology, 54(2): 407–419.
8. Jaszczak-Wilke, E.; Polkowska, Z.; Koprowski,M.; Krzysztof Owsianik, K.; Mitchell, A.E. and Bałczewski, P. (2021): Amygdalin: Toxicity, Anticancer Activity and Analytical Procedures for Its Determination in Plant Seeds. Molecules. 26(8): 2253.
9. Wang, Z. and Zhang, X. (2017): Chemopreventive activity of honokiol against 7, 12 Dimethylbenzanthracene-induced mammary cancer in female sprague dawley rats. Frontiers in Pharmacology, 8: 320.
10. Dogru, H.Y.; Isguder, C.K.; Arici, A.; Ozsoy, A.Z.; Delibas, I.B. and Cakmak, B. (2017): Effect of amygdalin on the treatment and recurrence of endometriosis in an experimental rat study. Periodicum biologorum, 119(3): 173–180.
11. Singhal, P.; Singh, N.N.; Sreedhar, G.; Banerjee, S.; Batra, M. and Garg, A. (2016): Evaluation of Histomorphometric Changes in Tissue Architecture in Relation to Alteration in Fixation Protocol – An In*vitro* Study. Journal of Clinical & Diagnostic Research. 10(8): 28–32.

12. Hussein, R.; Khalaf, M. and Mohamed, W. (2020): Hesperidin and eugenol attenuate cadmium-induced nephrotoxicity via regulation of oxidative stress, Bax/Bcl2 and cleaved caspase 3 expression. Turkish Journal of Biochemistry, 45(6): 767–775.

13. Bancroft, J.D. and Gamble, M. (2008): Theory and practice of histological techniques, 6th edition, Churchhill-Livingstone, London England. P: 121-135.

14. Mulyati, G.D.; Nurani,L.H. and Widyarini, S. (2017): Effects of co-chemotherapy ethyl acetate fraction of eurycoma longifolia jack roots and doxorubicin against apoptosis through expression p53 mutant and bcl-2.Indonesian Journal of Medicine and Health, 9(1):68-77.

15. Lohmann, C.M.; League, A.A.; Clark, W.S.; Lawson, D.; DeRose, P.B. and Cohen, C. (2000): Bcl-2: Bax and Bcl-2: Bcl-x ratios by image cytometric quantitation of immunohistochemical expression in ovarian carcinoma: correlation with prognosis. Cytometry, 42(1): 61-66.

16. Ribeiro, D.A.; Salvadori, D.M.F. and Marques, M.E.A. (2005): Abnormal expression of bcl-2 and bax in rat tongue mucosa during the development of squamous cell carcinoma induced by 4-nitroquinoline 1-oxide. International Journal of Experimental Pathology, 86(6): 375–381.

17. Bishayee, A.; Mandal, A.; Bhattacharyya, P. and Bhatia, D. (2016): Pomegranate exerts chemoprevention of experimentally induced mammary tumorigenesis by suppression of cell proliferation and induction of apoptosis. Nutrition and cancer, 68(1): 120–130.

18. Russo, J. (2015): Significance of rodent mammary tumors for human risk assessment. Toxicologic Pathology, 43(2): 145–170.

19. Karimi, B.; Ashrafi, M.; Shomali, T. and Yektaseresht, A. (2019): Therapeutic effect of simvastatin on DMBA-induced breast cancer in mice. Fundamental & Clinical Pharmacology, 33(1): 84–93.

20. Soliman, M.M. and Elfeky, A.M.S. (2016): Studies on the biochemical and molecular effects of some natural herbs on experimental-induced breast cancer in wistar rats. National Journal of Physiology, Pharmacy and Pharmacology, 6(5): 349-358.

21. Miquel, C.; Borrini, F.; Grandjouan, S.; Aupérin, A.; Viguier, J.; Velasco, V.; Duvillard, P.; Praz, F. and Sabourin, J.C. (2005): Role of bax mutations in apoptosis in colorectal cancers with microsatellite instability. American Journal of Clinical Pathology, 123(4):562-70.

22. Bishayee, A.; Mandal, A.; Thoppil, R.J.; Darvesh, A.S.; and Bhatia, D. (2013): Chemopreventive effect of a novel oleanane triterpenoid in a chemically induced rodent model of breast cancer. International Journal of Cancer, 133(5): 1054–1063.

23. Kulsoom, B.; Shamsi, T.S.; Afsar, N.A.; Memon, Z.; Ahmed, N. and Hasnain, S.N. (2018): Bax, Bcl-2, and Bax/Bcl-2 as prognostic markers in acute myeloid leukemia: are we ready for Bcl-2-directed therapy?. Cancer Management and Research, 10: 403–416.

24. Redza-Dutordoir, M. and Averill-Bates, D.A. (2016): Activation of apoptosis signalling pathways by reactive oxygen species. Biochimica et Biophysica Acta, 1863: 2977–2992.

25. Morales-Cruz, M.; Cruz-Montañez, A.; Figueroa, C.M.; González-Robles, T.; Davila, J.; Inyushin, M.; Loza-Rosas, S.A.; Molina, A.M.; Muñoz-Perez, L.; Kucheryavykh, L.Y.; Tinoco, A.D. and Griebenow, K. (2016): Combining stimulus-triggered release and active targeting strategies improves cytotoxicity of cytochrome c nanoparticles in tumor cells. Molecular Pharmaceutics, 13: 2844–2854.

26. Azimian, H.; Dayyani, M.; Toossi, M.T.B. and Mahmoudi, M. (2018): Bax/Bcl-2 expression ratio in prediction of response to breast cancer radiotherapy. Iranian Journal of Basic Medical Sciences, 21(3): 325–332.

27. Kubatka, P.; Kello, M.; Kajo, K.; Samec, M.; Jasek, K.; Vybohova, D.; Uramova, S.; Liskova, A.; Sadlonova, V.; Koklesova, L.; Murin, R.; Adamkov, M.; Smejkal,K.; Svajdlenka, E.; Solar, P.; Samuel, S.M.; Kassayova,M.; Kwon, T.K.; Zubor, P.; Pec, M.; Danko, J.; Büsselberg, D. and Mojzis, J. (2020): Chemopreventive and therapeutic efficacy of cinnamomum zeylanicuml bark in experimental breast carcinoma: mechanistic in *vivo* and in *vitro* analyses. Molecules, 25(6): 1399.

28. Nozawa, K.; Fritzler, M.J.; Takasaki, Y.; Wood, M.R. and Chan, E.K.L. (2009): Co-clustering of Golgi complex and other cytoplasmic organelles to crescentic region of half-moon nuclei during apoptosis. Cell Biology International, 33(2):148-157.

29. Sánchez-Pérez, R.; Belmonte, F.S.; Borch, J.; Dicenta, F.; Møller, B.L. and Jørgensen, K. (2012): Prunasin hydrolases during fruit development in sweet and bitter almonds. Plant Physiology, 158(4): 1916–1932.

30. Milazzo, S.; Lejeune, S. and Ernst, E. (2007): Laetrile for cancer: a systematic review of the clinical evidence. Supportive Care in Cancer, 15(6): 583–595.

31. Kwon, H.Y.; Hong, S.P.; Hahn, D.H. and Kim, J.H. (2003): Apoptosis induction of Persicae Semen extract in human promyelocytic leukemia (HL-60) cells. Archieves of Pharmacal Research, 26(2):157-61.

32. Chen, Y.; Ma, J.; Wang, F.; Hu, J.; Cui, A.; Wei, C.; Yang, Q. and Li, F. (2013): Amygdalin induces apoptosis in human cervical cancer cell line HeLa cells. Immunopharmacology and Immunotoxicology, 35(1):43-51.

33. Qadir, M. and Fatima, K. (2017): Review on pharmacological activity of amygdalin. Archives in cancer research, 5(4):160.

34. Zhou, C.; Qian, L.; Ma, H.; Yu, X.; Zhang, Y.; Qu, W.; Zhang, X. and Xia, W. (2012): Enhancement of amygdalin activated with β-D-glucosidase on HepG2 cells proliferation and apoptosis. Carbohydrate Polymers, 90: 516-523.

35. Song, Z. and Xu, X. (2014): Advanced research on anti-tumor effects of amygdalin. Journal of Cancer Research and Therapeutics, 10(5): 3-7.

36. Shi, J.; Chen, Q.; Xu, M.; Xia, Q.; Zheng,T.; Teng, J.; Li, M. and Fan, L. (2019): Recent updates and future perspectives about amygdalin as a potential anticancer agent: A review. Cancer Medicine, 8(6): 3004-3011.

37. Lee, H.M. and Moon, A. (2016): Amygdalin regulates apoptosis and adhesion in Hs578T triple-negative breast cancer cells. Biomolecules and Theraputics, 24(1):62-66.

38. O'Brien, B.; Quigg, C. and Leong, T. (2005): Severe cyanide toxicity from 'vitamin supplements'. Europian Journal of Emergency Medicine, 12:257-8.

39. Parker-Cote, J.L.; Rizer, J.; Vakkalanka, J.P.; Rege , S.V. and Holstege, C.P. (2018): Challenges in the diagnosis of acute cyanide poisoning. Clinical Toxicology (Philadelphia), 56(7):609-617.

40. Mirmiranpour, H.; Khaghani, S.; Zandieh, A.; Khalilzadeh. O.; Gerayesh-Nejad, S.; Morteza, A. and Esteghamati, A. (2012): Amygdalin inhibits angiogenesis in the cultured endothelial cells of diabetic rats. Indian Journal of Pathology and Microbiology, 55(2):211-4.

الملخص العربى

تأثير الأميجدالين (فيتامين B17)على سرطان الثدي المستحث في أنثى الفئران الصغيرة:دراسة نسيجية و شكلية قياسية

شيماء ممدوح مطاوع[١]، سامية عبد المسيح يوسف[١]، غادة أجمد عبد العليم[٢]، مابسة فهمي محمد[١]، مجدي سعيد مصطفى[١]

[١]قسم التشريح الآدمي وعلم الأجنة، [٢]قسم الكيمياء الحيوية، كلية الطب جامعة طنطا

الخلفية: يعتبر سرطان الثدي أحد الأسباب الشائعة للوفيات بين النساء في جميع أنحاء العالم. الأميجدالين هو مركب نباتي يوجد في نوى العديد من الفاكهة و يتم تكسير الأميجدالين بواسطة بكتيريا الأمعاء إلى السيانيد.

هدف من العمل: هو دراسة التأثير المحتمل للاميجدالين (فيتامين B١٧) على ورم الثديي المستحث في إناث الفئران البيضاء (دراسة نسيجية ووراثية).

المواد و الطرق: تم استخدام خمسين أنثى من الفئران البيضاء. تم تقسيم الحيوانات إلى مجموعتين: المجموعة الضابطة والمجموعة التجريبية: المجموعة الأولى (المجموعة الضابطة) تتكون من ٣٠ فأرًا. المجموعة الثانية (المجموعة التجريبية): تتكون من ٢٠ فأر وقسمت إلى: IIa: ضمت ١٠فئران. تلقى كل فأر ٨٠ مجم / كجم ٧ ، ١٢-ثنائي ميثيل بنزينثراسين (DMBA) مخفف في زيت السمسم (٠,٥ مل) جرعة واحدة عن طريق الفم. IIb: شمل ١٠ فئران. تلقى كل فأر نفس جرعة DMBA مثل المجموعة الفرعية IIa ثم تلقى الأميجدالين ٥ مجم / كجم. تم أخذ عينات من الغدد الثديية للدراسات النسيجية والوراثية.

النتائج: أظهر فحص المقاطع المصبوغة بالهيماتوكسيلين و الايوسين من المجموعة الفرعية IIa تضخمًا في الخلايا المبطنة للقنوات اللبنية التي أدت الى انسداد التجويف. أظهرت المجموعة الفرعية IIb طبقة منظمة جيدًا من الخلايا المبطنة المكعبة إلى العمودية ذات النواة البارزة و كانت هناك مواد ميتة في التجويف. بواسطة المجهر الإلكتروني النافذ ، أظهرت مقاطع في الغدد الثديية من المجموعة الفرعية IIa نوى كبيرة غير منتظمة الشكل مع نوية بارزة. كما لوحظ تكتل الكروماتين وزيادة كتلة الكروماتين الحقيقي والجيوب النووية. أظهرت المجموعة الفرعية IIb، نواة على شكل نصف قمر. في المجموعة الفرعية IIa ، زادت نسبة bcl-٢ مقارنة بالمجموعة الضابطة و كان هناك زيادة في نسبة bax. في المجموعة الفرعية IIb ظهر نقص في نسبة bcl-٢ و زادت نسبةbax بشكل كبير مقارنة بالمجموعة IIa.

الخلاصة: أثبت الأميجدالين و هو مركب طبيعي أن له تأثير علاجي على سرطان الثدي خاصة عن طريق زيادة bax بروتين