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Research Article

# Study of Toxic Effects of Adriamycin Drug on Female Sex Cells and Comparing Honey and Ginseng Ability to Treat These Effects

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## Abstract

**Objective:** This study aims to investigate the ability of a certain mixture of honey with royal jelly and ginseng root herb to prevent the toxic side effects, caused by the adriamycin drug. One of the most important side effects of this drug is the change in the structure of cell and tissue of the ovary, especially Graafian follicles, which may lead to sexual ovarian hormonal disorders. consequently, this will lead to menstrual disorders that lead in certain cases to early menopause (infertility), and decrease in the reproductive capacity.

**Methods:** First Study: field study, included 500 women who were treated with adriamycin. second study: An experimental study was conducted on 75 young adult female Syrian hamsters, twenty-five hamsters were injected with Adriamycin drug and another 25 females were injected with Adriamycin drug after oral administration with preventive dose of honey and royal jelly and another 25 females were injected with Adriamycin drug after oral administration with preventive dose of ginseng. histologically study of the ovaries and sex hormones.

**Results:** ginseng root herb is not considered a sufficient preventive supplement, as it did not protect the tissue structures. However, it contributes to raising the levels of sex hormones. but the honey with royal jelly has a clear role in renovating and They protect the tissue structure of the ovary, enhancing the hormonal balance, and prevents the hormonal disorders, and the occurrence of early menopause caused by adriamycin.

**Conclusion:** The Honey with royal jelly contributes, as a preventive dose, in preventing the side effects of adriamycin drug, and reducing the reproductive aging and early menopause.

**Keywords:** Adriamycin; Ginseng root herb; Graafian follicles; Honey; Menopause; Panax ginseng; Royal jelly; Sex hormones; Syrian hamster

## Introduction

The era of chemical treatment in the United States of America began in 1940 during the Second world war by the discovery of mustard nitrogen, which had shown to have anti-cancer properties, especially against lymphoma and leukemia. the initial results were very encouraging [1], and led to the development of many anti-cancer drugs, but the drug has many side effects, including cytological, Physiological, morphological, histological, and psychological effects. However, we will address the cytotoxic effects that may develop during the chemotherapy treatment of cancers, most notably adriamycin which is used to treats many malignant tumors, is considered as one of the first line medications used for treatment of cancer breast, which is in a continuous increase in Syria, despite all precautions used to limit its spreading. adriamycin drug belongs to the anthracycline glycosides which in turn belong to the antitumor antibiotic group. This drug generally works to kill mitotic cells at the end of the S-phase synthesis stage, a DNA replication phase. the drug has the chemical formula $C_{27}H_{29}NO_{11}$* HCl, in the form of four rings linked by a glycosidic band and amine sugar called Danocimyn (Figure 1), and has the molecular weight of 579.99 [2,3,4].

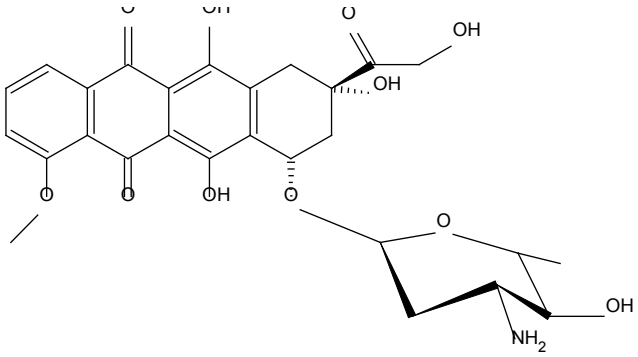



**Figure 1:** Chemical structure of Adriamycin.

Adriamycin is one of the most successful and most widely used chemotherapy drugs for various cancers. It was considered as the first line drug in the treatment of many tumors [5,6], including:

| | Tumour Type | Response Rate (%) | Duration (month) |
|---|---|---|---|
| **Established Activity** | Breast | 35 | 6-Mar |
| | Ovary | 38 | 6-Mar |
| | Lung | 30 | 3 |
| | Sarcoma | 30 | 4 |
| | Wilms | 66 | 4 |
| | Bladder | 28 | 6-Apr |
| | Neuroblastoma | 41 | 4 |
| | Hodgkin's | 36 | 6-Apr |
| | Non-Hodgkin's Lymphoma | 40 | 6-Apr |
| | Acute Leukemia | 35 | 3 |
| | Hepatoma | 32 | 6-Apr |
| | Thyroid | 30 | 10-Jun |
| **Some Response** | Stomach | 30 | 4-Feb |
| | Cervix | 32 | 6-Feb |
| | Head & Neck | 19 | 4-Feb |
| | Testicle | 20 | 6-Mar |
| | Myeloma | 33 | 3 |
| | Endometrial | 36 | 6-Apr |
| **Unresponsive** | Colorectal | | |
| | Pancreas | | |
| | Renal | | |
| | Melanoma | | |
| | Brain | | |

**Table 1:** Shows the ability of Adriamycin to treat various cancers.

## Materials and Methods

### First Study

Field study, a questionnaire was distributed at several cancer centers in Aleppo (Al-Kind hospital, Aleppo University Hospital, Hamayat Hospital, other centers), (A certified statement was taken by the presidency of the University of Aleppo to work on this field study in all the centers of Aleppo governorate). The study included 500 women who were treated with Adriamycin, whether married or unmarried in the age interval between 15 and 45 years. In order to be able to conduct a field study on the drug, and determine its effect on the menstrual cycle, ovarian cycle and reproductive activity in women, we exclude from this study woman who suffered from pre-treatment menstrual disorders or those who had one of their family members with genetic problems (to avoid genetic factors).

### Second Study

An experimental study was conducted on 75 young adult female Golden Hamsters (Syrian) *Mesocricetus auratus* during the breeding period. For better results, breeding animals were raised during spring (breeding season). Adult male hamsters were present around in order to allow female hamsters to meet them and smell the

pheromones from male hamster's urine because female hamsters do not have a specific time for ovulation cycle, which lasts only for four days, that coincide with meeting mature males or smelling the Pheromones from male hamster's urine. The Lab conditions were handled according to the guidelines of Helsinki declaration rights (1975) of using laboratory animals. Golden female hamsters were divided into three main groups according to the preventive substance, after calculating their weights and length:

- For the first group: Adriamycin (Doxorubicin-Ebewe-AUSTRIA) was injected as a single intraperitoneal dose (60-75 mg/m$^2$) without any preventive substance (A).
- The second group: A daily dose of Panax ginseng (200 mg/kg/day) has been given for 15 days, then adriamycin was injected as in group (G-A).
- The third group: A daily dose of Sidr honey (800 mg/kg/day) with royal jelly (10 mg/kg/day) has been given for 15 days, then adriamycin was injected as in group (H-A).

Each of the above groups was divided into secondary groups taken according to time, so that we could monitor the development of the toxic effect of the drug: (Time I: after three days of the injection, Time II: after five days of the injection, Time III: after 10 days of the injection and Time IV: after 15 days of the injection). These Times simulate the end time of the single dose given to the breast cancer patient. For each of the four-time groups, a blood sample was taken to assess levels of sex hormones, and ovarian excision was done to conduct the histological assessment.

## Results

From a previous field study [7], we found that adriamycin is used primarily for the treatment of malignant tumors, especially breast cancer, which is in a continuous increase in our country, with a percentage of more than 60.7% of malignant tumors occurring in female population (Figure 2).

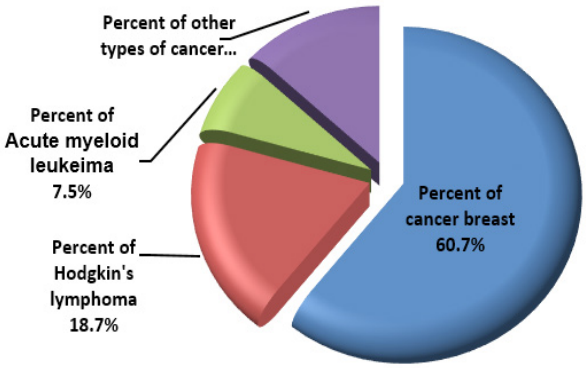



**Figure 2:** Types of cancers treated with adriamycin in females.

Despite adriamycin was proved to have high success rates in managing malignant tumors, it, unfortunately, exerts a burden on the reproductive capacity of people being treated with. It was found:

- That most of the women were observed to have a temporary menstrual interruption (92.5%) upon starting the treatment with the drug.
- During the treatment period, more than 86% of females were found to have menstrual cycle disorders (Figure 3).

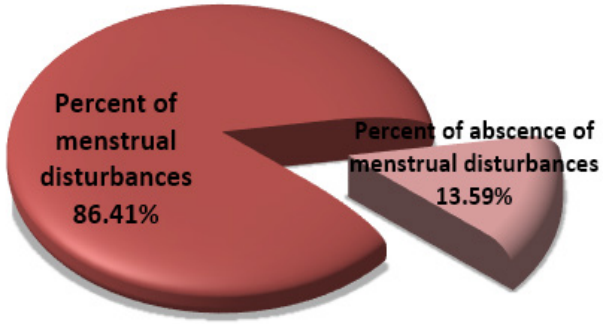



**Figure 3:** Percentage of menstrual disturbance in women treated by adriamycin.

That the vast majority suffered from a delayed menstrual cycle in the treatment period, exceeding 55%, while more than 23% of the irregular menstrual cycle and more than 15% reached early menopause at an advanced stage of their ages (Figure 4).

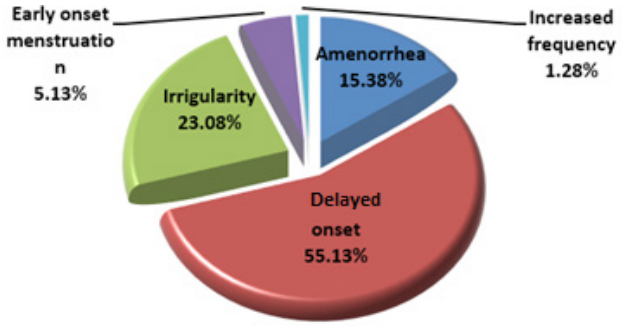



**Figure 4:** Types of menstrual disturbances in females treated by adriamycin.

- The high rate of failure to conceive in married women who are subjected to treatment by more than 91%.
- The remaining proportion was divided into two groups: most of them had a spontaneous miscarriage due to the low proportion of progesterone, which works on the implantation of embryos in the uterus. the second group includes those who gave birth to normal infants were caused by the interruption of treatment of the drug for more than two years but failed to reproduce again after returning to treatment with the drug, which led to the return of toxicity of the property (Figure 5).

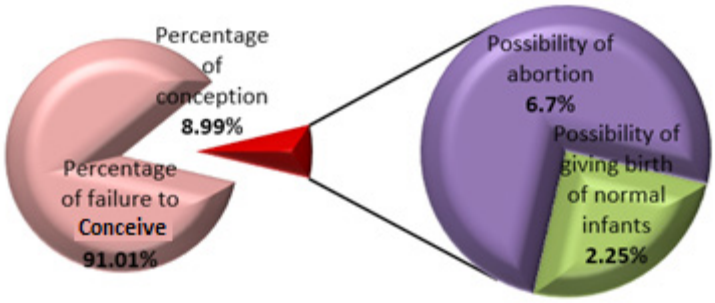



**Figure 5:** Shows the rates of pregnancy and miscarriage in females treated with adriamycin.

The experimental study also showed, according to the investigated sex hormones levels (FSH, LH, Estradiol, Progesterone), (Table 2), that it matches with the field study. It also showed the absence of functional activity of the ovary through the disappearance of ovulation stages. In addition, levels of hormones were suggestive of started ovarian disorder (i.e., the hormonal type) reaching early menopause. These investigations included:

| Group Home | Subject | Sample Number | F.S.H (MIu/ml) | L.H (MIu/ml) | Pro (Nmol/ml) | E.S (Pg/ml) | Characterization of the situation |
|---|---|---|---|---|---|---|---|
| Injected group with Adriamycin drug without protective (A) | Control | 1 | 14.89 | 67.56 | 3.04 | 259.9 | Ovulation |
| | | 2 | 13.65 | 45.78 | 5.84 | 258.7 | Ovulation |
| | | 3 | 15.67 | 54.65 | 7.09 | 423.5 | Ovulation |
| | | 4 | 1.08 | 2.01 | 22.42 | 156 | Luteal phase |
| | | 5 | 2.6 | 2.32 | 14.65 | 169.9 | Luteal phase |
| | First time (A-I) | 1 | 2.9 | 1.09 | 59.69 | 67.1 | Luteal phase |
| | | 2 | 21.98 | 53.01 | 1.29 | 13.65 | I started ovary disorder |
| | | 3 | 26.89 | 47.98 | 1.18 | 5 | postmenopause |
| | | 4 | 3.05 | 2.03 | 22.63 | 68.73 | Luteal phase |
| | | 5 | 2.99 | 8.4 | 12.72 | 55.11 | Luteal phase |
| | Second time (A-II) | 1 | 50.07 | 41.54 | 1.49 | 5.57 | postmenopause |
| | | 2 | 4.75 | 11.87 | 1.38 | 122.6 | Follicular phase |
| | | 3 | 23.67 | 43.89 | 1.35 | 15.37 | I started ovary disorder |
| | | 4 | 24.06 | 51.01 | 0.86 | 11.64 | I started ovary disorder |
| | | 5 | 46.06 | 57.09 | 0.38 | 6.33 | postmenopause |
| | Third time (A-III) | 1 | 3.47 | 9.35 | 4.2 | 163.5 | Follicular phase |
| | | 2 | 25 | 58.98 | 1.32 | 13.72 | I started ovary disorder |
| | | 3 | 25.04 | 53.6 | 2.06 | 14.32 | I started ovary disorder |
| | | 4 | 3.56 | 4.94 | 3.84 | 24.81 | Follicular phase |
| | | 5 | 3.78 | 3.2 | 4.02 | 30.41 | Follicular phase |
| | Fourth time (A-IV) | 1 | 1.5 | 1.57 | 2.39 | 45.24 | Follicular phase |
| | | 2 | 22.142 | 43.78 | 2.9 | 16.01 | I started ovary disorder |
| | | 3 | 3.89 | 3.67 | 13.87 | 168.07 | Luteal phase |
| | | 4 | 2.98 | 1.07 | 4.86 | 152.3 | Follicular phase |
| | | 5 | 4.02 | 2.41 | 0.56 | 50.01 | Follicular phase |

**Table 2:** Table of hormonal ratios in the group injected with adriamycin.

The histological study on the ovary also showed that there were necrotic changes of the whole ovaries in general, and at the level of ovarian follicles in particular up to total follicular atresia. These necrotic changes appeared in three main forms:

## First-Necrosis and Decomposition of Follicular Cells

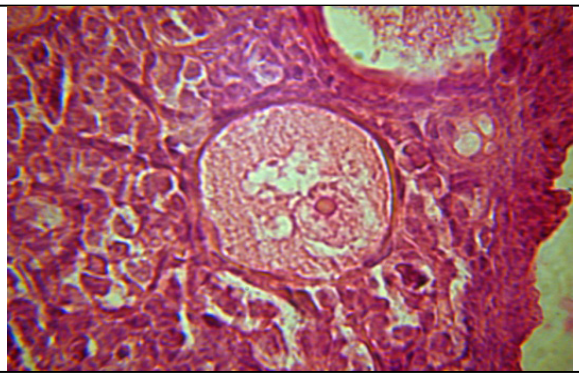
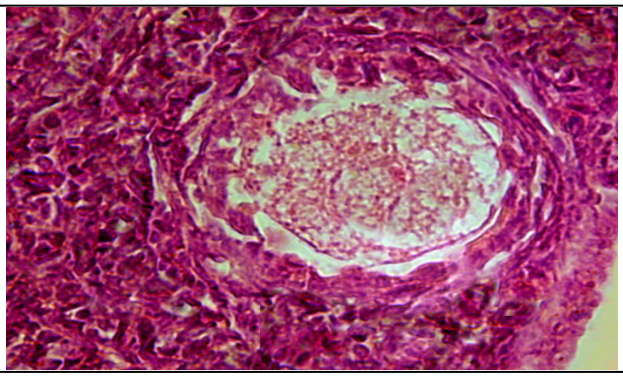
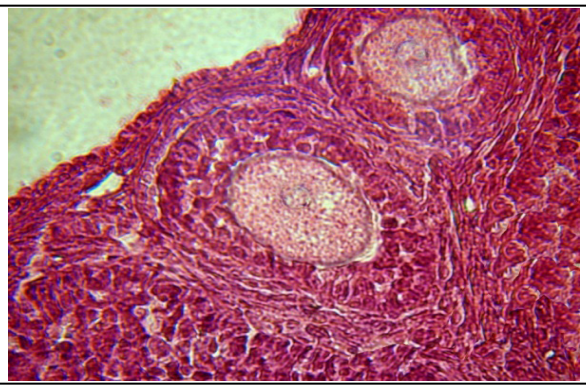
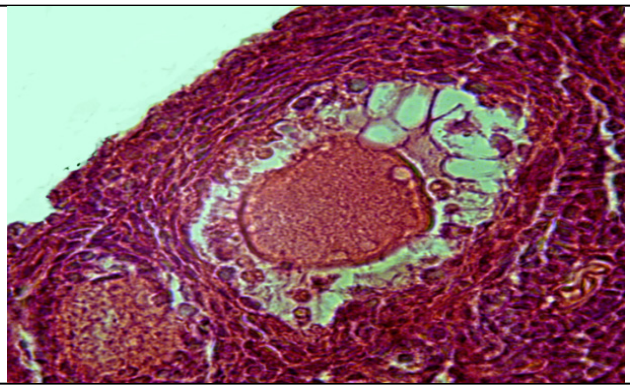
Among the manifestations of the decomposition of the nucleus of the follicular cells as that has been observed are: the thickening of the nuclei (pyknosis), their fragmentation into parts (karyorrhexis), condensation of chromatin around the cell membrane, and increase the intensity of cytoplasmic pigmentation due to the disappearance of the nucleus in some cells. At the level of the cytoplasm, some of the follicular cells had shrinking cytoplasm, which led to morphological changes and some of the nuclei was expelled outside of the cell. At the level of its relationship with the neighboring cells, there has been an increase in spacing between follicular cell, causing them to spread within the antrum and cumulus oophorous decomposition. It was noticed also a loss of microscopic vesicles between the follicular cells and oocyte, leading to widening of the area of the zona pellucida.

## Second-Necrosis and Decomposition of Oocytes

There is a loss of oocytes nuclei of their central position, and they displaced towards the cell membrane. in some follicles, nuclear membranes decomposed and disappeared in other stages. other modifications that occur are the decomposition of some parts of its cellular membranes and the filling of the cytoplasm with fatty gaps, and oocyte shrinking. In some follicles, complete loss of oocytes has been observed.

## Third-Necrosis and Decomposition of the Theca Follicular

It appears in the form of compression of the nuclei and cells as a whole, and attain a spindle shape more than the normal ones. partial disappearance of the cellular was noticed, casing some follicles and reached a full disappearance in some follicles. In addition, there was an increase in thickness and irregularity of the basement membrane.

<table>
<tr><th colspan="2">Histological study of the Ovary</th></tr>
<tr><td colspan="2">(Primordial follicles )</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td>The transverse section of primordial follicles in the control group. (H & E x400)</td><td>The transverse section in degradable primordial follicles in the ovary of females injected with adriamycin drug without a protective dose. (H & Ix400)</td></tr>
<tr><td colspan="2">(Primary follicle )</td></tr>
<tr><td>  </td><td>  </td></tr>
</table>

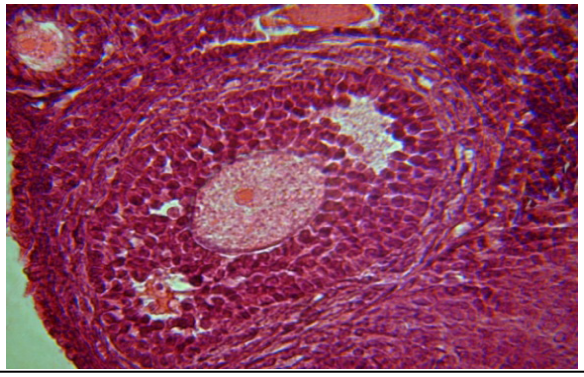
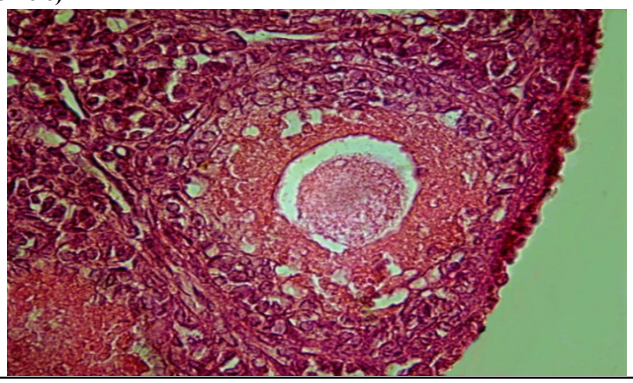
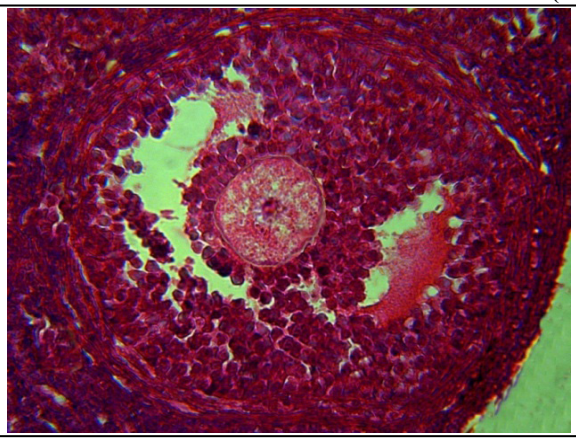
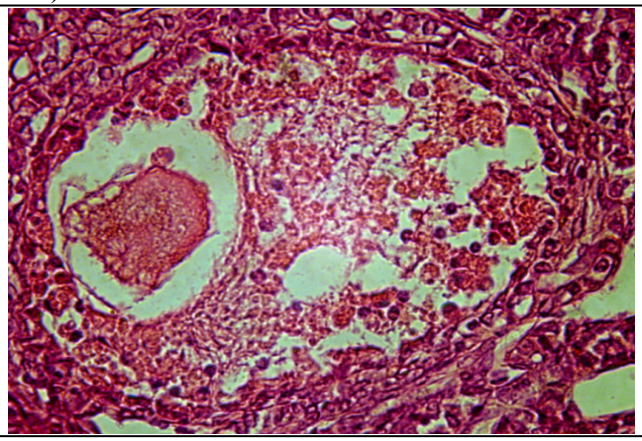
| The transverse section of primary follicles in the control group. (H & E x400) | The transverse section in degradable primary follicles in the ovary of females injected with adriamycin drug without a protective dose. (H & Ix400) |
|---|---|
| **(Secondary follicle)** | |
|  |  |
| The transverse section of secondary follicles in the control group. (H & E x400) | The transverse section in degradable secondary follicle follicles in the ovary of females injected with adriamycin drug without a protective dose. (H & Ix400) |
| **(Graafian follicle)** | |
|  |  |
| The transverse section of Graafian follicles in the control group. (H & E x400) | The transverse section in degradable Graafian follicle follicles in the ovary of females injected with adriamycin drug without a protective dose. (H & Ix400) |

**Table 3:** Shows the differences between the normal follicles and follicles that are degraded by Adriamycin toxicity.

It is clear from the above results, that although this drug has many therapeutic uses for cancer, it has deleterious effects on reproductive cells in the ovaries, in addition to other many side effects, which negatively affects the reproductive ability of population treated with this drug (according to both field and hormonal studies). Therefore, it might be necessary to find out effective solutions to protect cells against these side effects to override any social problems. This research focused on using Complementary and Alternative Medicine (CAM) to protect reproductive cells (ovarian cells), and protect them from the toxic effect of the drug. The materials used in this research are the roots of the red Korean ginseng herb and the Sidr honey with royal jelly.

Ginseng herb (*Panax ginseng*) is one of the widely spread herbal medications used in Korea. It has been used as a food additive, and its benefits are well known by the Chinese and Japanese as a magic formula because of its role in treating many diseases. Its name is derived from the Greek word Panax, which means medication for all diseases [8,9]. Ginseng contains a group of vitamins (A, $B_2$, $B_5$, H, D), some the Enzymes, Calcium, Camphor, Starch, Thyrosides, Glycosides, Paxosides, Triesster, Panaxcvide AF, Panaxatriol, essential oil Hoile, Salutin, Acetylene Compounds, Steroids, Hexane, and Amino acids [10-13]. However, the most important effective elements are Ginsenoside Triterpene which contains more than 30 compounds; Saponin compounds which contain 25 compounds of saponins; and Gelcosiedes which have steroidal nature [14-16].

Honey and royal jelly were also targeted. They are natural substances with various nutritive and therapeutic properties. They have been used since ancient times and they have even considered being more useful than mammalian milk. Honey contains useful natural sugars in the form of different carbohydrates such as monosaccharides (Fructose and Glucose), disaccharides (Sucrose, Maltose, and Trehalose), and polysaccharides (Oligosaccharide, Trisaccharides, Melezitose, and Raffinose), with some organic acids. Most importantly, it contains antioxidants, most of which are herbal in origin (=112 compounds), and polyphenolic acids (Uercetin, Luteolin, Kaempferol, Apigenin, Chrysin, Galangin). The most important antioxidant effect is possessed by Flavonoids, which comprises about (2-46 mg/g) of the honey, and these proportions increases if the honey is collected in the warm and dry areas [17-19].

In our study, we chose the Sidr honey as a preventive dose, which derived from Ziziphus Spina-Christi trees in warm mountains in areas of Yemen and Oman. Sidr honey is classified primarily among the most common types of honey because it contains a large percentage of antioxidants, most notably flavonoids acids [20]. The royal jelly contains many antioxidant compounds. It contains 22 amino acids which act as effective antioxidants against different mutant substances, as well as it contains a natural sex hormones (Testosterone, Estradiol, Progesterone, Prolactin), unsaturated fatty acids (6%), and many mineral salts ranging between (0.8% - 3%) which include : K, P, S, Na, Ca, Al, Mg, Zn, Fe, Cu and Mn, as well as the presence of vitamins ranging from (12% - 43%), including Riboflavin, Thiamine, Niacin, Folic acid, Pyridoxine, Pantothenic acid, and vitamins A, C, D, E and K, and E, which activates sexual function [21-24].

According to the properties possessed by these natural substances (ginseng herb, honey and royal jelly); the question emerges is: can these natural substances be a real solution to avoid the toxic effects of Adriamycin on the tissues of the ovary, and to maintain its function and prevent disturbances in sex hormones, preventing early menopause?

When compared results of hormonal levels shown in (Table 2), and the hormonal levels of other groups treated with preventive supplements (Table 4,5), at different Times, show clearly the stage of ovulation after its total disappearance in the group injected with the drug only, was significantly observed in the group that was treated with honey and royal jelly, (The stage of ovulation, Represent the functional activity of the ovary).As noted Absence of the initial ovarian disorders, as well as the absence of early menopause in the group latter (G-A & H-A).

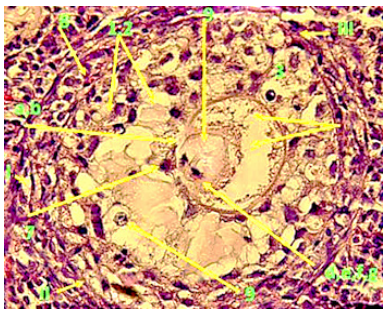
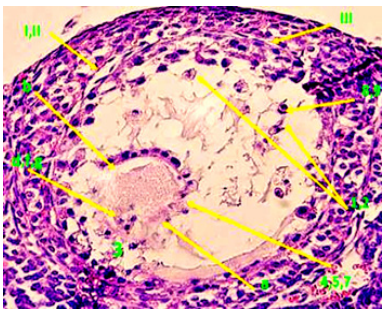
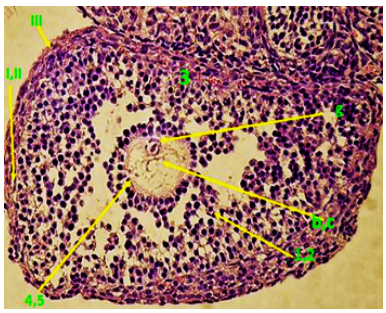
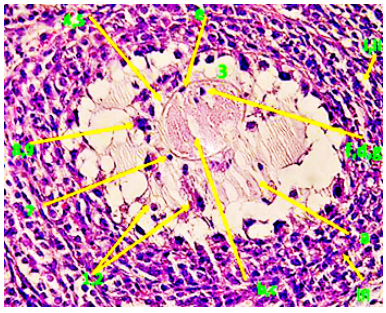
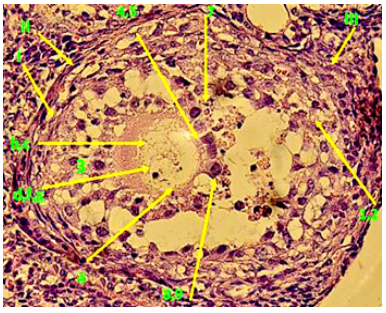
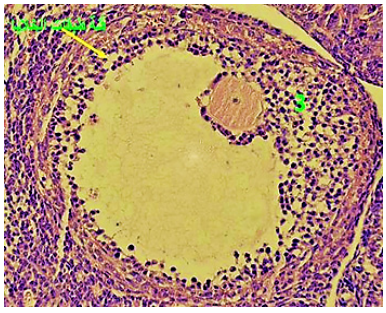
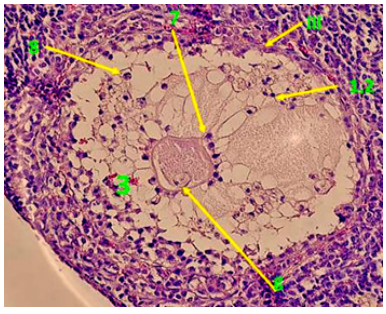
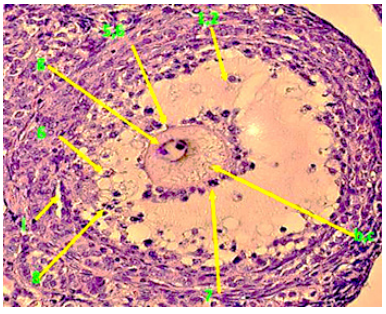
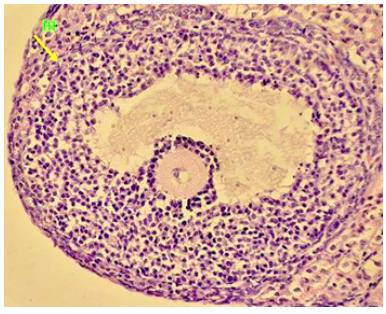
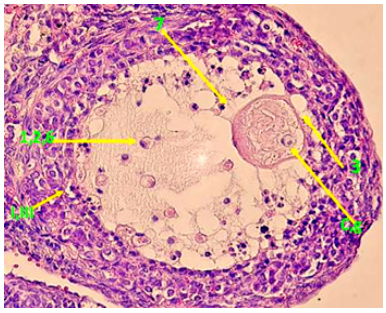
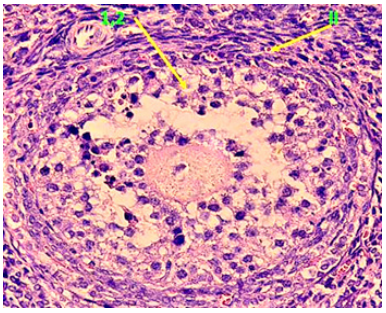
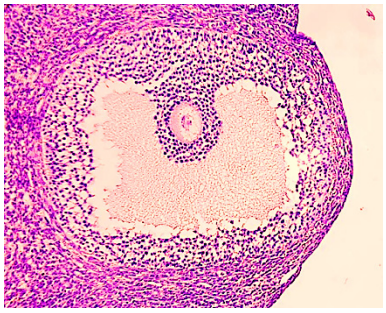
| Group Home | Subset | Sample number | F.S.H (MIu/ml) | L.H (MIu/ml) | Pro (Nmol/ml) | E.S (pg/ml) | Characterization of the situation |
|---|---|---|---|---|---|---|---|
| **Injected group with Adriamycin drug and a preventive dose ( Korean Ginseng) (G-A)** | Control "Korean Ginseng" | 1 | 19.08 | 90.45 | 9.5 | 498.6 (High) | Ovulation |
| | | 2 | 6.5 | 9.7 | 23.1 | 208.8 | Luteal phase |
| | | 3 | 6.78 | 8.97 | 21.09 | 210.3 | Luteal phase |
| | | 4 | 17.87 | 70.65 | 9.34 | 150.4 | Ovulation |
| | | 5 | 22.01(High) | 85.76 | 6.81 | 465.5 (High) | Ovulation |
| | First time (G-A-I) | 1 | 6.67 | 10.7 | 12.6 | 93.5 | Luteal phase |
| | | 2 | 15.98 | 89.02 | 5.31 | 129.5 | Ovulation |
| | | 3 | 7.6 | 10.67 | 9.18 | 97.4 | Luteal phase |
| | | 4 | 21.76 | 90.78 | 2.85 | 102.7 | Ovulation |
| | | 5 | 12.41 | 11.76 | 2.01 | 18.53 | Follicular phase |
| | Second time (G-A-II) | 1 | 7.08 | 10.65 | 5.05 | 50.5 | Luteal phase |
| | | 2 | 7.45 | 11.13 | 8.69 | 53.1 | Luteal phase |
| | | 3 | 12.97 | 11.98 | 1.22 | 23.33 | Follicular phase |
| | | 4 | 7.72 | 11.18 | 5.39 | 48.2 | Luteal phase |
| | | 5 | 12.51 | 12.06 | 0.9 | 33.49 | Follicular phase |
| | Third time (G-A-III) | 1 | 12.5 1 | 12.45 | 2.51 | 70 | Luteal phase |
| | | 2 | 16.42 | 11.6 | 12.96 | 95.7 | Luteal phase |
| | | 3 | 12.5 | 12.76 | 1.69 | 58.2 | Follicular phase |
| | | 4 | 26.56 | 10.67 | 7.67 | 71.27 | Luteal phase |
| | | 5 | 65.8 | 95.87(High) | 5.6 | 100.2 | Ovulation |
| | Fourth time (G-A-IV) | 1 | 86.87 | 95.07 | 6.53 | 251.04 | Ovulation |
| | | 2 | 100.45(High) | 96.01(High) | 5.11 | 234.73 | Ovulation |
| | | 3 | 98.56(High) | 13.98 | 23.2 | 150.09 | Luteal phase |

**Table 4:** Shows the hormonal levels in the group treated with preventive dose (Ginseng root herb) and injected with adriamycin.

| Group Home | Subset | Sample number | F.S.H (MIu/ml) | L.H (MIu/ml) | Pro (Nmol/ml) | E.S (Pg/ml) | Characterization of the situation |
|---|---|---|---|---|---|---|---|
| **Injected group with Adriamycin drug and a preventive dose (honey, royal jelly) (H-A)** | Control honey, royal jelly | 1 | 3.5 | 5.43 | 20.59 | 174.4 | Luteal phase |
| | | 2 | 12.32 | 16.87 | 22.62(High) | 209.7 | Ovulation |
| | | 3 | 18.5 | 55.7 | 9.29 | 423 | Ovulation |
| | | 4 | 13.67 | 15.43 | 28.33 | 276 | Ovulation |
| | | 5 | 19.67 | 85.36 | 9.12 | 480.01 | Ovulation |
| | First time (H-A-II) | 1 | 14.4 | 18.01 | 2.11 | 275.69 | Ovulation |
| | | 2 | 3.32 | 2.21 | 76.53 | 146.62 | Luteal phase |
| | | 3 | 2.45 | 2.13 | 75.07 | 134.33 | Luteal phase |
| | | 4 | 16.04 | 65.87 | 10.99 | 373.73 | Ovulation |
| | | 5 | 17.94 | 84.78 | 19.20(High) | 411.21 | Ovulation |
| | Second time (H-A-II) | 1 | 4.12 | 8.02 | 77.35 | 149.92 | Luteal phase |
| | | 2 | 1.73 | 10.13 | 79.44 | 165.6 | Luteal phase |
| | | 3 | 5.62 | 4.98 | 72.51 | 130.2 | Luteal phase |
| | | 4 | 14.83 | 69.56 | 17.32(High) | 318.21 | Ovulation |
| | | 5 | 8.5 | 12.3 | 4.61 | 157.84 | Follicular phase |
| | Third time (H-A-III) | 1 | 6.87 | 9.02 | 71.88 | 162 | Luteal phase |
| | | 2 | 11.94 | 9.45 | 4.15 | 165.42 | Follicular phase |
| | | 3 | 20.01 | 80.67 | 15.67(High) | 280.75 | Ovulation |
| | | 4 | 15.32 | 85.67 | 8.96 | 221.4 | Ovulation |
| | | 5 | 6.99 | 1.43 | 90.02(High) | 119.98 | Luteal phase |
| | Fourth time (H-A-IV) | 1 | 18.65 | 75.01 | 18.71(High) | 352.98 | Ovulation |
| | | 2 | 19.65 | 85.34 | 11.98 | 480.96 | Ovulation |
| | | 3 | 7.8 | 4.5 | 60 | 174.19 | Luteal phase |
| | | 4 | 20.45 | 80.11 | 14.37(High) | 445.94 | Ovulation |

**Table 5: S**hows the hormonal levels in the group treated with preventive dose (honey with royal jelly) and injected with adriamycin.

The histological study of the ovary in general, and of the follicular and oocytes in particular, has shown differences between groups at all times. The comparison will be limited to mature Graafian follicles as they are the most prone to Necrosis and have the most influential effect on ovarian activity [25] (Table 6).

| Histological study of Graafian follicle | | | |
|---|---|---|---|
| | Injected group with adriamycin drug without protective<br><br>(A) | Injected Group with adriamycin drug and a preventive dose (Korean Ginseng)<br><br>(G+A) | Injected Group with adriamycin drug and a preventive dose (honey, royal jelly)<br><br>(H+A) |
| **First time**<br><br>**(I)** |  |  |  |
| **Second time**<br><br>**(II)** |  |  |  |
| **Third time**<br><br>**(III)** |  |  |  |
| **Fourth Time**<br><br>**(IV)** |  |  |  |

| | |
|---|---|
| a) Decomposition of the Oocyte membrane.<br>b) Shrinkage of Oocyte cytoplasm.<br>c) Presence of the gaps in the Oocyte and the lipid bodies in its cytoplasm.<br>d) Decomposition of the nucleus of the Oocyte.<br>e) The distribution of the nucleus contents in the Oocyte cytoplasm.<br>f) Decomposition of the nucleic chromatin in the of Oocyte.<br>g) Decentralization of the nucleus.<br>h) Gaps between the basement membrane and the follicle's Surrounding tissues and the alienation of the Granulosa cells of the basement membrane.<br>i) Gaps in Theca follicular.<br>j) Thinning and decomposition of Theca follicular. | a) Loss of contact within the Granulosa cells<br>b) Disturbance of the Granulosa cells into the antrum.<br>c) Decomposition of the Cumulus oophorous.<br>d) Disappearance of the desmosomes crunching the Granulosa cells with the Oocyte.<br>e) Expansion of the Zona pellucid.<br>f) Shrinkage of the skeletal and cytoplasm of the Granulosa cells.<br>g) Decomposition of the cytoplasmic membrane, and rush of its nuclei to the Zona pellucid.<br>h) Karyorrhexis of the nucleus of the Granulosa cells.<br>i) Condensation of chromatin forward the membrane of the Granulosa cells. |

**Table 6:** Comparison of the severity of Necrosis in Graafian follicles between groups (A & G-A & H-A).

In this comparison, there was no significant difference between the adriamycin-only group (A) with the group that was supplemented with ginseng root herb and injected with the drug (G + A) at the level of decomposition and necrosis in the cells. In contrast, there was a significant positive difference, with even an increase in the divisional activity in the follicular cells in the group that was supplemented with honey and royal jelly and injected with the drug (H + A). Moreover, we have noticed that there were some mature follicles that exceeded the normal follicles in size**.**

## Discussion

### The Group Injected with the Drug Without Preventive Supplements (A)

Adriamycin will stimulate the degradation (Necrosis) of the various histological structures of the ovaries (follicular cells and oocytes) (Table 6). This is attributed to the adriamycin action mechanism which results in a DNA destruction through its non-specific binding with the coupled nitrogenous bases of DNA strands by forming covalent bonds, i.e., (Methylene linkage between N3 and C-strand guanine), and by forming a non-covalent bonding between the nitrogen bases (a bond between n-strand and N2 of the drug forming a formaldehyde derived bond), leading to DNA and probably RNA disintegration [26-29] (Figure 6). These changes, in turn, cause the deletion of a large number of genes in the dividing cells, i.e., it has a mutant effect on DNA and RNA [30].

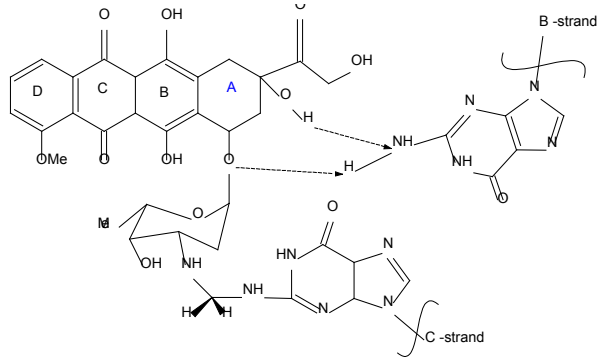



**Figure 6:** Shows how adriamycin is bound with DNA by covalent and non-covalent bonds.

In addition, the drug has the ability to inhibit the enzyme Topoisomerase II (this enzyme plays a role during the replication process of DNA, as it is responsible for recombination of DNA strands after their separation during the replication) (Figure 7) [31]. The drug binds with the DNA strands during their duplication while they are separate from each other, with subsequent failure of strands recombination. That results in a failure of the Topoisomerase II enzyme to recognize the DNA structure, and inhibiting the effect of this enzyme. As a result, restoration of the normal structure of the double-stranded DNA fails, with subsequent failure of cell division process, which causes the death of the programmed apoptotic cell [32,33].

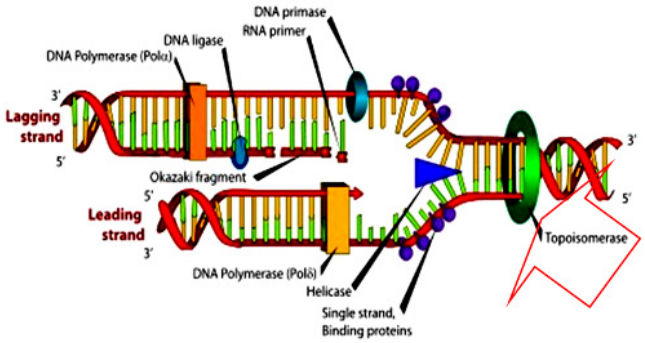



**Figure 7:** Shows the effect of Topoisomerase II enzyme on DNA strands during duplication.

The process of cell membrane destruction occurs as a result of the drug binding to cell membrane components, especially phospholipids, which results in changing the structure of the plasma membrane [34,35]. In addition, cell membrane decomposition may result from the effect of free radicals, since the interaction of the drug with the ($Fe^{3+}$), results in a series of oxidative and reductive reactions, producing an Iron-Adriamycin complex and hydrogen peroxide, $H_2O_2$, compounds that interact with the fats through a process called Lipid Peroxidation (LPO) causing damage to the plasma membranes [36,37].

This degradation and necrosis of the ovarian tissues will result in a disruption in the functional activity of the ovaries, leading to the absence of the ovulatory phase. In addition, some cases may reach early menopause (Table 2), due to the toxic effects of the drug on the ovarian follicles, especially on the mature hormones-producing follicles, especially the Estradiol and Progesterone, which in turn negatively affect other sex hormones (FSH and LH) through the Feedback mechanism. These hormonal changes cause disturbances in the menstrual cycle, or weak embryonic implantation in the endometrium, leading to spontaneous abortion [38]. These clinical findings were observed in this field study in women treated with adriamycin.

## Group Supplemented with Ginseng Herb Root and Injected with Adriamycin (G + A)

The histological study the group (A+G) confirmed that there are degradation and necrotic changes in ovarian follicular cells similar to that found in the group (A) injected with drug only. No significant difference between these groups is found (Table 6). In contrast, the hormonal study (Table 3) confirmed the presence of ovarian activity. Hormonal analysis showed neither sex hormones deficiency disturbances, nor did early menopause similar to that noticed in the previous group (A) (Table 4). It was even noted that some cases have reached the luteal and ovulation phases. There was a clear ovarian activity, despite the similar tissue destruction of the ovaries in both (A) and (G + A) groups. This difference between the histological study and the hormonal study can be explained as follows:

The roots of ginseng herb have been shown to increase the levels of sex hormones despite that the ovaries did not recover structurally. The ginseng herb roots have a stimulatory effect on sex hormones synthesis. Some studies have indicated that the herb contains active ingredients such as Saponine and glycosides, both have a stimulatory effect on the anterior lobe of the pituitary gland, leading to an increase in FSH and LH hormones secretion [40]. This might explain the increased FSH hormone levels in many members of this group.

Regarding ovary-secreted hormones, especially Estradiol, we noticed normal hormonal levels, despite the histological decomposition of the hormone-secreting follicular cells. The hormonal levels have even increased in some members of the control group, and that is attributed to:

- The binding occurs between the ovarian and pituitary hormones via a positive feedback mechanism. The increased concentration of the hormone is responsible for the development of follicles (F.S.H) which positively influence the synthesis of Estrogens [39].
- There is a similarity between estrogens hormone complex and the structure of some ginseng root herb Gelcosiedes that have steroidal nature. In addition, some ginseng ginsenosidic acids have a similar basic structure to estrogen hormones. This similarity leads to stimulation of the estrogen hormone receptors (including ER-α and ER-β), despite the absence of the hormone itself. These receptors are activated by binding of estrogen-similar compounds after bypassing the plasma membrane in the follicular cells resulting in stimulating the gene transcription to form RNA. Some of these compounds have also similar mechanisms by acting on the progesterone receptors [40,41], and thus the ginseng has effects similar to that of progesterone and estrogen hormones (Figure 8).

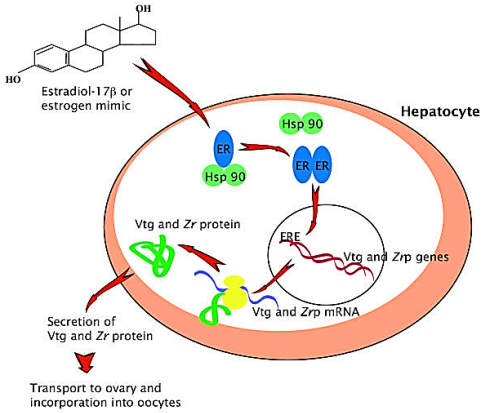



**Figure 8:** Mechanism of hormonal receptor stimulation in follicular cells.

This is confirmed by some studies [16,42] which demonstrate the ability of ginseng herb to treat menopausal disorders through treating low estradiol level that may cause these disorders.

### Group Supplemented with Honey and Royal Jelly and Injected with Adriamycin (H + A)

In this group, there is a significant difference between the (H+A) group on one hand, and both the group injected with the drug without preventive supplements (A) and the group supplemented with ginseng herb root and injected with adriamycin (G+A) groups on the other hand, in terms of preserving the histological structure of the ovary. The histological study (as presented in (Table 6) confirms the existence of partially normal follicles to completely normal follicles after their exposure to the toxic and mutant effect of adriamycin on the ovarian structure as that seen in both (A) and (G+A) groups. Thus, (H+A) group is more able to maintain the functional activity of the ovary more effectively than the group dosed with the roots of the ginseng herb (G+A). The ovulation stages, which represents the peak ovarian activity, is increased (Table 5), which is attributed to the protective effect of the supplemental doses (honey with royal jelly) in the form of two important roles:

The first role: To protect the histological structures of the ovary from the toxic effect of Adriamycin, because: Honey and royal jelly contain a total of 112 antioxidants, further to Polyphenols and Flavonoids are the most important antioxidants, which present with higher concentrations in the Sidr honey that have been used in this experiment [17-19]. Antioxidants antagonize the effects of mutant compounds through minimizing DNA breakdown and hence protecting the DNA from the mutant effect of adriamycin. In addition, Flavonoids increase the effectiveness of Glutathione reeducates, which helps to stimulate cell division and growth. They also activate Glutathione Peroxidase and Glutathione-S-Transferase (GST) enzymes, which are enzyme antioxidant. The honey also contains vitamin (C), which blocks vulnerable sites on the DNA, preventing the access of mutant compounds to these sensitive sites [43-48].

In addition, honey has the ability to prevent the binding of iron with mutant compounds, and thus prevent the formation of free radicals that are generated by the drug when linked to ($Fe^{+3}$) resulting in the formation of "Iron-adriamycin complex" and Hydrogen Peroxide ($H_2O_2$) free radical. These compounds interact with phospholipids of the cell membrane [49]. And thus, honey prevents the decomposition of the cell membrane that might be caused by the drug.

The second role: Maintaining the level of sex hormones despite the toxic effect of the drug on the cells secreting these hormones, due to: The ability of honey and royal jelly to preserve the follicular cells that are found in the mature follicles and to promote the division of follicular cells as demonstrated by the histological study through observation of large-sized mature follicles which are considered as the main producer of Estradiol & Progesterone hormones in the ovaries. The increased hormonal levels in some members of the group may be explained by the consideration that these hormones are naturally present in the royal jelly as we explained before [21].

In addition, royal jelly contains fatty acids which have a direct effect on the receptors of estrogen-producing cells (ERα and ERβ receptors), such as: (10-hydroxy decenoic acid, p-hydroxy benzoic acid, 24-cholesterol Matalan, 10-hydroxy-2-decenoic acid, 8-hydroxyoctanoic, 3-hydroxy decenoic, 3-1 Dextro, Lauric, Undecenoic, Capric, Nonanoic, Palmitoleic, Myristoleic, Tridecenoic, Linoleic, and Arachidic acids). This effect results in the stimulation of nuclear proteins, leading to increased mRNA replication rates of the gene ($Ps_2$) that produce the functional protein of natural estrogen [50,51]. Additionally, honey and royal jelly contain factors that stimulate the release of pituitary hormones, and thus indirectly stimulate the release of ovarian hormones [52].

## Conclusion

- Adriamycin causes decomposition and necrosis of ovarian follicles, especially mature follicles, which adversely affect the ovarian function and the reproductive capacity.
- Ginseng root herb is not considered a sufficient preventive supplement, as it did not protect the tissue structures. However, it contributes to raising the levels of sex hormones by stimulating the pituitary gland hormones (FSH, LH), and activating some estrogen & progesterone receptors instead of the real hormone. Ginseng root herb will maintain the functional role of ovaries rather than histological and structural protection, which may not positively contribute to the reproductive capacity.
- Sidr honey and royal jelly are considered to be the best preventive supplements. They protect the tissue structure of the ovary, especially mature follicles (Graafian follicles,) which positively contribute better than Ginseng root herb in maintaining sex hormones and the functional role of the ovary, as well as positively maintaining the reproductive capacity.

## Recommendations

The people who are treated with the chemical therapeutic doses should take the Sidr honey and royal jelly to maintain their sexual and reproductive capacities. It is preferable to start taking these supplements 15 days before commencing chemical treatment with the following doses:

- Sidr honey dose: 800 mg / kg / day.

- Royal jelly dose: 10 mg / kg / day.

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