THE ROLE OF HONEY WITH ROYAL JELLY IN PROTECTING THE GRAAFIAN FOLLICLES FROM THE TOXICITY OF THE ADRIAMYCIN DRUG

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INTRODUCTION

Chemical treatments of cancerous tumors are considered as a special kind of cellular poison (Cytotoxic) [1], for both normal and tumor tissues, especially the rapid reproductive tissues like ovaries and testes [2]. These effects on the genitals organs may lead to the destruction of the cells, or inhibition of its functions [3], especially the hormonal, menstrual cycle in the female, and the number of sperms in male [4].

Adriamycin is considered the best and the most commonly used anti-cancer drugs in chemotherapy for various cancer tumors [5, 6]. It belongs to the Anthracyclines compounds that follow a group of antibiotic, anti-tumors [7]. Many studies of the toxic effects of this drug have been concerned precisely on the DNA [8-10], the enzyme Topoisomerase II [11-13], the structure of phosphoric lipid membranes, and the formation of basic oxygenic roots through peroxide of lipids in cell membranes [14]. All of these mechanisms contribute in killing of reproductive cells, rather than the cells present in the resting phase [7]. Therefore, it is very important to minimize the side effects of these drugs.

The honey with royal food nutrient may be considered as a universal miracle, having several medical properties. This combination contains a wide diversity of, sugars, proteins, enzymes, and minerals, pollens, remains of fungi, algae, yeasts, antioxidants (the glucose oxidas, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives), amino acids, proteins [15, 16]. They also contain some hormones such as Estrogen, testosterone, Progesterone, prolactin [16], B complex vitamins (B-1, B-2, B-6), pantothenic acid, nicic acid. And some biological factors act as bio catalysts in the process of regeneration of cells within the human body [17]. The present study aims to determine the toxic effects caused by the drug Adriamycin on the Graafian follicles, the division possibility, the sexual hormones, Oocyt, reproductive ability, hence investigates the ability of honey with royal jelly in minimizing these side effects.

MATERIALS AND METHODS

Animals

Fifty young adult female Syrian hamsters (Mesocricetus auratus), weighing between 85 gm-110 gm, three to four months aged, and lengths between (12Cm-13Cm) were obtained from the breeding colony in the faculty of sciences during the breeding seasons (spring and summer). Animals were housed in plastic cages (16x 31x 42Cm). They were under the same laboratory conditions, including natural light supplemented with artificial light (12L: 12D). Ambient temperature in the animal facilities was held constant. The animals were divided into several groups in cages, and subjected to identical environmental conditions (22±2°C). Water and food were accessible to the cages. Some cages with several males were placed close to the females cages in order to fermonal incitement of males. Males were moved to the female cages, two days before bloodletting in order to incite the female ovulation and sexual activity. The animals were handled according to the guidelines of Helsinki declaration rights (1975) of using laboratory animals.

Females were divided into two main studied groups

The first major group (A) Injected with Adriamycin drug and did not take a preventive dose. This group consists of 25 females, divided into two subsections:

- The first group (natural control) contains five females, orally administrated with tap water for 15 days, and then injected with a physiological solution (Intraperitoneal Injection).
- The Second group (experimental group) contains twenty females, orally administrated with tap water for 15 days and then injected intraperitoneally with Adriamycin drug (Doxorubicin-EBewe_AUSTRIA) as a single dose (in terms of the dose given to an adult person 60-75 mg/m² as a single dose every 15 to 21 days [20].

The second major group (H+A)

This group consists of 25 females, injected with Adriamycin drug after the preventive, then divided into two subsections:

- Group I: (witness the preventive dose of honey with royal jelly) and consists of five females oral administrated for 15 days with Sidr honey mixed with royal jelly (was 800 mg/kg/day of Honey and 10 mg/kg/day of royal jelly), they were mixed and dissolved by tap water [15, 16]. This group was not injected with drugs. (References 5 m)
- The second group (group experiment): consists of twenty females administrated with preventive dose with the same ratios mentioned earlier, and then injected intraperitoneally with Adriamycin drug (proportions are similar to the group A).

**Laboratory study**

- Experimental animals were anesthetized and dissected, and then blood samples of the heart were collected in heparinized tubes, and finally the paired ovaries were collected at different time intervals as follows:
  I) Three days after the injection of the drug (A-I).
  II) Five days after the injection of the drug (A-II).
  III) Ten days after the injection of the drug (A-III).
  IV) Fifteen days after ingesting the drug (A-IV).

- The ovaries were fixed, dehydrated in graded alcohol, and embedded in paraffin. Five Micron sections were stained with Hematoxylin & Eosin (H & E) following the routine tissue preparation technique.

- Immunohistochemical study was applied through histological sections stained with immunocistain (Ki67) that specialized for disclosure of cell divisions. Antibody Ki67 (MIB) united with nuclear protein that appears in all stages of cell division (G1, S, G2, M), except the resting phase (G0), in order to determine the effectiveness of cell divisions within the Granulosa cells or reproductive cells (brownish color in the cell divided).

- The sexual hormones were analyzed (FSH & LH & Progesterone & Estradiol) using Roche Cobas e 411 hormones analyzer, (4th Generation) that belong to 4th generation, and work according to Chemiluminiscence (ECL) mechanism.

**RESULTS AND DISCUSSION**

In a previous study published in 2010 [18] about the effect of Adriamycin drug on the ovaries of white mice, we pointed out that the Graafian follicles are the most affected ovarian follicles by the toxicity of this drug. This was attributed to the high reproductive divisive ability as compared to the rest of the follicles. The toxicity of the drug appears clearly, whenever the cells are multiplied rapidly. Furthermore, the Graafian follicles predominate on the rest of the ovarian follicles, which are almost completely stopped from development and cell division. This is likely to be due to the role of the Graafian follicles hormonal control (hormone Estradiol) that effect indirectly the rest sexual hormones, such as the hormones of the anterior lobe of the pituitary gland (LH & FSH) as a result of the feedback mechanism that inhibits the development of other follicles. Thus, the decomposition of Graafian follicles was larger than other follicles, especially after placing the injected females with males, which stimulate the process of ovulation (the evolution of the Graafian follicles) through sexual attraction.

**First group:** injection group with Adriamycin drug without preventive dose (A)

**A. Histological study of the Graafian follicles**

**A-1. The normal control group**

The Theca folliculi of the Graafian follicles for the control group individuals is divided into two parts: Theca externa, which is composed of a thin layer of fusiform cells, through which the blood vessels pass toward the second part which is called Theca interna. This part consists of a large-sized, oval-shaped Fibrocystic cells, and interspersed with a dense network of capillaries, which distributed along its border in order to supply the follicle with blood. Inside the Theca folliculi there are several layers of cuboidal shape of Granulosa cells, with a cylindrical central nucleus. Among the Granulosa cell groups there is the antrum, and also any Granulosa cells are surrounded the Oocyte, forming the Cumulus opens, or Corona radiate. The Oocyte and the Cumulus are separated by a transparent area called the Zonapellucida, occupied by microscopic villi joining the Granulosa cells and the Oocyte.

The Oocyte has a large size and spherical shape with central positioning nuclei [19], fig. 1. Granulosa cells in the Graafian follicles have a high divisive-capacity with exceeding the rest of the developing ovarian follicles (fig. 2).

![Fig. 1: Transverse section of Graafian follicles in the control group](image1)

**A-2. The experiment group injected with the drug without preventive dose**

I) After three days of Adriamycin drug injection (A-I), it has been noted that the Graafian follicles were the most affected ovarian follicles. The toxicity of the drug caused the disappearance of the most of the natural Graafian follicles from the ovary. Furthermore, most of the follicles that have been seen in the ovary during this period are decomposed follicles with abnormal structures. In addition, a complete destruction of the Cumulus oophorous has been observed, with the spread of the Granulosa cells in the antrum. This is due to the loss of contact within the Granulosa cells, and the rush of its nuclei toward the antrum and the Zona pellucida. Consequently, this causes the destruction of the chromatin in some cells and its accumulation on the basement membrane of other cells, and thinning of the thickness of Theca folliculi. In addition, we noticed the presence of spaces in theca folliculi and migration of the Granulosa cells from the basement membrane, and partly the breadth of the Zonapellucida because of the loss of desmosomes between the oocyte and the cells. Regarding the Oocyte, the position of the nucleus is laterally with partial Granulosa decomposition of the nucleus membrane. In addition to the decomposition of the nucleus in some Graafian follicles (fig. 3), a clear reduction in the divisive ability of the Granulosa cells is seen compared to the normal control (fig. 4).

II) After five days of injecting the drug (A-II), a clear and complete decomposition in all the Graafian follicles has been observed. This decomposition, compared with the first time (A-I), is represented in a total destruction in Cumulus oophorous, and more spacing of...
Granulosa cells and increase in the spread towards the antrum. It is accompanied with the breadth of the Zona pellucida because of the loss of desmosomes among themselves, more spacing in Theca folliculi, and the increase in its thinning, and more condensation of chromatin to the degree of increase its Condensation on the Granulosa cell membrane. Concerning the Oocyte there will be an entire decomposition of the egg and the dissolution of the lateral nucleus. (fig. 5). This is in addition to increase in weakness, in the divisible ability in the Granulosa cells (fig. 6).

III) After ten days of Adriamycin drug injection (A-III), no maturity Graafian follicles were seen, but we noticed the emergence of a small number of the Graafian follicles in its early stages. The decomposition observed in the full maturity follicles is almost a full destruction of the cumulus oophorius has. Furthermore, a continuing spreading of the Granulosa cells in the antrum. Furthermore, we noticed a continues expansion of the Zona plleucida accompanied by the presence of some of the follicles, which still suffer from condensation of chromatin toward the cell membrane of the Granulosa cells, and then continues moving away of cells from the basement membrane, with thinning Theca folliculi and the presence of voids in it. Regarding the Oocyte, the nucleus still laterally with a fatty gaps in it (fig. 7). However, for the granulosa cell division, a light improvement in the division has been observed (fig. 8).

IV) Fifteen days after the drug injection (A-IV) the presence of a very few number of the semi-normal young Graafian follicles has been observed. This is produced from the development of previous ovarian follicles, with light decomposition in the Cumulus oophorius and thinning in the Theca folliculi. However, for the full maturity Graafian follicles, some sort of full decomposition appears; whereas the Oocyte in the Graafian follicles shows a very little degeneration with the persist of the lateral positioning of the nucleus, and the presence of greasy gaps in the Oocyte cytoplasm (fig. 9). Similarly, there have been a clear improvement in the level of the Granulosa cells divisive capacity. Nevertheless, these changes did not arrive at the normal level (fig. 10).

An investigation of the previous results shows that the highest pathological changes, ovarian tissue alteration, and the reduction divisive ability appear at the beginning of the injection process, and until the beginning of the third time. The second time (the fifth day of the time of injection) was the most affected time concerning the histological changes of the other times in the Graafian follicles. Then the gradual recovery stage started from the beginning of the third time and afterwards. This can be attributed to the strong trickonization attack, which occurred at the beginning of the Adriamycin drug injection of the tissues with divisive activity, such as the Granulosa cells and the Oocyte cells. In fact, the intraperitoneal injection helps in allowing the drug to reach the area surrounding ovary at the same level. Therefore, a clear reduction in the divisive activity took place in the beginning. This is demonstrated in sections stained by the immunohistochemistry dye (Ki57) where a complete halt in the cell division [in time II] is shown. Then, the body started to resist the poisoning of the drug, and dispose of toxic effects through gradual subtraction of the drug and its metabolic outputs (through urine, feces and bile) [20]. Therefore, start very slowly a gradual recovery, regeneration of tissue, and proliferative divisive activity. However, the disposal of the toxic is greater for the rapid reproduction than that of the moderate divisive activity tissues [2].

This, in fact explains the relatively high number of immature Graafian follicles in the third time, and the fourth time, because of the full decomposition of the Graafian follicles infected by the cell apoptosis. This led to the demise of the inhibitory effect of the other follicles development, and thus, incomplete follicles returned to the growth and development, forming a young Graafian follicle resulting from the development of immature follicles. Nevertheless, there will still be a number of Graafian follicles below the normal level, as compared with the control group. This is because of the remaining of the toxic effects of the drug.

The obtained result shows a good agreement with that of other studies [21, 22]. In other words, both studies confirm the negative impact of the Adriamycin drug on the supporting cells of Oocytes. Such as the Granulosa cells or the Theca follicle cells, which causes changes in the growth and maturation of ovarian follicles. This will eventually result in the decomposition of ovarian follicles. On the other hand, the results of this work agree with that of (the Andreeva et al. [23]), about the impact of Adriamycin drug in the female reproductive system in rats. Both studies show a decrease in the number of mature Oocytes and an increase in the rate of fertilized Oocytes death after a month of use of the single dose. This result is also inconsistent with the study of (Shima [24]) on mice, females injected with Adriamycin drug. The latter study showed a steady decline in the number of the Graafian follicles, and a decrease in the fertility rate as compared with the natural average in a direct proportion with the amount of the dose.

The obtained result agrees with that of (Borovskaya [25]). The latter work referred to the impact of the two on trasekidin (Adriamycin and Alvarmorubisan) in preventing the occurrence of any pregnancy for female rats, which matted after one day of the use of the drug. The study attributed the result to toxicity influence of these compounds on the Graafian follicles.

B. Changes study and sexual hormone disorders

Through the hormonal analysis conducted on the main group injected with the drug without preventive dose (A), and its comparison with the control group (table 2) and the standard field for each phase of the cycle of each of the sex hormones (FSH & LH & Estradiol & Progesterone) (table 1) in order to identify the phases of ovarian accurately, the following has been observed:

- The presence of hormonal disorder cases, which has some of them led to early menopause (infertility, sexual) with a ratio of (2/5), this event took place after the third day of the drug injection (in the first time (AI). It then increases until reaches (4/5), after the fifth day of the injection (in the second time (A-II). At the third time (A-III) it back down to the rate of (2/5), and reaches (1/5) in the fourth time (A-IV). This is attributed to the partial disposal of the drug as a result of the drug metabolized and its secretion in the urine and feces [20]. However, the mutation of effect continues.

- Removal of all members of this group of Ovulation phases or Luteal phase, except the appearance of a single case of the Luteal phase in the fourth time (A-IV). However, the rest of the follicular phases are primitive. This sexual hormonal disorder is related to the imbalance in the histological structure of the ovary. These findings are inconsistent with that of our previous study [18], which is conducted on women, used the Adriamycin drug. The results showed the presence of temporary menstruation outages, at the beginning of the usage of the drug with a ratio (92.5%). It then followed by disorders of menstruation during the treatment period with ratio reached (86.41%). This led 15.38 % to reach an early postmenopause, as a result of the sexual hormones (FSH & LH & Estradiol) disorders. These findings also agree with several studies about the impact of Adriamycin drug at the level of female hormones (Salmon and his colleagues [26]). The latter investigation showed disturbances in the level of hormones for women who undergone a chemotherapy that consists of a group of drugs (5-fluorouracil, Adriamycin Cyclophosphamide, and BCG). The results also consist with the results of (Shamberger and colleagues [27]). This study referred to the presence of disorder in the menstrual cycle, and a temporary menopause, when women had undergone chemotherapy with Adriamycin, Cyclophosphamide and Methotrexate drug.

To summer it up, the Adriamycin drug may cause the occurrence of decomposition and damage of all ovarian follicles, and especially Graafian follicles that secrete the Estradiol hormone. This hormone plays a fundamental role in the ovarian cycle, through the hormonal control on the exporter follicles hormone (FSH) (through the Negative Feed Back mechanism). Also the Ovulation hormone (LH) which plays an important role in the maturation of Graafian follicles and the occurrence of Ovulation (through the positive feedback mechanism) [29, 30]. The damage caused by the drug is a bug in the harmony and coordination, resulting from the toxic effects of the drug on the ovary, which led to the breakdown and killing of Granulosa cells in Graafian follicles, which is the main producers of Estrogen hormones, such as the Estradiol. This led to a defect in all sexual hormones, disorders of the ovaries session, and early post-menopause, which led to prevent ovary from reaction and phase of evolution.
granulosa cells lecration. Moreover, granulosa cells inside the cavity as a result of the loss of connection and all their nuclei had settled laterally (fig. 11). Furthermore, with the destruction of the cumulus operas, the spread of the basement membrane. However, the oocyte cytoplasm has shrunk, natural Graafian follicles has been obtained, in comparison with the control group. The decomposition, in decaying follicles, is represented with royal jelly) and injected with Adriamycin drug (H + A).

Table 1: shows the percentages of normative sex hormones developed for each of the phases [28]

<table>
<thead>
<tr>
<th>Characterization of the situation</th>
<th>E 2 (Pg/ml)</th>
<th>Pro (Nmol/ml)</th>
<th>LH (MIU/ml)</th>
<th>F.S.H (MIU/ml)</th>
<th>Sample number</th>
<th>Subset</th>
<th>Group</th>
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<tr>
<td>Ovulation</td>
<td>25.90</td>
<td>3.04</td>
<td>67.56</td>
<td>14.89</td>
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<td>Control</td>
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<td>Ovulation</td>
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<td>45.78</td>
<td>13.65</td>
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<td>7.09</td>
<td>54.65</td>
<td>15.67</td>
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<td>22.42</td>
<td>2.01</td>
<td>1.08</td>
<td>4</td>
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<tr>
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<td>14.65</td>
<td>2.32</td>
<td>2.60</td>
<td>5</td>
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<td>Luteal phase</td>
<td>67.10</td>
<td>59.69</td>
<td>1.09</td>
<td>2.90</td>
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<td>First time</td>
<td>(A-I)</td>
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<tr>
<td>I started ovary disorder</td>
<td>13.65</td>
<td>1.29</td>
<td>53.01</td>
<td>21.98</td>
<td>2</td>
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<td>47.98</td>
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<td>3.05</td>
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<td>55.11</td>
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<td>8.40</td>
<td>2.99</td>
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<tr>
<td>postmenopause</td>
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<td>1.49</td>
<td>41.54</td>
<td>50.07</td>
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<td>Second time</td>
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<td>Follicular phase</td>
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<td>1.38</td>
<td>11.87</td>
<td>4.75</td>
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<td>I started ovary disorder</td>
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<td>(A-III)</td>
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<td>1.32</td>
<td>58.98</td>
<td>25.00</td>
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<td>53.60</td>
<td>25.04</td>
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<td>2.39</td>
<td>1.57</td>
<td>1.50</td>
<td>1</td>
<td>Fourth Time</td>
<td>(A-IV)</td>
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<tr>
<td>I started ovary disorder</td>
<td>16.01</td>
<td>2.90</td>
<td>43.78</td>
<td>22.142</td>
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<tr>
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Second Group: oral administrated with preventive dose (honey with royal jelly) and injected with Adriamycin drug (H + A)

A. The study of histological decomposition and apoptosis

The histological study of Graafian follicles in this group showed

I) After three days of Adriamycin drug injection, with continued dosing of preventive dose (H + A-I), a decrease in the number of natural Graafian follicles has been obtained, in comparison with the control group. However, the decomposition was lower than the group (AI). The decomposition, in decaying follicles, is represented with the destruction of the cumulus operas, the spread of the granulosa cells inside the cavity as a result of the loss of connection with each other, and nuclei rush towards the zona pellucida as a result of the Granulosa cells Membranes laceration. Furthermore, there will be thinning in the theca folliculi, appearance of the few gaps inside, and migration of the granulosa cells away from the basement membrane. However, the Oocyte cytoplasm has shrunk, and all their nuclei had settled laterally (fig. 11). Moreover, the Granulosa cells’ divisive ability is moderate as compared to the control group given preventive dose (fig. 12).

II) After five days of Adriamycin drug injection with continuing oral administration preventive dose (H + A-II), an increase in the reduction and decomposition of natural Graafian follicles as compared to the control group has been observed (despite the presence of Graafian follicles in the early stage, which her morphology tends to be complete, but in a small size and a low number of the Granulosa cells). Furthermore, some follicles showed more degeneracy as compared to the first time (H + AII). Also, in addition to histological degeneration and decomposition appeared at the time I, new degenerations were found, such as the fragmentation in chromatin, Condensation of Granulosa cells, increased Cumulus oophorous damage as a result of the spacing Granulosa cells from each other, breadth of the Zona pellucida (a result of partial loss of contact between the Oocyte and Granulosa cells, and spacing itself from the Basement membrane), and the thinning of the theca folliculi. However, concerning the Oocyte nucleus, it takes a terminal positioning (fig. 13). On the other side, the Granulosa cells’ divisive ability remains moderate at the early stage of Graafian follicles, with decreases in the decaying Graafian follicles (fig. 14).

III) After ten days Adriamycin drug injection, with continued oral administration, dosing preventive dose (H + A-III), Graafian follicles showed a clear decline concerning the degradation as compared with the time II (H + A-II). In addition, there was an increase in the number of semi-natural Graafian follicles in early stages. Nevertheless, the morphology of Graafian follicles in the early stage in this group has a perfectly similar arrangement to the complete Graafian follicles, with the presence of some traces of decomposition, such as a little spread in the Granulosa cells within the antrum, and spaces in the thin theca folliculi. However, the Oocytes are semi-normal and its central nuclei are central (in spite of its terminal existence at some of the Oocytes in the Graafian follicles). This is in addition to the presence of fatty gaps, and partial shrinkage in its cytoplasm (fig. 15). As for the divisive ability of the Granulosa cells, there was a clear improvement as compared with the first and second time (H + AI) (H + A-II) (fig. 16).

IV) Fifteen days after drug Adriamycin injection, with a continues oral administration preventive dose, (H + A-IV) Graafian follicles seem normal and almost complete. However, there have been a few layers of the Granulosa cells. In addition, the oocytes are normal.
inhibitory ability to some mutagenic or toxic substances [38]. This is enzyme GST. This is considered one of the enzymes that have the warding off the effects. AL-Rubaie 2006 [41], referred to the role of stimulating the division and growth of cells, and contributes to the effectiveness of the enzyme Glutathione Reductase. It also helps Flavonoides play an important role in increasing the activity of the and amino acids in honey and royal jelly, which contribute in inhibition of the mitomycin -C drug - (more than 400 Pg / ml), and within the range of the control. However, cases of Ovulation at all times has been observed, despite the decline in the second time (H+A-II), as a result of an increase in decomposition of Graafian follicles. This indicates that the ovary could re-develop the rest of the ovarian follicles to youth Graafian follicles, and led to a rise in the number of Ovulation phases in the third and fourth time. The rate of Ovulation became 3/4 as compared with the group injected with Adriamycin drug without preventive dose (A). Some of these cases reached early menopause and were free from Ovulation phase.

B. Changes and sexual hormone disorders study

Through hormonal analysis conducted on the main group, which orally administered with preventive dose of honey with the royal food, and injected with Adriamycin drug (H+A) (table 4), and comparing it with drug injected group without preventive dose (A) (table 2), taking into account the standard range field for each phase of the ovary phases and of each of the sexual hormones (FSH & LH & Estradiol & Progesterone) (table 1), in order to identify accurately the ovarian phases; we conclude the following:

- Increase in the Ovulation phases of normal control group, orally administered with preventive dose. The most are Ovulation phases with a ratio (4/5) of ovulation as compared with the natural control group, where the rate of Ovulation is (3/5).

- The absence of disorder cases in sexual hormones or case approaching the early post-menopause (cases of infertility). However, cases of Ovulation at all times has been observed, despite the declines in the second time (H+A-II), as a result of an increase in decomposition of Graafian follicles. This indicates that the ovary could re-develop the rest of the ovarian follicles to youth Graafian follicles, and led to a rise in the number of Ovulation phases in the third and fourth time. The rate of Ovulation became 3/4 as compared with the group injected with Adriamycin drug without preventive dose (A). Some of these cases reached early menopause and were free from Ovulation phase.

- The ratio of the Progesterone hormone increased at a rate of (2/5) in the control group. In the second time (H+A-II) this rate had fallen to each (1 / 5), and then increased again to the rate of (2/5) at the third time III (H+A-III), and eventually in the fourth time (H+A-IV), the rate, reached the ratio (4/3). However, most of these heights are in the Ovulation phase.

- The increase is also observed in the Estradiol hormone ratio. This arrived at the normal upper limit of the phase, which represents (more than 400 Pg / ml), and within the range of the control. However, this rate fall in the second time (H+A-II), and back to increase in the fourth time (H+A-IV) within the scope of phase representation. However, In the main group injected with the drug only (A), this rate decreases.

The height of the Ovulation phase’s number and that of ovarian hormones rates after falling in earlier times can be attributed to the influence of intoxication shock at the beginning of the injection of the drug which led to partial a reduction in the Ovulation phase’s numbers, and hormonal ratios. This is unlike the group (A) which had the same action as that in the group (H+A), but with clearer and larger form. This result is related to the role of the preventive dose (honey with royal jelly) in protecting the tissue is the structures of the ovary, through which the ovary was able to re-developing the rest of the ovarian follicles to youth Graafian follicles after the decomposition of the Graafian follicles, which is...
completely affected by the drug and was working to curb the
development of the rest of the follicles (this is shown by
histological study). This helped the rest of ovarian follicles
to complete the development into Graafian follicles, and thus,
increased in the number of Ovulation phases in the group (H+A).
However, the group (A) did not show an Ovulation phase, despite
metabolizing and excreting the drug in the urine and feces [20]. In
addition, the preventive dose could protect the combinations,
stimulates the cells producing different sexual hormones to
produce these hormones, despite the influence of the toxic effec ts
on the cells. In addition to the presence of some sexual hormones
in the components of preventive dose in its natural forms, as in the
royal jelly.

As a result, the preventive dose of honey with royal jelly was able to
ward off the side effects of the drug. This is in addition to the presence sex hormones, which contributed in maintaining the vitality of the ovary and the ovarian cycle.

**Table 3: shows the analysis of sex hormones in female hamsters injected with the drug after oral administration with honey and royal jelly**

<table>
<thead>
<tr>
<th>Characterization of the situation</th>
<th>E. S (Pg/ml)</th>
<th>Pro (Nmol/ml)</th>
<th>L. H (Miu/ml)</th>
<th>F. S. H (Miu/ml)</th>
<th>Sample number</th>
<th>Subset</th>
<th>Group Home</th>
</tr>
</thead>
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<tr>
<td>Luteal phase</td>
<td>174.40</td>
<td>20.59</td>
<td>5.43</td>
<td>3.50</td>
<td>1</td>
<td>Control honey, royal jelly</td>
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<tr>
<td>Ovulation</td>
<td>209.7</td>
<td>22.62(High)</td>
<td>16.87</td>
<td>12.32</td>
<td>2</td>
<td>First time (H-A-I)</td>
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<tr>
<td>Ovulation</td>
<td>423.00</td>
<td>9.29(High)</td>
<td>55.70</td>
<td>18.50</td>
<td>3</td>
<td>Second time (H-A-II)</td>
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</tr>
<tr>
<td>Ovulation</td>
<td>276.00</td>
<td>28.33</td>
<td>15.43</td>
<td>13.67</td>
<td>4</td>
<td>Third time (H-A-III)</td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>480.01</td>
<td>9.12</td>
<td>85.36</td>
<td>19.67</td>
<td>5</td>
<td>Fourth time (H-A-IV)</td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>275.69</td>
<td>2.11</td>
<td>18.01</td>
<td>14.40</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteal phase</td>
<td>146.62</td>
<td>76.53</td>
<td>2.21</td>
<td>3.32</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteal phase</td>
<td>134.33</td>
<td>75.07</td>
<td>2.13</td>
<td>2.45</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>373.73</td>
<td>10.99(High)</td>
<td>65.87</td>
<td>16.04</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>411.21</td>
<td>19.20(High)</td>
<td>84.79</td>
<td>17.94</td>
<td>5</td>
<td></td>
<td></td>
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<tr>
<td>Luteal phase</td>
<td>149.92</td>
<td>77.35</td>
<td>80.02</td>
<td>4.12</td>
<td>1</td>
<td></td>
<td></td>
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<td>Luteal phase</td>
<td>165.60</td>
<td>79.44</td>
<td>10.13</td>
<td>1.73</td>
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<td>Luteal phase</td>
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<td>5.62</td>
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<td>Ovulation</td>
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<td>69.56</td>
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<td>Follicular phase</td>
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<td>12.30</td>
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<td>11.94</td>
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<td>Ovulation</td>
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<td>15.67(High)</td>
<td>80.67</td>
<td>20.01</td>
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<td></td>
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<td>Ovulation</td>
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<td>8.96</td>
<td>85.67</td>
<td>15.32</td>
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<td></td>
<td></td>
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<tr>
<td>Luteal phase</td>
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<td>90.02(High)</td>
<td>1.43</td>
<td>6.99</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>352.98</td>
<td>18.71(High)</td>
<td>75.01</td>
<td>18.65</td>
<td>1</td>
<td></td>
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</tr>
<tr>
<td>Ovulation</td>
<td>480.96</td>
<td>11.98(High)</td>
<td>85.34</td>
<td>19.65</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteal phase</td>
<td>174.19</td>
<td>60.00</td>
<td>4.50</td>
<td>7.80</td>
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<td>Ovulation</td>
<td>445.94</td>
<td>14.37(High)</td>
<td>80.11</td>
<td>20.45</td>
<td>4</td>
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<tr>
<td>death</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
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</table>

As a result, the preventive dose of honey with royal jelly was able to
ward off the side effects of the drug, and to avoid the hormonal
disorders or approach to impotence sexual by maintaining the
structure of the ovarian follicle tissue, particularly Graafian follicles.

Ovarian hormones (Estradiol& Progesterone) are naturally
present in honey and royal jelly in addition to some biological
factors that stimulate for more synthesis of these hormones [16, 17]. Many researchers [45, 46] have confirmed the similarity
between the role of honey with the royal food and some hormones
in stimulating the gonads, and raising the level of pituitary
hormones, and the sexual and somatic activity in both males and
females. Thus, they activate the gonads, with increasing the
weights of the ovaries and uteruses for rats female. Consequently,
they increase the number of Graafian follicles and corpora lutea.
All these results refer to the similarity between the effects of
honey with the royal food and that of female and male hormones
(Estradiol & Progesterone, and Testosterone) in rats male.
Townsen&Abott [47] confirmed these roles of honey with the
royal food in the revitalization of female reproductive glands.

Fig. 3: Transverse section in Degradable Graafian follicles in the
ovary of females injected with Adriamycin drug without protective
dose (the first time (AI)-(H&Ix400)

Fig. 4: Transverse section in Degradable Graafian follicles in the
ovary of females injected with Adriamycin drug without protective
dose (the first time (AI)-(H &Ix400)
Fig. 5: Transverse section in Degradable Graafian follicles in the ovary of females injected with Adriamycin drug without protective dose (the second time (A-II)-(H & Ix400))

Fig. 6: Transverse section in Degradable Graafian follicles in the ovary of females injected with Adriamycin drug without protective dose (the second time (A-II)-(Ki67x400))

Fig. 7: Transverse section in the early stage of Graafian follicles in females injected with Adriamycin drug without preventative dose (The third time (A-III)-(H & Ix400))

Fig. 8: Transverse section in the early stage of Graafian follicles in females injected with Adriamycin drug without preventative dose (The third time (A-III)-(Ki67x400))

Fig. 9: Transverse section in the Graafian follicles (incomplete) in females injected with Adriamycin drug without preventative dose (The fourth time (A-IV)-(H & Ix400))

Fig. 10: Transverse section in the Graafian follicles (incomplete) in females injected with Adriamycin drug without preventative dose (The fourth time (A-IV)-(Ki67x400))
Fig. 11: Transverse section in the most Graafian follicles decomposition in the females injected with the drug and administrated with the preventive dose (the first time (H+AI)-(H & Ix400))

Fig. 12: Transverse section in the most Graafian follicles decomposition in the females injected with the drug and administrated with the preventive dose (the first time (H+AI)-(H & Ix400))

Fig. 13: Transverse section in small size Graafian follicles in females injected with the drug and administrated with the preventive dose (the second time (H+A-II)-(H & Ix400))

Fig. 14: Transverse section in small size Graafian follicles in females injected with the drug and administrated with the preventive dose (the second time (H+A-II)-(H & Ix400))

Fig. 15: Transverse section in youthful Graafian follicles in females injected with the drug and administrated with the preventive dose (the third time (H+A-III)-(H & Ix400))

Fig. 16: Transverse section in youthful Graafian follicles in females injected with the drug and administrated with the preventive dose (the third time (H+A-III)-(H & Ix400))
Recommendations

Because honey and royal jelly are of natural foods and harmless to humans, it is desirable to women, treated with Adriamycin drug, to take honey with the royal food as the preventive dose. This compound protects the ovary from the drug toxicity, and maintains its histological and functional vitality. Consequently, get rid of hormonal disorders and menstrual, and the bothers related to menopause.

CONFLICT OF INTERESTS

Declared None

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Picazo RA, Cocero MJ, Barragan ML, Sebastian AL. Effects of LH administration at the rate of an FSH superovulatory regimen on ovulation rate and embryo Production in their breeds of sheep.] Theriogenol 1996;45(5):1065-73.


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Picazo RA, Cocero MJ, Barragan ML, Sebastian AL. Effects of LH administration at the rate of an FSH superovulatory regimen on ovulation rate and embryo Production in their breeds of sheep.] Theriogenol 1996;45(5):1065-73.


