



## CHEMOTHERAPEUTIC AGENT-INDUCED OVARIAN GONADOTOXICITY

Mona AM Helal

Department of Zoology, Faculty of women for Arts, Science & Education, Ain Shams University, Cairo, Egypt.

Received – October 16, 2014; Revision – October 26, 2014, Accepted – November 16, 2014  
Available Online – December 20, 2014.

### KEYWORDS

Ovary  
Chemotherapy agents  
Hormones  
Oxidative stress  
Apoptosis  
Histology

### ABSTRACT

Paclitaxel and Carboplatin have been considered as a combined chemotherapy agents for a variety of human tumors, including ovarian carcinomas. The objective of this study was to evaluate the ovarian toxicity of two chemotherapy agents either solely or in combination against the ovarian follicular reserve in young female rats. Administration of paclitaxel and/or carboplatin significantly reduced the levels of serum gonadotropin and steroid hormones and increased ovarian MDA concentration. Activities of SOD and Catalase were significantly ( $P < 0.001$ ) reduced by the combined effects of anticancer drug. The percentage of ovarian DNA fragmentation was elevated after the treatment with paclitaxel and carboplatin, when it compared with the control and other treated groups a significant ( $p < 00.001$ ) fragmentation was reported in the combined treated group. The numbers of healthy follicles per ovary were decreased and the numbers of atretic follicles were increased. Histologically, the ovaries of the treated groups were hypoplastic and characterized by atresia and follicular degeneration. The mechanism of ovotoxicity involves lowering levels of pituitary gonadotrophin secretion and production of oxygen free radicals that in turn related to a decreased number of healthy follicles and increased number of atretic follicles.

\* Corresponding author

E-mail: monaamhelal@yahoo.com (Mona AM Helal)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

## 1 Introduction

Physiological activities of the ovaries are associated with cyclic changes in their structure and function (Borovskaya et al., 2004). Infertility represents one of the major long-term consequences of combination chemotherapy in young women. The gonadotoxic effect of various chemotherapeutic agents is diverse, may involve variety of physiological mechanisms, and is not unequivocally understood (Blumenfeld, 2012).

Paclitaxel is effective against a variety of human tumours, including ovarian carcinomas (Wiseman & Spencer, 1998). Paclitaxel is classified as a "plant alkaloid," a "taxane" and an "antimicrotubule agent." The plant alkaloids are cell-cycle specific. This means they attack the cells during various phases of the cell division. Paclitaxel as antimicrotubule agents, inhibit the structures and formation of microtubule within the cell. Microtubules are part of the cell's apparatus for dividing and replicating itself. Inhibition of these structures ultimately results in cell death (Beck et al., 2000). Animal studies reported that, by inducing cell death, paclitaxel was shown to cause damage to healthy mature oocytes, and affects short-term reproductive potential in a dose-related manner (Wang et al., 2000).

Carboplatin [cis-diammine (1,1- cyclobutanedicarboxylate) platinum(II)] is a second generation platinum-containing anticancer drug. Platinating agents have been used clinically for nearly 30 years as part of the treatment of many types of cancers, including head and neck, testicular, ovarian, cervical, lung and relapsed lymphoma. The cytotoxic lesion of platinating agents is thought to be the platinum binds with DNA to form intrastrand cross links and adducts that cause changes in the conformation of the DNA and affect DNA replication (Rabik & Dolan, 2007), leading to embryotoxicity and embryonic demise (Fleischer et al., 2011). Experiments on Wistar rats showed that cisplatin and carboplatin induced similar morphological alterations in the ovaries. Both agents reduced the number of structural and functional elements, but the effect of cisplatin was more pronounced.

Combined chemotherapy with carboplatin and paclitaxel is a well-established treatment regimen in advanced ovarian cancers (Belani et al., 2008). Chemotherapy combinations, especially those containing alkylating agents, cause ovarian atrophy and injury to blood vessels, leading to follicular depletion (Fleischer et al., 2011). When follicular depletion is so significant, as in natural menopause, the woman experiences premature ovarian failure (POF). In 8% of young women, survivals of prepubertal chemotherapy, which resume normal cyclic ovarian function, POF may occur before the age of 40 years (Critchley and Wallace 2007).

The purpose of this study was to determine the effects of chemotherapy drugs paclitaxel and carboplatin either

individually and in combination with each other on the ovarian function and follicular reserves in rats and to study the cellular mechanisms of ovotoxicity.

## 2 Materials and Methods

### 2.1 Experimental Animals and experimental design

The present work has been carried out on young female albino Wistar rat weighing about 80-85 grams (6 weeks old). The animals were obtained from the Animal House of El Salam Farm, Giza-Cairo, housed under standard laboratory conditions ( $28 \pm 2$  °C and 12-hr light/dark cycle), and were fed on standard pellet diet and water ad libitum. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, Medical Research Center, Ain Shams University. Before starting experiment, animals were acclimatized for 7 days to the laboratory conditions. Rats were divided into five equal groups of 8 animals in each : group first is control group (without any treatment) feed on standard pellet diet ; second group rats were saline-treated control group which is i.p administered with saline solution (0.9% NaCl @ 1ml); third group of rats were i.p administered with paclitaxel (7.5 mg/kg b. wt); fourth group of rats were with administered carboplatin (30 mg/kg b.wt) and the fifth group of rats were i.p administered paclitaxel and caboplatin at the same doses. Rats were administered paclitaxel and/or caboplatin alone or incombination regardless of their estrous cycle stages as a single injection every week for 4 weeks (seven estrous cycles). High dose chemotheapeutic agents were administered to study groups. Paclitaxel (Laboratorios Filaxis S.A. Argentina) was injected at dose 7.5 mg/kg b. wt dissolved in sterile saline solution (0.9% NaCl, 1ml) according to Yucebilgin et al. (2004) and Ozcelik et al. (2010). While carboplatin (Hospira Australia Pty Ltd, Lexia Australia and Thissen Laboratories s.a., Belgium) was administered at the dose of 30 mg/kg b.wt dissolved in saline solution (0.9% NaCl, 1ml) according to Fuse et al. (1996) and Suzuki et al. (2008).

The mean length of rat estrous cycle was recognized as 4-5 days and in order to evaluate the influence of chemotherapeutic agents over follicles, all rats were sacrificed one week after the administration of the last single dose of chemotherapeutic agents. Animals were sacrificed one week after their 4<sup>th</sup> intraperitoneal dose of the chemotherapeutic agents and the blood was collected (3 mL/ rat) into non heparinized glass test tube, isolated blood samples were centrifuged at 10 000 g for 30 minutes and the serum obtained was aliquoted and stored at - 70°C for determination of LH, FSH, estradiol and progesterone hormone levels. Since the variation in the ovarian weight may be an indicator of the ovarian function, ovarian weight was recorded at the end of the treatment period. Right and left ovaries from each rat were carefully removed, dissected free of the surrounding adipose tissue and weighed.

Three ovaries from each group were stored (-70°C) for molecular analysis and determination of DNA fragmentation, ovaries samples (7-8 ovaries per group) were homogenized in cold buffer potassium phosphate (100 mM potassium phosphate, pH 7.0, containing 2mM EDTA) to give 20% homogenate and the supernatant was separated and stored (-70°C) used for carrying out biochemical studies. Whereas the rest of the ovaries samples (4-5 ovaries per group) were fixed in 10% neutral formalin solution for the histological examination.

## 2.2 LH, FSH, Estradiol and Progesterone hormone levels

Quantitative determination of Luteinizing (LH) concentration and Follicle-Stimulating hormones (FSH) were measured in serum by immunodiagnostic reagents and Elisa Kit test (DiaMetra kits, Via Giustozzi, Italy). Estradiol (E2) concentration was measured by immunoassay test kit (bioCheck, Inc, Foster City, Ca94404, California). Progesterone concentration was estimated by Elisa kit (Calbiotech, Inc, Catalog No.PG129S, Spring Valley, California).

## 2.3 Biochemical studies

The level of lipid peroxidation was indicated by the content of TBARS in the ovary. Tissue TBARS was determined by the method of Ohkawa et al. (1979), SOD was determined by the method of Nishikimi et al. (1972) and tissue catalase activity was assayed using the method described by Aebi (1984).

## 2.4 DNA extraction and agarose gel electrophoresis

Extraction of DNA from whole ovaries was performed using the DNA extraction and Purification Kit (GeneJET™, K0722, Sigma) according to the manufacturer's instructions. Genomic DNA extraction from the ovaries of control and treated animals was used for assessment of DNA fragmentation (ladder formation) qualitatively by agarose gel electrophoresis at 46 V for 2 h on a 1.0% agarose gel containing 0.4 µg/ml ethidium bromide. DNA bands were viewed using an ultraviolet transilluminator (UVP White/UV Transilluminator USA). The intensity of the bands on gel was converted into digital image with gel analyzer and was quantified by phoretix software (phoretix 1D gel analysis software version 4.01).

## 2.5 Ovarian histology and follicle counting

Ovaries fixed in formalin were taken out and fixed in 70% ethanol. The tissues were dehydrated by a graded series of ethanol concentrations, xylene: ethanol (1: 1), xylene (100%) and finally paraffin wax. Then, the tissues were serially sectioned at 5 µm thickness, mounted onto glass slides, and stained with hematoxylin and eosin (H&E) according to standard protocols and analyzed using a light microscope. Ovaries from each animal group were analyzed. Follicles were counted and classified as primordial, primary, preantral, antral or atretic, as described by Myers et al., 2004. In addition, the number of corpora lutea was counted in each analyzed section (nine sections / ovary).

Table 1 Effect of paclitaxel and/or carboplatin on body and ovary Weights.

Groups	Initial body weight (g)	Final body Weight (g)	Ovary weight (mg)
<b>Control</b>	83.00 ± 1.50	127.71 ± 3.66	<b>40 ± 2.6</b>
<b>Saline</b>	82.50 ± 4.51	126.16 ± 3.41	<b>37 ± 3.2</b>
<b>Paclitaxel</b>	83.50 ± 3.27	122.00 ± 6.22 <sup>e</sup>	<b>31 ± 3.4 a<sup>x</sup></b>
<b>Carboplatin</b>	83.80 ± 3.18	122.62 ± 3.45 <sup>e</sup>	<b>26 ± 2.0 a<sup>z</sup>b<sup>y</sup></b>
<b>Paclitaxel and carboplatin</b>	<b>84.00 ± 3.16</b>	<b>100.00 ± 6.76 a<sup>z</sup>b<sup>z</sup>c<sup>y</sup>d<sup>y</sup></b>	<b>25 ± 2.5 a<sup>z</sup>b<sup>z</sup></b>

Results are expressed as mean values ± SE (n=8); a: significantly different from control; b: significantly different from saline group; c: significantly different from paclitaxel group; d: significantly different from carboplatin group; e: significantly different from paclitaxel and carboplatin group; <sup>x</sup>p < 0.05; <sup>y</sup>p < 0.01; <sup>z</sup>p < 0.001

Table 2 Effects of paclitaxel and/or carboplatin on serum gonadotropin and steroid levels

Groups	LH (IU/ml)	FSH (IU/ml)	Estradiol (pg/ml)	Progesterone (ng/ml)
<b>Control</b>	1.35 ± 0.92	1.41 ± 0.48	1.38 ± 0.48	5.85 ± 1.48
<b>Saline</b>	1.71 ± 0.56	1.57 ± 1.13	1.41 ± 0.82	5.08 ± 0.96
<b>Paclitaxel</b>	0.78 ± 0.36	1.08 ± 0.23	0.75 ± 1.50	4.02 ± 0.45
<b>Carboplatin</b>	0.40 ± 0.18 <sup>b</sup>	0.93 ± 0.48	0.86 ± 0.40	4.90 ± 0.34
<b>Paclitaxel and carboplatin</b>	<b>0.10 ± 0.01<sup>ab</sup></b>	<b>0.62 ± 0.27<sup>ab</sup></b>	<b>0.28 ± 0.69<sup>ab</sup></b>	<b>3.63 ± 0.79<sup>ab</sup></b>

Results are expressed as mean values ± SE (n=8); a: significantly (p < 0.05) different from control; b: significantly (p < 0.05) different from saline group.

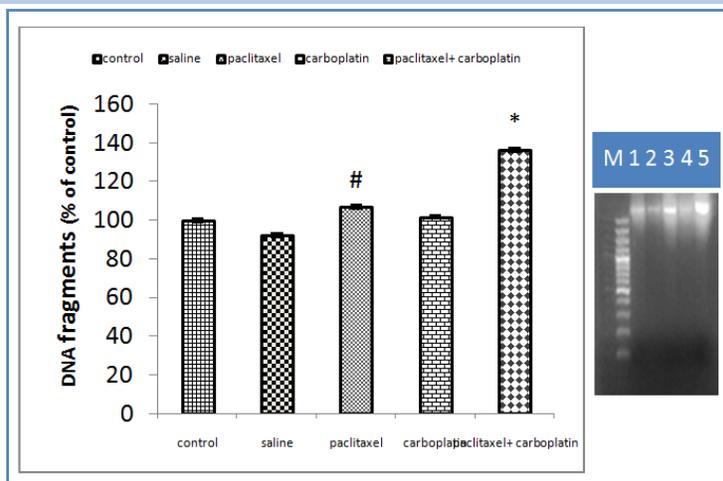


Figure 1:A

Figure 1:B

Figure 1:A Quantitative estimation of DNA fragmentation. Quantitative analysis of DNA fragmentation, Data are expressed as mean  $\pm$  SE (n=3). # p<0.05 (vs. saline group), \* p<0.001 (vs. control group).

Figure 1: B Agarose gel electrophoresis of genomic DNA extracted from ovary of rat in various treated groups: Lane M: protein marker 100 base pairs plus DNA ladder. Lane 1: Control rat. Rats treated with saline (lane 2), paclitaxel (Lane 3), carboplatin (Lane 4), rats treated with paclitaxel and carboplatin (lane 5).

Primordial follicles were characterized as oocytes surrounded by a single layer of flattened granulosa cells. Primary follicles were characterized as oocytes surrounded by a single layer of cuboidal granulosa cells. Preantral follicles were characterized as oocytes surrounded by two or more layers of cuboidal granulosa cells with no visible antrum. Antral follicles were identified by the presence of an antrum. Atretic follicles were characterized by the presence of degenerating oocytes, disorganized granulosa cell layers, folded zonapellucida (partially or completely separated from corona radiata and from the granulosa cells of the oocyte), and pyknotic nuclei (Muskhelishvili et al., 2005). The number of corpora lutea was determined in the same sections as the other types of follicles.

## 2.6 Statistical analysis

Data were expressed as mean  $\pm$  SEM. One-way analysis of variance followed by the LSD test was used to assess significant differences among the experimental groups (software package version 10, SPSS Inc., Chicago, Illinois, USA).

## 3 Results

### 3.1 Body and ovary weights

Combined treatment of Paclitaxel and Carboplatin showed significant decrease (P<0.001) in the mean body weights and absolute ovary weights as compared to normal control values (Table 1). Administration of Paclitaxel (p < 0.05) or Carboplatin (P<0.001) alone resulted in a significant reduction in the weight of ovaries as compared to the control group (Table 1). The body weight did not show any significant

change (P> 0.05) between the single agent treated groups at the end of the experiment.

### 3.2 Serum gonadotropin and steroid levels

Remarkable reduction in the levels of serum gonadotropin and steroid hormones were recorded in the all treated groups as compared with control and saline groups. Significant (p < 0.05) diminution in the serum level of LH was noticed after injection with carboplatin alone or combined with paclitaxel with respect to the control and saline treated groups. Whereas paclitaxel treated group exhibited non-significant decrease (p > 0.05) in the levels of LH as compared to the control. FSH, estradiol and progesterone levels significantly decreased (p < 0.05) with the double effects of the two chemotherapeutic agents (Table 2) and decreased non significantly (p > 0.05) with the single agent treated groups versus control values.

### 3.3 Ovarian antioxidant enzymes

The changes in antioxidant enzyme activities are presented in Table 3. Administration of paclitaxel and/or carboplatin significantly (P<0.001) increased ovarian MDA concentration which indicating elevation of ovarian membrane lipid peroxidation.

The percentages of changes were 115.8% , 113.6% and 74% of control in the group of both drugs, Carboplatin and paclitaxel treated groups, respectively. SOD activity significantly (P< 0.001) decreased (78.3% of control) with the combined effects of anticancer drugs, in the carboplatin treated group (73.8% of control) and in paclitaxel group (59.3% of control).

Table 3 Effects of paclitaxel and/or carboplatin on ovarian antioxidant enzymes.

Groups	Lipid peroxide (nmol/g.tissue)	SOD (U/gm tissue)	Catalase (U/gm tissue)
<b>Control</b>	4456.27 ± 100.08	2169.78 ± 200.36	8.35 ± 0.46
<b>Saline</b>	3394.05 ± 477.32	2387.24 ± 237.83	8.91 ± 0.70
<b>Paclitaxel</b>	7755.77 ± 376.94 a <sup>z</sup> b <sup>z</sup> d <sup>y</sup> e <sup>y</sup>	882.73 ± 173.37a <sup>z</sup> b <sup>z</sup>	4.72 ± 1.47 a <sup>y</sup> b <sup>y</sup>
<b>Carboplatin</b>	9521.45 ± 185.96 a <sup>z</sup> b <sup>z</sup> c <sup>y</sup>	568.29 ± 122.22 a <sup>z</sup> b <sup>z</sup>	4.76 ± 0.89a <sup>y</sup> b <sup>y</sup>
<b>Paclitaxel and carboplatin</b>	<b>9620.46 ± 1243.33 a<sup>z</sup>b<sup>z</sup>c<sup>y</sup></b>	<b>470.36 ± 293.80 a<sup>z</sup>b<sup>z</sup></b>	<b>4.20 ± 1.14 a<sup>z</sup>b<sup>z</sup></b>

Results are expressed as mean values ± SE (n=6); a: significantly different from control; b: significantly different from saline group; c: significantly different from paclitaxel group; d: significantly different from carboplatin group; e: significantly different from paclitaxel and carboplatin group; <sup>y</sup>p < 0.01; <sup>z</sup>p < 0.001

Catalase levels significantly (p < 0.01) decreased in all treated groups and recorded 49.7 % of control with paclitaxel plus carboplatin treated group, while it became 42.9% in carboplatin treated group and reached to 43.4% of in paclitaxel treated group.

### 3.4 Agarose Gel Electrophoresis and Quantitation of DNA Fragmentation

DNA fragmentation that was indicative of apoptosis was clearly represented in Figure 1. The cytotoxic effects of paclitaxel and carboplatin are shown by elevation in the percentage of apoptotic DNA fragmentation. Quantitative estimation of DNA cleavage from ovarian tissue revealed a significant elevation (p < 0.001) in percentage of DNA fragmentation after the double treatment with paclitaxel and carboplatin, when compared with the control and other treated groups. The percentages of changes were 136.51%, 107.15%, and 101.75% for combination of both chemotherapeutic agents, paclitaxel and carboplatin treated groups respectively.

### 3.5 Follicular and corpora lutea counts

The number of follicles per ovary counted in serial sections of all animals, revealed significant differences between control and rats exposed to paclitaxel and/or carboplatin. Follicle counts in all treated groups showed that all types of ovarian follicles were lower than control and saline groups (table 4).

The mean numbers of follicles for the control and study groups are shown in Table 4. The number of primordial and primary follicles after chemotherapy treatment showed significant reduction in the study groups compared to the control group. Carboplatin treated group showed the highest drop in the number of primordial follicles (control: 11.33 ± 1.76; carboplatin 4.50 ± 0.86) and primary follicles (control: 5.50 ± 1.50; carboplatin 3.00 ± 0.57) as compared to paclitaxel group and paclitaxel combined with carboplatin group (Table 4). The paclitaxel plus Carboplatin combination therapy group exhibited a significant reduction (p < 0.05) in number of preantral follicles compared to those of control, saline and single-agent groups (control: 5.75 ± 1.79; Paclitaxel and carboplatin: 1.50 ± 0.50).

The number of antral follicles in the study groups, showed statistically significant (P < 0.001) drops compared to that of control group and saline group. The most affected group in terms of the number of antral follicles, was the paclitaxel plus carboplatin combination group (control: 5.66 ± 1.85; paclitaxel plus carboplatin 0.75 ± 0.47). The number atretic follicles after chemotherapy, was found to show a statistically significant elevation in all study groups compared to the control group. The highest increase in number of atretic follicles was observed in group of combination therapy (control: 2.00 ± 0.63; paclitaxel plus carboplatin: 8.80 ± 1.46) (Table 4).

Table 4 Follicular and corpora lutea counts in the studied groups.

Groups	Primordial follicles	Primary follicles	Preantral follicles	Antral follicles	Atretic follicles	Corpora lutea
<b>Control</b>	11.33 ± 1.76	5.50 ± 1.50	5.75 ± 1.79	5.66 ± 1.85	2.00 ± 0.63	11.50 ± 1.94
<b>Saline</b>	9.66 ± 0.88	6.66 ± 0.88	5.33 ± 0.71	5.75 ± 0.85	3.40 ± 0.74	11.33 ± 1.76
<b>Paclitaxel</b>	6.40 ± 1.12 a <sup>y</sup>	4.00 ± 0.40 b <sup>y</sup>	4.20 ± 1.31	1.60 ± 0.50 a <sup>z</sup> b <sup>z</sup>	5.16 ± 1.92 a <sup>x</sup> e <sup>x</sup>	7.12 ± 0.74 a <sup>z</sup> b <sup>x</sup>
<b>carboplatin</b>	4.50 ± 0.86 a <sup>z</sup> b <sup>z</sup> e <sup>x</sup>	3.00 ± 0.57 a <sup>z</sup> b <sup>z</sup>	4.33 ± 0.88	1.00 ± 0.31 a <sup>z</sup> b <sup>z</sup>	4.50 ± 0.42 e <sup>y</sup>	10.00 ± 0.01 e <sup>x</sup>
<b>Paclitaxel and Carboplatin</b>	7.71 ± 1.18 a <sup>x</sup> d <sup>x</sup>	4.25 ± 0.62 b <sup>x</sup>	1.50 ± 0.50 a <sup>z</sup> b <sup>x</sup>	0.75 ± 0.47 a <sup>z</sup> b <sup>z</sup>	8.80 ± 1.46 a <sup>z</sup> b <sup>z</sup> c <sup>x</sup> d <sup>y</sup>	5.77 ± 0.70 a <sup>z</sup> b <sup>z</sup> d <sup>x</sup>

Results are expressed as mean values ± SE (n=6); a: significantly different from control; b: significantly different from saline group; c: significantly different from paclitaxel group; d: significantly different from carboplatin group; e: significantly different from paclitaxel and carboplatin group; <sup>x</sup>p < 0.05; <sup>y</sup>p < 0.01; <sup>z</sup>p < 0.001

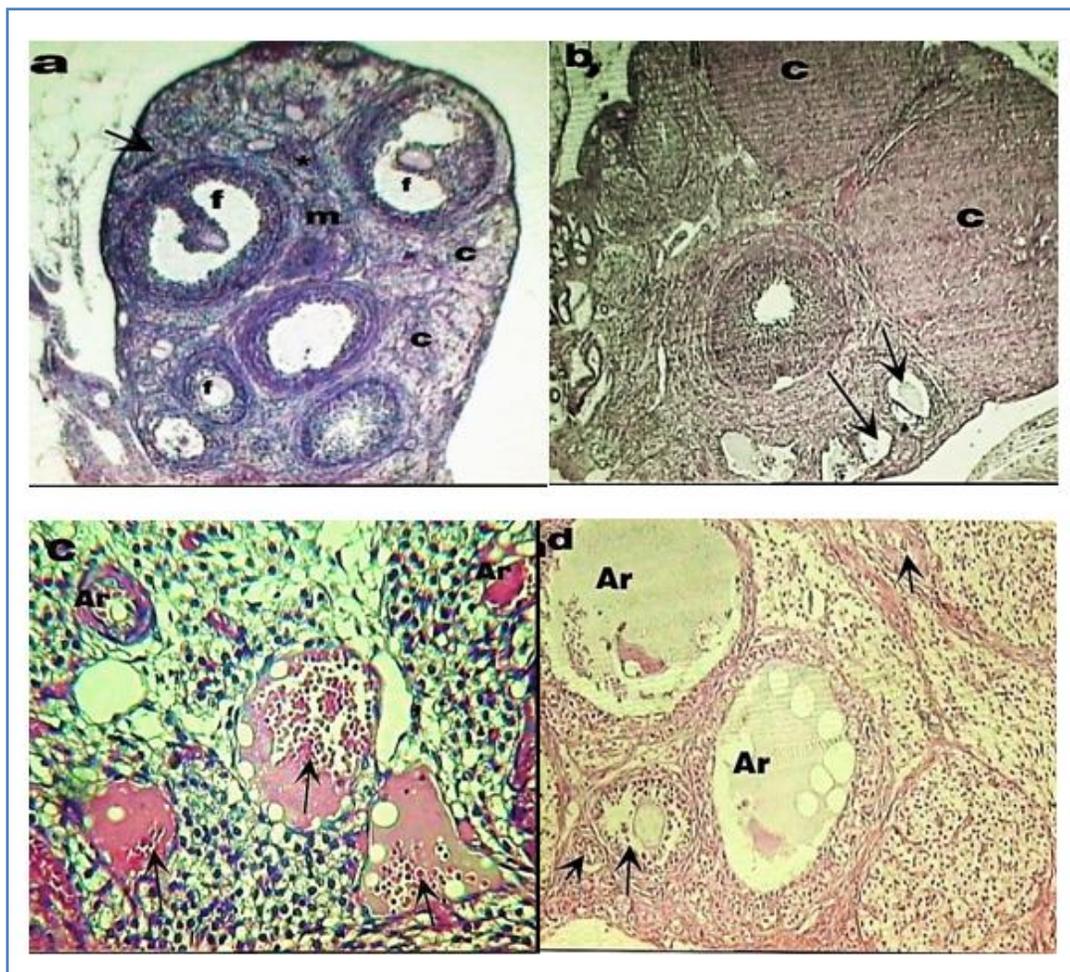


Figure 2 Light micrographs of ovarian sections stained with hematoxylin-eosin .

- (a) Control ovary is normal and characterized by outer cortex (arrow), inner medulla (m), follicles at various stages of development (f), corpora lutea (C), and interstitial tissue (\*) (X 50).
- (b) ovary of rat treated with paclitaxel showing, corpora lutea (C) and many atretic follicles (arrows) (X 50).
- (c) carboplatin treated ovary showing atretic follicle (Ar), vascular congestion (arrows) and vacuolar degeneration of the ovarian stromal cells (X100).
- (d) ovary of rat treated with paclitaxel and carboplatin showing degeneration of the developing follicle (arrows) and increase of atretic follicles (Ar) (X 100).

On contrast, remarkable reduction in corpora lutea counts were noticed in all treated groups compared to the control group but it were more pronounced in the group of rats treated with both paclitaxel and carboplatin as compared to control (control:  $11.50 \pm 1.94$ ; paclitaxel and carboplatin  $5.77 \pm 0.70$ ). There was a statistically significant difference ( $p < 0.05$ ) was detected in terms of decrease in number of corpora lutea between Carboplatin + paclitaxel group and Carboplatin group.

### 3.6 Histological examination

Ovary of control rat contains follicles of all developing stages, corpora lutea, and interstitial tissue (Figure 2 a). While the

ovaries of all treated groups were hypoplastic and characterized by atresia, few primordial, primary, antral follicles and corpora lutea. Ovary from rat exposed to paclitaxel showed many atretic follicles and few corpora lutea and developing follicles (Figure 2 b), whereas the carboplatin injected ovary generally exhibited the absence of the mature follicles and the presence of the corpora lutea, the ovary was deficient in small follicles, in addition to, vascular congestion and vacuolar degeneration of the ovarian stromal cells (Figure.2 c). Ovary from rat exposed to paclitaxel and carboplatin treated group showed follicular degeneration and many atretic follicles (Figure 2 d).

## Discussions

The present study was designed to demonstrate the effects of chemotherapy drugs paclitaxel and carboplatin exposure on the function of ovary and to examine the mechanism of ovotoxicity. It is well known that body weight provides information on the general health level of animals which can also be important interpretation of reproductive effects (Aly et al., 2009). Significant reduction in the body weight and ovary weight resulted from the use of paclitaxel and/or Carboplatin may be correlated with the possibility of low plasma levels of gonadotrophins (Kulin & Reiter, 1973) and increase in the number of atretic follicles (Ksheerasagar & Kaliwal, 2008).

In the present study, treatment of paclitaxel and carboplatin solely or in combination produced reduction in the production of gonadotropin releasing hormones, Luteinizing hormone (LH) Follicle stimulating hormone (FSH) and steroid hormones (estradiol and progesterone). It also decrease in differential follicle counts, the reason may be due to the hormonal imbalance in any of the stages in hypothalamohypophysial ovarian axis since the ovarian function is controlled by the hypothalamopituitary unit, which functions in a coordinated manner with appropriate signal provided by ovary via pituitary gland, and is responsible for the synthesis and storage of gonadotropins FSH and LH.

These glycoprotein hormones in turn play a key role in the regulation of folliculogenesis. Therefore, toxicants that interfere with the ovarian function could do so indirectly by acting on the level of hypothalamus or pituitary gland or both (Xu et al., 1997). The less secretion of estrogen from the ovary which, in turn might be reduction in FSH secretion, is likely to be consequence of decreased gonadotropins, thus causing the negative feedback on hormonal imbalance. Moreover, it is possible that reductions of estradiol and progesterone are related to a decreased number of healthy follicles. (Badraoui et al., 2010).

The possibility of low plasma levels of gonadotropins may be due to the elevated level of plasma glucocorticoids which may suppress the sensitivity of gonadotroph cells to gonadotrophin releasing hormone and therefore may prevent gonadotrophin secretion (Kamel & Kubujak, 1987). The decrease in the number of primary, secondary, and Graafian follicles seen in the current study could be due to a lack of available proteins necessary for cell division, growth, and differentiation of germ cells during oogenesis and hence a decrease in follicle-stimulating hormone (FSH) secretion (Xu et al., 1997). Cyclophosphamide has been found to inhibit the development of antral follicles in rats, thereby increasing the atretic follicles through interfering with hormonal ovarian follicular development and reduces estradiol (Ataya et al., 1988). Evans et al. (1997) have shown that the ovarian androgen and inhibin secretion by follicles may be an important part in the regulation of FSH secretion and follicular dynamics.

Paclitaxel and carboplatin in the present work have been shown to increase TBARS (thiobarbituric acid reactive substances) level in ovary of young rat consistent with possible ovotoxicity effect via oxidative stress associated with reduction in catalase and superoxide dismutase this phenomenon generally leads to excessive formation of reactive oxygen species (ROS). ROS could interact with the unsaturated lipids of membranes, destroying ovaries tissues (Badraoui et al., 2007). Superoxide dismutase (SOD), and catalase (CAT) constitute the enzymic antioxidant system, which scavenges ROS. SOD is the only enzymes that disrupts superoxide radicals (Beutlar & Gelbart, 1985). It protects the cells against superoxide- and hydrogen peroxide-mediated LPO. Cisplatin has been also shown to increase oxidative stress in normal tissues (Naziroglu et al., 2004) which resulted in the deterioration in the quality and quantity of ovarian follicles.

The present investigation showed increase of DNA fragmentation, percentage of apoptosis and atretic follicles with decrease of follicular counts after chemotherapy treatments. Hudson (2010) stated that at least three outcomes are possible for oocytes with DNA damage : elimination if damage is too severe, survival in spite of injury, or survival following repair of damage. The first outcome does occur, as shown by decreased follicle numbers following exposures to alkylating agents *in vitro* and in rodents. Lopez & Luderer (2004) found that cyclophosphamide treatment increased apoptosis in granulosa cells of secondary and antral follicles. The decreased number of healthy follicles and the elevation in the number of apoptotic follicles may be attributed to low FSH levels (Roy & Treacy, 1993). Borovskaya et al. (2004) showed that *in vivo* administration of cisplatin was associated with morphological damage of the ovarian follicles that was consistent with apoptosis, including evidence of degradation of cell nuclei and death of follicular epitheliocytes. Yeh et al. (2006) found decline in Mullerian inhibiting substance due to increase in apoptosis of ovarian follicles after cisplatin treatment.

In the present study, mature follicles showed higher sensitivity against chemotherapy compared to the primordial follicles. Similar results have been reported in rat (Tan et al., 2010) treated with chemotherapy. Lower doses of chemotherapy agents e.g., cyclophosphamide (CPA) (Jarrell et al., 1991), doxorubicin (Ben-Aharon et al., 2010), or paclitaxel (Ozcelik et al., 2010) induce severe loss of ovarian follicles, including primordial follicles and, consequently, persistent ovarian dysfunction in rodents. Hoyer et al., 2001 stated that, the mechanism of ovotoxicity to primordial and primary follicles in rats involves acceleration of the normal rate of atresia and intracellular targeting of mitochondrion-mediated pathways of apoptosis. *In vitro* studies, treatment with paclitaxel initiated apoptosis in granulosa cells, the most important component of follicles, inflicting destruction over primordial follicles (Wang et al., 2000). Moreover, paclitaxel causes a delay in meiotic maturation of mature oocytes and spindle defects, leading to formation of aneuploid oocytes and a reduction in number of ovarian follicles (Mailhes et al., 1999).

## Conclusion

From the results of the present study it is obvious that, both chemotherapeutic agent paclitaxel and carboplatin can interfere with the ovarian function by lowering the level of pituitary gonadotrophin secretion and production of oxygen free radical that in turn related to a decreased number of healthy follicles and increased number of atretic follicles. Therefore patients who seek fertility must minimize the use of platinum compounds during the treatment.

## Acknowledgments

The author thanks all staff members of the Medical Research Center, Ain Shams University for making all facilities available for the author's research.

## References

- Aebi H (1984) Catalase in vitro. *Methods in Enzymology* 105: 121–126.
- Aly HAA, Domènech O, Abdel-Naim AB (2009) Aroclor 1254 impairs spermatogenesis and induces oxidative stress in rat testicular mitochondria. *Food and Chemical Toxicology* 47: 1733–1738.
- Ataya K, Tadros M, Mohammed S (1988) Cyclophosphamide inhibits antral follicular development in the rat ovary. *Biology of Reproduction* 38: 157.
- Badraoui R, Abdelmoula NB, Feki M, Ben Nasr H, Rebai T (2010) Endocrine disruption and ovarian morphometric responses in rats following exposure to tetradifon. *General and Comparative Endocrinology* 166: 268–272.
- Badraoui R, Sahnoun Z, Abdelmoula N B, Hakim A, Fki M, Rebai T (2007) May antioxidants status depletion by Tetradifon induce secondary genotoxicity in female Wistar rats via oxidative stress? *Pesticide Biochemistry and Physiology* 88: 149–155.
- Beck WT, Cass CE, Houghton PJ (2000) Microtubule-targeting anticancer drugs derived from plants and microbes : Vinca Alkaloids, Taxanes, and Epothilones. In: Bast RC, Kufe DW, Pollock RE, Weichselbaum RR, Holland JF, Frei E, Gansler TS (Eds) *Holland-Frei Cancer Medicine*, 5th edition Hamilton (ON), BC Decker.
- Belani CP, Ramalingam S, Perry MC, LaRocca RV, Rinaldi D, Gable PS, Tester WJ (2008) Randomized, phase III study of weekly paclitaxel in combination with carboplatin versus standard every-3-weeks administration of carboplatin and paclitaxel for patients with previously untreated advanced non-small-cell lung cancer. *Journal of Clinical Oncology* 20: 468–473.
- Ben-Aharon I, Bar-Joseph H, Tzarfaty G, Kuchinsky L, Rizel S, Stemmer SM, Shalgi R (2010) Doxorubicin-induced ovarian toxicity. *Reproductive Biology and Endocrinology* 8: 20. doi: 10.1186/1477-7827-8-20.
- Beutler E, Gelbart T (1985) Plasma glutathione in health and in-patient with malignant disease. *Journal of Laboratory and Clinical Medicine* 105: 581–584.
- Blumenfeld Z (2012) Chemotherapy and fertility. *Best Practice & Research in Clinical Obstetrics and Gynecology* 26 : 379–390.
- Borovskaya TG, Goldberg VE, Fomina TI, Pakhomova AV, Kseneva SI, Poluektova ME, Goldberg ED (2004) Morphological and functional state of rat ovaries in early and late periods after administration of platinum cytostatics. *Bulletin of Experimental Biology and Medicine* 137: 331–335.
- Critchley HOD, Wallace WHB (2007) Do survivors of childhood cancer have increased incidence of premature menopause? *Nature Review Clinical Oncology* 4: 84–85.
- Evans AC, Komar CM, Wandji SA, Fortune JE (1997) Changes in androgen secretion and luteinizing hormone pulse amplitude are associated with the recruitment and growth of ovarian follicles during the luteal phase of the bovine estrous cycle. *Biology of Reproduction* 57: 394–401.
- Fleischer RT, Vollenhoven BJ, Weston GC (2011) The effects of chemotherapy and radiotherapy on fertility in premenopausal women. *Obstetrical Gynecological Survey* 66: 248–284.
- Fuse H, Fujiuchi Y, Junicho A, Iwasaki M, Katayama T (1996) Effect of carboplatin on rat spermatogenesis. *Urology International* 56: 219–223.
- Hoyer PB, Devine PJ, Hu X, Thompson KE, Sipes IG (2001) Ovarian toxicity of 4-vinylcyclohexene diepoxide: A Mechanistic Model. *Toxicological Pathology* 29 : 91–99.
- Hudson MM (2010) Reproductive outcomes for survivors of childhood cancer. *Obstetrics and Gynecology* 5: 1171–1183.
- Jarrell JF, Bodo L, YoungLai EV, Barr RD, O'Connell GJ (1991) The short-term reproductive toxicity of cyclophosphamide in the female rat. *Reproductive Toxicology* 5: 481–485.
- Kamel FA, Kubajak, CL (1987) Modulation of gonadotropin secretion by corticosterone interaction with gonadal steroids and mechanism of action. *Endocrinology* 121: 561–568.
- Ksheerasagar RL, Kaliwal BB (2008) Effects of carbosulfan administration schedules on estrous cycle and follicular dynamics in albino mice. *Industrial Health* 46: 210–216.

- Kulin, HE, Reiter EO (1973) Gonadotrophins during childhood and adolescence: A review. *Pediatrics* 51: 260–271.
- Lopez SG, Luderer U (2004) Effects of cyclophosphamide and buthionine sulfoximine on ovarian glutathione and apoptosis. *Free Radical Biology and Medicine* 36 : 1366 – 1377.
- Mailhes JB, Carabatsos MJ, Young D, London SN, Bell M, Albertini DF (1999) Taxol-induced meiotic maturation delay, spindle defects, and aneuploidy in mouse oocytes and zygotes. *Mutation Research* 423: 79-90.
- Muskhelishvili L, Wingard SK, Latendresse JR (2005) Proliferating cell nuclear antigen—a marker for ovarian follicle counts. *Toxicologic Pathology* 33 : 365–368.
- Myers M, Britt KL, Wreford NG, Ebling FJ, Kerr JB (2004) Methods for quantifying follicular numbers within the mouse ovary. *Reproduction* 127: 569–580.
- Naziroglu M, Karaoglu A, Aksoy AO (2004) Selenium and high dose vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats. *Toxicology* 195: 221-230.
- Nishikimi M, Roa NA, Yogi K (1972) Determination of superoxide dismutase. *Biochemical Biophysical Research Communication* 46: 845-854.
- Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 95 : 351-358.
- Ozcelik B, Turkyilmaz C, Ozgun MT, Serin IS, Batukan C, Ozdamar S, Ozturk A (2010) Prevention of paclitaxel and cisplatin induced ovarian damage in rats by a gonadotropin-releasing hormone agonist. *Fertility Sterility* 93: 1609–1614.
- Rabik CA, Dolan ME (2007) Molecular mechanisms of resistance and toxicity associated with platinating agents. *Cancer Treatment Reviews* 33: 9– 23.
- Roy SK, Treacy BJ (1993) Isolation and long-term culture of human preantral follicles. *Fertility and Sterility* 59: 783–790.
- Tan SJ, Yeh YC, Shang WJ, Wu GJ, Liu JY, Chen CH (2010) Protective effect of a gonadotropin-releasing hormone analogue on chemotherapeutic agent-induced ovarian gonadotoxicity: a mouse model. *European Journal of Obstetrics and Gynecology and Reproductive Biology* 149: 182-185.
- Suzuki Y, Tanaka K, Neghishi D, Shimizu M, Murayama N, Hashimoto T, Yamazaki H (2008) Increased distribution of carboplatin, an anti-cancer agent, to rat brains with the aid of hyperbaric oxygenation. *Xenobiotica* 38: 1471-1475.
- Wang TH, Wang HS, Soong YK (2000) Paclitaxel-induced cell death: where the cell cycle and apoptosis come together. *Cancer* 88: 2619.2628.
- Wiseman LR, Spencer CM (1998) Paclitaxel: An update of its use in the treatment of metastatic breast and ovarian and other gynecological cancers. *Drugs Aging* 12: 305–334.
- Xu Y, Yuan S, Xie Q (1997) Effects of fluorosis on induced secretion of rat prolactin in vivo and in vitro. *Fluoride* 30: 173-178.
- Yeh J, Kim B, Liang YJ, Peresie J (2006) Müllerian inhibiting substance as a novel biomarker of cisplatin-induced ovarian damage. *Biochemical and Biophysical Research Communications* 348: 337–344.
- Yucebilgin MS, Terek MC, Ozsaran A, Akercan F, Zekioglu O, Isik E, Erhan Y (2004) Effect of chemotherapy on primordial follicular reserve of rat: an animal model of premature ovarian failure and infertility. *Australian and New Zealand Journal of Obstetrics and Gynecology* 44: 6–9.