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Protective Effects of Imatinib and Ginkgo Biloba on Cisplatin-induced Ovarian Damage in Rats

İmatinib ve Ginkgo Bilobanın Sıçanlarda Sisplatin Kaynaklı Over Hasarı Üzerine Koruyucu Etkilerinin İncelenmesi

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ABSTRACT

Introduction: In our study we aimed to observe the protective effects of imatinib and ginkgo biloba (GB) on cisplatin (CP)-induced ovarian damage in rats.

Methods: Thirty-two female rats were included and assigned to four groups. Group 1 had no medication. Their ovaries were removed for examination and the serum Anti-Mullerian hormone (AMH) levels were measured. Group 2 received a single dose of 7.5 mg/kg intramuscular CP. Group 3 received a single dose of 7.5 mg/kg oral imatinib and 30 minutes later, a single dose of 7.5 mg/kg intramuscular CP was administered. Group 4 received 80 mg/kg oral GB for 10 days. Sixty minutes after the first administration of the GB, a single dose of 7.5 mg/kg intramuscular CP was administered. The ovaries and serum AMH levels of the rats were assessed after 10 days of observation.

Results: Comparing group 1 and 2 showed that the total histopathological ovarian damage scores increased in the latter (p=0.044). This group also had decreased primordial follicles, preantral follicles and serum AMH (p=0.001, p=0.004 and p<0.001 respectively). In group 3, total histopathological ovarian damage score increased (p=0.020), and a reduction in primordial follicles (p=0.008) and serum AMH levels (p<0.001) was observed. In group 4, total histopathological ovarian damage score increased (p=0.016) as in groups 2 and 3. There was also a reduction in primordial follicles, preantral follicles and serum AMH levels (p<0.00, p=0.010 and p<0.001 respectively).

Conclusion: It was concluded that imatinib and GB were not effective in preventing CP-induced ovarian damage in rats.

Keywords: Cisplatin, imatinib, ginkgo biloba, Anti-Mullerian hormone, ovary, rat

ÖZ

Amaç: Çalışmamızda, imatinib ve ginkgo bilobanın (GB) sıçanlarda sisplatin (CP) kaynaklı over hasarı üzerindeki koruyucu etkilerini gözlemlemeyi amaçladık.

Yöntemler: Çalışmamıza toplam 32 erişkin dişi rat alındı ve 4 gruba ayrıldı. İlk gruba ilaç verilmedi. Muayene için sıçanların overleri çıkarıldı ve serum Anti-Mullerian hormon (AMH) seviyeleri ölçüldü. Grup 2'ye tek doz 7,5 mg/kg intramüsküler CP verildi. On günlük gözlemden sonra, overler ve sıçanların serum AMH seviyeleri değerlendirildi. Grup 3'e tek doz 7,5 mg/kg oral imatinib verildi ve 30 dakika sonra, tek doz 7,5 mg/kg intramüsküler CP uygulandı. Grup 4 on gün boyunca 80 mg/kg oral GB aldı. GB ilk uygulamasından 60 dakika sonra, tek bir doz 7,5 mg/kg intramüsküler CP uygulanmıştır. Gruplar ve grup 4'ün overleri ve serum AMH düzeyleri, 10 günlük gözlemden sonra değerlendirildi.

Bulgular: Grup 2'de total histopatolojik over hasarı skoru grup 1'e göre arttı (p=0,044). Ayrıca grup 2'de primordiyal foliküller, preantral foliküller ve serum AMH düzeyleri azaldı (sırasıyla, p=0,001, p=0,004 ve p<0,001), 3. grupta, aynı zamanda toplam histopatolojik over hasarı skoru (p=0,020) arttı. Primordiyal foliküllerde (p=0,008) ve serum AMH düzeylerinde azalma gözlendi (p<0,001). Grup 4'te toplam histopatolojik over hasarı skoru grup 2 ve grup 3'te olduğu gibi arttı (p=0,016). Ayrıca primordiyal foliküllerde, preantral foliküllerde ve serum AMH düzeylerinde azalma olduğu gözlendi (p<0,00, p=0,010 ve sırasıyla p<0,001).

Sonuç: İmatinib ve GB'nin sıçanlarda CP'nin neden olduğu yumurtalık hasarını önlemede etkili olmadığı sonucuna varıldı.

Anahtar Kelimeler: Sisplatin, imatinib, ginkgo biloba, Anti-Mullerian hormon, over, sıçan

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Introduction

Cisplatin (CP) is one of the first chemotherapy drugs in cancer treatment. CP is also known as "the penicillin of cancer" as it is a widely used chemotherapeutic agent in the medical management of cancers worldwide. CP use in clinical practice has increased day by day after its approval of the Food and Drug Administration for cancer treatment in 1978 (1). Lung, head and neck, ovarian, cervical, bladder and testicular tumours are the most common tumours treated with CP (2). DNA is the main target of CP. CP interferes with DNA synthesis and repair mechanisms, causing DNA damage and subsequently inducing apoptosis in tumours. The damage in DNA synthesis affects especially blood cells, germ cells and young cells (3).

CP is one of the most effective chemotherapeutic agents especially in childhood cancers and the average cure rate is 85% in literature (4,5). On the other hand, CP has some disadvantages because it interferes with DNA repair mechanisms. The incidence of secondary tumours mostly in proliferative organs is higher in patients receiving CP, especially at young ages (6). This is one of the limiting features of CP. At this point CP-induced ovarian damage appears to be a very important side effect, especially for women who want to preserve ovarian functions (7). CP-induced ovarian damage may cause deterioration in quality of life and increase in treatment costs, from ovarian failure and infertility (8).

It is also known that CP induces the production of free oxygen radicals, which have cytotoxic effects on normal cells and causes oxidative stress throughout the body (9,10). Some evidence found that antioxidant substances reduce organ damage from oxidative stress caused by CP (11-13).

Imatinib is a competitive tyrosine kinase inhibitor (TKI) and generally used in cancer therapy (14). It is a TKI, inhibiting Abelson tyrosine kinase (c-abl), platelet derived growth factor receptor and receptor tyrosine kinase (15). Imatinib can affect all basic cellular functions (cell signalling, proliferation and differentiation), including ovarian follicles (16,17). In literature, it has been proposed as a medication to prevent primordial follicle loss induced by CP, based on its ability to inhibit c-abl kinase inhibitor (18,19). However, further studies are required on imatinib co-administration to prevent ovarian functions in CP treatment (20).

In recent studies, some antioxidant plants have been shown to have preservative effects against chemotherapy-induced reproductive organ damage (21). Ginkgo biloba (GB) has been used in traditional Chinese medicine for 5,000 years. It is a potent antioxidant and directly effective on free oxygen radicals (22). Besides the antioxidant effects of GB, its anticancer effects have been discussed in some publications (23,24).

In the current study, we aimed to investigate whether imatinib and GB, have protective effects on CP-induced ovarian damage.

Methods

This is an experimental animal study. In May 2019, the research was conducted after approval from the animal experiments local ethics committee of the Üsküdar University (no: 2019-05, date: 15.02.2019).

Animals Used in the Research

In this study, female wistar albino rats of the norvegicus species were used. The weighed from 219 to 265 grams, and were aged between 10 and 12 weeks. Four to five rats were placed in each cage. They received light for 12 hours between 8 am and 8 pm. They had unrestricted access to tap water and standard rodent pellet food at an average room temperature of 21 to 23 degrees. Humidity rate was kept between 40 and 50 percent.

Experimental Groups

Group 1 (the control group): These rats underwent a laparotomy at baseline and the ovaries were removed. Blood was drawn from the inferior vena cava for Anti-Mullerian hormone (AMH) testing.

Group 2 (the CP group): Rats received CP intramuscularly at a dose of 7.5 mg/kg at baseline (25) and underwent an oophorectomy at the end of day 10. At least 2-3 mm^3 of blood was drawn from the inferior vena cava for AMH testing.

Group 3 (the CP + imatinib group): Thirty minutes after the first dose of imatinib, rats received intramuscular CP at a dose of 7.5 mg/kg. They then received oral imatinib (Glivec®, Novartis, İstanbul, Turkey) for 10 days at a dose 7.5 mg/kg (18,20). Both ovaries were removed surgically at the end of day 10. At least 2-3 mm^3 of blood was drawn from the inferior vena cava for AMH testing.

Group 4 (the CP + GB group): Sixty minutes after the first administration of GB, rats received CP at a dose of 7.5 mg/kg intramuscularly. They additionally received GB (Ginkgo biloba leaf extract, Solgar, İstanbul, Turkey) orally, dissolved in distilled water, for 10 days at a dose 80 mg/kg (26). Both ovaries were removed surgically at the end of day 10, and at least 2-3 mm^3 of blood was drawn from the inferior vena cava for AMH testing.

Cisplatin Dose and Preparation

CP was administered intramuscularly only at baseline at a dose of 7.5 mg/kg. While preparing the drug; we used the central drug preparation unit of our hospital (with Robotic Chemotherapy Drug Preparation System) in a closed environment where microbiological contamination and employee exposure risks are eliminated under conditions that comply with national and international standards. These standards included: negative pressure indoor air environment complying with ISO 5, Class 100 and GMP Class A, double HEPA filter air cleaning system, safe waste management system, high capacity laminator current and dose sensitivity information (gravimetric and volumetric) measurement and the barcode system.

Operational Procedures

Powder free sterile latex gloves were used in all surgical procedures. After rats were decapitated, blood samples were taken for AMH hormone evaluation. Then laparotomy was performed in the supine position and oophorectomy was done. Operations were completed between 5 and 10 minutes to avoid the drying effects of room air (Figure 1).

Histopathological Examination

All examinations were performed by the same pathologist blindly. Removed ovaries were put into 10% formalin. Paraffin blocks were prepared within 24 hours after treatment. Five micrometre tissue sections were sampled and follicle examination in each ovarian tissue was made by taking five different sections. Tissues were stained with haematoxylin eosin and examined by light microscopy (Olympus Clinical Microscope, Tokyo, Japan). Paraffin blocks were sectioned using a microtome blade (Leica, Nussloch, Germany).

Figure 1. Excision of the ovary

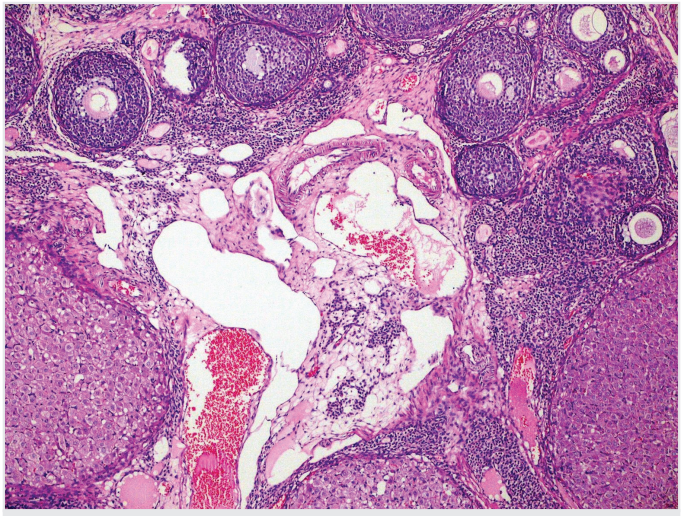

Figure 2. Oedema and vascular congestion x200 haematoxylin eosin

Histopathological injury scores were evaluated as described by Celik et al. (27). Cellular degeneration, vascular congestion, oedema, haemorrhage and inflammation were examined. The evaluations were graded from 0 to 4.

Grade 0: No abnormal findings were detected. Grade 1: mild vascular congestion, mild oedema, absence of haemorrhage or leukocyte infiltration. Grade 2: moderate vascular congestion, moderate oedema, absence of haemorrhage or leukocyte infiltration. Grade 3: severe vascular occlusion, severe oedema, minimal leukocyte infiltration and minimal haemorrhage. Grade 4: severe vascular occlusion, severe oedema, leukocyte infiltration and haemorrhage (Figure 2).

To evaluate ovarian reserves, all follicles were examined as described by Parlakgumus et al. (28). Primordial, primary, secondary (preantral), tertiary (antral) and atretic follicles were counted (Figure 3, 4). A primordial follicle was defined as oocyte with epithelial cell layer in

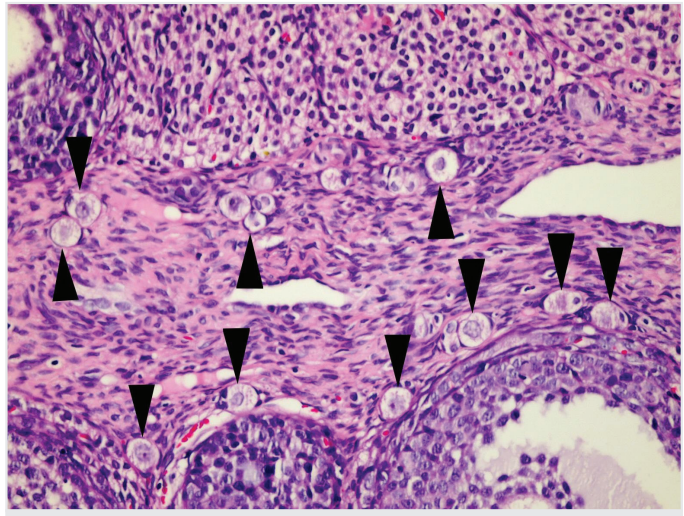

Figure 3. Primordial follicles x400 haematoxylin eosin

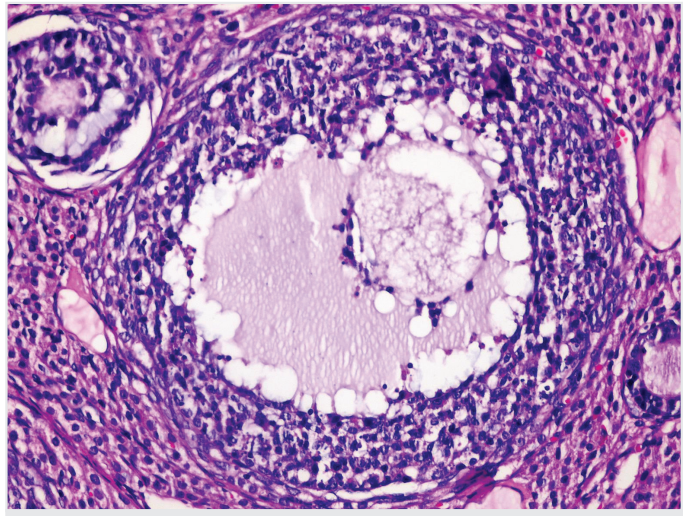

Figure 4. Degenerated follicle x400 haematoxylin eosin

only one layer. A primary follicle was defined as a follicle surrounded by one or more layers of cuboidal granulosa cells. A secondary (preantral) follicle was defined as a follicle consisting of antrum folliculi and zona pellucida surrounded by two or more cell layers. Tertiary follicles were defined as follicles with layers of antrum, stratum granulosum and surrounding cumulus oophorus. For the atretic follicle, the basement membrane that separated the oocyte from granulosa cells was often thickened to become the glassy membrane. Fibrous material replaced the granulosa cells and loss of cohesion could also be observed in granulosa cells.

Anti-Mullerian Hormone Assays

Blood samples were collected into tubes containing lithium heparin (BD Vacutainer Plasma tubes, Manchester, England). The concentration of the Lithium Heparin additive in these tubes was 17 international units of heparin/mL of blood. Blood samples were centrifuged within 30 minutes of sampling. After 15 minutes of centrifugation at 1000xg, serum was removed and remaining plasma was transferred into an eppendorf tube and stored frozen at -20 °C until the time of analysis AMH concentrations were measured in "ng/mL" of plasma using the enzyme-linked immunosorbent assay method. The rat AMH kit used in study had a sensitivity of 0.10 g/mL, a detection range of 0.16 to 10 ng/mL and a coefficient of variation less than 10% (Elabscience®, Rat AMH kit; Houston, Texas, ABD). The laboratory technician of the university hospital laboratory was blinded to the study groups All samples were analysed in the same assay.

Statistical Analysis

All the data were analysed by SPSS 25.0 (SPSS Inc., Chicago, IL, USA). Results were presented as number, percentage, average and standard deviation. One-way ANOVA, Kruskal-Wallis and Tamhane post hoc tests were used for comparison between the groups. The correlations between AMH and other variables were investigated by Spearman correlation analysis. Statistical significance level was accepted as $p<0.05$.

Results

There were no significant differences between the study groups concerning rat weights (ANOVA F=0.410; p=0.747) (minimum 219 grams, maximum 265 grams). Histopathological features of the groups were compared and shown in Table 1. The control group showed no increase in ovarian damage scores. Tamhane post hoc analysis revealed significant subgroup differences concerning oedema between the control and CP groups (p=0.032), concerning vascular congestion between control and both CP (p=0.023) and CP + GB groups (p=0.007). Also, the total damage score was significantly different between the control group and CP (p=0.044), CP + imatinib (p=0.020), as well as CP + GB groups (p=0.016). Tamhane post hoc comparisons are given in Table 2. Group 1 with normal ovaries (score: 0.13) had the lowest ovarian damage scores and group 2 with only CP had the highest scores (score: 3.47). In the follicle count, most follicles were seen in the control group (group 1), while the least follicles was seen in CP + GB group (group 4).

Follicle counts in the study groups were compared and is shown in Table 3. Tamhane post hoc analysis revealed significant subgroup differences in the number of primordial follicles between the control and CP + imatinib (p=0.008), CP alone (p=0.001), as well as CP + GB groups (p<0.001). There were significant differences in the number of secondary follicles between the control group and both CP (p=0.004) as well as CP + GB groups (p=0.010).

A significant correlation between AMH levels and over volume in the control group (Table 4). Also, positive correlations were detected between the total damage score and the number of atretic follicles in the CP group.

The mean AMH level was highest in the control group (2.73 ng/mL), and lowest in CP + GB group (0.11 ng/mL). AMH values were significantly lower in all groups compared to control rats (p<0.001 in all). A significant correlation between AMH levels and over volume in the control group (group 1). Also, positive correlations were detected between the total damage score and the number of atretic follicles in the CP group (group 3) (Table 5).

Discussion

Some chemotherapeutics used for cancer treatment are major causes of ovarian damage. Prevention of primordial follicle destruction and premature ovarian ageing will be beneficial for children, adolescents and young women with fertility desire. Primordial follicles are very sensitive to radiotherapy and chemotherapy. Follicular reserve decreases and premature ageing occurs (18), especially during chemotherapy. Both *in vitro* and *in vivo* studies have shown that CP administration clearly causes increased free oxygen radicals (9,10). In animals treated with CP,

Table 1. Comparison of histopathological damage scores of control vs Cisplatin, Cisplatin + Imatinib and Cisplatin + Ginkgo biloba groups

	Control			Cisplatin + Imatinib			Cisplatin			Cisplatin + Gingko biloba				
		Percentile			Percentile			Percentile			Percentile			
	Median	25	75	Median	25	75	Median	25	75	Median	25	75	H*	p
Oedema	0	0	0	1	0	2	2	1	2	0	0	1	12.067	0.007
Vascular congestion	0	0	0	1	0	1	2	1	2	1	1	1	12.535	0.006
Inflammation	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Cellular degeneration	0	0	0	0	0	0	0	0	1	0	0	1	3.576	0.311
Haemorrhage	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Total damage score	0	0	0	3	1	3	3	3	5	1	1	2	13.672	0.003

*Kruskal-Wallis test

free oxygen radicals cause multiple cellular changes and organ damage. Data from animal studies indicate that if oxidative stress is blocked, organs will be preserved (9,11).

In another study, imatinib was found to be effective in preventing primordial oocyte damage caused by CP or other c-abl inhibitors. Because of this, it was suggested that imatinib administration with chemotherapeutics might be considerable (5).

Imatinib acts by inhibiting c-abl, a TKI. Thus, imatinib has been shown to cause the accumulation of p63, which is an oocyte-specific homologue of p53 and activates apoptosis in DNA damage (18,29,30). Imatinib affects

Table 2. Post-hoc bi-variate comparison of the variables using the Tamhane's test

					95% Confidence Interval	
Dependent Variable	**(I) Group**	**(J) Group**	**Mean Difference (I-J)**	**p**	**Lower Bound**	**Upper Bound**
Oedema	Cisplatin + Imatinib	Cisplatin	-0.455	0.942	-1.99	1.08
		Cisplatin + Gingko biloba	0.657	0.501	-0.55	1.87
		Control	1.035	0.096	-0.16	2.23
	Cisplatin	Cisplatin + Gingko biloba	1.112	0.132	-0.24	2.47
		Control	1.490	0.032	0.13	2.85
	Cisplatin + Gingko biloba	Control	0.378	0.377	-0.28	1.03
Vascular congestion	Cisplatin + Imatinib	Cisplatin	-0.477	0.875	-1.81	0.86
		Cisplatin + Gingko biloba	-0.095	1.000	-1.19	1.00
		Control	0.892	0.108	-0.17	1.96
	Cisplatin	Cisplatin + Gingko biloba	0.381	0.909	-0.81	1.57
	Control	Control	1.369	0.023	0.20	2.54
	Cisplatin + Gingko biloba	Control	0.988	0.007	0.31	1.67
Cellular degeneration	Cisplatin + Imatinib	Cisplatin	-0.367	0.912	-1.54	0.80
		Cisplatin + Gingko biloba	-0.137	0.999	-1.11	0.84
		Control	0.244	0.695	-0.35	0.84
	Cisplatin	Cisplatin + Gingko biloba	0.230	0.995	-1.05	1.51
		Control	0.611	0.470	-0.56	1.78
	Cisplatin + Gingko biloba	Control	0.380	0.720	-0.57	1.33
Total damage score	Cisplatin + Imatinib	Cisplatin	-1.298	0.790	-4.57	1.98
		Cisplatin + Gingko biloba	0.425	0.983	-1.43	2.28
		Control	2.038	0.020	0.33	3.75
	Cisplatin	Cisplatin + Gingko biloba	1.723	0.499	-1.51	4.95
		Control	3.336	0.044	0.09	6.58
	Cisplatin + Gingko biloba	Control	1.613	0.016	0.30	2.93

Table 3. Comparison of ovarian follicle counts and Anti-Mullerian hormone levels between groups

	Control			**Cisplatin + Imatinib**			**Cisplatin**			**Cisplatin + Gingko biloba**				
		Percentile			**Percentile**			**Percentile**			**Percentile**			
	Median	**25**	**75**	**Median**	**25**	**75**	**Median**	**25**	**75**	**Median**	**25**	**75**	**H***	**p**
Number of primordial follicles	12.00	11.00	14.00	5.00	3.00	8.00	4.00	3.00	9.00	4.00	3.00	6.00	16.753	0.001
Number of primary follicles	13.00	10.00	14.00	11.00	7.00	13.00	8.00	8.00	11.00	7.00	6.00	9.00	8.659	0.034
Number of secondary follicles	10.00	8.00	10.00	7.00	5.00	8.00	5.00	5.00	8.00	5.00	3.00	8.00	13.164	0.004
Number of tertiary follicles	8.00	7.00	10.00	6.00	6.00	7.00	8.00	7.00	10.00	9.00	7.00	10.00	4.94	0.176
Number of atretic follicles	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	2.00	3.047	0.384

*Kruskal-Wallis test

early folliculogenesis in the postnatal period and decreases activation of primordial follicle pool in rat ovaries, causing an increase in the expression of AMH proteins (31). Another recent study showed a novel finding that CP-induced damage is associated with increased expression of TAp63 phosphorylated at Ser395 and Ser160/162 residues in human ovary. So, imatinib use could not provide protection for human ovarian cells. Besides, it was stated that imatinib itself may be a gonadotoxic agent for ovarian follicles (32). However, further studies are needed to investigate the long-term outcomes and their effects on fertility.

Conclusion

It is not possible to clearly describe the success of imatinib in reducing ovarian damage induced by CP. In our study, total damage score increased with the use of both CP and imatinib (group 3) compared to

Table 4. Correlations between Anti-Mullerian hormone levels and variables

			Weight (gram)	Over volume (mm^3)	Total damage score	Number of atretic follicles
Cisplatin + Imatinib	Anti-Mullerian hormone level (ng/mL)	r	-0.303	-0.354	-0.255	-0.064
		p	0.466	0.389	0.543	0.881
	Weight (Gram)	r	-	-0.340	0.568	-0.380
		p	-	0.410	0.142	0.353
	Over volume (mm^3)	r	-	-	0.013	-0.390
		p	-	-	0.976	0.340
	Total damage score	r	-	-	-	-0.325
		p	-	-	-	0.432
Cisplatin	Anti-Mullerian hormone level (ng/mL)	r	0.108	0.025	-0.313	-0.536
		p	0.799	0.953	0.450	0.171
	Weight (Gram)	r	-	-0.692	-0.248	-0.332
		p	-	0.057	0.553	0.422
	Over volume (mm^3)	r	-	-	-0.393	-0.173
		p	-	-	0.336	0.681
	Total damage score	r	-	-	-	0.877**
		p	-	-	-	0.004
Cisplatin + Gingko biloba	Anti-Mullerian hormone level (ng/mL)	r	0.494	-0.037	-0.007	0.368
		p	0.213	0.931	0.988	0.370
	Weight (Gram)	r	-	0.393	-0.308	0.032
		p	-	0.336	0.458	0.941
	Over volume (mm^3)	r	-	-	-0.508	-0.594
		p	-	-	0.199	0.121
	Total damage score	r	-	-	-	0.311
		p	-	-	-	0.454
Control	Anti-Mullerian hormone level (ng/mL)	r	-0.012	0.717*	0.412	-0.620
		p	0.978	0.046	0.310	0.101
	Weight (Gram)	r	-	0.056	0.581	0.057
		p	-	0.895	0.131	0.894
	Over volume (mm^3)	r	-	-	0.257	0.000
		p	-	-	0.539	1.000
	Total damage score	r	-	-	-	-0.293
		p	-	-	-	0.482

*: Kruskal-Wallis test, **: Spearman correlation analysis

control ovaries (group 1) (p=0.020), and the number of primary follicles in group 3 decreased compared to group 1 (p=0.008). Also, the mean AMH level (0.19 ng/mL) significantly decreased (p<0.001).

GB has been shown to be an antioxidant with protective effects on CP-induced cellular damage. In a study by Chang et al. (33), the effect of GB on total ovarian follicle count, apoptotic indices and cytoplasmic protein levels were examined and its protective effects on ovarian reserve have been showed.

On the other hand, it was reported that GB inhibits growth in ovarian cancer cells and triggers apoptosis. It has been stated that GB combination with chemotherapeutics could provide a preventive strategy for infertility (21,34,35). CP and protective agents against CP-induced organ damages are current issues in cancer treatment in patients of reproductive age, but none of the studies in literature have examined the effect of CP treatment on ovarian damage scores, AMH levels, ovarian, preantral, antral and atretic follicles. In our study, vascular congestion (p=0.007) and total damage score (p=0.016) were increased compared to control

Table 5. Correlations between rat weights, over volume, total damage score, number of atretic follicles, and Anti-Mullerian hormone levels

			Weight (gram)	Over volume (mm^3)	Total damage score	Number of atretic follicles
Cisplatin + Imatinib	Anti-Mullerian hormone level (ng/mL)	r	-0.303	-0.354	-0.255	-0.064
		p	0.466	0.389	0.543	0.881
	Weight (Gram)	r	-	-0.340	0.568	-0.380
		p	-	0.410	0.142	0.353
	Over volume (mm^3)	r	-	-	0.013	-0.390
		p	-	-	0.976	0.340
	Total damage score	r	-	-	-	-0.325
		p	-	-	-	0.432
Cisplatin	Anti-Mullerian hormone level (ng/mL)	r	0.108	0.025	-0.313	-0.536
		p	0.799	0.953	0.450	0.171
	Weight (Gram)	r	-	-0.692	-0.248	-0.332
		p	-	0.057	0.553	0.422
	Over volume (mm^3)	r	-	-	-0.393	-0.173
		p	-	-	0.336	0.681
	Total damage score	r	-	-	-	0.877**
		p	-	-	-	0.004
Cisplatin + Ginkgo biloba	Anti-Mullerian hormone level (ng/mL)	r	0.494	-0.037	-0.007	0.368
		p	0.213	0.931	0.988	0.370
	Weight (Gram)	r	-	0.393	-0.308	0.032
		p	-	0.336	0.458	0.941
	Over volume (mm^3)	r	-	-	-0.508	-0.594
		p	-	-	0.199	0.121
	Total damage score	r	-	-	-	0.311
		p	-	-	-	0.454
Control	Anti-Mullerian hormone level (ng/mL)	r	-0.012	0.717*	0.412	-0.620
		p	0.978	0.046	0.310	0.101
	Weight (Gram)	r	-	0.056	0.581	0.057
		p	-	0.895	0.131	0.894
	Over volume (mm^3)	r	-	-	0.257	0.000
		p	-	-	0.539	1.000
	Total damage score	r	-	-	-	-0.293
		p	-	-	-	0.482

*: Kruskal-Wallis test, **Spearman correlation analysis

ovaries after the use of CP in combination with GB. The mean number of ovarian follicles was the least in the CP + GB group. Both primary ($p<0.001$) and preantral follicles ($p=0.01$) decreased and AMH levels were also significantly lower in the CP + GB group ($p<0.001$).

It is important how all the apoptotic indices, cytoplasmic protein levels, antioxidant mechanisms, enzymatic changes, histopathological damage scores, follicular examinations and ovarian reserve tests help us in cancer treatment in young patients of reproductive ages. And more importantly, these parameters have real clinical implications for reproductive organs and fertility. We investigated whether follicle count and AMH levels, which are the two most used parameters in the evaluation of fertility in the clinic, can be maintained with imatinib and GB in CP-treated rats.

In our study, we concluded that imatinib and GB were not effective in preventing CP-induced ovarian damage.

Ethics

Ethics Committee Approval: The study was conducted after approval from the animal experiments local ethics committee of the Üsküdar University (no: 2019-05, date: 15.02.2019). This study was conducted at the Animal Testing Laboratory of the University after the approval of the Ethics Committee.

Informed Consent: Experimental animal study.

Peer-review: Externally peer-reviewed.

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