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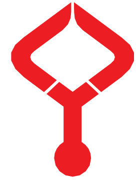

Research Article

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Evaluation of protective effects of mirtazapine and mesna on cisplatin-induced ovarian damage in rats

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Abstract
To evaluate whether mirtazapine and mesna have protective effects on cisplatin-induced ovarian injury. A total of 32 female Wistar Albino rats were divided into 4 groups (8 rats per group) and included in the study. No medication was administered to the first group; only intervention was that their ovaries were removed and anti-mullerian hormone (AMH) values were measured. The second group received intramuscular cisplatin at a single dose of 7.5 mg/kg. The third group received a single dose of 200 mg/kg mesna intraperitoneally, and 30 minutes later, a single dose of 7.5 mg/kg intramuscular cisplatin was administered. The fourth group received oral 30 mg/kg mirtazapine, and 60 minutes later, a single dose of 7.5 mg/kg intramuscular cisplatin was administered. Oral 30 mg/kg mirtazapine was continued for ten days. Ovaries and AMH values of all groups were evaluated at the end of tenth day. In the cisplatin group when compared to normal ovarian tissue total histopathological damage score increased ($p=0.037$), preantral follicle count decreased ($p=0.003$) and AMH levels decreased ($p<0.001$). In the cisplatin + mesna group total ovarian damage score was also increased ($p=0.005$), preantral and antral follicles decreased ($p<0.001$ and $p=0.001$, respectively), and AMH levels decreased ($p<0.001$). In the cisplatin + mirtazapine group, total ovarian damage score ($p<0.001$), preantral follicle count ($p=0.002$) and AMH values were decreased ($p<0.001$). It was concluded that mesna and mirtazapine were not effective in preventing ovarian damage due to cisplatin.

Keywords: Cisplatin, mirtazapine, mesna, anti-mullerian hormone, ovary, rats

1. Introduction

Cisplatin (cis-diamminedichloroplatinum) is a platinum-containing compound that inhibits RNA, DNA and protein synthesis in cells (Khalaf et al., 2019). It is the first-line chemotherapeutic agent for lung and ovarian cancer (Qian et al., 2019). There are various signaling pathways involved in apoptosis caused by cisplatin in granulosa cells (Wu et al., 2018).

Chemotherapeutic treatments used for cancers can damage the ovaries within a range of minimal damage to total ovarian failure. Up to 40% of patients can have a complete ovarian failure (Yeh et al., 2008a). Cisplatin is a chemotherapy agent with adverse side effects such as ovarian toxicity, uterine toxicity, myelosuppression; nephrotoxicity, neurotoxicity, ototoxicity; hepatotoxicity and gastrointestinal toxicity (Domitrovic et al., 2014; Pandir et al., 2014; Omar et al., 2016; Saribas et al., 2016; Kaygusuzoglu et al., 2018). Ovarian toxicity due to chemotherapy is one of the main concerns of young women at a reproductive age (15–44 years) (Morgan et al., 2012) and the most important problem is the limited number of oocytes present in prenatal ovaries. To date, many chemotherapeutic agents have been reported to damage the ovarian follicles and increase the risk of premature ovarian failure, early menopause and infertility. This may lead to a decrease in the quality of life of patients and an increase in medical costs (Stroud et al., 2009; Morgan et al., 2012; Roness et al., 2014; Xiao et al., 2017).

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Cisplatin causes a decrease in plasma antioxidant concentrations in cancer patients (Weijl et al., 1998; Srivastava et al., 2010) Thus, agents capable of directly cleaning free radicals may have the potential to reduce the side effects of such chemotherapeutics (Cheki et al., 2019).

Mesna (2-mercaptoethane sulfonate), which is a Food and Drug Administration (FDA) approved agent, plays an important role in the prevention of urotoxicity, ototoxicity and intestinal damage by sweeping reactive oxygen species (ROS) and using anti-oxidative inhibitors (Siu and Moore, 1998; Ypsilantis et al., 2004; Rybak et al., 2007; Yeh et al., 2008b). Recently, mesna administration during cisplatin treatment has been reported to protect the ovaries by reducing follicle loss in them (Yeh et al., 2008b). However, there is still some uncertainty as to how mesna can protect against cisplatin-induced damage (Li et al., 2013).

Mirtazapine is an agent used to treat major depression. In recent years, however, researchers have begun to conduct studies on its antioxidant activity. The Scientific World Journal reported that this agent could be used as a cell protector because of its inhibitory effects and its effects on antioxidant parameters (Berger et al., 2004; Kopelman et al., 2009). Mirtazapine prevents cisplatin-induced oxidative stress in ovarian tissue and infertility dose-dependently. It is also assumed to block infertility by its antioxidant, sedating and antidepressant activities (Altuner et al., 2013).

Reducing ovarian damage due to chemotherapy is one of the important clinical issues for young women for whom fertility is greatly significant. In this study, we aimed to assess whether mesna and mirtazapine, which are considered to reduce ovarian damage due to cisplatin; have protective effects on ovarian and follicular damage and anti-mullerian hormone (AMH) levels.

2. Materials and methods

This study was approved by the animal testing laboratory ethics committee in July 2019.

2.1. Animals used in the research

Female Wistar albino rats of the *Norvegicus* species were used in our study. They were aged between 10-12 weeks and weighed from 180 to 260 grams. We placed four or five rats in each cage. The rats received light for twelve hours. They were provided access to standard rodent pellet foods and tap water at an average room temperature of 21-23 degrees Celsius. The humidity rate was kept between 40 and 50 percent.

2.2. Study groups

Group 1 (the control group): This group underwent a laparotomy procedure at baseline and their ovaries were removed. Blood was drawn from the inferior vena cava for AMH testing.

Group 2 (the cisplatin group): This group received cisplatin intramuscularly at a dose of 7.5 mg/kg at baseline (Pandir et al., 2014) and underwent an oophorectomy procedure at the end of the 10th day of the study. At least 2-3 mm^3 of blood was drawn from the inferior vena cava for AMH testing.

Group 3 (the cisplatin + mesna group): This group received 200 mg/kg mesna (Uromitexan® ampule, Eczacibasi Baxter, Istanbul, Turkey) intraperitoneally (Yeh et al., 2008a) thirty minutes after cisplatin was injected at a dose of 7.5 mg/kg intramuscularly. 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 cisplatin + mirtazapine group): The rats initially received cisplatin at a dose of 7.5 mg/kg intramuscularly 60 minutes after the first administration of mirtazapine. Additionally, they orally received mirtazapine dissolved in distilled water (Remeron®, Schering Plough, Istanbul, Turkey) for 10 days at a dose of 30 mg/kg (Altuner et al., 2013; Gulec et al., 2013) and 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.

2.3. Cisplatin dose and preparation

Cisplatin was administered intramuscularly only at baseline at a dose of 7.5 mg/kg. 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 were eliminated under conditions that comply with national and international standards while preparing the drug. 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 was employed.

2.4. Surgical procedures

Ovaries and blood samples of rats were taken when the study was completed. The rats were decapitated. Blood samples were obtained. Laparotomy was performed and ovaries were excised (Fig. 1). All surgical procedures were performed with powder-free latex gloves.

2.4. Histopathological examinations

All examinations were performed by the same pathologist who was blinded to the study. Removed ovaries were put into 10% formalin. Paraffin blocks were prepared within 24 hours after treatment. Five-micrometer tissue sections were taken and follicle examinations in each ovarian tissue were made by taking five different sections. Tissues were stained with hematoxylin-eosin and examined using a light microscope (Olympus Clinical Microscope, Tokyo, Japan). Paraffin blocks were sectioned using a microtome blade (Leica, Nussloch, Germany).

Histopathological injury scores were evaluated as described by Celik et al. (Celik et al., 2004). Cellular degeneration, vascular congestion, edema; hemorrhage and inflammation were examined. The evaluations were graded from 0 to 4.

Grade 0: Normal findings were observed, no abnormal findings were detected. Grade 1: mild vascular congestion, mild edema, absence of hemorrhage or leukocyte infiltration. Grade 2: moderate vascular congestion, moderate edema, absence of hemorrhage or leukocyte infiltration. Grade 3: severe vascular occlusion, severe edema, minimal leukocyte infiltration and minimal hemorrhage. Grade 4: severe vascular occlusion, severe edema, leukocyte infiltration and hemorrhage (Fig. 2).

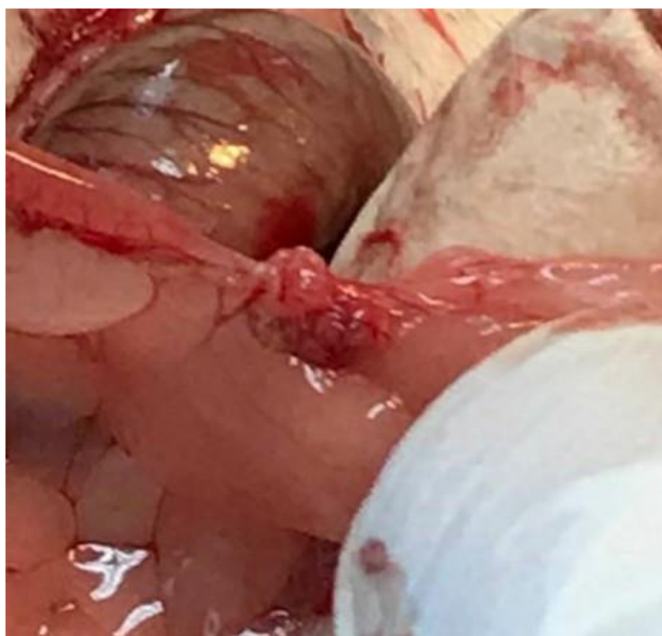

Fig. 1. Excision of the ovary

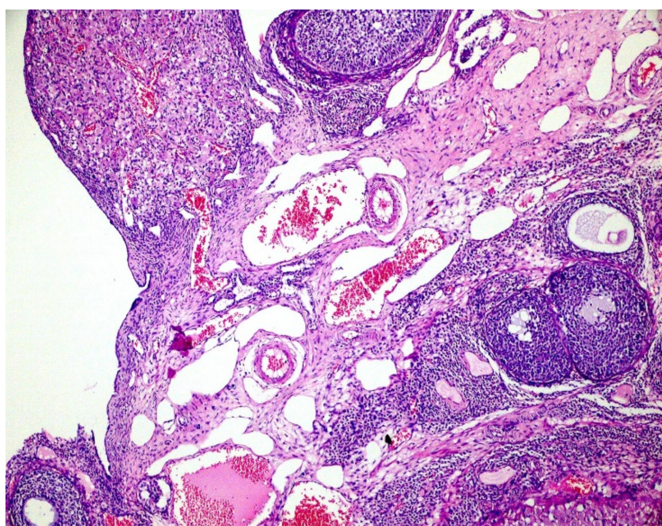

Fig. 2. Vascular congestion (Hematoxylin-eosin staining, x200)

All follicles were examined as described by Parlakgumus et al. (Parlakgumus et al., 2014) to evaluate ovarian reserves. Primordial, primary, secondary (pre-antral); tertiary (antral) and atretic follicles were counted (Figures 3 and 4). Primordial follicle was defined as oocyte with the epithelial cell layer in only one layer. The primary follicle was described as a follicle surrounded by one or more layers of cuboidal granulosa cells. The secondary (pre-antral) 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 the antrum, stratum granulosum and surrounding cumulus oophorus. Atretic follicle was described as the basement separating the oocyte from granulosa cells that often thickens to become the glassy membrane. Fibrous material replaced the granulosa cells and loss of cohesion may also be observed in granulosa cells.

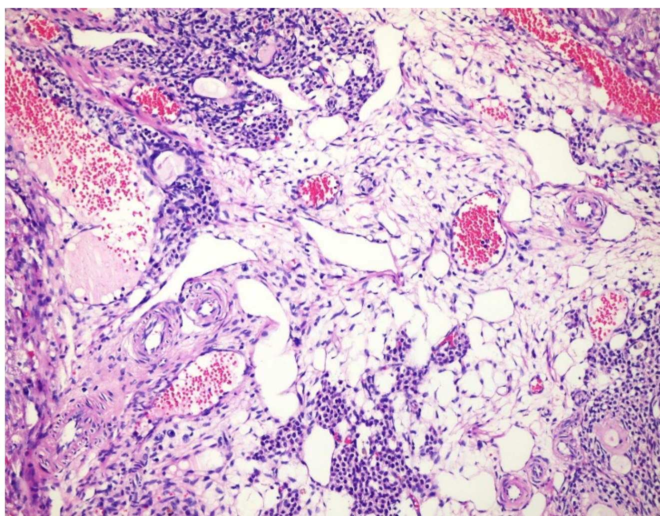

Fig. 3. Edema in the medullary region - dilated vessels (Hematoxylin-eosin staining, x200)

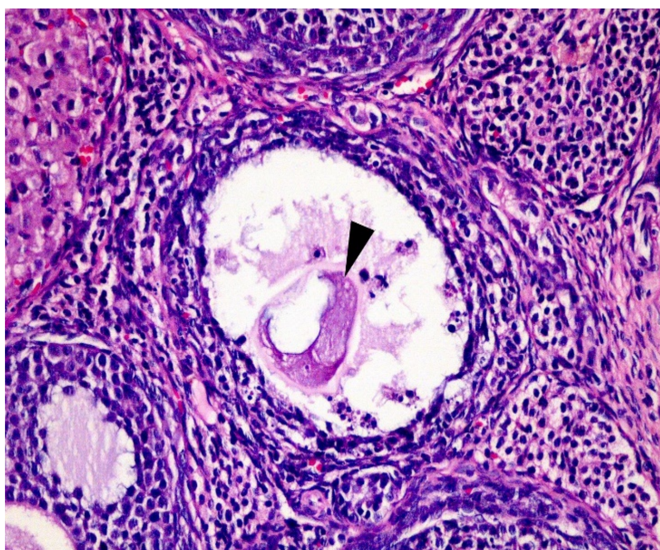

Fig. 4. Defragmented oocyte in the degenerated follicle (Hematoxylin-eosin staining, x400)

2.5. AMH 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. The serum was removed after 15 minutes of centrifugation at

1000xg, remaining plasma was transferred into an Eppendorf tube and stored frozen at -20 ºC until the time of analysis. The AMH concentrations were measured in “ng/ml” plasma using the ELISA method. The rat AMH kit had a sensitivity of 0.10 g/mL, a detection ranging from 0.16 to 10 ng/mL and a coefficient of variation less than 10% (Elabscience, Rat AMH kit, Houston, Texas, ABD). The laboratory technician at the laboratory of the university hospital was blinded to the study groups and unaware of which samples belonged to which rat. All samples were analyzed in the same assay.

2.6. Data analysis

Data were analyzed using the SPSS 25.0 (SPSS Inc., Chicago, IL, USA) software package program. Results were expressed in numbers, percentages, averages and standard deviation., The Kruskal-Wallis and post hoc Tamhane tests and one-way ANOVA were used to compare the groups. The significance level was accepted as p <0.05.

Table1. Comparison of histopathological damage scores of normal ovary vs Cisplatin, Cisplatin + Mesna and Cisplatin + Mirtazapine groups

	Normal ovary	Cisplatin	p*	Cisplatin +Mesna	p**	Cisplatin +Mirtazapine	p***
Edema							
Mean	0.00	1.51	0.028	1.26	0.034	1.00	0.044
SD	0.00	1.08		0.88		0.75	
Median	0.00	2.00		2.00		1.00	
IQR	0.00	2.00		2.00		1.00	
Vascular congestion							
Mean	0.00	1.38	0.019	1.26	0.001	1.25	0.01
SD	0.00	0.92		0.47		0.70	
Median	0.00	2.00		1.00		1.00	
IQR	0.00	2.00		1		1	
Inflammation							
Mean	0.00	0.00	-	0.13	0.928	0.00	-
SD	0.00	0.00		0.36		0.00	
Median	0.00	0.00		0.00		0.00	
IQR	0.00	0.00		0.00		0.00	
Cellular degeneration							
Mean	0.00	0.64	0.413	1.01	0.048	1.64	<0.001
SD	0.00	0.92		0.76		0.51	
Median	0.00	0.00		1.00		2.00	
IQR	0.00	2.00		2.00		1	
Hemorrhage							
Mean	0.00	0.00	-	.13	0.922	0.00	-
SD	0.00	0.00		.36		0.00	
Median	0.00	0.00		0.00		0.00	
IQR	0.00	0.00		0.00		0.00	
Total score							
Mean	0.12	3.53	0.037	3.79	0.005	3.89	<0.001
SD	0.34	2.58		1.84		1.34	
Median	0.00	4.00		4.00		4.00	
IQR	0.00	5.00		3		3	

p-values were calculated with one-way ANOVA followed by post hoc Tamhane. *Normal ovary vs. Cisplatin. **Normal ovary vs. Cisplatin +Mesna. ***Normal ovary vs. Cisplatin + Mirtazapine.

3. Results

There was a significant difference between the weights at the beginning of the experiment (F=3.804, p=0.021) although the rats were randomly assigned to the groups. The different

groups were the "cisplatin + mesna" and "control" groups (Tamhane, p= 0.038). Hence, the findings were presented after the weight of the rats were adjusted.

There was a significant difference between the mean ovarian volumes of the groups. The post hoc analysis revealed that only the cisplatin and the control groups had significantly different mean ovarian volume. The control group had the highest volume while the cisplatin group had the lowest (F=4.964, p=0.007). There was no significant difference between the groups in terms of ovarian area.

Table 2. Comparison of normal ovary vs Cisplatin, Cisplatin + Mesna and Cisplatin + Mirtazapine groups in terms of follicle counts and AMH values.

	Group						
	Control	Cisplatin	p*	Cisplatin+ Mesna	p**	Cisplatin+ Mirtazapine	p***
Primordial follicle			<0.001		<0.001		0.086
Mean	13.17	5.23		4.10		7.53	
Standard Deviation	2.71	3.09		2.28		4.77	
Median	12.00	5.00		4.00		7.00	
IQR	4	6		3		8	
Primary follicle			0.129		0.031		1.000
Mean	12.13	8.23		8.09		12.18	
Standard Deviation	2.65	3.46		2.23		5.85	
Median	12.00	8.00		7.00		15.00	
IQR	4	4		2		10	
Secondary (pre-antral) follicle			0.003		<0.001		0.002
Mean	10.01	5.72		3.65		5.26	
Standard Deviation	2.09	1.82		2.41		2.05	
Median	9.00	5.00		4.00		4.00	
IQR	3	3		4		4	
Tertiary (antral) follicle			0.886		0.001		0.996
Mean	8.24	9.66		3.53		7.66	
Standard Deviation	1.92	3.26		1.51		2.48	
Median	8.00	9.00		3.00		8.00	
IQR	4	6		2		3	
Atretic follicle			0.981		0.371		0.001
Mean	0.37	0.64		1.01		1.64	
Standard Deviation	0.52	0.92		0.76		0.51	
Median	0.00	0.00		1.00		2.00	
IQR	1	2		2		1	
AMH (ng/mL)			<0.001		<0.001		<0.001
Mean	2.73	0.41		0.29		0.37	
Standard Deviation	0.52	0.33		0.32		0.35	
Median	2.86	0.29		0.17		0.22	
IQR	0.87	0.56		0.3		0.7	

p-values were calculated with one-way ANOVA followed by post hoc Tamhane. *Normal ovary vs. Cisplatin. **Normal ovary vs. Cisplatin +Mesna. ***Normal ovary vs. Cisplatin + Mirtazapin

3.1. Histopathological damage scores

It is important to note that the significant difference in total damage score between groups is caused by the control group and not the intervention groups (Kruskal-Wallis

H=15.078, p=0.002). The damage scores of the intervention groups were close. Edema and vascular congestion damage were mostly observed in the cisplatin group. The third and fourth groups did not have a decrease in congestion damage. Hemorrhage and inflammation damages were slightly increased in the cisplatin group; however, it did not differ significantly. Cellular degeneration was mostly observed in the cisplatin + mirtazapine group. Mesna and mirtazapine were not able to reduce this damage (Table 1).

Table 3: Comparison of subgroups according to the mean follicle count

					95% GA	
			Difference	**P**	**Alt**	**Üst**
Primordial follicle count	Cisplatin+Mesna	Cisplatin+Mirtazapine	-3,.427	0.469	-9.61	2.76
		Cisplatin	-1.123	0.963	-5.31	3.07
		Control	-9.061	<0.001	-12.89	-5.24
	Cisplatin+Mirtazapine	Cisplatin	2.304	0.856	-4.06	8.67
		Control	-5.635	0.086	-11.88	0.61
	Cisplatin	Control	-7.939	<0.001	-12.31	-3.56
Primary follicle count	Cisplatin+Mesna	Cisplatin+Mirtazapine	-4.088	0.473	-11.61	3.43
		Cisplatin	-0.132	1.000	-4.66	4.39
		Control	-4.039	0.031	-7.77	-0.31
	Cisplatin+Mirtazapine	Cisplatin	3.955	0.563	-3.74	11.65
		Control	0.049	1.000	-7.49	7.59
	Cisplatin	Control	-3.907	0.129	-8.58	0.77
Secondary follicle count	Cisplatin+Mesna	Cisplatin+Mirtazapine	-1.611	0.692	-5.11	1.89
		Cisplatin	-2.072	0.382	-5.42	1.28
		Control	-6.364	<0.001	-9.85	-2.88
	Cisplatin+Mirtazapine	Cisplatin	-0.461	0.998	-3.43	2.51
		Control	-4.752	0.002	-7.90	-1.61
	Cisplatin	Control	-4.291	0.003	-7.24	-1.35
Tertiary follicle count	Cisplatin+Mesna	Cisplatin+Mirtazapine	-4.132	0.012	-7.42	-0.84
		Cisplatin	-6.133	0.004	-10.22	-2.04
		Control	-4.715	0.001	-7.36	-2.07
	Cisplatin+Mirtazapine	Cisplatin	-2.001	0.712	-6.44	2.43
		Control	-0.584	0.996	-4.01	2.84
	Cisplatin	Control	1.418	0.886	-2.75	5.59
Atretic follicle count	Cisplatin+Mesna	Cisplatin+Mirtazapine	-0.627	0.400	-1.66	0.41
		Cisplatin	0.372	0.950	-0.92	1.67
		Control	0.639	0.373	-0.39	1.67
	Cisplatin+Mirtazapine	Cisplatin	0.999	0.114	-0.17	2.17
		Control	1.266	0.001	0.48	2.05
	Cisplatin	Control	0.267	0.981	-0.90	1.44

3.2. Ovarian follicle counts

There was a decrease in the primordial, primary, preantral and antral follicles in the cisplatin group. However, only the primordial and preantral follicles had a significant difference (p<0.001 and p=0.003, respectively). Atretic follicles were similar to normal ovarian tissue and no differences were present (Table 2). The cisplatin + mesna group had a significant decrease in all primordial, primary, preantral and antral follicles. Atretic follicles were similar to normal ovaries (p= 0.371). The preantral follicles decreased (p= 0.002) and atretic follicles increased (p= 0.001) in the cisplatin + mirtazapine group. Other follicles were similar to normal ovarian tissue. The most significant difference was in terms of tertiary follicles (Table 3).

3.3. AMH levels

AMH mean values were significantly higher in the control group (F=75.018, p<0.001) but similar in other groups. The AMH was 2.73 ng/ml in the normal ovarian group while it significantly decreased in all groups treated with cisplatin. The AMH was 0.41 ng/ml in the cisplatin only group and lower than rats with normal ovary (p<0.001). It was 0.29 ng/ml in the cisplatin + mesna group and lower than the normal ovary group (p<0.001). The AMH was 0.37 ng/ml and lower in the cisplatin + mirtazapine group compared to the normal ovary group (p <0.001). There was a significant relationship between the AMH levels, ovarian volume and primary follicle count (Table 4).

4. Discussion

The toxic effects of cisplatin are attributed to many factors such as peroxidation of the cell membrane, DNA damage, mitochondrial dysfunction; inhibition of protein synthesis and the ability to influence host immune response (Jordan and Carmo-Fonseca, 2000). Mesna is a small nucleophilic synthetic molecule and contains a sulfhydryl group with the potential to purge reactive oxygen reagents (Jost et al., 2017). Yeh et al. (Yeh et al., 2008b) emphasized that Mesna can be

Table 4: Correlations between rat weights, over volume, total damage score, number of follicles, and AMH levels

Group			OV	THH	PFS	PrFS	SFS	TFS	AFS	AMH
Cisplatin + Mesna	**Wt**	**r**	0.026	-0.655	0.201	0.315	0.042	-0.421	-0.349	-0.289
		p	0.952	0.078	0.633	0.448	0.921	0.299	0.396	0.487
	OV	**r**		-0.118	0.814	0.463	-0.135	0.236	-0.575	-0.230
		p		0.781	0.014	0.248	0.750	0.573	0.136	0.584
	TDS	**r**			0.119	-0.665	0.224	0.791	0.676	0.111
		p			0.779	0.072	0.594	0.019	0.066	0.793
	PFC	**r**				0.293	-0.025	0.471	-0.275	-0.415
		p				0.482	0.954	0.238	0.510	0.307
	PrFC	**r**					-0.750	-0.218	0.828	-0.450
		p					0.032	0.605	0.011	0.264
	SFC	**r**						-0.128	0.429	0.491
		p						0.762	0.288	0.217
	TFC	**r**							0.370	0.032
		p							0.367	0.940
Cisplatin & Mirtazapine	**Wt**	**r**	0.648	-0.109	-0.636	-0.442	0.116	-0.455	-0.397	-0.443
		p	0.082	0.797	0.090	0.272	0.785	0.258	0.330	0.272
	OV	**r**		0.058	-0.116	0.024	0.013	0.012	-0.456	-0.530
		p		0.891	0.785	0.954	0.976	0.977	0.256	0.177
	TDS	**r**			-0.065	0.142	-0.281	0.084	0.484	-0.153
		p			0.879	0.737	0.500	0.843	0.225	0.717
	PFC	**r**				0.573	0.433	0.543	0.228	0.253
		p				0.137	0.284	0.165	0.587	0.545
	PrFC	**r**					-0.259	0.872	-0.057	-0.133
		p					0.536	0.005	0.893	0.754
	SFC	**r**						-0.187	0.181	0.128
		p						0.657	0.667	0.763
	TFC	**r**							-0.342	0.217
		p							0.407	0.606
Cisplatin	**Wt**	**r**	-0.692	-0,.248	0.181	0.377	0.432	-0.434	-0.332	0.108
		p	0.057	0.553	0.668	0.358	0.285	0.283	0.422	0.799
	OV	**r**		-0.393	-0.050	-0.103	-0.284	0. 353	-0.173	0.025
		p		0.336	0.906	0.808	0.496	0.392	0.681	0.953
	TDS	**r**			-0.139	0.205	0.006	0.370	0.877	-0.313
		p			0,.742	0.626	0.988	0.367	0.004	0.450
	PFC	**r**				0.179	0.753	0.265	-0.249	-0.431
		p				0.671	0.031	0.526	0.552	0.286
	PrFC	**r**					0.538	0.445	0.298	-0.196
		p					0.169	0.270	0.474	0.641
	SFC	**r**						0.198	0.000	-0.516
		p						0.639	1.000	0.191
	TFC	**r**							0.228	0.060
		p							0.587	0.888
Control	**Wt**	**r**	0.056	0.581	-0.540	-0.073	-0.030	0.195	0.057	-0.012
		p	0.895	0.131	0.167	0.864	0.943	0.643	0.894	0.978
	OV	**r**		0.257	0.380	0.700	0.434	0.579	0.000	0.717
		p		0.539	0.353	0.053	0.283	0.133	1.000	0.046
	TDS	**r**			-0.423	0.334	0.168	0.000	-0.293	0.412
		p			0.297	0.419	0.691	1.000	0.482	0.310
	PFC	**r**				0.630	0.410	0.534	-0.173	0.537
		p				0.094	0.313	0.173	0.682	0.170
	PrFC	**r**					0.804	0.736	-0.399	0.928
		p					0.016	0.037	0.327	0.001
	SFC	**r**						0.772	-0.344	0.655
		p						0.025	0.404	0.078
	TFC	**r**							0.057	0.546
		p							0.893	0.162

Wt: Weight (Gram), OV: Ovary volume (mm^3), TDS: Total damage score, PFC: Primordial follicle count, PrFC: Primary follicle count, SFC: Secondary follicle count, TFC: Tertiary follicle count, AMH: Anti-mullerian Hormone

effective in preventing damage caused by free radicals to protect the anti-mullerian hormone (AMH) and ovarian follicles. Mirtazapine is a noradrenergic and specific serotonergic antidepressant drug. It affects antioxidant

parameters and has protective effects on cisplatin-induced neurotoxicity and nephrotoxicity and indomethacin-induced gastrotoxicity (Bilici et al., 2009; Gulec et al., 2011; Sener et al., 2012; Gulec et al., 2013). Its effects should be assessed with further research for the prevention of cisplatin-induced damage (Gulec et al., 2013). Consequently, we examined the protective effects of mesna and mirtazapine on cisplatin-induced ovarian damage. We primarily investigated tissue damage of cisplatin in the ovarian injury evaluation. We evaluated edema, vascular congestion, hemorrhage; inflammation, cellular degeneration and total damage scores. There was a significant increase in the damage scores of the cisplatin-treated groups. So, ovarian tissue is damaged due to chemotherapy. However, such damage could not be reduced in both groups that used mesna and mirtazapine and they were inadequate in this regard.

Mesna has been used with chemotherapeutic agents to reduce cytotoxicity by removing oxygen-free radicals with the sulfhydryl group (-SH) (Siu and Moore, 1998; Kabasakal et al., 2004). In their study, Li et al. assessed the effects of cisplatin and mesna use on AMH-positive follicles (Li et al., 2013). No significant increase was observed in the AMH-positive follicles and they mentioned that the same dose of mesna and duration of administration may not reverse intensive damage and mesna played a role in the cisplatin-induced ovarian damage (Li et al., 2013). Our results also showed that mesna can protect the ovaries from cisplatin-induced damage through antioxidants without inhibiting the anti-tumor effects of cisplatin; however, there was no significant success in the histopathological damage, follicular damage and protection of AMH hormone values.

Primordial, primary, preantral and antral follicles were counted on hematoxylin and eosin-stained slides according to the criteria of Oktay et al. (Oktay et al., 1995). There was no significant difference in the number of AMH-positive follicles between normal ovarian and cisplatin groups.

AMH was considered a reliable marker for ovarian reserve assessment (Yeh et al., 2006; Streuli et al., 2008) and often used as an indicator of ovarian damage (Visser et al., 2006). Ovarian reserve is a term used to describe the ability of an ovary to produce a sufficient number of mature oocytes for adequate fertility. Evaluation of ovarian reserve after chemotherapy is very important in demonstrating reproductive potential. The targets of the chemotherapeutic agents may be granulosa cells that regulate and support the developing follicles. Any damage to these dividing cells affects oocyte maturation by resulting in follicular destruction and ovarian dysfunction (Li et al., 2013).

Ovarian follicles can also be damaged and lose their function after chemotherapy. Li et al. revealed that the percentage of AMH positive follicles reduced significantly compared to normal ovarian group and cisplatin damaged ovarian follicles when it was administered (Li et al., 2013). They did not observe a significant increase in the AMH-positive follicle percentage with mesna when cisplatin was administrated. In the present study, the number of all types of follicles decreased in the cisplatin group, however; only the primordial and preantral follicles had a difference. Cisplatin + mesna administration did not prevent this decrease in follicles. There was a significant decrease in all follicle values. Only preantral follicles decreased ($p = 0.002$) while side atretic follicles increased ($p = 0.001$) when cisplatin + mirtazapine was administrated.

There was a significant decrease in the AMH values of all groups treated with cisplatin. The cisplatin (0.41 ng/ml, $p < 0.001$), cisplatin + mesna (0.29 ng/ml, $p < 0.001$) and cisplatin + mirtazapine groups (0.37 ng/ml) had lower AMH levels compared to the normal ovarian group ($p < 0.001$). There was no relationship between the rat weight, total damage score, atretic follicle count; pre-antral + antral follicle count and AMH values.

Cisplatin damaged normal ovarian tissue and resulted in increased damage scores, decreased functional follicle counts, increased atretic follicle counts and consequently decreased AMH values. Mesna and mirtazapine, which were thought to have protective effects against ovarian damage, were not successful in preventing these adverse effects. In conclusion, mesna and mirtazapine were not effective in preventing ovarian damage due to cisplatin

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Conflicts of Interests

The authors have no conflicts of interest to declare.

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