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Protective effects of Dehydroepiandrosterone (DHEA) vs Caffeic Acid Phenethyl Ester (CAPE) against Ischemia-Reperfusion injury in Rat Ovaries

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Abstract

Objective: In this study, the effectiveness of caffeic acid phenethyl ester (CAPE) and Dehydroepiandrosterone (DHEA) in preventing ischemia reperfusion injury associated with ovarian torsion have been investigated.

Materials and Methods: Twenty four adult female Wistar Albino rats were randomly divided into four groups. Ovaries were not twisted, and only healthy ovarian tissues were removed from the rats in the first group, while ovaries were twisted for 3 hours in the other groups. The second group did not receive any medications before the ovaries were untwisted, while 20 micromole/kg of CAPE was applied on peritoneal surface to the third group, and 60 mg/kg of DHEA was administered intraperitoneally to the fourth group.

Results: The level of primordial follicles was higher in the third group compared to the second group after the torsion of the ovary ($p=0.017$). The mean level of primary follicles was higher in the first group compared to the number of follicles in the third and fourth groups after the torsion of the ovary ($p<0.001$). The median hemorrhage level was higher in the second group following ovarian torsion compared to that in the first group ($p=0.005$).

Conclusion: Agents that have been considered to reduce injury resulting from ischemia-reperfusion proved ineffective during the early stages in terms of the number of follicles in the ovaries; however, we believe that long-term studies may be more beneficial.

Keywords: Caffeic Acid Phenethyl Ester, Dehydroepiandrosterone, Ischemia-Reperfusion, Ovary, Rat

Introduction

Early detection and treatment is of paramount importance for the preservation of ovarian functions and the prevention of serious morbidities in ovarian torsion (1). There are reports indicating that this condition is more likely to occur in the pediatric population compared to adult women (2), which demonstrates how important the preservation of fertility is. Oxidative processes leading to injury in ovarian torsion and significant increases in oxidative markers in twisted ovaries have been demonstrated in various studies (3-5). To date, a number of antioxidant agents have been used to reduce oxidative injury and cell loss. However, none of these agents has been introduced into routine clinical practice.

Caffeic acid phenethyl ester (CAPE) is one of the most active compounds of honey bee product propolis and has proven beneficits in oxidative injury in various tissues. The effects of CAPE on the brain, kidney, testis, genital organs, ovary and various tissues have been investigated (6-11). Dehydroepiandrosterone (DHEA) has been widely used to increase ovarian reserves in infertile women. Previous studies reported encouraging outcomes with DHEA in terms of improved oocyte and embryo yields and live birth rates in women with diminished ovarian reserves (12-15). In this study, we aimed to analyze the effectiveness of CAPE and DHEA in preventing ovarian injury associated with ischemia-reperfusion and their protective effects on ovarian reserves and against follicle loss.

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Materials and methods

Ethics committee approval for this study was obtained from the animal testing laboratory (Protocol code: 103.2018.mar).

Animals used in the research: Female Wistar albino rats of the Norvegicus species were used in our study. The rats used in the study weighed from 200 to 250 grams. They aged between 10-12 weeks. 4-5 rats were placed in each cage. The rats received light for twelve hours. Rats were provided access to standard rodent pellet foods and tap water at an average room temperature of 21-23 degrees Celsius. Humidity was kept between 40 and 50 percent.

Groups: Group 1 (normal ovary group – Group N): no chemical agents were applied to this group. Decapitation was applied first, and laparotomy was performed afterwards. During laparotomy, the ovaries were removed and fixed in 10% formaldehyde.

Group 2 (the twisted ovary group – Group T): The first laparotomy was performed after anesthesia. Ovarian tissue was found, and a 720-degree torsion procedure was performed. A hypoxia of 3 hours was achieved. The second laparotomy was done, and ovarian tissue detorsion was performed. A 6-hour reperfusion was achieved after detorsion. Decapitation was performed at the end of the second 6 hours. Ovarian tissues were excised by laparotomy.

Group 3 (CAPE group– Group C): The first laparotomy was performed after anesthesia. The left ovary was found, and torsion was performed with 720-degree rotation. Then, it was fixed to the lower abdominal wall. After fixation, the abdomen was closed and exposed to ischemia for 3 hours. At the end of this period, the abdomen was opened again, and detorsion was performed. After detorsion, 20 micromole/kg of CAPE (Sigma-Aldrich Chemie GmbH®, Saint Louis, USA) was applied to the ovarian surface (2). Then, the abdomen was closed again. Reperfusion was achieved for 6 hours, and decapitation was performed at the end of this period. Laparotomy was performed, and both ovaries were excised.

Group 4 (PRP group– Group P): The first laparotomy was performed after anesthesia. The left ovary was found, and torsion was performed with 720-degree rotation. Then, it was fixed to the lower abdominal wall. After fixation, the abdomen was closed and exposed to ischemia for 3 hours. At the end of this period, the abdomen was opened again, and detorsion was performed. The surgical wound was closed following the application of DHEA to the peritoneal surface at a dose of 60 mg/kg diluted in 0.1 ml of sesame oil. Reperfusion was achieved for 6 hours, and decapitation was performed at the end of this period. Laparotomy was performed, and both ovaries were excised.

Operations: In all procedures, latex powder-free gloves were used. Ketamine hydrochloride (10%) 80 mg / kg (Ketalar; Eczacıbaşı Warner Lambert, Istanbul, Turkey) and xylazine hydrochloride (2%) 15 mg / kg (Rompun, Bayer Health Care LCC, Kansas City, KS), were used for anesthesia. Surgical site cleaning was performed with 10% Povidone-iodine solution (Batticon's; Adeka Laboratories, Istanbul, Turkey). 5 cm median incision was used for laparotomy. The left ovary was twisted with blood vessels to achieve a torsion of 720 degrees (Figure 1). The sprained ovarian tissue was sutured to the abdomen with 5/0 silk sutures. After bleeding control, abdominal wall was closed with 2/0 polyglactin 910 sutures. Surgical procedures were completed before 15 minutes to prevent the drying effect of room air.

Histopathological examinations: 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 micrometer tissue sections were taken, and follicle examinations were made in each ovarian tissue by taking 5 different sections. Tissues were stained with hematoxylin eosin and examined by 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. (2). Cellular degeneration, vascular congestion, edema, hemorrhage and inflammation were examined (Figure 1). 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.

To evaluate ovarian reserves, all follicles were examined as described by Parlakgumus et al. (16) Primordial, primary, secondary (pre-antral), tertiary (antral) and atretic follicles were counted. Primordial follicle is defined as an oocyte with epithelial cell layer in only one layer. Primary follicle is defined as a follicle surrounded by one or more layers of cuboidal granulosa cells. The secondary (pre-antral) follicle is defined as a follicle consisting of antrum follicles and zona pellucida surrounded by two or more cell layers. Tertiary follicles are defined as follicles with layers of antrum, stratum granulosum and surrounding cumulus oophorus. In atretic follicles, the basement that separates the oocyte from granulosa cells often thickens to become the glassy membrane. Fibrous material replaces the granulosa cells, and loss of cohesion may occur in granulosa cells (Figures 2-4).

Statistical analysis:

SSPS Version 15.0 was used for statistical analysis. Kolmogorov-Smirnov test and histogram examinations were used to evaluate the normality of distribution of variables. Mean ± standard deviation or median (interquartile range) values are used to present descriptive analyses. One-way ANOVA test was used to analyze numerical data showing normal distribution. Kruskal-Wallis test was used to analyze numerical data showing non-normal distribution. P value <0.05 was accepted as the statistical significance limit.

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Results

The mean level of primordial follicles in Group C was higher than that in Group T before the torsion of the ovary (p=0.002). The mean level of primary follicles in Group N before the torsion of the ovary was higher than those in groups C, D and T (p=0.002). The mean level of inflammatory cell infiltration in Group N was higher than those in groups C, D and T before the torsion of the ovary (p=0.003) (Table 1).

The mean level of primordial follicles in Group C was higher than that in Group T after the torsion of the ovary (p=0.002). The mean level of primary follicles in Group N was higher than those in groups C and D after the torsion of the ovary (p<0.001). The mean level of hemorrhage in Group T after the ovarian torsion was higher than that in Group N after the torsion of the ovary (p=0.005). The mean level of vascular congestion in Group N was lower than those in Groups T and C after the torsion of the ovary (p=0.007). The mean level of edema in Group N was lower than those in groups C, D and T after the torsion of the ovary (p=0.02) (Table 2). The histopathological appearances of follicles are seen in figures 2-4.

Table 1. Comparison of normal ovary examinations according to groups

		Groups				p
		C	D	T	N	
Primordial Follicle	**Mean**	20,3	11,5	5,7	14,2	0,002[a]
	SD	±5,6	±7,8	±3,9	±4,2	
	Minimum	14,0	4,0	1,0	8,0	
	Maximum	28,0	22,0	11,0	18,0	
Primary Follicle	**Mean**	6,5	8,7	7,5	15,2	0,002 [a]
	SD	±3,3	±2,8	±2,5	±5,0	
	Minimum	3,0	5,0	4,0	7,0	
	Maximum	12,0	12,0	10,0	21,0	
Secondary Follicle	**Mean**	7,7	10,8	7,7	7,2	0,248 [a]
	SD	±2,5	±4,9	±2,1	±3,4	
	Minimum	5,0	4,0	5,0	4,0	
	Maximum	12,0	18,0	10,0	12,0	
Tertiary Follicle	**Mean**	3,5	4,8	4,0	3,8	0,756 [a]
	SD	±1,5	±2,9	±2,7	±1,3	
	Minimum	2,0	1,0	1,0	2,0	
	Maximum	6,0	9,0	7,0	5,0	
Hemorrhage	**Median**	0,0	0,0	0,0	0,0	0,553 [b]
	Percentiles -25	0,0	0,0	0,0	0,0	
	Percentiles -75	0,0	0,0	0,0	0,0	
Vascular Congestion	**Median**	1,0	1,0	1,0	1,0	0,716 [b]
	Percentiles -25	1,0	1,0	1,0	1,0	
	Percentiles -75	2,0	2,0	2,0	1,0	
Cellular degeneration	**Median**	0,5	0,0	1,0	0,5	0,638 [b]
	Percentiles -25	0,0	0,0	0,0	0,0	
	Percentiles -75	1,0	0,0	1,0	1,0	
Inflammatory cell infiltration	**Median**	0,0	0,0	0,0	1,0	0,003 [b]
	Percentiles -25	0,0	0,0	0,0	0,0	
	Percentiles -75	0,0	0,0	0,0	1,0	
Edema	**Median**	0,0	1,0	0,0	0,0	0,282 [b]
	Percentiles -25	0,0	0,0	0,0	0,0	
	Percentiles -75	0,0	1,0	1,0	1,0	

SD: standard deviation, [a]One-way ANOVA test, [b]Kruskal-Wallis test

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Table 2. Comparison of ovarian examinations after torsion according to groups

		Groups				p
		C	D	T	N	
Primordial Follicle	Mean	15,5	8,0	5,5	14,2	0,017[a]
	SD	±7,3	±6,5	±4,0	±4,2	
	Minimum	5,0	1,0	2,0	8,0	
	Maximum	24,0	18,0	12,0	18,0	
Primary Follicle	Mean	4,7	5,7	9,3	15,2	<0,001 [a]
	SD	±1,4	±4,4	±3,3	±5,0	
	Minimum	3,0	1,0	6,0	7,0	
	Maximum	7,0	13,0	14,0	21,0	
Secondary Follicle	Mean	7,7	7,3	9,2	7,2	0,736 [a]
	SD	±4,2	±3,0	±2,9	±3,4	
	Minimum	4,0	4,0	5,0	4,0	
	Maximum	15,0	11,0	13,0	12,0	
Tertiary Follicle	Mean	3,5	2,5	4,7	3,8	0,318 [a]
	SD	±2,8	±1,0	±2,2	±1,3	
	Minimum	0,0	1,0	2,0	2,0	
	Maximum	8,0	4,0	8,0	5,0	
Hemorrhage	Median	2,0	2,0	2,5	0,0	0,005 [b]
	Percentiles -25	1,0	1,0	2,0	0,0	
	Percentiles -75	3,0	2,0	3,0	0,0	
Vascular Congestion	Median	3,0	2,0	2,5	1,0	0,007 [b]
	Percentiles -25	2,0	2,0	2,0	1,0	
	Percentiles -75	3,0	3,0	3,0	1,0	
Cellular degeneration	Median	1,0	1,5	1,0	0,5	0,455 [b]
	Percentiles -25	1,0	1,0	1,0	0,0	
	Percentiles -75	1,0	2,0	2,0	1,0	
Inflammatory cell infiltration	Median	1,0	0,5	1,0	1,0	0,382 [b]
	Percentiles -25	1,0	0,0	1,0	0,0	
	Percentiles -75	2,0	1,0	1,0	1,0	
Edema	Median	2,0	1,5	1,5	0,0	0,027 [b]
	Percentiles -25	1,0	1,0	1,0	0,0	
	Percentiles -75	3,0	2,0	3,0	1,0	

SD: standard deviation, [a]One-way ANOVA test, [b]Kruskal-Wallis test

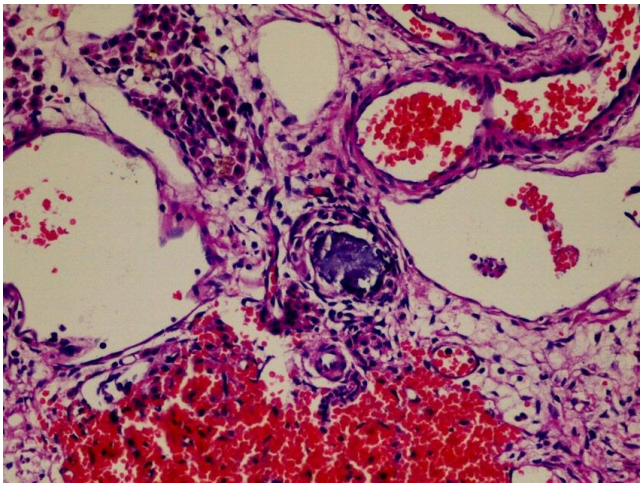

Figure 1: Bleeding in ovarian stroma, congested vessels and secondary follicle x400 hematoxylin eosin

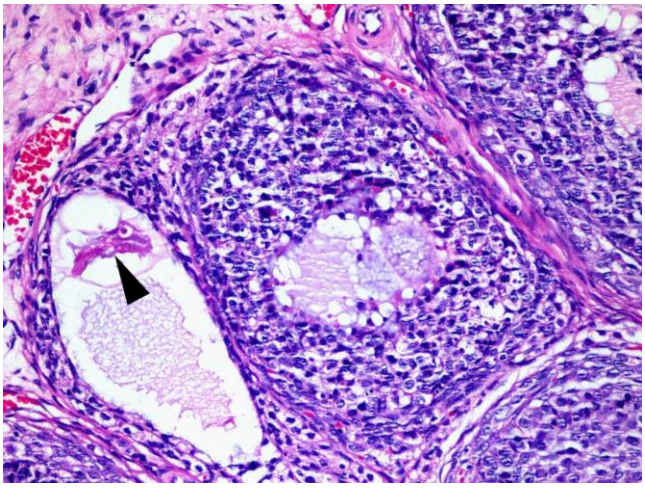

Figure 2: Degenerate follicle in fragmented oocyte x400 hematoxylin eosin

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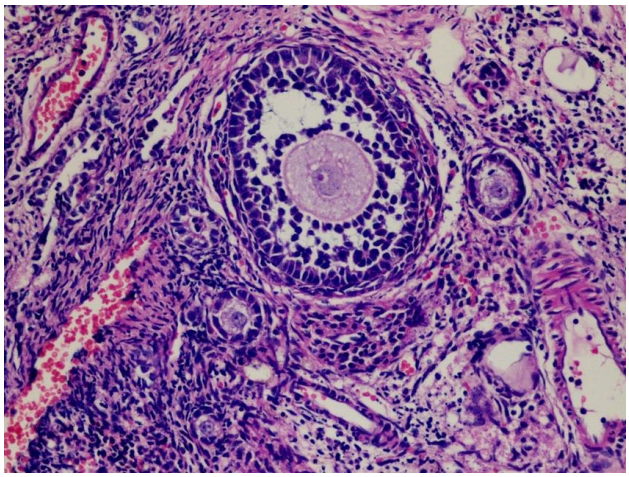

Figure 3: Antral follicles and primary follicles x400 hematoxylin eosin

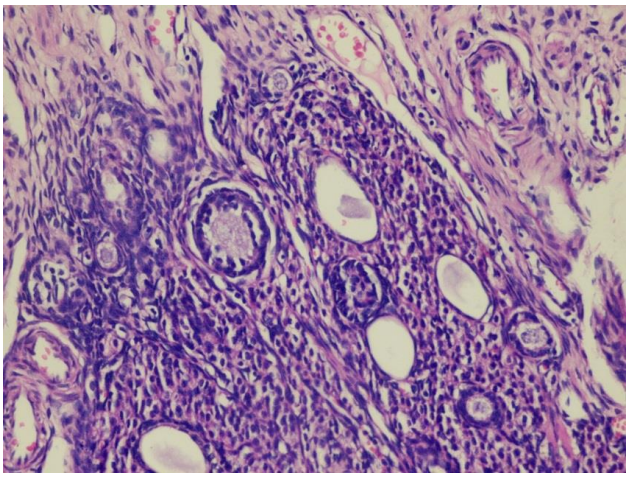

Figure 4: Primordial and primary follicles x400 hematoxylin eosin

Discussion

The examination of normal ovaries revealed statistically significant variations in the numbers of primordial follicles and primary follicles before the torsion of the ovary. These variations appear to be associated with inborn characteristics of these rats. In the examinations after the torsion of the ovary, these significant variations in the numbers of primordial follicles and primary follicles did not change.

However, the level of hemorrhage in the CAPE and DHEA groups after the torsion of the ovary was significantly lower than the group of rats that did not receive medications ($p<0.005$). Vascular congestion and edema was lower in the normal ovary group than the other groups ($p=0.007$ and $p=0.027$, respectively).

In a previous study, Kart et al. investigated factors indicating oxidative damage in ischemia reperfusion (I/R) such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). However, follicle counts were not investigated in this study. The authors concluded that although CAPE had no significantly superior effects, it might have protective effects against I/R injury (5).

In another study, Celik et al. investigated xanthine oxidase activity, malondialdehyde levels and reduced glutathione levels. The effectiveness of CAPE in preventing I/R injury was also investigated, but follicle counts were not assessed. In this study, the effectiveness of CAPE remained indefinite, and the authors concluded that CAPE might only be effective in reducing I/R injury (2).

In another study investigating the effects of CAPE in I/R injury, Ozler et al. reported torsion-related decreases in the numbers of preantral and small antral follicles (8). The investigator did not use any therapeutic agents. This study investigated the effects of an ovarian torsion of 360 degrees with a reperfusion time of 3 hours. We believe that the differences between the results of this study and our study might be related to the differences in surgical techniques and duration of reperfusion.

Favorable effects of DHEA on ovaries have been established (3). However, the effects of DHEA on follicles in I/R injury were not investigated in any previous studies. In our study, no significant effect of DHEA was observed on follicle counts during the early stages after I/R. However, it would be meaningful if these results were assessed along with long-term outcomes.

In this study, CAPE and DHEA – which are believed to reduce I/R injury as indicated by biochemical and/or histopathological studies demonstrating oxidative injury – made no differences in follicle counts during the early stages following I/R. Based on these results, one may conclude that follicle loss associated with I/R injury may not occur during the early stages after I/R. It may be rational to search for an answer to whether a significant long-term effect would be observed.

Furthermore, we believe that in clinical practice, preventing complete or partial loss of follicles is rather important than seemingly protecting follicles against cellular damage at molecular level. Surely, whether the quantitative presence of these follicles indicates their functionality in terms of fertility is an important matter needing further investigation for an accurate answer.

Conclusion

Agents that have been considered to reduce injury resulting from ischemia-reperfusion proved ineffective in terms of the number of follicles in the ovary during the early stages; however, we believe that long-term studies may be more beneficial.

What is known about this topic

- Ovarian torsion is more common in young women
- Ischemia reperfusion injury is preventable with antioxidant agents, and CAPE is a potent antioxidant agent
- The number of ovarian follicles collected in the treatment of infertility can be increased by DHEA, and DHEA is

one of the most commonly used medical agents by infertility specialists worldwide.

What this study adds

- Ovarian torsion both causes histopathological damage to ovarian tissue and causes follicle loss
- The antioxidant properties of CAPE cannot prevent the damage in ovarian torsion
- The follicle-enhancing effect of DHEA does not provide protective effects on ovarian torsion.

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Ethical issues: Author declare, originality and ethical approval of research. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

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