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Effects of allium cepa on ovarian torsion-detorsion injury in a rat model

Ratlarda ovaryan torsiyon-detorsiyon modelinde allium cepanın etkilerinin incelenmesi

Hakan Kula[1], Orkun İlgen[1], Sefa Kurt[1], Filiz Yılmaz[2]

[1]*Dokuz Eylül University Faculty of Medicine, Department of Obstetrics and Gynecology, İzmir, Turkey*

[2]*Hitit University, Çorum Erol Olçok Training and Research Hospital, In Vitro Fertilization Center, Çorum, Turkey*

Abstract

Objective: Ischemia/reperfusion (I/R) damage following detorsion treatment, tissue fibrosis, and adhesions cause secondary tissue damage in the ovaries. Many studies have been evaluated to minimize antioxidant damage in ovarian reserve loss while minimizing I/R damage. However, no study observed long-term effects on the ovarian torsion model in rats. In this study, we evaluated the profibrotic effects of A. cepa on an ovarian torsion model on rats.

Materials and Methods: Group I (n=7) rats were the sham group. Group II (n=7) rats were the torsion group and Group III (n=7) rats were the torsion + A. cepa group. To observe the long-term effects of allium cepa, rats were fed for 21 days. Cellular damage I/R is evaluated by histopathological damage score, and transforming growth factor-beta 1 (TGF-β1) and alpha-smooth muscle actin (α-SMA) is measured to analyze the profibrotic effect.

Results: A. cepa altered cellular damage due to improvement in the histopathological damage score with A. cepa intake. However, the profibrotic mediators TGF-β1 and α-SMA are non- significantly changed by the A. cepa (p=0.477 and p=0.185 respectively).

Conclusion: A. cepa is a potent protective on cellular tissue, minimizing I/R damage on ovarian tissue histologically. Our study implies that A. cepa does not affect fibrosis-related mediators in the rat ovary.

Keywords: Allium cepa, fibrosis, ovarian torsion, ischemia-reperfusion injury, TGF-β1, α-SMA

Öz

Amaç: Over torsiyonunda tedavi amaçlı uygulanan detorsiyon işlemine sekonder oluşan iskemi/reperfüzyon hasarının (I/R); dokuda oluşan fibrozis ve adezyonlara bağlı olarak over dokusuna sekonder hasar verebilir. Over torsiyonunda literatürde birçok çalışma oxidan hasara bağlı over rezervini minimalize etmeye yönelik birçok çalışma mevcuttur. Ancak, literatürde iskemi hasarının torsiyon sonrası uzun dönemde ovarian yapısal değişikliklerine dair bir çalışma mevcut değildir. Bu çalışmada A. cepa'nın profibrotik mediatörler üzerine etkisinin incelenmesi rat modeli üzerinde amaçlanmıştır.

Gereç ve Yöntemler: Grup I (n=7) ratlar kontrol grubu olarak belirlenmiştir. Grup II (n=7) torsiyon-detorsiyon modeli ve Grup III (n=7) torsion-detorsiyon + A. cepa rejimi uygulanacak gruplar olarak belirlenmiştir. Adezyon ve fibrotik değişimlerin izlenmesi için ratlar prosedur sonrası 21 gün beslendi. Hücresel hasar düzeyi "Histopatolojik Hasar skoru" ile ölçüldü. Fibrotik değişiklikler için dönüşen büyüme faktörü-beta 1 (TGF-β1) ve alfa-düz kas aktini (α-SMA) düzeyleri ölçüldü.

Bulgular: A. cepa ile beslenen ratlarda Histopatolojik Hasar skorunda anlamlı düşüş izlendi. Ancak TGF-β1 (p=0,477) ve α-SMA (p=0,185) düzeylerinde istatistiksel olarak anlamlı değişim izlenmedi.

Sonuç: A. cepa, hücresel düzeyle hasarı minimalize etmede potent bir mediatör olarak öngörülürken, profibrotik mediatörler olan TGF-β1 ve α-SMA düzeylerinde anlamlı bir değişiklik oluşturmamıştır.

Anahtar Kelimeler: Allium cepa, fibrozis, over torsiyonu, iskemi-reperfüzyon hasarı, TGF-β, 1, α-SMA

PRECIS: Whether allium cepa has an improving mediator for fibrosis and ovarian damage on ovarian torsion-detorsion injury in a rat model investigated in this study.

Address for Correspondence/Yazışma Adresi: Hakan Kula MD,
Dokuz Eylül University Faculty of Medicine, Department of Obstetrics and Gynecology, İzmir, Turkey
Phone: +90 535 823 06 85 **E-mail:** hkula95@gmail.com **ORCID ID:** orcid.org/0000-0003-1443-5796

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Introduction

Ovarian torsion is an acute pathology of reproductive age that causes a decreased ovarian reserve[1]. Approximately 2.5% to 7.4% of acute abdominal pain cases are diagnosed with ovarian torsion[2]. The pathophysiology of the disease is stasis in arterial and venous blood flow following the rotation of the ovarian tissue from the pedicle itself. Sudden -onset pelvic pain without relief by analgesics, leukocytosis, vomiting, fever, and nausea are the most common symptoms of the disease[3]. Ultrasound imaging findings are the medialized and increased ovarian size, decreased vascular flow, and diagnostic for ovarian torsion[4]. Benign ovarian masses and cysts, particularly dermoid cysts, predispose ovarian torsion incidence[5].

Laparoscopic or laparotomic surgery is the mainstay of treatment, and early diagnosis is essential for minimizing ischemic damage to the ovarian tissue[6]. Moreover, following the laparoscopic detorsion procedure, the reversibility of ovarian tissue is limited because of the ischemia/reperfusion damage (I/R damage) by oxidative stress[7]. The management of ovarian torsion is also important for fertility preservation at reproductive age. According to the studies, providing detorsion within 24 h following ovarian torsion is related to a better ovarian reserve and minimizing ovarian tissue necrosis[8]. Reactive oxygen species (ROS), cause cellular damage by interaction with biomolecules of cells during reperfusion damage[9]. Increased ROS levels stimulate inflammasome expression [Nucleotide-binding and leucine-rich repeat (NLR) genes] by NF-kB mediators. Increased inflammasome expression stimulates profibrotic macrophages and inflammation together[10].

Transforming growth factor-beta 1 (TGF-β1) and alpha-smooth muscle actin (α-SMA) are potential biomarkers to analyze myofibroblast activity and fibrosis in human tissues[11,12]. Moreover, increased ROS and inflammatory mediators may increase fibrosis. Hepatic fibrosis after CCl_4 intoxication in nicotinamide adenine dinucleotide phosphate oxidase deficient mice models reported with ROS[13]. Moreover, in previous studies silibinin that is an antioxidant molecule, improved hepatic fibrosis and regeneration[14].

After ovarian torsion, antioxidant molecules may help minimize I/R damage mechanisms[15]. Many antioxidant molecules have been studied for minimizing ovarian tissue damage for maintaining ovarian functions. Allium Cepa Liliaceae (A. cepa) is a widely known onion bulb a plant that belongs to the botanical family Amaryllidaceae[16]. Quercetin, flavonoids, saponins, and organosulfur are the main components of A. cepa[17]. With these rich derivatives, A. cepa has plenty of therapeutic benefits. Antibiotic, antidiabetic, anti-teratogenic, and anti-inflammatory effects of A. cepa has been widely studied[17,18]. Moreover, A. cepa is compared with Alfa-Tocopherol and Vitamin-C, which are proven antioxidant molecules, and reported that A. cepa is a potential antioxidant molecule[19].

In this study, we investigated whether A. cepa impacts profibrotic mediator levels and histological improvement. We designed a rat ovarian torsion-detorsion model to measure profibrotic mediator levels and evaluate histological evaluation scores for irreversible cellular damage on ovarian tissue.

Materials and Methods

This study was approved by the institutional review board at Dokuz Eylül University Laboratory Animals Local Ethics Committee (no: 40-2020). Twenty-one adult Sprague-Dawley rats (180-250 grams) were collected from Dokuz Eylul University Experimental Animal Laboratory. Rats were sheltered in standard steel cages at a room temperature of 22 °C±2 °C, with 12 h light/dark cycles. Standard rat chow and tap water were provided to rats with ad libitum. Vaginal smears were performed at 6-12-hour intervals[20]. Rats that are all in the estrus phase are included in the study.

Study Protocol

Twenty-one rats were randomly divided into three groups that consisted of seven animals. Surgical procedures were performed under sterile conditions. Anesthesia conditions were provided with an intraperitoneal injection of 50 mg/kg ketamine hydrochloride (Ketalar, 50 mg/kg, Pfizer) and 7 mg/kg xylazine hydrochloride (Alfazyne; Alfasan International BV, Holland).

Under anesthesia, a 2 cm-midline sterile incision opened in the lower abdomen of the rats. Uterine horns, adnexa, and ovaries were identified. In Group I, only a sterile abdomen incision was made. Both ovaries of rats were rotated to the right 360 degrees and clamped over 2.5 h in Group II and III rats. Tissues were detorsioned after 3 h. A. cepa powder was obtained from fresh onion bulbs. Onion bulbs were peeled and dried on air within one week. Normal feeds of rats fortified with A. cepa powder in specific groups.

Group I (n=7) rats received only saline (0.9% NaCl) with oral gavage daily. Group II (n=7) (torsion/detorsion group) received only saline (0.9% NaCl) with oral gavage daily. Group III (n=7) (torsion/detorsion+ A. cepa group) rats received 20% Allium cepa powder + 80% normal feed with oral gavage per day. Each group was fed for 21 days with planned regimens to observe the long-term effects of allium cepa on ovarian torsion models. Rats were sacrificed on day 21st under anesthesia and the ovaries were collected for histopathological and biochemical examination. During the trial, none of the rats died and any adverse effects (hair loss, fatigue, loss of appetite, etc.) did not observed in rats due to A. cepa intake.

Histopathological Examination

The ovaries were embedded in paraffin blocks after formalin fixation. 5 µm thick tissue sections were obtained, stained in hematoxylin-eosin, and evaluated with light microscopy (CX-41, Olympus). Follicular degeneration, vascular congestion, hemorrhage, inflammatory cell presence, and primordial, primary, secondary, and tertiary follicle count scores were evaluated on histopathological examination.

Follicle counting was performed according to the study by Parlakgumus et al.[21]. The ovarian histopathological damage score was evaluated based on the following parameters: follicle cell degeneration, vascular congestion, hemorrhage, and inflammation (0: None, 1: Mild, 2: Moderate, 3: Severe)[22].

Biochemical Examination

The ovarian tissues were collected to detect TGF-beta1 and alfa-SMA levels in rats. TGF-β1 and α-SMA (BTLAB, catalog numbers E1688Ra and E2330Ra) were quantitatively assessed using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis of the data obtained in the process of the study was done with SPSS (Statistical Package for Social Sciences) 26.0 computer package program. Mean ± standard deviation was determined in the evaluation. The biochemistry and parametric data were summarized as mean ± standard deviation. The difference between the groups was analyzed using the Kruskal-Wallis test, from which group the difference originated was analyzed with the Mann-Whitney U test. A p-level of <0.05 was accepted to demonstrate statistical significance.

Results

Results of the Histopathological Examination

The mean scores of the ovarian damage scores are shown in Table 1. Follicular degeneration was significantly higher in Group II than in Groups I and III [(1±0) vs. (2.5±0.5) vs. (1.5±0.7), p<0.01 respectively]. Vascular congestion was significantly higher in Group II than in Groups I and III [(1.7±0.7) vs. (2.5±0.5) vs. (1.2±0.7), p<0.05 respectively].

Table 1. The mean scores of ovarian damage scores

	Group I	Group II	Group III	p-value
Follicular degeneration	1±0	2.5±0.5*	1.5±0.7	p<0.01
Vascular congestion	1.7±0.7	2.5±0.5**	1.2±0.7	p<0.05
Hemorrhage	1±0	2.1±0.6*	1.4±0.7	p<0.01
Inflammatory cell	1.4±0.7	2.4±0.5**	1.7±0.4	p<0.05
Primordial follicle	5.7±0.7	3±0.8*	5.2±0.7	p<0.01
Primary follicle	4.8±0.3	2±0.8*	4.4±0.7	p<0.01
Secondary follicle	4±1.2	1.8±0.6*	3.5±0.9	p<0.01
Tertiary follicle	3.1±0.3	1.7±0.7*	2.5±0.5	p<0.01

*: p<0.01, Group 2 compared with Groups I and III, **: p<0.05, Group 2 compared with Groups I and III

Hemorrhage was significantly higher in Group II than in Groups I and III [(1±0) vs. (2.1±0.6) vs. (1.4±0.7), p<0.01 respectively]. Inflammatory cell levels were significantly higher in Group II than in Groups I and III [(1.4±0.7) vs. (2.4±0.5) vs. (1.7±0.4), p<0.05 respectively].

Primordial follicle count was significantly lower in Group II than in Groups I and III [(5.7±0.7) vs. (3±0.8) vs. (5.2±0.7), p<0.01 respectively]. Primary follicle count was significantly lower in Group II than in Groups I and III [(4.8±0.3) vs. (2±0.8) vs. (4.4±0.7), p<0.01 respectively]. Secondary follicle count was significantly lower in Group II than in Groups I and III [(4±1.2) vs. (1.8±0.6) vs. (3.5±0.9), p<0.01 respectively]. Tertiary follicle count was significantly lower in Group II than in Groups I and III [(3.1±0.3) vs. (1.7±0.7) vs. (2.5±0.5), p<0.01 respectively]. Histopathologic images of the ovaries are shown in Figure 1.

Results of the Biochemical Examination

TGF-β1 and α-SMA levels were compared between the groups. Levels of TGF-β1 and α-SMA are shown in Table 2. α- SMA levels did not change statistically significantly with A. cepa among Groups A, B, and C (34.8±14 vs. 43.6±5.2 vs. 52.6±18.9, p=0.185, respectively). Moreover, there was no statistically significant decrease observed in TGF-β1 levels among Groups A, B, and C (506.9±109.5 vs. 472.4±131.9 vs. 537.6±101.7 p=0.477, respectively).

Discussion

Ovarian torsion is a critical emergent situation that causes ovarian damage, followed by decreased fertility potential and follicular reserve. Early diagnosis and detorsion are the primary

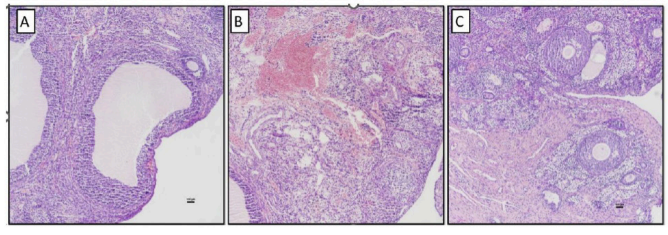

Figure 1. Histopathologic images of the ovaries (H&E staining) (4×). A: Group I: Sham-operated group, B: Group II Torsion/detorsion group, C: Group III Torsion/detorsion + A. cepa group

Table 2. Levels of α-SMA and TGF-β1

	Group I	Group II	Group III	p-value
α-SMA (mean ± standard deviation)	34.8±14	43.6±5.2	52.6±18.9	0.185
TGF-β1 (mean ± standard deviation)	506.9±109.5	472.4±131.9	537.6±101.7	0.477

steps of treatment to minimize this loss. I/R damage with ROS after detorsion is another tissue -damaging factor in ovarian torsion. Excessive ROS production stimulates lipid peroxidase production and SOD, catalase (CAT), and glutathione peroxidase levels for irreversible injury[23,24]. Although the I/R damage mechanism on ovaries with detorsion has not been fully understood, many antioxidants have been used to minimize ovarian tissue loss with reperfusion injury.

Ovarian histopathological evaluation is one of the most decisive methods for estimating I/R tissue damage[25]. We used histopathological tissue damage scores to estimate whether A. cepa has an antioxidant effect. This study demonstrates that A. cepa is a potential alleviating antioxidant in follicular degeneration ($p<0.01$), vascular congestion ($p<0.05$), hemorrhage ($p<0.01$), inflammatory cell presence ($p<0.05$), and primordial, primary, secondary, and tertiary follicle count scores ($p<0.01$). No study in the literature evaluated the antioxidant potential of A. cepa on the ovarian tissue. According to our results, A. cepa improves the histological findings of ovarian I/R damage. Previous studies have reported that A. cepa has an antioxidant effect on the liver, kidney, and brain tissues with decreased malondialdehyde (MDA) levels and increased amino acid levels[26].

Polymorphonuclear leucocytes and macrophages migrate to the ischemia zone because of increased signaling on the damaged tissue[27]. Under ischemic conditions, myofibroblasts may trigger the granulation tissue and fibrosis by secreting platelet-derived growth factors (PDGF), epidermal growth factor (EGF), TGF-β1, and α-SMA[28]. Overexpressed myofibroblast activity may predispose fibrosis and adhesions in damaged tissues[29]. TGF-β1 is the main activating mediator of fibroblast activity with induced myofibroblast migration and activation to inflammation with increasing α-SMA levels[30]. This shows that TGF-β1 is one of the most important profibrogenic mediators of wound tissue remodeling and increases structural stability in damaged tissues[28]. Moreover, TGF-β1 impacts fibrosis and scarring with induced extracellular matrix (ECM) production by myofibroblast activity[31]. Elongated or overexpressed myofibroblast activity may result in fibrosis and organ function abnormalities[29].

Fujishita et al.[32] reported that following laparoscopic ovarian detorsion operation, tubal occlusion, and pelvic adhesions occurred at second-look laparoscopy. Fibrosis negatively affects tissue healing properly and plays a key role in pelvic adhesions. These conditions may cause pelvic structural abnormalities, chronic pelvic pain, and decreased vascular perfusion on I/R damaged ovary.

The TGF-β1 expression has been investigated in many studies. A study reported that atypical prostate hyperplasia decreased TGF-β1 levels and increased IGF levels by A. cepa intake may induce hyperplasia in the gland[33]. Increased hyperplasia levels based on that increased proptosis and inhibited tissue proliferation provided by TGF-β1[34]. However, in our study, there was no statistically significant difference in TGF-β1, and α-SMA levels between Groups I, II, and III ($p=0.477$ and $p=0.185$, respectively). Our results were inconsistent with the previous study. This means that profibrotic mediator levels TGF-β1 and α-SMA might not be hindered by the A. cepa.

Flavonoids such as kaempferol , quercetin, and feruli, cysteine sulfoxides have anti-inflammatory and antioxidant effects on A. cepa[17]. Anticancer, anti-asthmatic, and hepatoprotective features are provided by multiple micronutrients in A. cepa[35]. A. cepa is an important alternative medical nutrient to prevent cell damage based on the histologic evaluation score data. However, there was no correlation observed between the profibrotic mediators TGF-β1 and α-SMA and A. cepa in the ovarian torsion-detorsion model in rats.

Strengths and Limitations of Our Study

This study is an important pilot study observing the long-term effects of ovarian torsion I/R damage and the evaluation of profibrotic mediator levels in the damaged ovarian tissue. These findings should be supported by further studies that will elucidate the fibrotic pathways associated with these mechanisms. However, our study was designed on an experimental rat model. During the administration period, the appropriate dosage may change in the female reproductive system.

Conclusion

The overall results showed that A. cepa may improve the antioxidant cell damage scores histologically. However, the antifibrotic mechanism of A. cepa is still debatable due to non-significant differences in TGF-β1, and α-SMA levels. If our study is supported by further studies, A. cepa is a potential and easily accessible antioxidant nutrition for ovarian detorsion reperfusion injury. Second, a limited number of animals were included in the study due to ethical restrictions.

Ethics

Ethics Committee Approval: This study was approved by the institutional review board at Dokuz Eylül University Laboratory Animals Local Ethics Committee (no: 40-2020).

Informed Consent: Not necessary.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.K., O.İ., S.K., Concept: H.K., Design: H.K., Data Collection or Processing: H.K., S.K., F.Y.,Analysis or Interpretation: H.K., Literature Search: H.K., Writing: H.K.

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