Brusatol Mitigates Ovarian Tissue Oxidatif Injury Induced by Ovarian Ischemia Reperfusion

Brusatol Over İskemi Reperfüzyonu ile İndüklenmiş Over Dokusu Oksidatif Yaralanmasını Azaltır

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ABSTRACT

Objective: In the scope of this study, it was tried to examine the probable benefits of brusatol against ovarian tissue injury originating from bilateral ovarian torsion/detorsion (T/D).

Material and Methods: In this study, experimental animals were randomized to 4 groups. Groups were programmed as sham group I (control), group II (T/D), group III (DMSO+T/D) and group IV (Brusatol+T/D). Group I; the abdomen region was barbered, disinfected, opened by incision and closed respectively, but no T/D model was performed. Group II, as described in group I, after opening and closing abdominal incision, 3 hours of torsion was applied and it was followed by a 3-hour of detorsion. Group III, 1% DMSO was applied intraperitoneally 0,3 ml as every other day for 10 days and the last dose was given 30 minutes prior to detorsion. Group IV; brusatol was administered intraperitoneally 0,5 mg/ml once every 2 days for 10 days and the last dose was applied 30 minutes prior to detorsion. Then, all processes were carried out as described in group II. Following detorsion period, in pursuit of sacrificing the rats, ovarian tissues were removed.

Results: Oxidant parameters (MDA, MPO, TOS, OSI) and pro-inflammatory cytokine (TNF-α, IL-1β) levels increased significantly while antioxidant levels (SOD, TAS) reduced in group II and III compared to group I ($p<0.05$). On the contrary, brusatol application (group IV) reversed all of these values compared to group II and III ($p<0.05$).

Conclusion: As a conclusion, brusatol was evaluated to have protective effects against T/D-induced ovarian injury in rats.

Keywords: Brusatol, Ovary, Torsion-Detorsion, Ischemia Reperfusion, Rat

ÖZ

Amaç: Bu çalışmanın kapsamında bilateral over torsiyon/detorsiyonundan (T/D) kaynaklanan over dokusu yaralanmasına karşı brusatolün olası yararları araştırılmaya çalışıldı.

Gereç ve Yöntemler: Bu çalışmada deney hayvanları 4 gruba randomize edildi. Gruplar grup I (sham-kontrol), grup II (T/D), grup III (DMSO+T/D) ve grup IV (Brusatol+T/D) olarak programlandı. Grup I; karın bölgesi traşlanıp, dezenfekte edildi, kesi ile açıldı ve tekrar kapatıldı, ancak T/D modeli uygulanmadı. Grup II'de, grup I'de tarif edildiği gibi, abdominal insizyon yapıldıktan sonra 3 saat torsiyon uygulandı ve bunu 3 saatlik detorsiyon izledi. Grup III, %1 DMSO, 10 gün boyunca her gün olduğu gibi intraperitonal olarak 0,3 ml uygulandı ve son doz detorsiyondan 30 dakika önce uygulandı. Daha sonra, tüm işlemler grup II' de tarif edildiği gibi gerçekleştirildi. Grup IV; brusatol, 10 gün boyunca her 2 günde bir 0,5 mg/ml intraperitonal olarak uygulandı ve detorsiyondan 30 dakika önce son dozu verildi. Detorsiyon süresinin ardından sıçanların sakrifiye edilerek over dokuları çıkarıldı.

Bulgular: Grup II ve III' te grup I ile karşılaştırıldığında, oksidan parametreler (MDA, MPO, TOS, OSI) ve proinflamatuar sitokin (TNF-α, IL-1β) düzeyleri artarken, antioksidan (SOD, TAS) parametreler önemli ölçüde azaldı ($p<0.05$). Aksine, brusatol uygulaması (grup IV), grup II ve III' e kıyasla bu değerlerin hepsini tersine çevirdi ($p<0.05$).

Sonuç: Sonuç olarak, brusatol' ün sıçanlarda T/D kaynaklı over hasarına karşı koruyucu etkileri olduğu değerlendirildi.

Anahtar Sözcükler: Brusatol, Over, Torsiyon-Detorsiyon, İskemi Reperfüzyon, Sıçan

INTRODUCTION

Ovarian cysts, pregnancy, polycystic over syndrome and transient or permanent obstruction of the ovarian artery may cause ischemia. However, the most common pathologic condition that eventuates as ovarian ischemia is the ovarian torsion (OT) (1). Most of the OT cases occur at reproductive age (2). When the either or both of ovaries and fallopian tubes round own vascular axis as partially or totally, this leads to OT (3). Prevalence of OT is about 3% and this is rising due to infertility treatments (1, 4). As an unusual gynecological emergency, OT performs a 5.9 per 100.00 people incidence; but in case of late diagnosis, this may cause ovarian necrosis and even infertility (5). Following OT, the oxygen concentrations in ovarian tissues increase as a result of vascular reperfusion. Increased numbers of oxygen molecules react with hypoxanthine and xanthine, triggering reactive oxygen species (ROS) generation and subsequently causing tissue damage (6-8). Lipid peroxidation causes malondialdehyde (MDA) production as end-product, an indicator for oxidative damage in cell membrane, resulted from ROS during ischemia reperfusion (I/R) state (9). When the balance between oxidant (ROS, free radical generation) and antioxidant (radical scavenging) conditions changed for the good of oxidant side, oxidative stress occurs (10). Total antioxidant effect is demonstrated with total antioxidant status (TAS) and total oxidant status (TOS) represents the total oxidant effect in body fluids and plasma (11). When the antioxidants were insufficient to able to alleviate ROS production, this condition takes charge in ovarian damage development (12). Tissue damage much more occurs in reperfusion phase than the ischemic stage (13). Activated neutrophil accumulation, responsible for releasing ROS, is a suggested pathogenesis for tissue damage in reperfusion stage (14). At the early stages of inflammation tumor necrosis factor alpha (TNF-a), interleukin-1 beta (IL-1b) and other pro-inflammatory cytokines arise which conduce to oxidative burst of neutrophils and free radical releasing (15, 16). Myeloperoxidase (MPO), as a product of macrophages and neutrophils, acts as a catalyst for peroxide and chlorine reaction. This reaction results in hypochlorous acid formation that plays role in hydroxyl radical production (17). If timely and adequate surgical/medical intervention is done, fertility can be maintained by avoiding tissue damage (18).

Brusatol, a medicine herb acquired from *B. javanica*, has been commonly used to contribute tumor, malaria and inflammation treatment. It also demonstrates various properties such as antitumor, antiviral, antimalarial, anti-inflammatory and insecticide activities (19). *B. javanica* seed oil and fruit obtained from *B. javanica* has been used as a traditional medicine in China (20).

It was seen that as a result of our research in scientific search motors such as sciencedirect and pubmed, there is no scientific study investigating the effectiveness of brusatol to reduce or eliminate ovarian oxidative tissue damage from torsion detorsion has been encountered. For this purpose, in this research, it was purposed to evaluate the effect of brusatol, which has antioxidant and anti-inflammatory effects on ovary to ease the oxidative damage in ovarian torsion/detorsion (T/D) rat model.

MATERIAL and METHODS

Atatürk University Experimental Animal Research and Application Center was used for experiment. The animals were also procured by the same place. Standard cages were used for animal housing in laboratory medium including temperature, humidity and 12 h light/dark cycle control. They were fed with standard pellet feed and water but famished prior to the experiment for 12 hours to avoid anesthesia complications. The study was carried out with the consent of Atatürk University Experimental Animals Local Ethics Committee (28.03.2019/62).

Groups and Torsion Detorsion Model

In this study, thirty-two Sprague Dawley type female rats were weighted (250-270 g) and randomly assigned to 4 groups. Group I (Sham-control group); the abdomen region was barbered, disinfected, incised and closed respectively, but no T/D model was or medical application was performed. Group II (T/D group); as described in group I, in torsion for 3 h, abdominal incision was opened was applied and again abdominal insicion closed. Later, detorsion for 3 h was started by opening again abdominal region and end of the detorsion, ovarian tissue was quickly removed. Ovarian T/D model was selected from previous study (21). Ovarian veins and arteria, fallopian tube and ovaries were spun in clockwise 360 degrees and fixed with atraumatic microvascular clamp for 3 hours and bilateral torsion was created. Then, allowing blood stream for 3 hours by releasing the clamps in the detorsion period was performed. The incision area was repaired with silk 3/0 suture. At the turn of the detorsion, the ovarian tissues were excised. Group III (DMSO+T/D group); DMSO was purchased from Sigma Aldrich Co. 1% DMSO was administered to rats intraperitoneally at 0.3 ml dose once every 2 days during 10 days before the experiment and the last dose was applied 30 minutes before detorsion. In group IV (Brusatol+T/D group), brusatol was purchased from Sigma Aldrich Co. Brusatol was applied to rats intraperitoneally at the dose of 0.5 mg/ml once twice in days for 10 days until the experiment and the last application was applied 30 minutes before detorsion. Then, the T/D model was carried out. The brusatol dose used in a previous study was preferred (22). All procedures were done using anesthesia of 10 mg/kg i.p xylazine

hydrochloride (Rompun®, Bayer, Istanbul) and 60 mg/kg i.p ketamine (Ketalar®, Pfizer, Istanbul). In the upshot of the experiment, ovarian samples were prepared washed and kept as frozen at -80 °C for the biochemical analysis.

Biochemical Analysis

In tissue samples, MDA level was measured to define lipid peroxidation status with the method presented by Ohkawa et al. (23). The values were presented as µmol/g tissue. The activity of superoxide dismutase (SOD) was determined by specification protocol detected by Sun et al (24). SOD activity results were shown as U/mg protein. MPO activity was measured using a method improved by Bradley et al (25). The results were presented as U/g protein. TOS and TAS measurements were carried out with appropriate kits (Rel Assay Diagnostics). TAS and TOS values were demonstrated as nmol/L. The ratio of TOS to TAS is the oxidative stress index (OSI). OSI level was determined as: OSI = [(TOS, µmol H_2O_2 equivalent/L)/(TAS, mmol Trolox equivalent/L) × 10]. The levels of TNF-α and IL-1β were evaluated with an appropriate kit (Elabscience, Wuhan, China).

Statistical analysis

The outcomes were presented as Mean ± Standard Error of Mean (SEM) and analyzed using One-way ANOVA and then Tukey test for pairwise comparisons of groups. The differences were admitted significant when P<0.05.

RESULTS

When OSI, TAS and TOS levels of this study were evaluated, it was observed that TOS and OSI values of group II and group III were significantly elevated compared to group I. Same parameters did not increase in treatment group (group IV) compared to group II and III (Table 1, p<0.05). On the other hand, TAS level was significantly decreased in group II and III compared to group I, but it was significantly elevated in treatment group (group IV) compared to group II and III (Table 1, p<0.05).

In addition, MPO activity and MDA values were significantly elevated in group II and group III compared to group I group but contrary to this, did not increase in group IV due to brusatol treatment compared to group II and group III (Table 2, p<0.05). However, SOD activity was significantly reduced in group II and group III compared to group I, whereas in group IV there was a significant elevation in SOD value compared to group II and group III (Table 2, p <0.05).

When IL-1β and TNF-α levels in group II and group III were analyzed, they were elevated significantly compared to group I, whereas these values decreased significantly in our treatment group compared to group II and group III (Figure 1, p<0.05).

DISCUSSION

When OT is detected, tissue reperfusion is performed via detorsion to achieve a protection against infertility even in cyanotic tissues. There are several risk factors such as hyperlaxity of ligamentum ovary proprium, adnexal cysts, pregnancy, infundibulopelvic ligaments and ovarian hyperstimulation which may lead to enlargement of ovaries (1, 2). Due to torsion, a decrease is observed in ovarian tissue venous return. Then stromal edema and internal haemor-

Table I: Mean±SEM results of Total Antioxidant Status (TAS) (mmol/L), Total Oxidant Status (TOS) (µmol/L) and Oxidatif Stress Index (OSI) parameters belong to experiment groups.

Groups		TAS (mmol/L)	TOS (µmol/L)	OSI
Group I	Mean± SEM	0.70±0.06*	6.12±0.83*	1.20±0.39*
Group II	Mean± SEM	0.29±0.00$^{*,\#}$	9.90±0.31$^{*,\#}$	3.36±0.16$^{*,\#}$
Group III	Mean± SEM	0.28±0.00$^{*,\#}$	10.64±0.35$^{*,\#}$	3.68±0.13$^{*,\#}$
Group IV	Mean± SEM	0.76±0.01	5.45±0.17	0.71±0.02

$^{*,\#}$: p<0.05, represents a statistically significant difference between the groups with the same letters. **SEM:** Standart Error of Mean.

Table II: Mean±SEM results of Superoxide Dismutaase (SOD) (U/mg protein), Myeloperoxidase (MPO) (U/g protein) activities and Malondialdehyde (MDA) (µmol/g tissue) level belong to experiment groups.

Groups		SOD (U/mg protein)	MPO (U/g protein)	MDA (µmol/g tissue)
Group I	Mean± SEM	336.78±30.08*	309750.31±50433.27*	68.44±6.78*
Group II	Mean± SEM	166.42±2.72$^{*,\#}$	568237.57±15748.81$^{*,\#}$	129.99±3.06$^{*,\#}$
Group III	Mean± SEM	167.77±2.53$^{*,\#}$	585346.48±17735.80$^{*,\#}$	126.08±4.41$^{*,\#}$
Group IV	Mean± SEM	362.13±8.66$^{\#}$	253140.89±10397.24$^{\#}$	64.61±2.26$^{\#}$

$^{*,\#}$: There is a statistically significant difference between the groups with the same symbols (p<0.05).

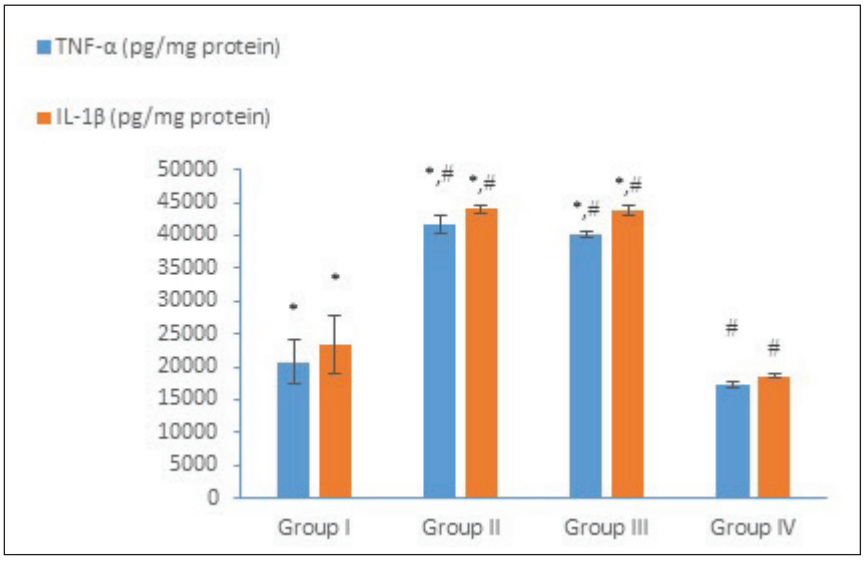

Figure 1: TNF-α and IL-1β levels of all experiment groups. ***,#:** There is a statistically significant difference between the groups with the same symbols ($p<0.05$).

rhage occur. In the event that blood flow stops, ischemic and necrotic phases occur in the tissues. Early diagnosis and control are necessary to preserve ovarian functions (1, 2,5). OT induces a reduction in blood stream resulting from the rotation of adnexa. Lipid peroxidation is a critical situation in cell membrane injury stemming from free oxygen radicals (9). It has been reported by Demiryilmaz et al. that in I/R-induced rat ovarian tissue, there were high levels of MDA (26). Reperfusion caused to increase of the ischemic damage at the cellular level via ROS (9). Reperfusion has been notified as a therapy in order to preserve ovarian functions and infertility (27) but I/R has been shown as a reason of infertility in rats (28). SOD is one of the most important antioxidant enzymes. There are several studies that demonstrate decline in SOD activity due to ovarian I/R procedure (29). Oxidative stress demonstrates the imbalance between oxidant and antioxidant status for the benefit of oxidant side. OSI, TOS to TAS ratio, is a determinant for the degree of oxidative stress (11). Accumulation of neutrophils play role as a part of I/R injury response. MPO demonstrates neutrophil status such as accumulation, activity, etc. (30). Neutrophils release TNF-α and IL-1β, two important cytokines. TNF-α is well known as one of the key cytokines mediating inflammatory responses (31). TNF-α has been reported to be one of the most prominent mediators involved in the development of inflammation and apoptosis induced by I/R injury (32).

Many brusatol-related studies are available in the literature supporting the results of present study. In current study, reduction of TNF-α, IL-1β levels in ovarian I/R model in rats by brusatol, suggesting that brusatol decreases I/R-induced ovarian injury. Brusatol has been shown to inhibit amyloid-induced neurotoxicity and decrease ROS in U-251 cells (33). Brusatol has been shown to improve colitis in rats by decreasing levels of TNF-α and IL-1β by increasing antioxidant levels (35). It was reported that brusatol reduced TNF-α and IL-1β levels against chronic obstructive pulmonary disease in mice (19). Brusatol has been determined to normalize glucose intolerance due to high fat diet in mice and to have anti-inflammatory properties (35). Brusatol has been demonstrated to be a potent antitumor agent in the colorectal cancer model in mice (36). Similar with these studies, in this search, anti-inflammatory and antioxidant features of brusatol have been indicated in ovarian I/R model in rats. In I/R group, TAS and SOD decreased while TOS, MDA, IL-1β, MPO, TNF-α, OSI levels were elevated compared to sham control group and brusatol treatment reversed these levels compared to I/R group.

We evaluated oxidative stress in ovarian tissue to investigate the potent mechanisms of the protective effects of brusatol against I/R-induced ovarian injury. It was observed as oxidative stress decreased with brusatol. To contribute important data to the clinical management of I/R, the mechanism of I/R-related organ injury should be evaluated. Various I/R studies demonstrated that suppression of oxidative stress and inflammation can help to the treatment of I/R. In current study oxidative stress and inflammation pathways were suppressed by brusatol and this may be a new molecule in the treatment of I/R.

CONCLUSION

Brusatol performs a protection against I/R-related ovarian injury by its antioxidants and anti-inflammatory features. Here, it was demonstrated that treatment with brusatol decreases ovarian injury in rats exposed to I/R model. Further studies will be essential to explain the different protective mechanisms on I/R-related ovarian tissue injury.

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Author roles:

FNEA: Designing the study and writing the article

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MCG. Collecting data, working with statistics

EE: Collecting data, reviewing the article

EE and MCG: collecting data, reviewing the article

FNEA and AT: reviewing the article, mentore

Etichal approval

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