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# The effects of sildenafil and tadalafil on ischemia–reperfusion injury in rat ovarian torsion model

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**Summary**

*Objective:* In cases of ovarian torsion (OT), reperfusion injury may further damage ovarian tissue. Tadalafil and sildenafil are phosphodiesterase-5 (PDE-5) inhibitors that are primarily used for treatment of erectile dysfunction and pulmonary hypertension. In this study, the authors investigated the effects of these agents in preventing ovarian ischemia-reperfusion injury in a rat OT model. *Materials and Methods:* A total of 48 young adult female Wistar Albino rats were randomized into six groups (n=8 in each group); group 1: control-sham operated, group 2: sildenafil one mg/kg, group 3: tadalafil one mg/kg, group 4: sildenafil one mg/kg + ischemia/reperfusion, (I/R) two hours of ischemia and two hours of reperfusion and a single dose of oral one mg/kg sildenafil one hour before I/R, group 5: I/R, and group 6: tadalafil one mg/kg + I/R two hours of ischemia and two hours of reperfusion and a single dose of oral one mg/kg tadalafil one hour before I/R. Histopathologic evaluation was performed and scored according to the degree of congestion, edema, and hemorrhage. Total histological score (THS), tissue protein carbonyl (PC), malondialdehyde (MDA) levels, and catalase (CAT) activity were also calculated. *Results:* Group 4, group 5, and group 6 had similar congestion, edema and THS ($p > 0.05$). Group 6 had significantly lower hemorrhage score, when compared with group 5 ($p = 0.03$). Mean PC, MDA levels, and CAT were similar between groups 4, 5, and 6 ($p > 0.05$). *Conclusions:* Tadalafil was associated with a decreased tissue hemorrhage score in I/R injury in rat ovarian torsion model.

*Key Words:* Reperfusion injury; Phosphodiesterase inhibitors; Ovary.

## Introduction

Ovarian torsion (OT) is an important gynecologic surgical emergency condition [1]. Although it is observed more commonly in reproductive-aged women due to the increased incidence of functional ovarian cyst formation, it may be encountered in women of any age. In case of ovarian neoplasia, the risk for OT increases. The major concern in women with OT is that it may result in ovarian necrosis, and a subsequent decrease in ovarian reserve may follow. When rotation around the infundibulopelvic and ovarii proprium ligaments occurs, ovarian venous structures are initially obstructed. Continued arterial circulation phase results in ovarian edema and enlargement during this process. Subsequently, local hemorrhage and necrosis occur in the ovarian tissue. In case of detorsion and restoration of the ovarian blood flow, reperfusion injury may further damage ovarian tissue. In contemporary practice, a trend towards ovarian salvage in cases with OT is observed [2-4]. Ischemic ovarian tissues, which were once deemed non-viable and that would be treated with adnexectomy, are now preserved, if obvious signs of tissue necrosis are absent.

Patients with OT generally refer to the emergency departments complaining of pelvic pain with an acute onset. In past medical history, a previously diagnosed ovarian cyst may be stated by the patient. The presumptive diagnosis is generally made preoperatively with a high accuracy by gray scale and Doppler ultrasound evaluation [5]. The definitive diagnosis and treatment is performed by surgical intervention. In addition to shifts in clinical practice towards ovarian preservation in OT cases, additional approaches to further prevent ovarian damage are being investigated [6-9].

Tadalafil and sildenafil are phosphodiesterase-5 (PDE-5) inhibitors that are primarily used for treatment of erectile dysfunction and pulmonary hypertension. In this study, the authors investigated the effects of these agents in preventing ovarian ischemia-reperfusion injury in a rat OT model.

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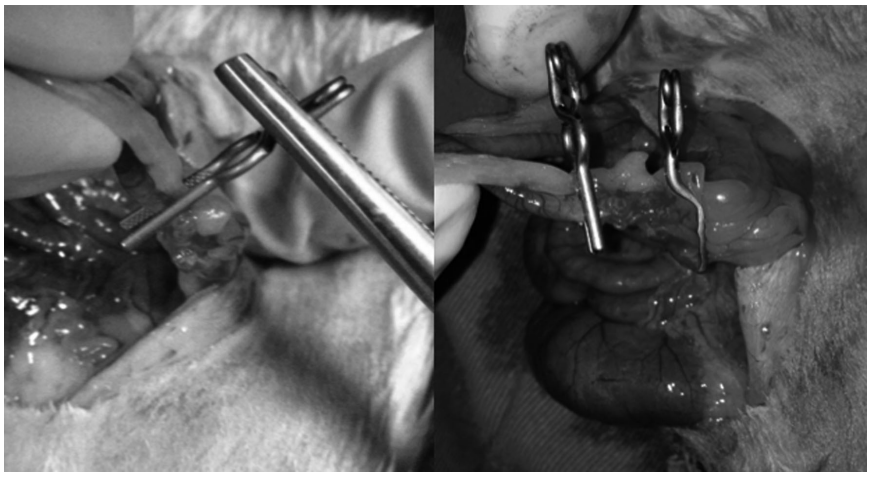

Figure 1. — Formation of the ovarian ischemia model by applying vascular clips on both sides of the rat ovary.

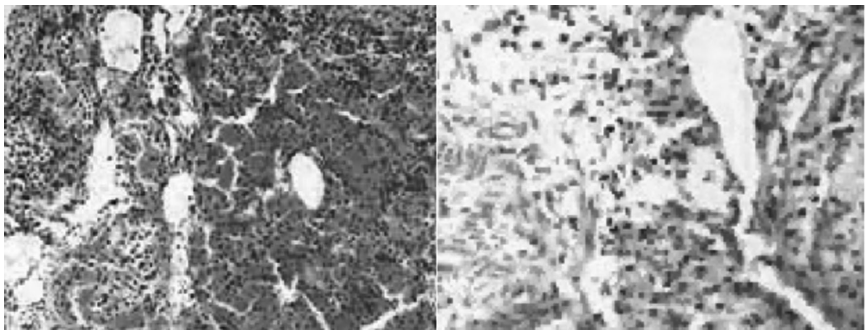

Figure 2. — Microscopic appearance of ovarian tissues after ischemia on the left and reperfusion on the right).

## Materials and Methods

The study was conducted at Diskapi YB Hospital Animal Experiments Laboratory after gaining approval from the local ethics committee. A total of 48 young adult female Wistar Albino rats weighing between 200 and 250 grams were randomized into six groups (eight rats in each group). The experimental drugs tadalafil and sildenafil were dissolved in sterile 0.9% NaCl in water and homogenized before oral administration. The assigned drugs in each group were administered via oral gavage one hour before laparotomy and formation of the ischemia/reperfusion model.

The ischemia/reperfusion (I/R) model was developed as described below: the rats were anesthetized using intraperitoneal ketamine 40 mg/kg and xylazine ten mg/kg. With the rats in dorsal recumbent position, a two-cm mid-line laparotomy was performed. In each rat, the vessels of right adnexa were occluded from above and below with vascular clips and were exposed to ischemia for two hours (Figure 1). After ischemia period, the sutures were removed and a two-hour reperfusion period was allowed. After the reperfusion period, right adnexa were removed. The retrieved ovarian tissues were divided into two equal-sized pieces, and were stored for further biochemical and histological analysis.

Study groups are summarized as follows: group 1 – 0.9 % NaCl solution in oral administration + sham operation (Only laparotomy), group 2 – sildenafil (one mg/kg oral administration) + laparotomy (one hour after drug administration), group 3 – tadalafil (one mg/kg) oral administration + laparotomy one hour after drug administration, group 4 – sildenafil (one mg/kg) oral administration + laparotomy one hour after drug administration + I/R model; group 5 – laparotomy + I/R model, and group 6 – tadalafil (one mg/kg) oral administration + laparotomy one hour after drug administration + I/R model.

*Histological examination*

A pathologist that was blinded to study groups performed all histological and immunohistochemical examinations. Half of the right ovary of each rat was placed separately into a formaldehyde media. Tissues were detected in 10% buffered formalin, after which a routine tissue follow-up was performed and were embedded in paraffin. Sections of five-μm thickness were cut from the paraffin blocks using a microtome and were deparaffinized. The samples were dyed with hematoxylin-eosin stain and were examined with a light microscope (Figure 2). A scoring system was utilized to determine the degree of vascular congestion, edema, and hemorrhage within the tissue samples. Each parameter was scored between 0 and 3. Score 0 represented absence of the investigated pathologic finding within the specimen; scores 1, 2, and 3 represented < 33%, 33- 66%, and > 66% of the investigated pathologic finding within the specimen, respectively. The sum of scores for each histological parameter was calculated as total histological score (THS). In addition, immunohistochemical expression of the angiogenesis related factors endothelial nitric oxide synthase (eNOS) and p53 were determined. Immunohistochemistry staining results were evaluated semi-quantitatively with light microscopy, where scores 0, 1, 2, and 3 represented none, weak, intermediate, and strong expressions, respectively.

Table 1. — *Comparison of congestion, hemorrhage, edema and THS between groups 1, 2, and 3.*

| | | Mean ± SD | Min - max | *p* |
|---|---|---|---|---|
| Congestion | Group 1 | 0.25 ± 0.46 | 0 - 1 | |
| | Group 2 | 1.125 ± 0.35 | 1 - 2 | 0.001 |
| | Group 3 | 1.5 ± 0.53 | 1 - 2 | |
| Hemorrhage | Group 1 | 0 ± 0 | 0 - 0 | |
| | Group 2 | 0.125 ± 0.35 | 0 - 1 | 0.000 |
| | Group 3 | 0.875 ± 0.35 | 0 - 1 | |
| Edema | Group 1 | 0.125 ± 0.35 | 0 - 1 | |
| | Group 2 | 0.875 ± 0.83 | 0 - 2 | 0.019 |
| | Group 3 | 0.875 ± 0.35 | 0 - 1 | |
| THS | Group 1 | 0.375 ± 0.74 | 0 - 2 | |
| | Group 2 | 2 ± 1.07 | 0 - 3 | 0.000 |
| | Group 3 | 3.25 ± 0.70 | 2 - 4 | |

SD: standard deviation; THS: total histological score.

Table 2. — *Comparison of congestion, hemorrhage, edema and THS between groups 4, 5, and 6.*

| | | Mean ± SD | Min - max | *p* |
|---|---|---|---|---|
| Congestion | Group 4 | 2.6 ± 0.74 | 1 - 3 | |
| | Group 5 | 2.5 ± 0.76 | 1 - 3 | 0.096 |
| | Group 6 | 2 ± 0.53 | 1 - 3 | |
| Hemorrhage | Group 4 | 2.6 ± 0.74 | 1 - 3 | |
| | Group 5 | 2.5 ± 0.76 | 1 - 3 | 0.032 |
| | Group 6 | 1.62 ± 0.74 | 1 - 3 | |
| Edema | Group 4 | 1.75 ± 0.46 | 1 - 2 | |
| | Group 5 | 1.5 ± 0.76 | 1 - 3 | 0.173 |
| | Group 6 | 1.25 ± 0.46 | 1 - 2 | |
| THS | Group 4 | 7 ± 1.93 | 3 - 8 | |
| | Group 5 | 6.5 ± 1.85 | 3 - 9 | 0.069 |
| | Group 6 | 4.87 ± 1.55 | 3 - 8 | |

SD: Standard deviation; I/R: ischemia/reperfusion;
THS: total histological score.

*Biochemical analysis*

Protein carbonyl (PC) and malondialdehyde (MDA) levels with catalase (CAT) activity were assessed in ovarian tissues. The tissues were kept at -80 $^0$C prior to biochemical analyses. After thawing, the tissue samples were weighed with a scale of 0.001-gram sensitivity. After being divided into small pieces, the tissues were homogenized in 0.2 M Tris-HCl buffer solution.The homogenate was centrifuged for 60 minutes at 3,500 rpm, and a supernatant was obtained. Catalase activity was determined by the assessment of the rate constant of hydrogen peroxide ($H_2O_2$) decomposition at 240 nm [10]. The MDA levels in ovarian tissues were determined according to the reaction with thiobarbituric acid at 90$^0$C to -100$^0$C [11]. The PC levels were determined with a spectrophotometric method which relies on the reaction of the carbonyl group with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazine [12].

*Statistical analysis*

Statistical analyses were performed using the SPSS 15 program. Continuous numeric variables were reported as mean ± standard deviation and discrete numeric variables were reported as median (minimum-maximum). Categorical variables were reported as number of cases and percentage (%). The significance of the difference of means between groups was tested with ANOVA variance analysis, and significance of the difference between medians were tested using Kruskal-Wallis test. Categorical parameters were evaluated with Pearson Chi-Square or Fisher tests, where appropriate. *P* values less than 0.05 were considered statistically significant.

## Results

A total of 48 rats were included in the study. Mean weight of rats was 224 ± 11.4 grams and no statistically significant difference was observed between the study groups in terms of their weights.

*Histological results*

When all groups were evaluated for the histological scores, group 5 (I/R) had significantly higher congestion, hemorrhage, edema, and THS when compared with group 1 (control). Group 4 (sildenafil+ I/R) had significantly higher congestion, hemorrhage, edema, and THS when compared with group 2 (sildenafil) ($p < 0.05$). Group 6 (tadalafil + I/R) had significantly higher congestion, hemorrhage, and THS than group 3 (tadalafil) ($p < 0.05$). However, edema score did not significantly differ between groups 6 and 3 ($p > 0.05$).

Group 1 (control) had the lowest congestion, hemorrhage, edema, and THS when compared to group 2 (sildenafil) and group 3 (tadalafil) groups ($p < 0.05$) (Table 1). THS of group 3 (3.25 ± 0.7) was significantly higher than group 2 (2 ± 1.07) ($p = 0.017$). Group 4 (sildenafil+I/R), group 5 (I/R), and group 6 (tadalafil + I/R) had similar congestion, edema, and THS ($p > 0.05$) (Table 2). Group 6 had significantly lower hemorrhage score, when compared with group 5 ($p = 0.03$).

Table 3. — *Comparison of eNOS and p53 levels between groups 1, 2, and 3.*

| | | Mean ± SD | Min - max | *p* |
|---|---|---|---|---|
| eNOS | Group 1 | 8.37 ± 3.58 | 0 -12 | |
| | Group 2 | 20.37 ± 5.75 | 13 -29 | 0.037 |
| | Group 3 | 26.37 ± 21.8 | 16 -80 | |
| p53 | Group 1 | 0 ± 0 | 0 -0 | |
| | Group 2 | 4.5 ± 1.07 | 3 -6 | 0.000 |
| | Group 3 | 4.12 ± 0.8 | 3 -5 | |

eNOS: endothelial nitric oxide synthase; SD: standard deviation.

*Immunohistochemical results*

When groups 1, 2, and 3 were compared eNOS expression, group 1 had the lowest score ($p = 0.03$). Similarly, group 1 had the lowest p53 score when compared with groups 2 and 3 ($p < 0.001$) (Table 3). eNOS expression scores were similar between groups 4, 5, and 6 ($p > 0.05$) (Table 4). p53 expression scores were lowest in group 6 ($p < 0.001$).

Table 4. — *Comparison of eNOS and p53 levels between groups 4, 5, and 6.*

| | | Mean ± SD | Min - max | *p* |
|---|---|---|---|---|
| eNOS | Group 4 | 136.37 ± 46.44 | 43 -168 | |
| | Group 5 | 133 ± 41.91 | 55 -172 | 0.098 |
| | Group 6 | 93.87 ± 35.12 | 28 -155 | |
| p53 | Group 4 | 15.12 ± 2.69 | 11 -19 | |
| | Group 5 | 16.12 ± 2.29 | 13 -19 | 0.000 |
| | Group 6 | 7.125 ± 0.64 | 6 -8 | |

eNOS: endothelial nitric oxide synthase; SD: standard deviation.

Table 5. — *Comparison of PC, MDA, and CAT levels between groups 1, 2, and 3.*

| | | Mean ± SD | Min - max | *p* |
|---|---|---|---|---|
| PC | Group 1 | 10.47 ± 2.27 | 7.13 - 13.80 | |
| | Group 2 | 13.85 ± 3.85 | 9.19 - 19.82 | 0.041 |
| | Group 3 | 9.12 ± 4.55 | 3.41 - 18.62 | |
| MDA | Group 1 | 0.31 ± 0.18 | 0.17 - 0.71 | |
| | Group 2 | 0.29 ± 0.07 | 0.20 - 0.40 | 0.008 |
| | Group 3 | 0.99 ± 0.75 | 0.08 - 2.45 | |
| CAT | Group 1 | 7.21 ± 4.95 | 2.72 - 17.48 | |
| | Group 2 | 4.63 ± 4.73 | 0 - 14.55 | 0.431 |
| | Group 3 | 8.18 ± 12.16 | 0 - 37.82 | |

PC: protein carbonyl; MDA: malondialdehyde; CAT: catalase activity.

Table 6. — *Comparison of PC, MDA, and CAT levels between groups 4, 5, and 6.*

| | | Mean ± SD | Min - max | *p* |
|---|---|---|---|---|
| PC | Group 5 | 12.55 ± 4.68 | 6.083 - 20.175 | |
| | Group 4 | 9.003 ± 2,187 | 5.937 - 11.657 | 0.147 |
| | Group 6 | 10.907 ± 3.073 | 6.786 - 14.526 | |
| MDA | Group 5 | 0.453 ± 0.297 | 0.111 - 0.976 | |
| | Group 4 | 0.307 ± 0.224 | 0.107 - 0.772 | 0.302 |
| | Group 6 | 0.544 ± 0.364 | 0.154 - 1.118 | |
| CAT | Group 5 | 11.077 ± 8.587 | 2.216 - 26.174 | |
| | Group 4 | 5.590 ± 5.801 | 2.028 - 19.204 | 0.093 |
| | Group 6 | 3.983 ± 4.163 | 0 - 12.496 | |

PC: protein carbonyl; MDA: malondialdehyde; CAT: catalase activity.

*Biochemical results*

When groups 1, 2, and 3 were compared in terms of mean PC levels, group 3 had the lowest PC value ($p < 0.05$) (Table 5). Mean MDA level was highest in group 3 ($p = 0.008$). CAT activity was similar between groups 1, 2, and 3 ($p > 0.05$). Mean PC, MDA levels, and CAT were similar between groups 4, 5, and 6 ($p > 0.05$) (Table 6). All groups were compared together for biochemical parameters. Group 2 (sildenafil) had significantly higher mean PC level, significantly lower MDA level, and CAT activity than group 4 ($p < 0.05$).

## Discussion

OT is a gynecological surgical emergency, which constitutes about 3% of all gynecologic emergencies, and may be associated with diminished ovarian reserve in case of delayed diagnosis and treatment [2, 13]. The blood is supplied to the ovaries by two main arteries. The first of these are the ovarian arteries, which run through the infundibulopelvic ligaments and are connected to the ovary from its lateral aspect within the pelvis. The other system is the branch coming from the uterine artery that runs within the ovarian ligament (lig. ovarii proprium). When ovarian torsion occurs, these vessels are occluded, and this results in ischemic damage on the ovarian tissue [1]. Furthermore, even when the torsion is corrected and the ovary is resupplied with blood, a reperfusion injury might further increase tissue loss [14]. Although the ischemic damage may not be prevented, numerous studies on prevention of reperfusion injury due to oxidative stress are being conducted, each investigating the effect of various drugs with antioxidant properties that are administered concomitantly with surgical detorsion [15-19].

In a previous study, the possible protective effects of caffeic acid phenethyl ester (CAPE) were investigated in a rat ovarian I/R model [7]. The levels of oxidative stress markers MDA and xanthine oxidase activities were found to be significantly lower in the group that was treated with CAPE [7]. In another study, iloprost, which is a prostacyclin analogue has been shown to be associated with less histological injury after ovarian reperfusion in a similar rat ovarian torsion model [8]. Curcumin, a curcuminoid of the popular South Asian spice turmeric and a member of the ginger family, was also found to be associated with decreased histological scores in a similar rat model [9]. Furthermore, total oxidant status (TOS) and oxidative stress index (OSI) were lower in the curcumin treated group. In the study by Oral *et al.*, the protective effects of montelukast, which is a leukotriene receptor antagonist, were investigated. In the group that was treated with montelukast, a lesser degree of hemorrhage in the ovarian tissue after I/R was observed, and this effect was dose-dependent [6]. In a recent study, it has been demonstrated that omegaven, which is a omega-3 fatty acid, decreases the detrimental effects of I/R injury in a rat OT model [20]. Ethyl pyruvate, which is an antioxidant agent, has also been found to be effective in decreasing ovarian tissue injury scores [20]. However, the authors of this study did not observe a difference between groups that were treated with 50 mg/kg or 100 mg/kg doses.

In the present study, the authors investigated the effects of sildenafil and tadalafil in a rat ovarian I/R model. In terms of histological scores, they did not observe any significant effects of sildenafil or tadalafil on congestion, edema, and THS after I/R. On the other hand, the group that was treated with tadalafil experienced lower hemor-

rhage when compared with untreated I/R group. Immunohistochemical analyses revealed that eNOS expression were similar between the I/R untreated (group 5) and treated groups (group 4-sildenafil and group 6-tadalafil). On the other hand, p53 expression was lowest in tadalafil treated group before I/R. In terms of biochemical changes during I/R, the authors did not observe any significant effects of sildenafil or tadalafil on mean PC, MDA or CAT levels.

To the best of the present authors' knowledge, this study was the first to investigate and compare the effects of phosphodiesterase-5 inhibitors sildenafil and tadalafil in a rat OT model. The group that was treated with tadalafil had significantly reduced hemorrhage within the ovarian tissue after reperfusion. These changes may be associated with decreased ovarian damage. Also p53 expression was lowest in the tadalafil treated group. These findings should be further investigated in future studies to reach a final conclusion on the utility of these drugs in clinical practice.

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