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Experimental Research

Protective effect of curcumin on ovarian reserve in a rat ischemia model: an experimental study

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Summary

Purpose of investigation: To determine the protective effect of curcumin on ovarian reserve in a rat ischemia model. *Materials and Methods:* Thirty female Albino rats were randomly divided into two groups by time of unilateral, left ovary ischemia/reperfusion (group 1: two-hour ischemia / two-hour reperfusion; group 2: four-hour ischemia / four-hour reperfusion). Each group was subdivided into three subgroups, sham, control, and curcumin (intraperitoneal curcumin (200 mg/kg) simultaneously with reperfusion). Histological grading of ischemic indices of paraffin-embedded ovarian tissue using hematoxylin and eosin (H&E), and anti-Müllerian hormone (AMH) levels by enzyme-linked immunosorbent assay (ELISA), were measured 40 days later. *Results:* No difference was found between groups 1 and 2 or among subgroups within either group for right and left ovary grades. AMH levels were significantly higher in the curcumin subgroup compared to sham and control within group 2 and in group 2 versus group 1 curcumin subgroups. *Conclusion:* Curcumin maintains and protects ovarian functions in an ischemia-reperfusion rat model.

Key words: Curcumin; Ovarian reserve; Rat-ischemia model; Ischemia-reperfusion; Anti-Müllerian hormone.

Introduction

Ovarian torsion, also known as adnexal torsion, accounts for approximately 2.7% of gynecological emergencies in women, placing it in the top five of such emergencies [1, 2]. Incidence of ovarian torsion increases in pregnancy, with approximately 20% of ovarian torsion cases occurring in pregnant women [1, 2]. Growing prevalence of ovarian stimulation treatment has added to the prevalence of ovarian torsion in pregnancy [3, 4]. Ovarian torsion is initially associated with a reduction in venous ovarian blood flow but it can develop into restriction of arterial blood flow, ischemia, and infarction [5, 6]. Late diagnosis and delayed management may lead to ovarian necrosis and decreased ovarian functions, however, the diagnosis and treatment is often delayed because of the non-specificity of the symptoms [5]. Treatment is carried out both to achieve blood flow and to restore tissue reperfusion. However the treatment itself can result in reperfusion injury, causing microvascular and parenchymal cell dysfunction of ischemic organs, during restoration of tissue reperfusion [7-10]. This reperfusion injury is mediated by reactive oxygen species (ROS), which promote the release of inflammatory agents [10, 11]. Thus if the impact of these detrimental ROS could be limited, ischemia-reperfusion injury could be reduced.

Extracted from a perennial herbaceous plant called Curcuma Longa, curcumin is used as a pigment, spice, and additive in most Asian countries [12]. In common with other flavonoid family members, curcumin possesses significant antioxidant and anti-inflammatory functions [12-15]. Recently, curcumin has been shown to reduce tissue damage caused by reperfusion injury in multiple rat models, including cardiac dysfunction due to renal ischemia and reperfusion [16], lung, renal, and cardiac damage in an abdominal aorta ischemia-reperfusion injury model [17] and neuroprotection in a cerebral ischemia reperfusion injury model [18]. Another recent study has also suggested that curcumin may have a protective effect against ovarian reperfusion injury in rats [19].

Ovarian reserve is the capacity of the ovary to produce viable oocytes to enable successful and healthy gestation. Anti-Müllerian hormone (AMH) decreases with advancing age, and it can be used as a putative marker for ovarian aging [20]. It is a sensitive marker of ovarian reserve and early-follicular phase and AMH appears to be associated with natural fertility [20, 21]. Recently a decrease in ovarian reserve, reflected in reduced AMH, has been shown in a rat model of ischemia-reperfusion injury [22]. Other rat studies suggest that curcumin may help maintain AMH lev-

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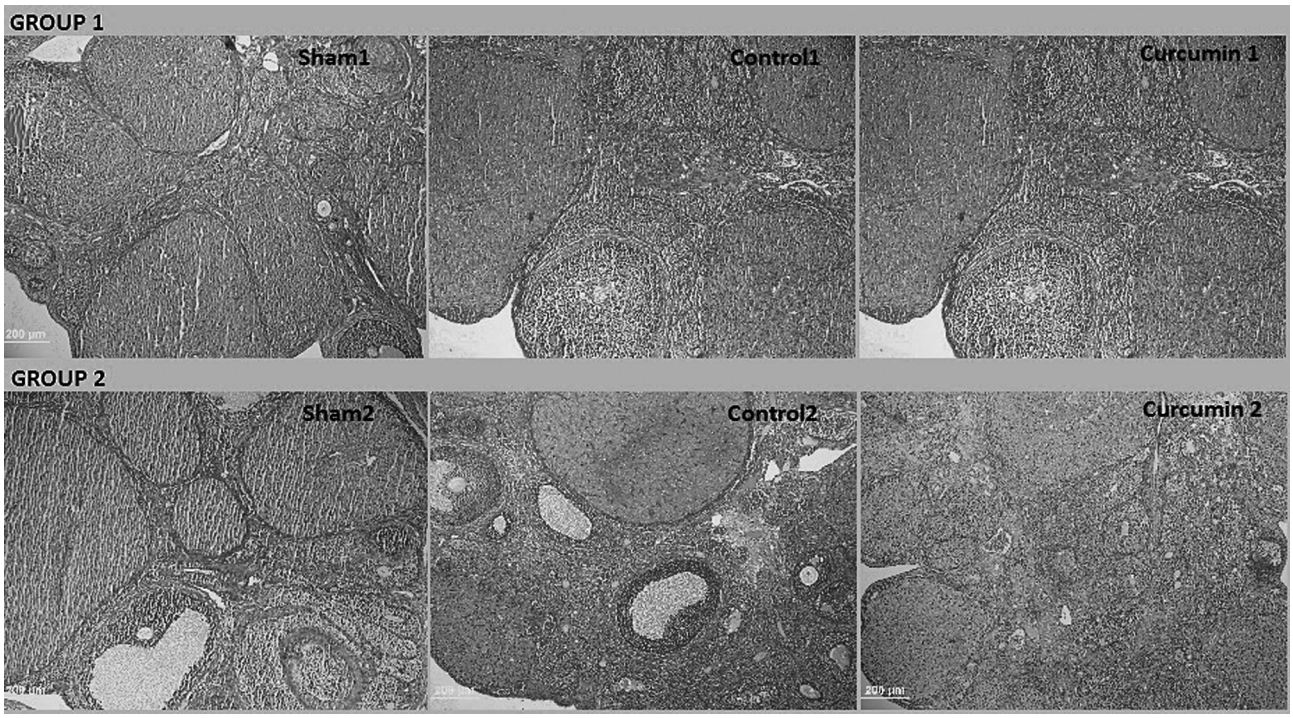

Figure 1. — Histologic morphologies of ovaries from group 1 and group 2. Sham 1 and sham 2: normal ovarian tissue structures (H&E, ×100). Control 1 and control 2: untreated groups, moderate edema, moderate vascular congestion, no hemorrhage, no PMN infiltration (H&E 100). Curcumin 1 and curcumin 2: treated groups; curcumin 1 (H&E ×100), moderate edema, moderate vascular congestion, no hemorrhage, no PMN infiltration; curcumin 2 (H&E ×100), mild edema, mild vascular congestion, no hemorrhage, no PMN infiltration.

els and protect ovaries [23]. However, further evidence is needed on the potential protective effect of curcumin before any applications in human beings could be considered. The present experimental, randomized, and controlled study was designed to determine what, if any, protective effect curcumin exerts on the ovarian reserve in a rat ischemia model.

Materials and Methods

The study protocol was approved by the Gazi University Ethics Committee for animal research (GUHADEK). Thirty female Wistar Albino rats, weighing 170-245 grams, were used. The rats were kept for at least seven days under appropriate conditions of temperature / humidity and a 12-hour light cycle, with sufficient water and food. They were randomly divided into two main groups: group 1 (two-hour ischemia + two-hour reperfusion group) and group 2 (four-hour ischemia + four-hour reperfusion group), with 15 rats in each. Groups 1 and 2 were subdivided into three subgroups each as follows: group 1: sham I (n=3), control I (n=6), and curcumin I (n=6); group 2: sham II (n=3), control II (n=6), and curcumin II (n=6).

Each rat was weighed and anesthetized with 50 mg/kg intramuscular ketamine hydro-chloride and ten mg/kg xylazine hydrochloride. Following preoperative sterilization, a longitudinal incision of 2.5 cm was performed in the midline area of the lower abdomen.

Group 1

Sham I: after laparotomy, the abdominal incision was covered with surgical gauze sluiced with sterile saline solution for four hours and then the abdominal wall was closed. Peritoneal and muscular layers were sutured in a continuous manner and the skin was sutured stich style with 3/0 atraumatic suture.

Control I: after laparotomy, the uterus and left ovary were visualized and a unilateral (left) complete ovarian ischemia model was obtained by locking the left ovarian vessels topknot style with atraumatic one silk suture (two-sided). After two hours, the complete ischemia silk sutures were opened and reperfusion was provided for two hours. The abdominal incision was closed similar to the sham group.

Curcumin I: The same surgical procedure was applied as with the control group, except that 200 mg/kg of curcumin was intraperitoneally administered simultaneously with reperfusion.

Group 2

Sham II: as for Sham I, except that the abdominal incision was covered with surgical gauze and soaked with sterile saline solution for eight hours. Control II: as for control I, except ischemia and reperfusion were carried out for four hours. Curcumin II: as for curcumin I, except ischemia and reperfusion/curcumin were carried out for four hours.

In total, three rats died due to anesthetical complications; two rats (in control I and control II) died at the fourth hour of the operation, and one rat (sham I) died in the first day after operation. A total of 27 rats survived for 40 days (three ovarian cycles). All rats were kept under appropriate conditions for 40 days and then operated on under the same anesthetic conditions as above. Bilateral

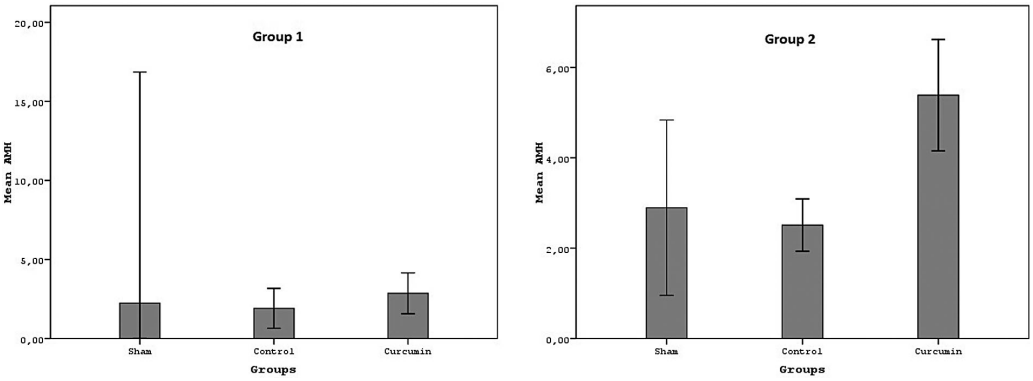

Figure 2. — AMH levels of group 1 and group 2. There was no significant variation in AMH levels among subgroups of group 1, while AMH levels were significantly higher in the curcumin group than sham and control subgroups of group 2.

Table 1. — *AMH and histological grade data for group 2 (two-hour ischemia - two-hour reperfusion)*

	Sham I	Control I	Curcumin I	*p*
Rat weight (grams)	210.5 (192-229)	211.5 (201-231)	188.5 (184-207)	0.021*
Rat weight 40th day (grams)	222.5 (203-242)	230.5 (212-253)	208.5 (199-227)	0.064
LO grade	1(1-1)	1.5(1-2)	2(1-2)	0.290
RO grade	1(1-1)	1(1-2)	1(1-2)	0.591
AMH	2.2±1.7	1.9±1.2	2.9±1.2	0.445

$p<0.05$ taken as significant; *there is a variation between control and curcumin.

Table 2. — *AMH and histological grade data for group 2 (four-hour ischemia - four-hour reperfusion)*

	Sham II	Control II	Curcumin II	p
Rat weight (gr)	179.5 (172-187)	208 (170-257)	189 (183-245)	0.153
Rat weight 40th. day (gr)	206 (204-208)	203 (194-256)	204 (198-275)	0.714
LO grade	2(2-2)	2(1-2)	1(0-2)	0.210
RO grade	1(1-1)	1(1-2)	1(1-1)	0.749
AMH	2.9±0.8	2.5±0.2	5.4±1.2	0.001*

$p<0.05$ taken as significant; *there are differences between curcumin II subgroup compared to both sham II and control II subgroups.

oophorectomy was performed on all rats and they were then sacrificed by intracardiac blood-letting. Blood samples were collected in serum separator tubes (SST) and allowed to clot for two hours at room temperature, before centrifugation for 15 minutes at 1,000 ×*g*. Serum was collected and subjected to assay immediately or stored at -80°C. Ovarian tissues were preserved in 10% formaldehyde solution for histopathologic examination. All the samples were labeled with consecutive numbers, and then transferred to the laboratory. Investigators carrying out biochemical and histological analyses were blinded to the randomization till the end of the study.

Histological evaluation

Paraffin-embedded ovarian tissue samples were stained with hematoxylin and eosin (H&E). For quantitative measures, all the sections were analyzed and photographed by a light photomicroscope in minimally five microscopic fields. The histologic sections were examined for the presence of interstitial edema, vascular congestion, hemorrhage, and polymorphonuclear (PMN) infiltration. Each specimen was scored on a scale ranging from 1 to 4 (1: none; 2: mild; 3: moderate; 4: severe) as follows [19, 24]: grade 1; mild edema / mild vascular congestion / no hemorrhage /no PMN; grade 2: moderate edema / moderate vascular congestion / no hemorrhage / no PMN; grade 3: severe edema / severe vascular congestion / minimal hemorrhage / minimal PMN; grade 4: severe edema / severe vascular congestion / severe hemorrhage / severe PMN.

Biochemical analyses

Serum samples were defrosted at room temperature and then the rat AMH concentrations were measured using a rat AMH enzyme-linked immunoassay (ELISA) kit according to the manufacturer's instructions, using a microplate reader at 450 nm. Serum AMH levels were expressed as ng/ml and the minimum detectable level of AMH was typically 0.05 ng/ml.

Statistical analysis

Using SPSS program, data compatibility of the normal distribution was controlled graphically and by Shapiro-Wilk test. In the representation of continual data, mean ± SD was used for normally distributed parameters, median (minimum-maximum) in not normally distributed parameters and number and percentage in categorical data. While one-way ANOVA with Bonferroni was used between normally distributed data in comparison of three independent groups, Kruskal Wallis test was used in not normally distributed data. Bonferroni revision Mann-Whitney test was applied for dual comparisons on data that were established as significant by Kruskal Wallis test. Correlation between parameters was controlled with Spearman correlation test. $P < 0.05$ was considered to be significant.

Results

Histologic morphologies of ovaries from groups 1 and 2 are illustrated in Figure 1. AMH levels of groups 1 and 2 are illustrated in Figure 2. There were no significant differences in terms of right and left ovary grades or AMH levels for any pairwise comparisons of group 1 subgroups (Table 1; Figures 1 and 2). For group 2, AMH levels were approximately doubled in the curcumin II group compared

Table 3. — *Comparison of AMH and histological grade data for curcumin I versus curcumin II groups.*

	Curcumin I	Curcumin II	*p*
Rat weight (grams)	188.5(184-207)	189(183-245)	0.937
Rat weight 40th day (grams)	208.5(199-227)	204(198-275)	0.589
LO grade	2(1-2)	1(0-2)	0.310
RO grade	1(1-2)	1(1-1)	0.394
NO	30.0(23.0-107.8)	47.3(11.8-97.8)	0.818
NOS	7.7(4.1-13.6)	7.1(3.9-11.1)	0.818
AMH	2.9±1.2	5.4±1.2	0.005*

$p < 0.05$ taken as significant.

to either the sham II or control II groups ($p = 0.007$ and $p = 0.001$. respectively; Table 2; Figure 2). No difference was identified between group 2 subgroups in terms of right and left ovary grades (Table 2; Figure 1). When the curcumin I subgroup was compared to the curcumin II subgroup, no differences were observed in terms of ovary grades (Table 3; Figure 1). However, the AMH levels were significantly higher in the curcumin II compared to the curcumin I subgroup ($p = 0.005$; Table 3; Figure 2). Correlation analysis showed no correlations between AMH values and ovary grades ($p > 0.05$)

Discussion

The results of this study suggest that curcumin can play a role in maintaining and enhancing ovarian functions, particularly ovarian reserve, after ischemia. Irrespective of histological grade, AMH levels were higher in the presence of curcumin compared to sham and control groups in a rat model of ischemia/reperfusion. Duration of ischemia and reperfusion was however significant, as AMH levels were significantly higher in the curcumin group compared to the sham or control groups only after four hours each of ischemia and reperfusion, not after two hours. AMH levels were also significantly higher when the curcumin subgroup in the four-hour ischemia / four-hour reperfusion group was compared to the curcumin subgroup in the two-hour ischemia / two-hour reperfusion group. The results demonstrate a beneficial role for curcumin where ovarian ischemia-reperfusion injury was suspected.

The literature suggests that AMH levels in ovaries are a predictor of ovarian reserve and fertility and can be used, for example, in in-vitro fertilization programs to determine the expected response [25]. AMH continues to be expressed in growing follicles until they reach the size when they are selected for dominance. In the male embryo, it inhibits Müllerian ducts and thus favors the development of male reproductive organs. In females, it is secreted by the granulosa cells of small ovarian follicles, whose numbers reflect the ovarian reserve, and hence it declines with age [26, 27].

Antioxidative, anti-inflammatory, and anti-ageing effects of curcumin have been established in rat models and other systems [13-15, 28]. Consistent with the results of this study, curcumin has also been suggested to reduce and reverse tissue damage in a rat ischemia-reperfusion ovarian model [19]. In that study, bilateral adnexal torsion was carried out for three hours only, whereas in the present study, both two-hour and four-hour treatments were included. There was strong correlation between ischemia / reperfusion injury and oxidative stress index and it was concluded that curcumin administration reversed ischemia / reperfusion injury in the ovarian torsion models of rats [19].

Results of another study suggested that curcumin can maintain AMH levels and protect ovaries against damage [23]. In that study [23], rats were divided into four groups, one of which underwent laparotomy and sham surgery while other three underwent bilateral tubal sterilization. Group 4 was also given curcumin. AMH levels were measured 50 days after sterilization and it was reported that while postoperative AMH levels were significantly lower in the non-curcumin-treated groups, there was no difference observed between the preoperative and postoperative values of AMH in the curcumin-treated group.

The present study is the first to evaluate the protective effect of curcumin on ovarian reserve in a rat ischemia-reperfusion model over different time periods in terms of AMH levels. Serum AMH levels were measured 40 days after the ischemia-reperfusion procedure, which were carried out for both two and four hours, with comparisons between both intra-group and inter-group subgroups. The most striking effect of curcumin was observed when the rats' ovaries were subjected to ischemia-reperfusion for four hours; the AMH values were significantly raised compared to the same subgroup of the two-hour ischemia-reperfusion. This suggests that curcumin protects ovarian reserves and maintains AMH levels, preventing certain ovarian damage.

The results from this ischemia- reperfusion model, which predicts curcumin's protective effect, could be extended to other procedures, such as cystectomy, adhesiolysis, and tubal sterilization, which may cause ovarian ischemia. Decrease of ovarian reserve and AMH are also a feature of aging and declining fertility in women after the age of about 30 years [26, 27]. This suggests that in the future, use of curcumin may be beneficial in older adult females for fertility in terms of preservation of ovarian reserve. However, further large-scale studies on animals and humans are required before any firmer conclusions can be drawn.

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