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The modulating effects of Resveratrol on the expression of MMP-2 and MMP-9 in endometriosis women: a randomized exploratory trial

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ABSTRACT
Endometriosis is an inflammatory disease; the hallmark of inflammation is over-activation of matrix metalloproteinases (MMPs). The regulatory effects of Resveratrol on MMPs were formerly depicted in other cell lines. This study aimed at investigating the effects of Resveratrol on expression of MMP-2 and -9 in endometriosis patients. This trial was carried out on endometriosis patients (n = 34) who were randomly divided into treatment (i = 17) and control (n = 17) groups. Alongside the routine protocol, the control and treatment groups took placebo and Resveratrol (400 mg), respectively, for 12–14 weeks. Endometrial tissue and fluid as well as blood sampling from both groups were done before and after the intervention. The level of mRNA and protein of both MMP-2 and -9 reduced in the endometrium of treatment group following intervention. Also, the serum and the endometrial fluid concentration of them lowered within the treatment group. Moreover, the serum and endometrial fluid levels of MMP-2 as well as MMP-9 were also diminished following the surgical removal of endometritic lesions. We showed that Resveratrol can modify the inflammation process in the endometrium of women with endometriosis at least in the level of MMP-2 and -9 expressions. The therapeutic potency of Resveratrol in endometriosis needs more clinical studies.

INTRODUCTION
Endometriosis, an invasive gynecological disorder, is defined as the presence of endometrial-like tissue outside the uterus, mainly within the peritoneal cavity [1]. It affects almost 10–15% of reproductive-age women and 50% of infertile women [2].

There are several theories about the pathogenesis of endometriosis. It is well known that in addition to retrograde menstruation, many other additional factors may influence the development of the disease. Lagana et al. hypothesized that abnormal expression of some genes involved in Wnt signaling pathway within mesoderm causes the aberrant placement of endometrial stem cells. In endometriosis, changes in the peritoneal microenvironment by pro-inflammatory cytokines, matrix metalloproteinase, and immune cells provide an environment for proliferation and differentiation of endometrial stem cells to endometrial cells [3]. Also, the breakdown of peritoneal homeostasis results in the failure of scavenging mechanisms via a significant enhancement in apoptosis of peritoneal fluid mononuclear cells and decline in apoptosis of endometriotic cells [4]. In recent years, some studies focused on the epigenetic changes in the pathogenesis of endometriosis. Dioxins bind to aryl hydrocarbon receptor (AhR) and interrupt some physiological processes in female reproductive system via epigenetic changes. The complex of dioxin-AhR alters peritoneal microenvironment and provokes progesterone resistance damaging the endometrial function and immune response against the endometriotic cells [5].

It has been revealed that some matrix metalloproteinases (MMPs) play a pivotal role in the endometrial physiologic functions including uterine tissue remodeling during the proliferative and secretory phases and in particular, during the embryo implantation [6]. A dysregulated expression of MMPs in relation to progesterone resistance in endometriosis patients has been reported during the period of implantation window, which may partly explain the higher rate of implantation failure after ART cycles [7]. The MMP-2 and MMP-9 expression are up-regulated in the endometriotic lesions as well as the eutopic endometrium of women with endometriosis [8,9].

Resveratrol, a naturally occurring phytoalexin and polyphenolic compound, affects some cellular processes like angiogenesis [10], apoptosis, proliferation, as well as oxygen radical formation [11]. Moreover, its in vitro inhibitory effects on the formation of endometriotic lesions in animal models have been reported [12]. Resveratrol reduces the resistance of endometriotic stromal cells against apoptosis via down-regulation of the expression of Survivin [13]. Also, it decreases the activity of MMP-2 and MMP-9 in the animal models of aortic aneurysm [14] and prostate cancer [15].

Growing evidence support the effect of endometriosis on the eutopic endometrium resulting in failure of implantation; however, the associated cellular or molecular mechanisms remain not
well understood; it seems that the disturbance in the expression of MMPs is involved. Thus, our objective was to examine the inhibitory effects of Resveratrol, which has the capacity for remodeling of tissue via the extracellular matrix [16], on the endometrial fluid (EF) and serum levels of MMP-2 and -9 as well as their genes and proteins expression in the eutopic endometrium of infertile patients with endometriosis within the window of implantation.

Materials and methods

This is a placebo-controlled, parallel, randomized, double-blind exploratory clinical trial with a 1:1 ratio.

Patients and samples collection

The trial took place at Arash and Shariati Hospitals (Tehran University of Medical Science, Iran) from May 2016 to July 2017. The study inclusion criteria were the following: women with endometriosis aged 18–37 years, having regular menstrual cycle, 18.5 ≤ body mass index ≤ 30 kg/m², no usage of hormonal treatment and intrauterine devices for at least 3 months before first sampling. Women were excluded if they have immune system diseases, chronic infections, diabetes mellitus, or any pathological condition in the cavity of uterus such as polyps and fibroids. A total of 34 women with endometriosis-associated infertility, all stages III and IV, who met our inclusion criteria agreed to participate in the study. In all women, endometriosis was diagnosed using laparoscopic and histopathologic examinations.

In our hospital, the protocol used routinely for infertility treatment in endometriosis women is as follows: after laparoscopic surgery, the patients are given a chance of getting pregnant spontaneously. If during 3 months, the patient is not become pregnant, she will enter the in vitro fertilization cycle. To schedule the menstrual cycle and coordinate the development of follicles, oral contraceptives (OCPs) are taken for 2–4 weeks prior to the GnRH antagonist gonadotropin stimulation.

First, we identified the infertile women who were candidate to undergo diagnostic laparoscopy. In total, 67 women agreed to take part in the study. Monitoring of urinary LH surge (LH + 6 to LH + 10) and the date of last menstrual period were used to determine the mid-secretory phase of the menstrual cycle; the laparoscopic surgery was planned for the mid-secretory phase of the menstrual cycle. An independent pathologist confirmed this after sampling the endometrial tissue based on the Noyes’ criteria [17]. In all participants the EF, endometrial tissue and blood samples were taken before induction of anesthesia for surgery. In 34 patients, endometriosis stages III and IV were confirmed by histo-pathologist; they were randomly and equally divided into control and treatment groups. Both groups were treated based on the routine protocol of hospital; the control group (n = 17) took placebo and the treatment group (n = 17) took 400 mg Resveratrol (99% pure trans-resveratrol Mega Resveratrol, Southampton, UK) twice daily for 12–14 weeks along with OCP in the last three weeks. Proper use of medications and their probable side effects were followed up using weekly phone calls and monthly visits. At the end of the intervention period, the second endometrial tissue and fluid as well as blood sampling was done in the mid-secretory phase of menstrual cycle.

The Consolidated Standards of Reporting Trials (CONSORT) diagram (Figure 1) shows the distribution of participants through the trial. To randomize allocation of participants into control or treatment groups, sealed and uniform envelopes were elicited by an independent hospital staff. Two authors (A. S. and M. K.) were blinded to grouping the participants during the whole period of the study.

All sampling steps were performed according to the instructions suggested by World Endometriosis Research Foundation (WERF) Endometriosis Phenome and Biobanking Harmonisation Project (EPHect) [18,19]. For blood sampling, 8 ml venous blood was centrifuged for 15 min at 2500 g at a temperature of 4 °C; serum was separated and stored in aliquots at −80 °C until further assessment. Before endometrial tissue sampling, we obtained the EF using uterine aspiration technique explained by Boomsma et al. [20]. First, a speculum was placed in, the cervix was gently
cleaned with a dry cotton swab and then an embryo transfer catheter (Frydman, 1306045, CCD Laboratories, Paris, France) softly entered the uterus cavity. A syringe was connected to the catheter and the uterine secretion was slowly aspirated. The content of the catheter was stored at −80 °C.

Pipelle curette (Pipelle de Cornier Mark II, Paris, France) was utilized to collect the endometrial tissue samples. The endometrial samples were divided into three portions, the first portion submerged in RNAlater (76104, QIAGEN, Hilden, Germany) and stored at −80 °C for RNA extraction, the second portion washed with sterile cold phosphate buffered saline (PBS) and stored in liquid nitrogen for western blot, and the last one was fixed in formalin for endometrial dating.

This study was approved by Tehran Medical University Research Ethical Committee (IR.TUMS.REC.1394.838). The participants gave their informed written consent to take part in the study.

Real-time quantitative polymerase chain reaction (RT-PCR)

The transcripts of MMP-2 and MMP-9 were quantified by using the RT-qPCR. The RNeasy Mini Kit (74104, Qiagen, Hilden, Germany) was used to extract and purify the RNA from homogenized endometrial tissues. To remove the residual genomic DNA, we incubated the RNA samples with DNase I (RNase-free) (EN0521, Fermentas, Opelstrasse, Germany). The RNA concentrations were analyzed by using Nanodrop2000 spectrophotometer (Thermo Scientific, Copenhagen, Denmark). Five μg RNA from each sample was individually reverse transcribed into complementary DNA (cDNA) using RevertAid First Strand cDNA Synthesis Kit (K1622, Fermentas, Opelstrasse, Germany). PRIMERBLAST software (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) was used to design the primers sequences. The following primer sequences were used: MMP-2: forward, 5′-TGGATGGCGCTTTAATCGT-3′; reverse, 5′-TCTCCTCCTCATTGTATCTC-3′; MMP-9: forward, 5′-AACAATACTCAGACGACGG-3′; reverse, 5′-CGACTCTCACCAGGCATCTC-3′ and β-actin: forward, 5′-CTCAGGATGATGATGATCGC-3′; reverse, 5′-CAGATGGAATCCTCTTGACCCA-3′. The total reaction volume was 20 μl containing 0.25 mM of each primer, 12 μl Ampliqon and diluted cDNA 1:100.

The apparatus of Real-Time PCR System (Applied Biosystems, Darmstadt, Germany) was used to measure all transcripts. Amplification was carried out with an initial denaturation for 15 min at 95 °C followed by 40 cycles of 95 °C for 15 s, 60 °C for 20 s, and 72 °C for 20 s. All experiments were performed in triplicate and the mean value was computed. β-Actin was used as control housekeeping gene. The PCR products were run on agarose gels (3%) and single bands with appropriate size were obtained for MMP-2, MMP-9, and β-actin. Also, the specificity of PCR reactions was confirmed by analyzing the melting curves. We utilized the Livak method (2−ΔΔCt) [21] to calculate the mRNA expression of MMP-2 and MMP-9 relative to calibrator sample. The 2−ΔΔCt reported as mRNA fold change.

Western blot

The endometrial tissues were individually homogenized and their total protein was extracted by using RIPA lysis buffer containing protease inhibitors (sc-24948, Santa Cruz Biotechnology, Heidelberg, Germany). The mixtures were centrifuged for 15 min at 13,000g/4 °C. The Bradford assay (23236, Pierce, IL) method was utilized to determine the total protein concentration of supernatants. The samples (20 μg of protein per lane) were electrophoresed on 10% SDS–polyacrylamide gel and then, proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad, Hercules, CA). The membranes were blocked in 5% non-fat dry milk in 0.1% TBS-T (Tris-buffered saline/Tween-20) and incubated, overnight at 4 °C, with mouse monoclonal anti-β-actin antibody (sc-517582, 1:200; Santa Cruz Biotechnology, Heidelberg, Germany), mouse monoclonal anti-MMP-9 antibody (sc-21733, 1:200; Santa Cruz Biotechnology, Heidelberg, Germany), and mouse monoclonal anti-MMP-2 antibody (sc-135595, 1:200; Santa Cruz Biotechnology, Heidelberg, Germany). After washing with TBS-T, the membranes were incubated with HRP-labeled secondary antibodies against mouse (sc-2005, 1:5000; Santa Cruz Biotechnology, Heidelberg, Germany) at room temperature for 1h and washed again with TBS-T. Immunodetections were followed by using ECL detection kit (34080, ThermoScientific, Schaumburg, IL) and chemiluminescence was captured using X-ray films (Eastman Kodak), Densitometric analysis software (Image) version 1.50, NIH Image J system, Bethesda, MD) was utilized to calculate the signal intensity of MMP-2 and MMP-9 relative to the density of β-actin band.

Measurement of EF and serum concentration of MMP-2 and MMP-9

The concentration of MMP-2 and MMP-9 in EF and serum of all participants were measured by using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Quantikine ELISA Kit; R&D Systems, Minneapolis, MN), in accordance to the manufacturer’s instructions. The assays measured total MMP-2 and MMP-9. The assay sensitivity for MMP-2 and MMP-9 was 0.082 ng/ml and 0.156 ng/ml, respectively. The inter- and intra-assay coefficients of variation were, respectively, 6% and 7% for MMP-2 and 3% and 7% for MMP-9. All analysis was performed in duplicate.

Statistical analyses

The Kolmogorov–Smirnov test was used to check the normality of parameters. Data were presented as means ± SD. Statistical analyses, using SPSS version 22 software (SPSS, Chicago, IL), were made by the paired Student’s t-test or independent sample t test. Significance was set at p < .05.

Results

No significant difference was seen in the mean age (31.32 ± 1.71 versus 30.19 ± 2.40 years) and BMI (28.43 ± 1.27 versus 27.77 ± 1.12 kg/m^2) between the control and treatment groups.

The effects of Resveratrol on the expression of MMP-2 and its endometrial fluid and serum concentration in the endometriosis patients

There was no significant difference between the control and treatment groups in the mRNA as well as protein expression of MMP-2 before the intervention (p = .75). The significantly regulatory effects of Resveratrol on the mRNA expression of MMP-2 are shown in Figure 2(A). In the treatment group, the mRNA expression of MMP-2 significantly decreased when compared with the control group after the intervention period (p < .05). Additionally, at the end of the intervention period, compared with before the intervention, the expression of MMP-2 mRNA significantly decreased within
the treatment group \( (p < .001) \) as well as within the control group \( (p < .05) \). After the intervention, the expression level of MMP-2 protein significantly decreased in the treatment group when compared with before the intervention \( (p < .001) \) as well as with the control group \( (p < .001) \) (Figure 2(C)).

Compared with before the intervention, at the end of intervention period, the mean serum level of MMP-2 decreased significantly within both the control \( (487.2 \pm 102.9 \text{ versus } 482.1 \pm 98.6 \text{ ng/ml}, p < .05) \) and treatment \( (485.0 \pm 92.4 \text{ versus } 394.0 \pm 87.9 \text{ ng/ml}, p < .001) \) groups; however, the difference between two groups was significant \( (482.1 \pm 98.6 \text{ versus } 394.0 \pm 87.9 \text{ ng/ml}, p < .01) \), too.

Following the intervention, the mean EF level of MMP-2 in the treatment group was significantly lower than in the control group \( (722.2 \pm 102.3 \text{ versus } 896.2 \pm 122.3 \text{ ng/ml}, p < .001) \), denoting that Resveratrol down-regulated the shedding of MMP-2 in the uterus cavity of endometriosis patients (Table 1). Furthermore, its mean level statistically altered within the treatment group \( (896.9 \pm 127.8 \text{ versus } 722.2 \pm 102.3 \text{ ng/ml}, p < .001) \) as well as the control group \( (909.0 \pm 136.1 \text{ versus } 896.2 \pm 122.3 \text{ ng/ml}, p < .05) \).

The effects of Resveratrol on the expression of MMP-9 and its endometrial fluid and serum concentration in the endometriosis patients

The significantly regulatory effects of Resveratrol on the mRNA expression of MMP-9 are shown in Figure 3(A). At the end of intervention period, the mRNA expression of MMP-9 \( (p < .05) \) significantly decreased in the treatment group when compared with the control. Moreover, MMP-9 mRNA expression
significantly decreased within the treatment group following intervention (p < .001).

Also, the expression levels of MMP-9 protein significantly reduced within the treatment group following the intervention (p < .001) as well as in comparison with the control group (p < .001) (Figure 3(C)).

The mean EF (347.5 ± 43.0 versus 422.3 ± 44.9 ng/ml) and serum (591.2 ± 70.1 versus 738.5 ± 101.1 ng/ml) levels of MMP-9 in the treatment group were significantly lower than in the control group (p < .001); also their significant changes within the treatment [EF (347.5 ± 43.0 versus 429.2 ± 36.8 ng/ml), p < .001; serum (591.2 ± 70.1 versus 739.4 ± 114.2 ng/ml), p < .001] as well

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### Table 1. EF and serum concentration of MMP-2 in control and treatment groups.

<table>
<thead>
<tr>
<th>MMP-2 concentration (ng/ml)</th>
<th>Control group (n = 17)</th>
<th>Treatment group (n = 17)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum, before intervention</td>
<td>487.2 ± 102.9</td>
<td>485.0 ± 92.4</td>
<td>.950</td>
</tr>
<tr>
<td>Serum, after intervention</td>
<td>482.1 ± 98.6</td>
<td>394.0 ± 87.9</td>
<td>.010</td>
</tr>
<tr>
<td>EF, before intervention</td>
<td>909.0 ± 136.1</td>
<td>896.9 ± 127.8</td>
<td>.790</td>
</tr>
<tr>
<td>EF, after intervention</td>
<td>896.2 ± 122.3</td>
<td>722.2 ± 102.3</td>
<td>.000</td>
</tr>
</tbody>
</table>

EF: endometrial fluid; control group: endometriosis women managed with the hospital routine protocol and took placebo twice daily for 12 weeks; treatment group: endometriosis women managed with the hospital routine protocol and took 400 mg Resveratrol twice daily for 12 weeks. Data are expressed as mean ± standard division.

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Figure 3. The mRNA and protein expression of MMP-9 (92 kDa) in the endometrium of control and treatment groups. Following intervention, the mRNA and protein expression of MMP-2 significantly lowered in the treatment group compared with the control group. Control group, women with endometriosis managed with the hospital routine protocol and took placebo twice daily for 12–14 weeks; treatment group, women with endometriosis managed with the hospital routine protocol and took 400 mg Resveratrol twice daily for 12–14 weeks. I: Control before intervention; II: control after intervention; III: treatment before intervention; IV: treatment after intervention. *p < .05; **p < .01, and ***p < .001.
inflammatory mediators such as cytokines, chemokines, MMPs, the inflammatory process causing the excessive production of some down of the endometrium during the different phases of menstrual cells and establishment of endometriosis [23]. The cells produce and secrete the inactive forms (pro-enzymes) of MMPs into ECM and biological fluids [22]. The expression and activity of MMPs are regulated by their specific inhibitors, some cytokines, growth factors [24], and hormones [25]. A fine balance in the endometrial matrix turnover, regulated by MMPs and their inhibitors, regulates the proliferation, stabilization and breakdown of the endometrium during the different phases of menstrual cycle. This balance disrupts in endometriosis [26]. Probably, free radicals and reactive oxygen species trigger an inflammatory process causing the excessive production of some inflammatory mediators such as cytokines, chemokines, MMPs, and prostaglandins, facilitating the implantation and proliferation of endometrial cells in the ectopic sites [27]. In recent years, some researchers have focused on the role of MMP-2 and 9, known as gelatinases, in the development of endometriosis. The preceding studies showed that the MMP-2 [28] and -9 [8] expression and activation were up-regulated in the eutopic endometrium of women with endometriosis. Tang et al. found that the pattern of DNA methylation of the promoter region of the MMP2 gene changes in endometriosis which may justify its over-expression in the endometrium of the patients [29]. Additionally, the serum and peritoneal fluid concentration of both MMPs in endometriosis patients were significantly higher than in healthy women [28,30].

The findings of a study, by suppressing MMPs in nude mice, revealed that the expression of MMPs has a key in vivo role in the establishment of endometriosis [31]. Also, in an animal model of endometriosis, inhibition of MMP-2 activity reduced the quantity of endometriotic fragments, which the authors tried to link it with the relationship between MMP-2 activity and neo-angiogenesis [32].

This study is first attempt to show the in vivo modulating effects of Resveratrol on expression of MMP-2 and -9 and their serum as well as EF concentration in endometriosis patients. Our results indicated that the mRNA and protein expression of MMP-2 and MMP-9 were down-regulated following Resveratrol treatment. Although the regulatory mechanism of Resveratrol on the expression of MMP-2 and -9 in women with endometriosis requires more researches, depending on the cell type, various mechanisms have been reported in other pathological conditions. Most importantly, the extensive biological effects of Resveratrol are mediated through its anti-oxidant, anti-cancer, and anti-inflammatory activities via down-regulation of COX-2 through suppression of NF-κB activation [33]. Moreover, in another study, the lung adenocarcinoma cell metastasis has been reduced by Resveratrol through the down-regulation of MMPs expression following suppression of the activation of NF-κB pathway [34]. It should be noted that the signaling of NF-κB, activating by pro-inflammatory cytokines, growth factors, kinases, and ROS [35], is impaired in endometriosis [36]. The other probable mechanism of action of Resveratrol arises from its anti-angiogenic activity [10] possibly via inhibition in VEGF, MMP-2, and MMP-9 gene expression, which reduced the size and number of endometriotic implants in an animal model [37]. The inhibitory effects of Resveratrol on other fundamental modulators of inflammation such as TNF-α and inflammatory cytokines have been also shown previously [38]. It is known that TNF-α mediates MMP-9 expression [39] and its expression is disturbed in endometriosis [40]. Likewise, the invasion of the human hepato-cellular carcinoma cells has been meaningfully prevented by Resveratrol via regulation of TNF-α-mediated MMP-9 expression [41]. The inhibitory effect of Resveratrol on MMP-9 expression via the suppression of the STAT3 pathways in breast cancer cells has also been documented [42]. Finally, it has been shown that Resveratrol reduces the activity of Wnt/β-catenin signaling pathway and thus decreases the expression of its target genes such as MMP-2 and MMP-9 [43]. The Wnt/β-catenin signaling is over-activated in the endometrium of endometriosis patients during the window of implantation [44].

Interestingly, surgical removal of endometriotic lesions decreased the serum and EF levels of both MMP-2 and MMP-9; Resveratrol reduced their concentration further. Previous studies showed that the peritoneal fluid and serum concentrations of both MMP-2 [28] and MMP-9 [45] in endometriosis patients were higher than in healthy women. There is controversy regarding surgical intervention including excision of endometrioma in infertile women with endometriosis because of a growing evidence of decreasing ovarian reserve after the surgery [46]. However, surgical removing of endometriotic lesions improves the spontaneous pregnancy rate [47] as well as the pregnancy rate following IVF cycles [48]. In this regard, we agree with Celik et al. [36] that surgical removal of endometriotic lesions may improve the endometrium condition at the molecular level during the window of implantation.

The definitive diagnosis of endometriosis by using laparoscopic and histological examinations, before sampling, also, the standard sampling, storage, and processing techniques were the strengths of the present study. All participants in this study were infertile women with endometriosis stages III and IV, which may

Table 2. EF and serum concentration of MMP-9 in control and treatment groups.

<table>
<thead>
<tr>
<th>MMP-9 concentration (ng/ml)</th>
<th>Control group (n = 17)</th>
<th>Treatment group (n = 17)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum, before intervention</td>
<td>754.5 ± 104.4</td>
<td>739.4 ± 114.2</td>
<td>.872</td>
</tr>
<tr>
<td>Serum, after intervention</td>
<td>738.5 ± 101.1</td>
<td>591.2 ± 70.1</td>
<td>.000</td>
</tr>
<tr>
<td>EF, before intervention</td>
<td>427.9 ± 44.4</td>
<td>429.2 ± 36.8</td>
<td>.042</td>
</tr>
<tr>
<td>EF, after intervention</td>
<td>422.3 ± 44.9</td>
<td>347.5 ± 43.0</td>
<td>.000</td>
</tr>
</tbody>
</table>

EF: endometrial fluid; control group: endometriosis women managed with the hospital routine protocol and took placebo twice daily for 12 weeks; treatment group: endometriosis women managed with the hospital routine protocol and took 400 mg Resveratrol twice daily for 12 weeks. Data are expressed as mean ± standard division.

Discussion
The findings of present study showed that Resveratrol can affect the expression of MMP-2 and MMP-9 in the endometrium of endometriosis patients. MMPs are proteolytic enzymes produced by various cells that breakdown several proteins of ECM and basal membrane and trigger the ECM remodeling process in physiological and pathological conditions [22]. In fact, MMPs facilitate the cells migration and invasion through ECM degradation and are extensively involved in vascular remodeling [22]; all are essential processes in the ectopic implantation of endometrial cells and establishment of endometriosis [23]. The cells produce and secrete the inactive forms (pro-enzymes) of MMPs into ECM and biological fluids [22]. The expression and activity of MMPs are regulated by their specific inhibitors, some cytokines, growth factors [24], and hormones [25]. A fine balance in the endometrial matrix turnover, regulated by MMPs and their inhibitors, regulates the proliferation, stabilization and breakdown of the endometrium during the different phases of menstrual cycle. This balance disrupts in endometriosis [26]. Probably, free radicals and reactive oxygen species trigger an inflammatory process causing the excessive production of some inflammatory mediators such as cytokines, chemokines, MMPs, and prostaglandins, facilitating the implantation and proliferation of endometrial cells in the ectopic sites [27]. In recent years, some researchers have focused on the role of MMP-2 and 9, known as gelatinases, in the development of endometriosis. The preceding studies showed that the MMP-2 [28] and -9 [8] expression and activation were up-regulated in the eutopic endometrium of women with endometriosis. Tang et al. found that the pattern of DNA methylation of the promoter region of the MMP2 gene changes in endometriosis which may justify its over-expression in the endometrium of the patients [29]. Additionally, the serum and peritoneal fluid concentration of both MMPs in endometriosis patients were significantly higher than in healthy women [28,30].

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This study is first attempt to show the in vivo modulating effects of Resveratrol on expression of MMP-2 and -9 and their serum as well as EF concentration in endometriosis patients. Our results indicated that the mRNA and protein expression of MMP-2 and MMP-9 were down-regulated following Resveratrol
limit the generalization of the findings to all women with endometriosis.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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