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Ellagic Acid potential use for endometriosis treatment: its effect on human endometrial cell cycle, adhesion and migration.

Short title: Ellagic acid possible endometriosis treatment.

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Abstract

Endometriosis is a common and challenging condition of reproductive-aged women that is defined as the presence of endometrial-like tissue outside the uterine cavity. Despite its prevalence, there is still no effective therapeutics so we aim to evaluate the ellagic acid (EA) effect on the most relevant aspects that are known to be altered in endometriosis. Endometrial primary cultures from women with and without endometriosis and endometrial cell lines were incubated with EA (50 and 100μM) for 24 and 48 h. The results demonstrated that EA arrest endometrial stromal cell cycle on G2 / M phase, after 48 h. In addition, EA 100μM treatment significantly decreased ECC-1 cell migration at 20 h and T-HESC cell migration at 10 h and 20 h; while EA 50μM caused a significant decreased on T-HESC cell migration at 20 h. On the other hand, we proved that treatment with EA for 24 h reduces T-HESC and ECC-1 adhesion to plastic. However, we did not find an effect of EA on cell proliferation. EA has an inhibitory effect on endometrial cell adhesion, migration and cell cycle progression *in vitro*. These highlight the idea to investigate natural compounds as a novel and promising therapeutic treatment for endometriosis.

Keywords: endometriosis - Ellagic Acid - migration - adhesion - cell cycle

1. Introduction

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Endometriosis is one of the most common benign gynecological diseases in women of reproductive age and is defined by the presence of endometrial-like tissue (epithelial and stromal elements) outside the uterine cavity[1]. It affects approximately 10% of the female population causing severe pelvic pain and infertility in 30-50% of the patients who suffer it. This is a disease with a complex etiology and the most accepted theory for endometriosis development is Sampson`s implantation theory based on retrograde menstruation[2]. According to this theory, for the endometriotic lesions establishment and maintenance, it is necessary that the endometrial cells reach the peritoneal cavity, adhere to the peritoneum, invade it, vascularize it and have the capacity to proliferate, therefore the migration, adhesion, and proliferation are crucial processes in endometriosis development. In addition, endometriosis is underdiagnosed because a large proportion of affected women are asymptomatic or their strong pelvic pain cannot be considered as specific symptoms of a disease. In general, the first doctor's visit are due to difficulty conceiving and, at present, laparoscopy is the only diagnostic method, which causes long delays before women acquire a definitive diagnosis [3].

Current treatments for endometriosis involve the surgical removal of implants and/or the induction of an hypoestrogenic state using combined oral contraceptives, progestagens alone or GnRH analogues, because it is an estrogen-dependent disease. That's why current medical therapies for endometriosis do not allow conception in women under treatment [4]. In addition, these therapies are not completely effective and have several adverse side effects leading to high recurrence rates [5,6] and avoiding their long term use [7–9].

The search for novel treatments for endometriosis, more accessible, with no side effects and that allow pregnancy, guided us to evaluate natural compounds. The consumption of berries and other polyphenol-enriched foods or juices has been associated with positive health effects like antioxidant properties, prevention of cardiovascular diseases and cancer [10,11]. Ellagitannins (ETs) and EA are polyphenols[12] present in those fruits, nuts and seeds[13–15]. Taking into account the diverse effects that the EA exerts on different cell types[16–20], we think that this natural compound could be a good option as an alternative therapy for endometriosis. In addition to the known anti-inflammatory, antiglycolytic, antioxidant and antimicrobial effects of its metabolites[21], it has been described that the EA has an antiproliferative and proapoptotic effect on colon, breast, and prostate[22] cancer cell lines. It also inhibits cell migration and the production of pro-matrix-metalloproteases 2 and 9 and gelatin. It has anti-angiogenic action since it decreases the levels of vascular endothelial growth factor 165. (VEGF 165)[23,24].

In addition, considering that endometriosis is an estrogen-dependent pathology, another factor that led us to evaluate this compound as a possible treatment for endometriosis is the fact that it has been described that one of the metabolites of EA, urolithin B is an antagonist of the aromatase [25], and this antiestrogenic activity of EA has been proven by other authors[26,27].

However, EA has not been tested as a therapeutic alternative for endometriosis.

The aim of this work was to assess the effect of EA on proliferation, cell cycle progression, adhesion and migration in human endometrial cells in an in vitro model of endometriosis.

2. Experimental methods

2.1. Patients

In this study participated women on reproductive age who underwent diagnostic laparoscopies due to infertility, tubal obstruction or other pathology, and who had not received treatment during the last six months. They were classified into two groups: a) Patients with endometriosis diagnosed by laparoscopy and confirmed by histological studies (the stages I, II, III, and IV were determined according to the Revised American Society for Reproductive Medicine Classification[28]) and b) Control women who did not suffer endometriosis or other pathology that could alter the cell population to be evaluated.

After written consent from the patients, endometrial biopsies were taken during the laparoscopy with diagnostic and therapeutic purposes, yielding to our group a small fraction of the material. Biopsies of eutopic endometrium were obtained from all subjects as described previously [29,30]. Patient characteristics are provided in Table 1.

This study was approved by the Ethics and Research Committee of the Instituto de Biología y Medicina Experimental - Consejo Nacional de Investigaciones Científicas y Técnicas (IBYME-CONICET) of Buenos Aires, Argentina, on 26 May 2015 (reference CE 005 - April/2015). Office of Laboratory Animal Welfare (OLAW) Assurance indentification number: F16-00065 (A5072-01).

2.2. Isolation and culture of endometrial stromal and epithelial cells

We obtained epithelial and stromal cells from eutopic endometrial biopsies. The cells were enzymatically separated and isolated by successive centrifugations, and primary cultures were established for in vitro studies. Briefly, tissue was minced, washed and placed in Dulbecco's

Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12, Gibco) supplemented with antibiotic-antimycotic (penicillin 100 IU/ml, streptomycin 100 mg/ml and amphotericin B 25 mg/ml, Gibco) and collagenase 0.5 mg/ml (type I, Gibco). After 2 h of incubation at 37°C in a 5% CO2 atmosphere, the resulting suspension was centrifuged at 100 x g for 5 minutes, and the pellet and supernatant were separated and reserved. The pellet containing epithelial glands was resuspended in culture medium and spun again at 100 x g for 5 minutes, so the final pellet mainly contained epithelial cells. This enriched epithelial fraction was cultured with MEM D-Val supplemented with 10% fetal bovine serum (FBS) (Gibco) and grown to sub-confluence (70–80%) at 37°C before the experiments. On the other hand, the supernatant containing mainly stromal cells was centrifuged at 400 x g for five minutes and the pellet containing mainly stromal cells were resuspended and the cells counted and plated with DMEM/F12 supplemented with 10% FBS and antibiotic-antimycotic to grow up to sub-confluence (70–80%) in a humidified environment with 5% CO2 at 37°C. It has been previously shown that this method guarantees a high purity of each type of cells in culture 31.

2.3. Cell line and culture conditions

T-HESC (ATCC® CRL4003™) was derived from the stromal cells obtained from an adult woman with myomas[32]. The primary stromal endometrium cells were immortalized by infection with supernatant from the packaging cell line pA317-hTERT (Geron Corp.; Menlo Park, CA), which expressed the hTERT and the puromycin resistance genes. They were cultured in DMEM/F-12 supplemented with 10% FBS (PAA Laboratories, USA) in a phosphate buffered saline (PBS) modified environment with 5% CO_2 at 37°C.

ECC-1 (ATCC® CRL2923™) was derived from endometrial epithelial cells from a human adenocarcinoma[33]. The cultures were maintained with Roswell Park Memorial Institute (RPMI)-1640 med supplemented with 10% fetal bovine serum, 1% pyruvate at 37°C in the presence of 5% CO_2.

2.4. Cell proliferation assay

For cell proliferation assays, $5x10^3$ ECC-1 cells/well, $5x10^3$ T-HESC cells/well, $5x10^4$ primary endometrial epithelial cells/well or $2x10^4$ primary endometrial stromal cells/well from eutopic tissue of women with endometriosis and controls were plated in 96 well culture plates with their

corresponding media culture supplemented with 10 % FBS and incubated at 37°C in a 5% CO_2 atmosphere. When cells reached a 70% confluence, cultures were washed with PBS and incubated for 24 or 48 h with 50 µM and 100 µM EA in fresh medium supplemented with 1% fetal bovine serum. Basal conditions were obtained by incubating cells with the vehicle used to dissolve the EA: 1% sodium hydroxide (NaOH, Sigma-Aldrich). We based on previous in vitro studies to fix the effective dose of EA; hence we arrived at these concentrations[22,34–39]. Each treatment condition was carried out in quadruplicate. Cell proliferation was determined by a colorimetric assay using the WST-1 Cell Proliferation Kit according to the manufacturer instructions (Roche Applied Science). Absorbance was measured at 450 nm using a multi-well plate reader. Cell proliferation was expressed as percentage of basal conditions in each experiment.

2.5. Cell Cycle Analysis

For cell cycle analysis, 2.5 x 10^5 T-HESC and ECC-1 cells/well were plated in 6 well culture plates with their corresponding culture medium supplemented with 10% fetal bovine serum. After 24 h, cultures were washed and incubated with different concentrations of EA (50 and 100 µM) in medium supplemented with 1% FBS for 24 or 48 h. Then, cells were harvested using 0.25% trypsin (Gibco) and centrifuged at 300 x *g* for 5 minutes. The supernatants were removed; cells were washed with ice-cold PBS, and fixed by slowly adding ice-cold 70% ethanol while mixing the solution in a vortex at low speed. Cells were kept at -20°C until assayed. On the day of the assay, tubes containing cells were centrifuged at 100 x *g* for 5 minutes and the supernatants were removed. Pellets were carefully resuspended adding the DNA-staining solution (50 µg/ml propidium iodide (Sigma-Aldrich) in PBS) and kept in the dark for 15 minutes at room temperature. Finally, cell cycle distribution was determined using a flow cytometer (FACS Canto II, BD Biosciences). The results were analyzed using Cyflogic 1.2.1 software. All treatments were compared to the basal condition.

2.6. Scratch (wound healing) assay

The wound healing assay was carried out using an established procedure [40]. Cells were seeded in a 6 well plate and allowed to adhere for 48 h. Then the monolayer was wounded by cross scratching with a 200 µl pipette tip. The detached cells were removed by rinsing with PBS. Immediately after wounding, 50 or 100 µM EA was added in culture media supplemented with 1% FBS. Images of the scratch were acquired immediately after wounding (0 h) and 5, 10 and 20 h later. All treatments

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were compared to the basal condition. The healing rate was calculated using the closure ratio analysis method [41]:

Healing rate (%) = [(0 h scratch area - X h scratch area)/0 h scratch area] × 100.

2.7. Adhesion assay to plastic.

Cell adhesion to plastic was evaluated in ECC-1 and T-HESC cultures treated with EA. Briefly, cells were cultured in their respective culture medium supplemented with 10% fetal bovine serum, at 37° with 5% CO_2 until reach a 70% confluence. Then, the cells were treated for additional 24 h with vehicle or 50-100 μM EA in culture medium supplemented with 1% fetal bovine serum. Following, the cells were harvested with trypsin 0.25% (Gibco), resuspended in serum-free medium and leave to recover for 40 minutes. Following recovering, cell suspension was added to 6 well culture plates (10^4 cells/ml/well) and cultured in a 5% CO_2 atmosphere at 37°C for 60 minutes. After that, the wells were washed four times with PBS, and the adherent cells were harvested and counted. Cell adhesion was determined staining cells with trypan blue and counting them under a phase-contrast microscope. All treatments were compared to the basal condition.

2.10. Statistical analysis

Statistical analysis was performed using GraphPad PRISM software V4.0 (GraphPad Software Inc, San Diego, California). Statistical comparisons among groups were carrying out by the Student t-test or the one-way analysis of variance (ANOVA) followed by Tukey´s multiple comparison tests. Results were expressed as mean ± SEM. In all cases, only a p value < 0.05 was considered significant.

3. Results

3.1. Effect of EA on endometrial cell proliferation.

The effects of both assayed concentrations of EA (50 and 100 μM) on cell proliferation are displayed in **Figure 1**. We did not find a statistically significant difference between EA and Basal, on cell proliferation in human endometrial epithelial (Figure 1A) or stromal (Figure 1B) cells. In the same way, we did not observe statistically significant effects of EA on ECC-1 (Figure 1C) and T-

HESCs (Figure 1D) cell proliferation. As shown in Figure 1, both cell lines and primary cultures behaved similarly, and facing the complexity conferred by the management and establishment of primary cultures [32]; from this point we decided to continue using the ECC-1 and T-HESC cell lines as a representative in vitro experimental model.

3.2. Effect of EA on endometrial cell cycle progression.

The effects of both assayed concentration of EA (50 and 100 μM) on the progression of the cell cycle are displayed in **Figure 2**. Cell cycle distribution profiles of 24 and 48 h EA-treated cells were evaluated via flow cytometry. Only exposure to 100 μM EA for 48 h caused a significant arrest of cell cycle in G2/M phase in T-HESCs cells (Figure 2B; $p<0.05$); we have no observed significant differences with EA treatment in the other conditions in T-HESs or ECC-1 cells.

3.3. Effect of EA on endometrial cell migration.

We evaluated the effects of EA on ECC-1 and T-HESCs cell migration by the wound healing technique (**Figure 3**). Treatment with 100 μM EA significantly decreased ECC-1 cell migration at 20 h (Figure 3A; $p<0.05$) and T-HESC cell migration at 10 h and 20 h (Figure 3B; $p<0.05$ and $p<0.001$ respectively). In addition, 50 μM EA caused a significant decreased on T-HESC cell migration at 20 h (Figure 3B; $p<0.01$) even though no significant effects on ECC-1 cell migration was observed (Figure 3A).

3.4. Effect of EA on endometrial cell adhesion to plastic.

We examined the adhesion of EA-treated ECC-1 and T-HESCs cells to plastic culture plates and compared this to attachment of untreated cells. As shown in **Figure 4**, the efficacy of EA-treated cells attachment was reduced on both cell lines. ECC-1 pre-treated with 50 and 100 μM EA significantly reduced their attachment competence ($p<0.01$ and $p<0.001$ vs. Basal respectively), and in the same way T-HESCs cells pre-treated showed a significant reduced attachment competence ($p<0.05$ and $p<0.01$ vs. Basal respectively).

4. Discussion

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Endometriosis is one of the most common benign chronic hormonal woman diseases with poor prognosis and a high recurrence rate. Long-term therapy is required, and nowadays the current treatments are surgical and/or medical approaches. The pharmacological strategy is based on drugs that are generally ineffective because there is no balance between their clinical efficacy and the personal needs of patients [42]. The choice between the treatments is influenced by several factors, including the type of lesion suspected, the personal insights of the patient, and the already known adverse effects that appear when hormonal drugs are used for a long period[43]. The variety of strategies and modalities demonstrates that treatment of endometriosis is constantly evolving and no single therapy is ideal for all patients.

Over the last years, evidence has been accumulated to suggest that medicinal botanicals have anti-inflammatory and pain-alleviating properties and hold promise for treatment of endometriosis[44]. Taking into account previous results obtained in our laboratory[45,46] and earlier promising results obtained in cancer[15], we focus on possible natural therapies that prevent recurrences after laparoscopy. In this sense, EA is a polyphenol usually found in berries and nuts; and is one of the natural options that have lately been considered to treat different diseases. It affects a large range of biological activities and its mechanisms of action are varied. At a systemic level, EA have shown to successfully inhibit angiogenesis, cell migration and cell invasion in ovarian, colon and bladder cancer cell lines[15]; some of the crucial processes for the infiltrative behavior and metastatic process as well as endometriosis patophisiology[47,48]. Accordingly to Sampson`s implantation theory[2], endometriotic cells may migrate, attach, proliferate, and invade. These sequential cellular events are involved in the initiation, progression, and growth of ectopic endometriotic lesions, and therefore in the development of the disease.

In the present study, we analyze cell proliferation and cell cycle progression to assess the effect of EA on endometrial growth. Our data indicate that EA does not exert any effect nor in endometrial primary epithelial and stromal cells, neither on endometrial ECC-1 and T-HESC cell

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lines (Figure 1). These results are in agreement with a previous work which demonstrates that EA in doses up to 50μM has no effect on cell proliferation in ECC-1 and T-HESCs cell lines[49]. However, it has been shown that 50 and 100μM EA suppresses the cell viability of U251 cells glioblastoma[50].

In addition our cell cycle results showed that EA causes an arrest in the G2/M phase in T-HESCs after 48 h treatment (Figure 2). These results are congruent with González-Sarrías et al. investigation[51] in which the exposure of Caco-2 cells to a mixture of EA and its metabolites (10 μM EA, 40 μM Urolithin A and 40 μM Urolithin B (Mix)) arrested cell growth at G2/M-phase since day 2 of treatment, associated to a downregulation of cyclins A and B1. On the other hand, we demonstrated that ECC-1 cell cycle progression is not affected by treatment with EA. This is consistent with previous works[24, 52] in which it is demonstrated that EA has cell type-dependent effects on cell metabolism, suggesting that there is no a single target of action for this compound.

Interestingly, we observed that EA arrests cell cycle at G2/M-phase in T-HESCs cells but has no effect on cell proliferation. We think that this may be due to the fact that the arrest was observed at 48h, the same time that cell proliferation was evaluated. Therefore it would be reasonable to find a decrease in cell proliferation at a later time, which was not analyzed in this work. In this regard, it has been reported that endometrial carcinoma KLE and AN3CA cell lines treated with EA 20μM for 24, 48 and 72 h, showed a significant decrease in cell viability only after 48 h[53]. However, during the same investigation, the arrest of the cell cycle in the G1 phase was evident at 24 h of treatment. Likewise, a study performed with apigenin, another natural compound, on pancreatic cancer cells[54] detected a cell cycle arrest in the G2/M phase at 24 h of treatment, although the decrease in the number of cells was confirmed only at 72 h.

Studies of the cellular mechanisms involved in the pathology of diseases are ideally carried out on primary cultured cells. However, the limited availability of tissue, the difficulties in establishing the culture, and the short useful life of these cells in subsequent passages leads to low reproducibility of assays[32]. Consequently, in this study we mainly use ECC-1 and T-HESCs cell

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lines since they have been widely utilized as a model of endometriosis due to the close physiological[55] and molecular[32, 56-60] similarities they share with primary cultures.

As we mentioned before, cell migration and adhesion are key processes for the establishment of the lesions and the development of endometriosis[1, 61, 62]. Accordingly, we assessed the effect of EA on these cell abilities. We demonstrated that EA treatment reduces cell migration in both ECC-1 and T-HESCs cells. In agreement with our results, Huidi Liu and col.[63] demonstrates that the ovarian cancer cells (A2780) underwent inhibition of cell migration upon exposure to 5, 10 and 15µg/ml EA. In addition, our results showed that pretreatment with EA decreases the ability of both cell lines to adhere to plastic (Figure 4). Congruently, a previous study conducted on neuroblastoma SH-SY5Y cells has already demonstrated that EA treatment (30µM) induces detachment and lower viability of adherent cells[64].

It is know that many intracellular and soluble cellular adhesion molecules are differentially expressed in endometrial cells from women with and without endometriosis [65, 66] which may facilitate the implantation of endometrial cells in an ectopic place. Moreover, Zhao Q. and col.[67] demonstrated that endometrial stromal cells from ectopic endometrium have a higher rate of migration than stromal cells from normal endometrium. These reports highlight the importance of targeting cell migration and adhesion as they are central processes in endometriosis pathophysiology and support the use of EA as a putative compound for the treatment of this disease.

Nevertheless, EA is a polyphenolic compound from the family of ellagitannins, which have low water solubility and absorption leading to a poor bioavailability[68, 69]. This disadvantage could be a difficulty thinking it as a possible treatment for endometriosis. However, new promising approaches are being developed. Recently, poor water soluble drugs have been successfully delivered by the use of nanoscale systems[70-72]. Growing evidence demonstrates that this strategy allows the delivery of higher local concentrations of drugs, which can enhance their therapeutic efficacy[73, 74]. In this sense, novel drug-delivery systems that overcome the low EA solubility and bioavailability (i.e., biocompatible polymers-based nanoparticles/ microcapsules/ biofilms/ micelles) are being tested *in*

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vitro and *in vivo*[75-79]. In summary, we found that EA affects cell cycle progression of endometrial stromal cells through the arrest at G2/M phase. Moreover, EA also inhibited the migration and adhesion of both stromal and epithelial cells suggesting that it could interfere with the early stages of lesion establishment. To the best of our knowledge our report constitutes the first study to test the effect of EA on endometriosis. Our results are promising even though more studies are needed to better understand the mechanisms of action of EA and it is the potential use as a preventive or therapeutic agent for endometriosis. Full characterization of this natural compound will likely provide new insights into alternative medicines with potential for therapeutic applications in women. In this context, further evaluation of the EA and its active metabolite(s) are currently being carry out by our group, in order to provide therapeutic alternatives for the inhibition of the development of endometriotic-type lesions.

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Conflict of Interest

Authors declare no conflict of interest. NONE

Authorship

BMC, did all the experimental work and contributed new ideas. MAB taught and collaborated in the realization of the cell cultures, DM and AGR collaborated in the processing of the samples, JJS provided the endometrial tissue biopsies; RIB proposed the evaluation of the ellagic acid as a possible treatment for endometriosis, elaborated the work plan and direct the laboratory.

5. References

1. L.C. Giudice, L.C. Kao, Endometriosis, *Lancet*.,2004,**364,**1789.

2. J.A.Sampson, Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity, *Am J Obs Gynecol*., 1927,**14**,422.

3. S. Kennedy, A. Bergqvist, C. Chapron, T. D'Hooghe, G. Dunselman, R. Greb, L. Hummelshoj, A. Prentice, E. Saridogan, ESHRE guideline for the diagnosis and treatment of endometriosis, *Hum Reprod.*, 2005, **20,**2698.

4. P. Vercellini, P. Viganò, E. Somigliana, L. Fedele, Endometriosis: pathogenesis and treatment, *Nat Rev Endocrinol.*, 2014,**10**, 261.

5. S.W. Guo, Recurrence of endometriosis and its control, *Hum Reprod Update*., 2009, **15,** 441.

6. G. Bozdag, Recurrence of endometriosis: risk factors, mechanisms and biomarkers, *Womens Heal*.(Lond), 2015,**11,** 693.

7. F. Barra, C. Scala, V. Mais, S. Guerriero, S. Ferrero, Investigational drugs for the treatment of endometriosis, an update on recent developments, *Expert Opin Investig Drugs*., 2018, **27,** 445.

8. A.K. Rodgers, T. Falcone, Treatment strategies for endometriosis, *Expert Opin Pharmacother*., 2008, **9,** 243.

9. V. Selak, C. Farquhar, A. Prentice, A. Singla, Danazol for pelvic pain associated with endometriosis, *Cochrane database Syst Rev.*, 2007, Issue **4**, 1.

10. R.H. Liu, Potential Synergy of Phytochemicals in Cancer Prevention: Mechanism of Action, *J Nutr*, 2004, **134,** 3479.

11. L. Wang, M. Martins-Green, Pomegranate and Its Components as Alternative Treatment for Prostate Cancer, *Int J Mol Sci.*, 2014, **15**, 14949.

12. R. Press, D. Hardcastle, Some physico-chemical properties of ellagic acid, *J Appl Chem.*, 2007, **19**, 247.

13. J.M. Landete, Ellagitannins, ellagic acid and their derived metabolites: A review about source, metabolism, functions and health, *Food Res*., 2011, **44**, 1150.

14. A.K. Kiss, J.P. Piwowarski, Ellagitannins, Gallotannins and their Metabolites- The Contribution to the Anti-Inflammatory Effect of Food Products and Medicinal Plants, *Curr Med Chem*., 2019, **25**, 4946.

15. C. Ceci, P.M. Lacal, L. Tentori, M.G. De Martino, R. Miano, G. Graziani, Experimental Evidence of the Antitumor, Antimetastatic and Antiangiogenic Activity of Ellagic Acid, *Nutrients*., 2018, **10**, 1756.

16. J.L. Ríos, R.M. Giner, M.C. Marín, M. Recio, A Pharmacological Update of Ellagic Acid, *Planta Med*., 2018, **84**, 1068.

17. I. Kang, T. Buckner, N.F. Shay, L. Gu, S. Chung, Improvements in Metabolic Health with Consumption of Ellagic Acid and Subsequent Conversion into Urolithins: Evidence and Mechanisms, *Adv Nutr*., 2016, **7**, 961.

18. D. Gramec Skledar, T. Tomašič, M. Sollner Dolenc, L. Peterlin Mašič, A. Zega, Evaluation of endocrine activities of ellagic acid and urolithins using reporter gene assays, *Chemosphere*., 2019, **220**, 706.

19. S.H. Park, J.L. Kim, E.S. Lee, S.Y. Han, J.H. Gong, M.K. Kang, Y.H. Kang, Dietary ellagic acid attenuates oxidized LDL uptake and stimulates cholesterol efflux in murine macrophages, *J Nutr*., 2011, **141**, 1931.

20. L. Mele, P. Mena, A. Piemontese, V. Marino, N. López-Gutiérrez, F. Bernini, F. Brighenti, I. Zanotti, D. Del Rio, Antiatherogenic effects of ellagic acid and urolithins in vitro, *Arch Biochem Biophys.*, 2016, **599**, 42.

21. K.N.M. Abdelazeem, Y. Singh, F. Lang, M.S. Salker, Negative Effect of Ellagic Acid on Cytosolic pH Regulation and Glycolytic Flux in Human Endometrial Cancer Cells, *Cell Physiol Biochem.*, 2017, **41**, 2374.

22. P. Pitchakarn, T. Chewonarin, K. Ogawa, S. Suzuki, M. Asamoto, S. Takahashi, T. Shirai, P. Limtrakul, Ellagic Acid Inhibits Migration and Invasion by Prostate Cancer Cell Lines, *Asian Pacific J Cancer Prev.*, 2013, **14**, 2859.

23. J.N. Lossoa, R.R. Bansode, A. Trappey, H.A. Bawadi, R. Truax, In vitro anti-proliferative activities of ellagic acid, *J Nutr Biochem.*, 2004, **15**, 672.

24. R. Vicinanza, Y. Zhang, S.M. Henning, D. Heber, Pomegranate Juice Metabolites, Ellagic

Acid and Urolithin A, Synergistically Inhibit Androgen-Independent Prostate Cancer Cell Growth via Distinct Effects on Cell Cycle Control and Apoptosis, *Evidence-Based Complement Altern Med.*, 2013, **2013**, 1.

25. L.S. Adams, Y. Zhang, N.P. Seeram, D. Heber, S. Chen, Pomegranate ellagitannin-derived compounds exhibit antiproliferative and antiaromatase activity in breast cancer cells in vitro, *Cancer Prev Res.*, 2010, **3**, 108.

26. H.S. Aiyer, R.C. Gupta, Berries and ellagic acid prevent estrogen-induced mammary tumorigenesis by modulating enzymes of estrogen metabolism, *Cancer Prev Res.*, 2010, **3**, 727.

27. K.P.L. Bhat, J.M. Pezzuto, Natural modulators of estrogen biosynthesis and function as chemopreventive agents, A*rch Pharm Res.*, 2001, **24**, 473.

28. ASRM. Revised American Society for Reproductive Medicine classification of endometriosis: 1996, *Fertil Steril.*, 1997, **67**, 817.

29. G.F. Meresman, S. Vighi, R.A. Buquet, O. Contreras-Ortiz, M. Tesone, L.S. Rumi, Apoptosis and expression of Bcl-2 and Bax in eutopic endometrium from women with endometriosis, *Fertil Steril*., 2000, **74**, 760.

30. H. Cho, H. Jung, H. Lee, H.C. Yi, H.K. Kwak, K.T. Hwang, Correction: Chemopreventive activity of ellagitannins and their derivatives from black raspberry seeds on HT-29 colon cancer cells, *Food Funct.*, 2015, **6**, 2861.

31. G.F. Meresman, M. Bilotas, R.A. Buquet, R.I. Barañao, C. Sueldo, M. Tesone, Gonadotropin-releasing hormone agonist induces apoptosis and reduces cell proliferation in eutopic endometrial cultures from women with endometriosis, *Fertil Steril.*, 2003, **80**, 702.

32. G. Krikun, G. Mor, A. Alvero, S. Guller, F. Schatz, E. Sapi, M. Rahman, R. Caze, M. Qumsiyeh, CJ. Lockwood, A Novel Immortalized Human Endometrial Stromal Cell Line with Normal Progestational Response, Endocrinology., 2004, **145**, 2291.

33. P.G. Satyaswaroop, R.J. Zaino, R. Mortel, Human endometrial adenocarcinoma transplanted into nude mice: growth regulation by estradiol, *Science*., 1983, **219**, 58.

34. K.L. Khanduja, P.K. Avti, S. Kumar, N. Mittal, K.K. Sohi, C.M. Pathak, Anti-apoptotic activity of caffeic acid, ellagic acid and ferulic acid in normal human peripheral blood

mononuclear cells: a Bcl-2 independent mechanism, *Biochim Biophys Acta*., 2006, **1760**, 283.

View Article Online
DOI: 10.1039/D0FO00267D

35. A.R. Sudheer, S. Muthukumaran, N. Devipriya, V.P. Menon, Ellagic acid, a natural polyphenol protects rat peripheral blood lymphocytes against nicotine-induced cellular and DNA damage in vitro: With the comparison of N-acetylcysteine, *Toxicology*., 2007, **230**, 11.

36. D. Wang, Q. Chen, Y. Tan, B. Liu, C. Liu, Ellagic acid inhibits human glioblastoma growth in vitro and in vivo, *Oncol Rep.*, 2017, **37**, 1084.

37. L. Vanella, C. Di Giacomo, R. Acquaviva, I. Barbagallo, G. Li Volti, V. Cardile, N.G. Abraham, V. Sorrenti, Effects of ellagic Acid on angiogenic factors in prostate cancer cells, *Cancers.*, 2013, **5**, 726.

38. A. Promsong, W.O. Chung, S. Satthakarn, W. Nittayananta, Ellagic acid modulates the expression of oral innate immune mediators: potential role in mucosal protection, *J Oral Pathol Med.*, 2015, **44**, 214.

39. A. Strati, Z. Papoutsi, E. Lianidou, P. Moutsatsou, Effect of ellagic acid on the expression of human telomerase reverse transcriptase (hTERT) alpha+beta+ transcript in estrogen receptor-positive MCF-7 breast cancer cells, *Clin Biochem.*, 2009, **42**, 1358.

40. C.C. Liang, A.Y. Park, J.L. Guan, In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro, *Nat Protoc.*, 2007, **2**, 329.

41. Y. Chen, W. Peng, Y. Lu, J. Chen, Y.Y. Zhu, T. Xi, MiR-200a enhances the migrations of A549 and SK-MES-1 cells by regulating the expression of TSPAN1, *J Biosci.*, 2013, **38**, 523.

42. S. Ferrero, G. Evangelisti, F. Barra, Current and emerging treatment options for endometriosis, *Expert Opin Pharmacother.*, 2018, **19**, 1109.

43. A.M. Quaas, E.A. Weedin, K.R. Hansen, On-label and off-label drug use in the treatment of endometriosis, *Fertil Steril*, 2015, **103**, 612.

44. F. Wieser, M. Cohen, A. Gaeddert, J. Yu, C. Burks-Wicks, S.L. Berga, R.N. Taylor, Evolution of medical treatment for endometriosis: back to the roots?, *Hum Reprod Update.*, 2007, **13**, 487.

45. A.G. Ricci, C.N. Olivares, M.A. Bilotas, J.I. Bastón, J.J. Singla, G.F. Meresman, R.I.

Barañao, Natural therapies assessment for the treatment of endometriosis, *Hum Reprod.*, 2013, **28**, 178.

View Article Online
DOI: 10.1039/D0FO00267D

46. L. Ferella, J.I. Bastón, M.A. Bilotas, J.J. Singla, A.M. González, C.N. Olivares, G.F. Meresman, Active compounds present in Rosmarinus officinalis leaves and Scutellaria baicalensis root evaluated as new therapeutic agents for endometriosis, *Reprod Biomed Online.*, 2018, **37**, 769.

47. M.D. Spuijbroek, G.A. Dunselman, P.P. Menheere, J.L. Evers, Early endometriosis invades the extracellular matrix, *Fertil Steril.*, 1992, **58**, 929.

48. C.A. Witz, S. Cho, V.E. Centonze, I.A. Montoya-Rodriguez, R.S. Schenken, Time series analysis of transmesothelial invasion by endometrial stromal and epithelial cells using three-dimensional confocal microscopy, *Fertil Steril.*, 2003, **79**, 770.

49. W. Zhang, J.H. Chen, I. Aguilera-Barrantes, C.W. Shiau, X. Sheng, L.S. Wang, G.D. Stoner, Y.W. Huang, Urolithin A suppresses the proliferation of endometrial cancer cells by mediating estrogen receptor-α-dependent gene expression, *Mol Nutr Food Res.*, 2016, **60**, 2387.

50. D. Wang, Q. Chen, B. Liu, Y. Li, Y. Tan, B. Yang, Ellagic acid inhibits proliferation and induces apoptosis in human glioblastoma cells, *Acta Cir Bras.*, 2016, **31**, 143.

51. A. González-Sarrías, J.C. Espín, F.A. Tomás-Barberán, M.T. García-Conesa, Gene expression, cell cycle arrest and MAPK signalling regulation in Caco-2 cells exposed to ellagic acid and its metabolites, urolithins, *Mol Nutr Food Res.*, 2009, **53**, 686.

52. A.L. Boehning, S.A. Essien, E.L. Underwood, P.K. Dash, D. Boehning, Cell type-dependent effects of ellagic acid on cellular metabolism, *Biomed Pharmacother.*, 2018, **106**, 411.

53. Y. Wang, F. Ren, B. Li, Z. Song, P. Chen, L. Ouyang, Ellagic acid exerts antitumor effects via the PI3K signaling pathway in endometrial cancer, *J Cancer.*, 2019, **10**, 3303.

54. M.B. Ujiki, X.Z. Ding, M.R. Salabat, D.J. Bentrem, L. Golkar, B. Milam, M.S. Talamonti, R.H. Jr. Bell, T. Iwamura, T.E. Adrian, Apigenin inhibits pancreatic cancer cell proliferation through G2/M cell cycle arrest, *Mol Cancer.*, 2006, **5**, 76.

55. R.J. Zhang, R.A. Wild, D. Medders, S.R. Gunupudi, Effects of peritoneal macrophages from patients with endometriosis on the proliferation of endometrial carcinoma cell line ECC-1,

Am J Obstet Gynecol., 1991, **165**(6 Pt 1), 1842.

View Article Online
DOI: 10.1039/D0FO00267D

56. R. Zhang, R.A. Wild, D. Medders, S.R. Gunupudi, Effects of peritoneal macrophages from patients with endometriosis on the proliferation of endometrial carcinoma cell line ECC-l, Am J Obstet Gynecol., 1991, **165**, 1842.

57. B. Mo, A.E. Vendrov, W.A. Palomino, B.R. DuPont, K.B.C. Apparao, B.A. Lessey, ECC-1 Cells: A Well-Differentiated Steroid-Responsive Endometrial Cell Line with Characteristics of Luminal Epithelium, Biology of Reproduction., 2006, **75**, 387.

58. S.S. Tabibzadeh, P.G. Statyaswaroop, P.N. Rao, Antiproliferative Effect of Interferon-7 in Human Endometrial Epithelial Cells in Vitro: Potential Local Growth Modulatory Role in Endometrium, Journal of Clinical Endocrinology and Metabolism., 1988, **67**, 131.

59. M. Annunziata, C. Grande, F. Scarlatti, F. Deltetto, E. Delpiano, M. Camanni, E. Ghigo, R. Granata, The growth hormone-releasing hormone (GHRH) antagonist JV-1-36 inhibits proliferation and survival of human ectopic endometriotic stromal cells (ESCs) and the T HESC cell line, Fertil Steril., 2010, **94**(3), 841.

60. X. Wen, Y. Xiong, L. Jin, M. Zhang, L. Huang, Y. Mao, C. Zhou, Y. Qiao, Y. Zhang, Bisphenol A Exposure Enhances Endometrial Stromal Cell Invasion and Has a Positive Association with Peritoneal Endometriosis, Reproductive Sciences., 2019, **27**, 704.

61. P.A. Klemmt, J.G. Carver, P. Koninckx, E.J. McVeigh, H.J. Mardon, Endometrial cells from women with endometriosis have increased adhesion and proliferative capacity in response to extracellular matrix components: towards a mechanistic model for endometriosis progression, *Hum Reprod*., 2007, **22**, 3139.

62. C.A. Witz, M.R. Thomas, I.A. Montoya-Rodriguez, A.S. Nair, V.E. Centonze, R.S. Schenken, Short-term culture of peritoneum explants confirms attachment of endometrium to intact peritoneal mesothelium, *Fertil Steril*. 2001, **75**, 385.

63. H. Liu, Z. Zeng, S. Wang, T. Li, E. Mastriani, Q.H. Li, H.X. Bao, Y.J. Zhou, X. Wang, Y. Liu, W. Liu, S. Hu, S. Gao, M. Yu, Y. Qi, Z. Shen, H. Wang, T. Gao, L. Dong, R.N. Johnston, S.L. Liu, Main components of pomegranate, ellagic acid and luteolin, inhibit metastasis of ovarian cancer by down-regulating MMP2 and MMP9, *Cancer Biol Ther.*, 2017, **18**, 990.

64. C. Fjaeraa, E. Nånberg, Effect of ellagic acid on proliferation, cell adhesion and apoptosis in

SH-SY5Y human neuroblastoma cells, *Biomed Pharmacother.*, 2009, **63**, 254.

View Article Online
DOI: 10.1039/D0FO00267D

65. B.A. Lessey, A.J. Castelbaum, S.W. Sawin, C.A. Buck, R. Schinnar, W. Bilker, B.L. Strom, Aberrant integrin expression in the endometrium of women with endometriosis, *J Clin Endocrinol Metab*., 1994, **79**, 643.

66. C.M. Kyama, L. Overbergh, A. Mihalyi, C. Meuleman, J.M. Mwenda, C. Mathieu, T.M. D'Hooghe, Endometrial and peritoneal expression of aromatase, cytokines, and adhesion factors in women with endometriosis, *Fertil Steril.*, 2008, **89**, 301.

67. Q. Zhao, M. Ye, W. Yang, M. Wang, M. Li, C. Gu, L. Zhao, Z. Zhang, W. Han, W. Fan, Y. Meng, Effect of Mst1 on Endometriosis Apoptosis and Migration: Role of Drp1-Related Mitochondrial Fission and Parkin-Required Mitophagy. *Cell Physiol Biochem*, 2018, **45**, 1172.

68. A. González-Sarrías, R. García-Villalba, M.A. Núñez-Sánchez, J. Tomé-Carneiro, P. Zafrilla, J. Mulero, F.A. Tomás-Barberán, J.C. Espín, Identifying the limits for ellagic acid bioavailability: A crossover pharmacokinetic study in healthy volunteers after consumption of pomegranate extracts, *J Funct Foods*., 2015, **19**, 225.

69. J. Long, Y. Guo, J. Yang, S.M. Henning, R.P. Lee, A. Rasmussen, L. Zhang, Q.Y. Lu, D. Heber, Z. Li Z,Bioavailability and bioactivity of free ellagic acid compared to pomegranate juice, *Food Funct*, 2019, **10**, 6582.

70. Z. Aytac, S. I. Kusku, E. Durgun, T. Uyar, Encapsulation of Gallic Acid/cyclodextrin Inclusion Complex in Electrospun Polylactic Acid Nanofibers: Release Behavior and Antioxidant Activity of Gallic Acid, Mater. *Sci. Eng. C.*, 2016, **63**, 231.

71. H. Wang, Y. Zhang, Zh. Tian, J. Ma, M. Kang, Ch. Ding, D. Ming, Preparation of β-CD-Ellagic Acid Microspheres and Their Effects on HepG2 Cell Proliferation, *Molecules.*, 2017, **22**, 2175.

72. Ch-Y. Qu, Min Zhou, Y-w. Chen, M-m. Chen, F. Shen, l-M. Xu, Engineering of Lipid Prodrug-Based, Hyaluronic Acid-Decorated Nanostructured Lipid Carriers Platform for 5-Fluorouracil and Cisplatin Combination Gastric Cancer Therapy, *Int J Nanomedicine.*, 2015, **10**, 3911.

73. N. Fomina, J. Sankaranarayanan, A. Almutairi, Photochemical Mechanisms of LightTriggered Release from Nanocarriers, *Adv. Drug Deliv. Rev.*, 2012, **64**, 1005.

74. W. Wu, W. Yao, X. Wang, C. Xie, J. Zhang, X. Jiang, Bioreducible heparin-based nanogel drug delivery system, *Biomaterials*, 2015, **39**, 260.

75. Y. Jeong, R.P. Yv, T. Ohno, Y. Yoshikawa, N. Shibata, S. Kato, K. Takeuchi and K. Takada, Application of Eudragit P-4135F for the delivery of ellagic acid to the rat lower small intestine, *J Pharma Pharmacol.*, 2001, **53**, 1079

76. A.M. Avachat, V.G. Patel, Self-nanoemulsifying drug delivery system of stabilized ellagic acid–phospholipid complex with improved dissolution and permeability, Saudi Pharm J., 2015, **23**, 276

77. C. Ding, H. Bi, D. Wang, M. Kang, Z. Tian, Y. Zhang, H. Wang, T. Zhu, and J. Ma, Preparation of Chitosan/Alginate-ellagic Acid Sustained-release Microspheres and their Inhibition of Preadipocyte Adipogenic Differentiation, *Curr Pharm Biotechnol*, 2019, **20**, 1213.

78. K. Sonaje, J. L. Italia, G. Sharma, V. Bhardwaj, K. Tikoo, M. N. Kumar, Development of Biodegradable Nanoparticles for Oral Delivery of Ellagic Acid and Evaluation of Their Antioxidant Efficacy against Cyclosporine A-Induced Nephrotoxicity in Rats, *Pharm Res.*, 2007, **24**, 899

79. O.M. Ali, A.A. Bekhit, S.N. Khattab, M.W. Helmy, Y.S. Abdel-Ghany, M. Teleb, A.O. Elzoghby, Synthesis of Lactoferrin Mesoporous Silica Nanoparticles for Pemetrexed/Ellagic Acid Synergistic Breast Cancer Therapy, *Colloids Surf B Biointerfaces*, 2020, **188**, 110824.

View Article Online
DOI: 10.1039/D0FO00267D

Figure captions

Figure 1: Effects of EA on cell proliferation in epithelial (A) and stromal (B) primary cell cultures from patients with Endometriosis and controls; and in ECC-1 (C) and T-HESCs (D) cells. Cell cultures were treated with EA 50 and 100 µM or vehicle (Basal group) for 24 or 48 h and cell proliferation was assessed by WST assay. N is expressed between parentheses in each bar.

Figure 2: Effect of EA on cell cycle distribution in ECC-1 (A) and T-HESCs (B) cells. Cell cultures were treated with EA 50 and 100 µM or vehicle (Basal group) for 24 or 48 h. Cell cycle was assessed by staining DNA with propidium iodide and analyzed with flow cytometer. Fluorescence-activated cell sorting (FACS) analysis of the cell cycle distribution for the Basal condition and for 50 and 100µM EA treatment for 24 h (i) and 48 h (iii). Comparison of percentage of cells in each stage of the cell cycle after EA treatment for 24 h (ii) and 48 h (iv). N=5. *P < 0.05 versus Basal.

Figure 3: Effect of EA on cell migration in ECC-1 (A) and T-HESCs (B) cells. Cell cultures were treated with EA 50 and 100 µM or vehicle (Basal group) and images were captured at 0, 5, 10 and 20 h after scratch. Quantitative analysis of the open wound was performed using Image J software and expressed as the percentage of 0 h wound for each treatment. Each point represents the mean of five experiments, performed in duplicate. *P < 0.05 and ***P < 0.001 100 µM EA versus Basal; ## P < 0.01 50 µM EA versus Basal.

Figure 4: Effect of EA on ECC-1 (A) and T-HESCs (B) cell attachment to plastic. Cell cultures were treated with EA 50 and 100 µM or vehicle (Basal group). After 24 h cells were harvested and let them to adhere to plastic for 1 h, and the number of attached cells was counted under a microscope. **P < 0.01 and ***P < 0.001 versus Basal. N is expressed between parentheses in each bar.

Table1: Characteristics of biopsies.

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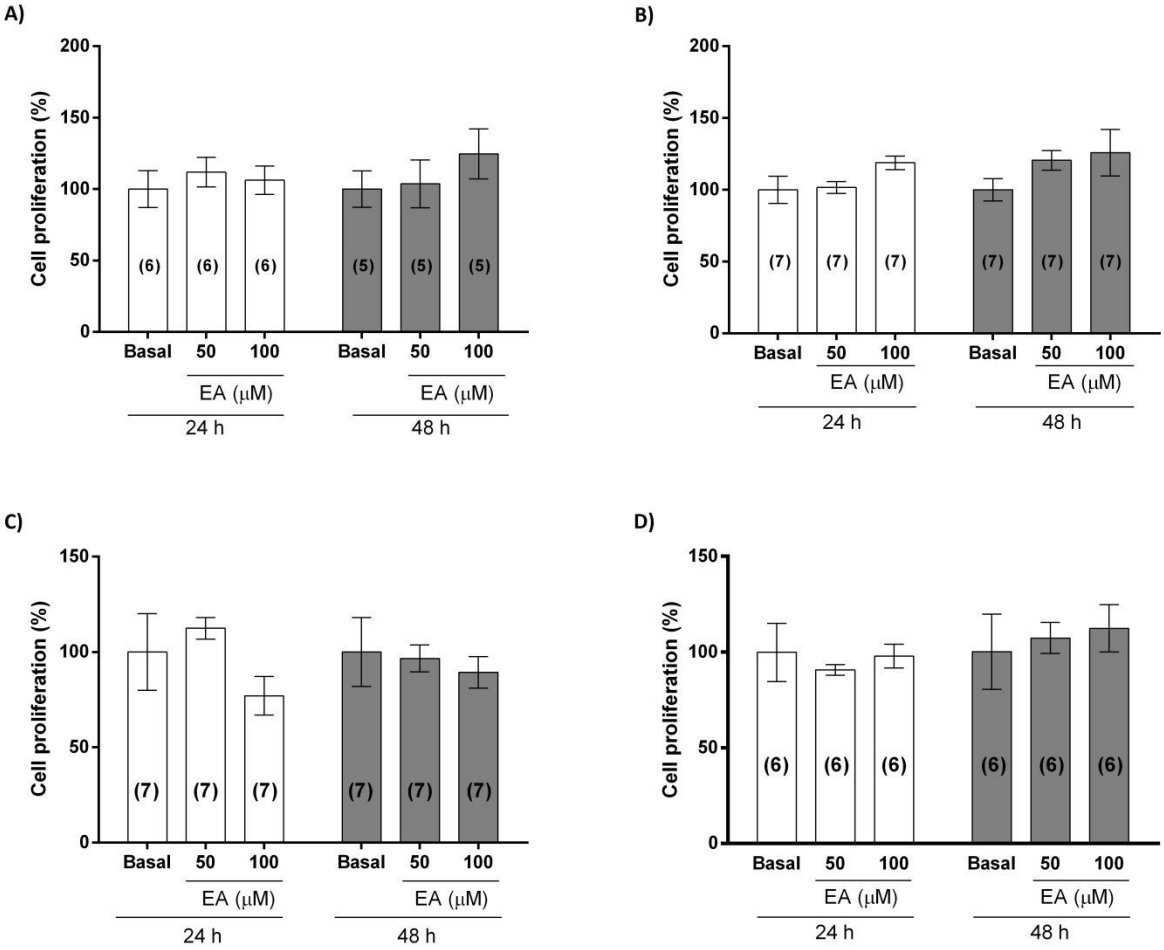
Figures

Figure 1:



574 ***Figure 2:***

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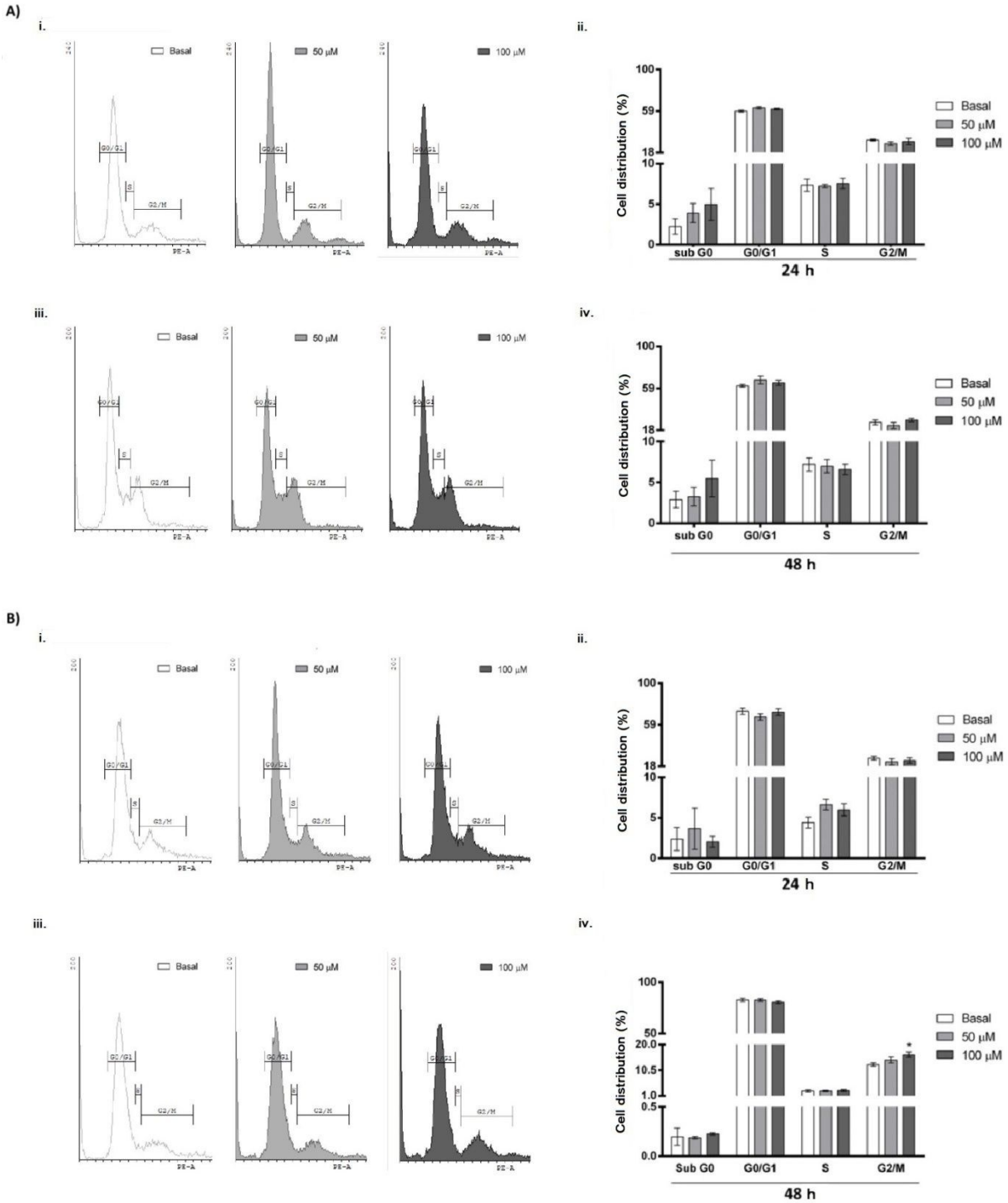

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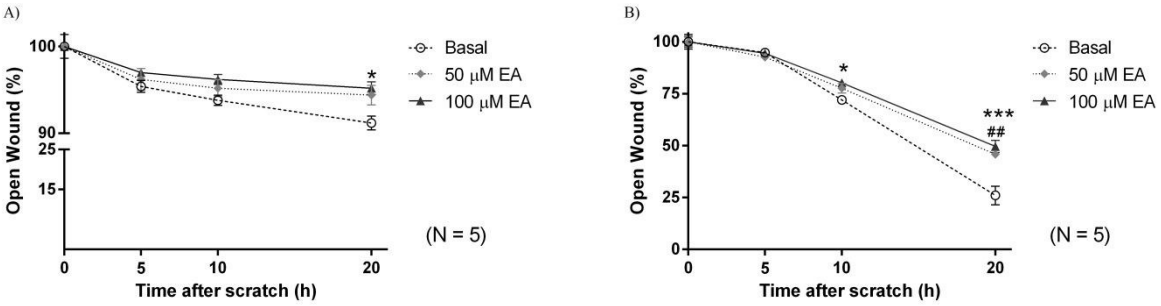

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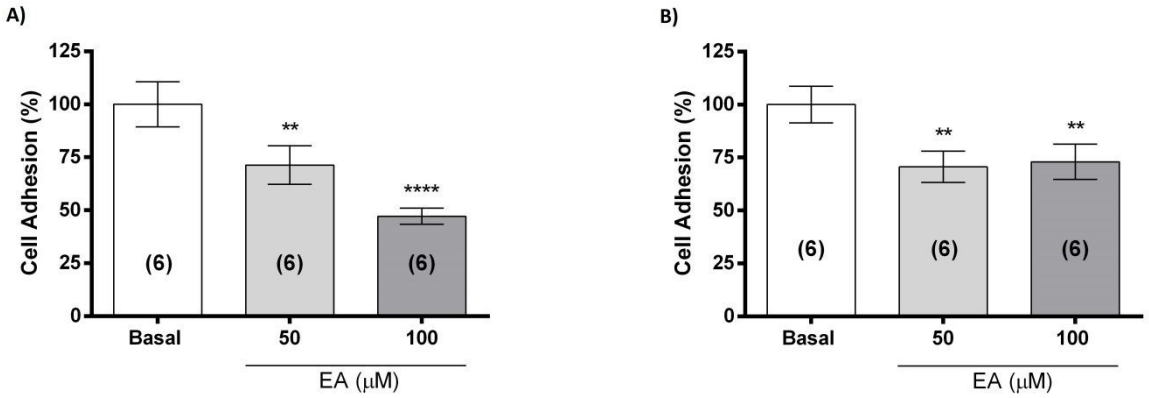
581 ***Figure 4:***



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584 **Tables**

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585 ***Table1:***

Laboratory Code	Patient age at biopsy	Endometriosis manifestations	Location of biopsy
C99a	42	EDT stage III Ovaric endometrioma	Uterus (eutopic)
C110a	28	EDT Ovaric endometrioma	Uterus (eutopic)
C113a	30	EDT stage II Ovaric endometrioma	Uterus (eutopic)
C123a	33	EDT stage II - Sterility Pouch of Douglas	Uterus (eutopic)
C126a	34	Control 2 years Sterility	Uterus (eutopic)
C149a	no data	Control	Uterus (eutopic)
C150a	40	Control	Uterus (eutopic)
C151a	26	EDT stage IV Ovaric endometrioma	Uterus (eutopic)
C152a	42	EDT stage I Bladder-Uterine	Uterus (eutopic)
C115a	40	EDT stage IV – 4 years Sterility Ovaric endometrioma	Uterus (eutopic)
C146a	36	Control	Uterus (eutopic)
C147a	40	Control Sterility	Uterus (eutopic)
C148a	34	EDT stage III Ovaric endometrioma	Uterus (eutopic)
C155a	41	EDT stage III - Sterility Ovaric endometrioma	Uterus (eutopic)
C164a	46	Control Endometrial polyp	Uterus (eutopic)

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EA treatment decreases cell adhesion and migration of endometrial cells, and alters the progression of endometrial stromal cell line cycle.

Treatment / Experimental Model	Ellagic Acid (EA) 
Stromal and Epithelial Pirmary Culture	We did not find an statistical effect of EA on cell proliferation in human endometrial cells
T-HESCs	No statistical effect on cell proliferation G2/M cell cycle arrest ↓ Migration ↓ Adhesion to plastic
ECC-1	No statistical effect on cell proliferation No statistical effect on cell cycle ↓ Migration ↓ Adhesion to plastic