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The Effect of Green Tea Extract Supplementation on Superoxide Dismutase Levels and Endometriosis Lesions: A Clinical Trial in Mice

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ABSTRACT

Endometriosis is a gynaecological disorder characterised by the presence of endometrial tissue outside the uterine cavity. It affects approximately 10-15% of females of reproductive age. Oxidative stress has been identified as a significant contributing factor to the development and progression of endometriosis. In this study, we investigated the effect of green tea extract on the endometriotic implant area and serum superoxide dismutase (SOD) levels in a rat model of endometriosis. Twenty-six female Balb/c rats were randomly divided into the experimental group (EG) and the control group (CG). All subjects in the EG received green tea extract (3 mg daily) for 14 days. On the fourteenth day, endometriosis was induced in all subjects (both EG and CG). The endometriotic implant area and serum SOD levels were assessed 14 days after induction. The endometriotic implant area in the green tea extract-treated group was significantly lower than that in the control group (p=0.022, RR=0.455, 95% CI=0.065-0.96). Serum SOD levels in the EG and CG were 5.33±0.52 ng/ml and 5.20±0.91 ng/ml, respectively (p=0.507). Green tea extract effectively inhibited the progression of endometriotic implants and increased serum SOD levels in the endometriosis-induced rat model. These findings suggest that the anti-angiogenic and antioxidant properties of green tea may have therapeutic potential in managing endometriosis.
Keyword : Green tea; endometriosis; antioxidant; superoxide dismutase

Introduction

Endometriosis is a gynaecological disorder characterised by the growth of endometrial tissue outside the uterus[1],[2]. Most cases are found in reproductive-age women, and it is estimated that 9-50% of them become infertile[3],[4]. The exact etiology of endometriosis remains unknown [3],[6]. However, it has been suggested that increased oxidative stress may promote angiogenesis and proliferation of endometriotic implant tissues in the peritoneal cavity, thus contributing to endometriosis[7],[8]. Several studies have reported a decline in total antioxidant levels, including Superoxide Dismutase (SOD), in patients with endometriosis[8],[10].

Antioxidant properties can be found in beverages such as tea, which are consumed daily. The catechins, notably Epigallocatechin gallate (EGCG) in green tea, are potential candidates for suppressing angiogenesis and appear to possess the most potent antioxidant activity among other catechins[11],[13]. EGCG suppresses E2-stimulated activation, proliferation, and VEGF expression of endometrial cells in vitro, while selectively inhibiting angiogenesis and blood perfusion of endometriotic implants in vivo [11],[14]. Currently, available treatments for endometriosis still focus on alleviating the symptoms rather than curing the

disease. Therefore, there is a need to develop more efficient and specific therapeutic alternatives to eliminate the lesions, prevent recurrence, and preserve fertility [2],[15],[16].

Regarding its content, green tea presents a potential alternative for treating endometriosis, as it is low cost, low toxicity, and widely available[11],[12],[17],[18]. Unfortunately, the interactions between green tea and endometriosis have not been extensively studied [11],[15],[19]. This study aims to investigate the effect of green tea extract on serum SOD levels and endometriotic implant area in endometriosis-induced rats.

Methods

Our animal model study has received approval from the ethics committee, and all animals were housed in a facility compliant with the National Health and Medical Research guidelines (No. 103/EC/H/FK-RSDK/II/2019).

Experimental subjects

Twenty-six female Balb/c rats aged 12 weeks and weighing between 15 and 20 grams were randomly allocated into two experimental groups of equal size (n = 13 rats/group): the control group (CG) and the experimental group (EG). All rats were provided standard chow and clean water ad libitum and kept at a controlled temperature of 20°C with a twelve-hour day-night cycle. All subjects underwent acclimatisation for seven days prior to the start of the intervention (fig. 1). The study was conducted from January to May 2019.

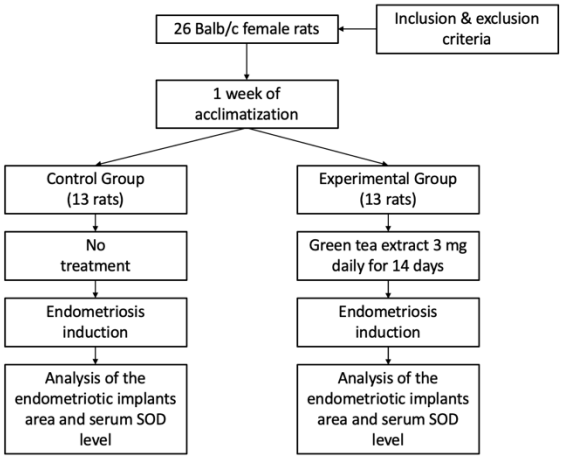

Figure 1. *The flowchart of the study*

Green tea extract

Green tea leaves are diluted in ethanol and processed into powder using the maceration method. At room temperature, five hundred grams of green tea powder were defatted in 5 litres of petroleum ether for three 1-hour sessions. After filtering the petroleum ether, the residue was macerated in methanol (1:5) for 12 hours. The results are stored in an Erlenmeyer flask. The maceration process was repeated three times until the maceration was obtained from three immersions. The macerate was evaporated to yield a gel extraction.

Green tea extract gavage

The green tea extract given to the rats was allometrically scaled from human equivalent doses of 13.3 mg/kg body weight to a rat dose of 163.99 mg/kg. Since each rat in the experimental group weighs 15-20 grams, the dose administered to the rats was 3 mg/rat/day for 14 days.

Endometriosis induction

Human endometrial tissues were obtained from a benign uterine tumour sample, washed twice with phosphate-buffered saline (PBS), and mixed as crude fragments in PBS containing Penicillin at 200 IU/ml and Streptomycin at 200 µg/ml. Each rat received an intramuscular injection of 0.2 ml of Cyclosporine and an intraperitoneal injection of 0.1 ml of endometrial tissue solution using a 16G needle on the first day. An injection of Estrogen (17β-estradiol) at 5.4 µg was administered to each rat in the experimental group on the first and fifth days.

Biochemical and Macroscopic Analysis

All rats were euthanized on the fourteenth day after inhalation anesthesia with ether. A blood sample was collected from the heart to examine the level of SOD in serum using the NWLSS NWK-SODO2 Superoxide Dismutase Activity Assay (Northwest Life Science Specialties, LLC, Vancouver, WA). The peritoneal cavity was opened to obtain the endometriotic implants, which were measured using the computerized tracing method with Motic Image® software (fig 2).

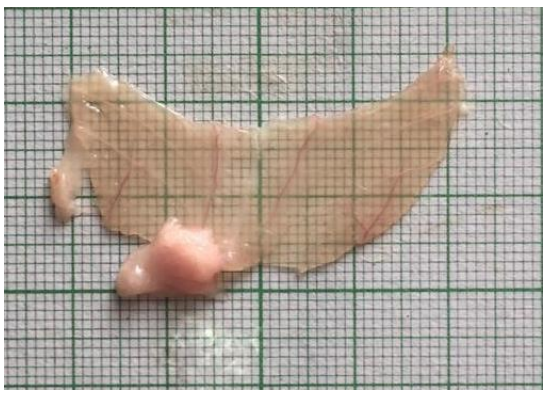

Figure 2. *Endometriotic implants in the peritoneum*

Statistical Analysis

Statistical analysis was conducted using SPSS (IBM Corporation, USA). Data were assessed

for normality with the Shapiro-Wilk test. The endometriotic implants were analysed using the Kruskal-Wallis test and the Mann-Whitney test. The SOD level in serum was evaluated with an independent t-test. Values of P<0.05 were deemed statistically significant. Data are expressed as mean ± standard deviation (SD) unless stated otherwise.

Results and Discussion

Endometriosis is a benign gynaecological condition commonly found in women. Approximately 176 million women worldwide are affected by endometriosis, with 30-40% of them at risk of becoming infertile [20],[24]. The currently available endometriosis treatments focus on symptom management rather than addressing the root cause of the disease [25],[26]. Because oxidative stress has been suggested as a potential factor in the pathophysiology of this condition, there is a need for new medical treatments that can reduce this stress and are as effective as hormonal therapies while also having acceptable side effects [25],[27]. Research indicates that green tea possesses anti-inflammatory, anti-angiogenic, anti-mitotic, and antioxidant properties in both in vivo and in vitro studies. The significant effects of tea catechins, particularly EGCG, on angiogenesis and oxidative stress have also been shown to be beneficial for cancer prevention [20],[23],[28],[29].

Thirteen female Balb/c rats in each group were generally observed to be similar and survived the experiment until the scheduled time. Our study investigated the effect of green tea extract on serum SOD levels and the development of endometriotic implants in the endometriosis rat model.

The endometriotic implant sizes in the control group (CG) and experimental group (EG) were 7.12±8.99 mm² and 2.12±6.01 mm², respectively (Fig. 3). The comparison of endometriotic implant sizes showed a statistically significant increase in the control group compared to the experimental group (p=0.022, RR=0.455, 95% CI=0.065-0.96). These results indicate that the likelihood of endometriosis implant formation in the experimental group was 0.455 lower than in the control. Furthermore, our findings suggest that green tea extract may serve as a beneficial dietary supplement in mitigating the severity of endometriosis. Future studies are needed to explore the underlying mechanisms and potential clinical applications of these results.

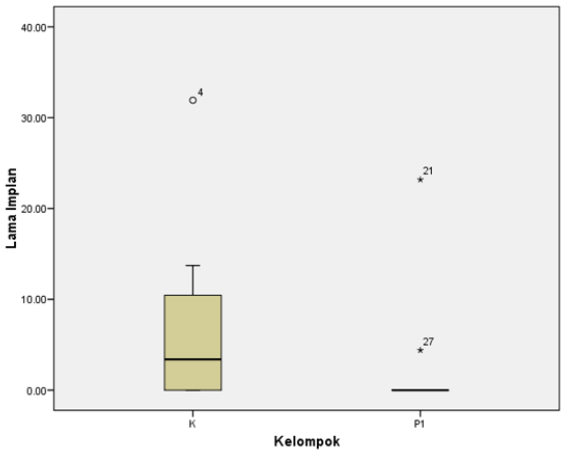

Figure 3. *Endometriotic implant area of endometriosis rat models in CG and EG.*

The results demonstrated a statistically significant difference (p=0.044) between the control group (CG) and the experimental group (EG) in the development of endometriotic implants. Among thirteen EG rats, only two (15.4%) developed an endometriotic implant, compared to eight (61.5%) rats in the control group. The proposed mechanism to explain the effect of green tea on the development of endometriotic implants involves the selective inhibition of ECGC towards Vascular Endothelial Growth Factor C (VEGFC) and VEGF receptor 2 (VEGFR2), which subsequently hinders angiogenesis and blood perfusion of endometriotic tissue implants [30].

The mean level of serum SOD in the control group (CG) and experimental group (EG) was 5.33±0.52 ng/ml and 5.20±0.91 ng/ml, respectively (fig. 4). The serum SOD level is slightly higher in the experimental group that received green tea extract. The comparison between the two groups showed no statistically significant difference in serum SOD levels (p>0.05).

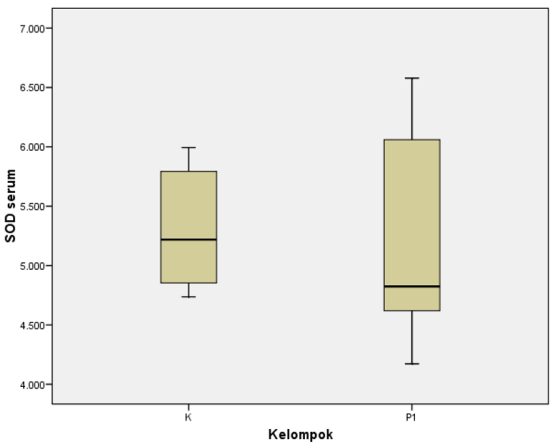

Fig 4. Serum SOD level of endometriosis rat models in CG and EG

Superoxide dismutase (SOD) is an enzyme that plays a vital role in breaking down free radicals and reactive oxygen species (ROS), thereby preventing oxidative stress. Women with endometriosis have significantly lower levels of superoxide dismutase and glutathione peroxidase in peritoneal fluid [7],[9]. Recent studies suggest a potential role for oxidative stress in stimulating inflammation and cell proliferation, thereby inhibiting the apoptosis of endometriotic cells and significantly impacting the implantation and growth of endometriotic foci. Santanam et al. employed a combination of vitamin C and vitamin E supplementation as antioxidants and observed a decrease in peritoneal markers in the group that received these antioxidants [8],[30],[31]. In this study, the level of serum SOD in the experimental group was slightly higher, but there was no statistically significant difference ($p>0.05$) between the two groups.

The type of biospecimen (serum versus peritoneal fluid) may result in potential limitations in this study. The formation of oxidative stress in peritoneal fluid is initiated by inflammatory cells. At the same time, cellular debris serves as a substrate, and the products of this process are subsequently exported to serum, where the oxidised metabolites are incorporated into carriers. Murphy et al. reported that the level of vitamin E in peritoneal fluid is significantly lower than in plasma, suggesting that the peritoneal cavity provides less antioxidant protection than serum. Consequently, peritoneal fluid may be more vulnerable to oxidative stress than serum. In this study, we measured SOD as a marker of oxidative stress in serum instead of peritoneal fluid; our results may have been biased towards the null.

Moreover, serum levels of oxidative stress may be influenced by other factors, such as endometriosis. Additionally, measurements in the peritoneal fluid could provide a more localized assessment of SOD related to endometriosis[7–9,14,31,32]. The limited sample size and short duration of the investigation in this study may impact the results. Future long-term studies with larger sample sizes are needed to confirm the safety and efficacy of green tea as a preventive and alternative treatment for endometriosis.

Conclusion

Green tea extract administration reduced the area of endometriotic implants in rat models of endometriosis by increasing serum superoxide dismutase (SOD) levels; the likelihood of endometriosis implant formation was 0.455 times lower in the experimental group compared to the control group. Further scientific studies are required to investigate the antioxidant effects of green tea extract on the growth and expansion of endometriosis tissue in humans, which could serve as an additional therapy for endometriosis.

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