

ORIGINAL ARTICLE:**Green tea extract reduces insulin level and folliculogenesis in insulin-resistant PCOS rats model****Irma Maya Puspita^{1*}, Budi Santoso², Budi Utomo³**¹Faculty of Health Sciences, Muhammadiyah University, Surabaya, Indonesia, ²Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, ³Department of Public Health and Preventive Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia**ABSTRACT**

Objectives: Polycystic ovary syndrome (PCOS) is an endocrine disorder with still unknown pathophysiology. Insulin resistance is one of major factors that affect the pathogenesis of PCOS (69%). Green tea has compounds that can be used to improve the condition of insulin resistance, so that folliculogenesis may recur in the case of PCOS.

Materials and Methods: The subjects in this study were rats which were divided into 5 groups, consisting of 2 groups serving as a control group (negative and positive) and 3 groups as the treatment group. Each group consisted of 7 experimental animals. Negative control group was given with distilled water for 14 days. Positive control group was given with injections of testosterone propionate 1 mg/100 g BW intramuscularly in the thigh for 28 days to obtain a model of insulin-resistant PCOS and the distilled water was used as therapy. The treatment groups were given with injections of testosterone propionate 1 mg/100gBW intramuscularly for 28 days and then treated with green tea extract in doses of each 200 mg/kg, 400 mg/kg, and 800 mg/kg for 14 days. Rats were dissected on day 42 for harvesting the right and left ovary and heart blood sample was taken for examining insulin levels.

Results: The results showed that administration of green tea extract on K5 can reduce insulin levels and increase folliculogenesis significantly (significance $p < 0.05$).

Conclusion: Green tea extract can reduce insulin levels and increase folliculogenesis in rats. PCOS - Insulin resistance at a dose of 800 mg/kg.

Keywords: green tea; insulin levels; folliculogenesis; polycystic ovary syndrome

ABSTRAK

Tujuan: Sindroma ovarium Polistik (SOPK) merupakan kelainan endokrin dengan patofisiologi masih belum diketahui secara jelas. Resistensi insulin merupakan salah satu yang terbesar dalam mempengaruhi patogenesis SOPK (69%). Teh hijau memiliki kandungan yang dapat digunakan untuk memperbaiki kondisi resistensi insulin sehingga folikulogenesis dapat terjadi kembali pada kasus SOPK.

Bahan dan Metode: Subjek penelitian menggunakan tikus yang dibagi dalam 5 kelompok terdiri dari 2 kelompok sebagai kelompok kontrol (negatif dan positif) dan 3 kelompok sebagai kelompok perlakuan. Setiap kelompok terdiri atas 7 hewan coba. Kelompok kontrol negatif diberikan aquades selama 14 hari. Kelompok kontrol positif diberikan injeksi testosteron propionat 1 mg/100grBB secara intra muskular di paha selama 28 hari untuk mendapatkan model SOPK – resistensi insulin dan aquades sebagai terapinya. Kelompok perlakuan diberikan injeksi testosteron propionat 1 mg/100gBB secara intra muskular selama 28 hari agar didapatkan model SOPK – resistensi insulin kemudian diterapi dengan ekstrak teh hijau dengan masing-masing dosis 200 mg/kgBB, 400 mg/kgBB, 800 mg/kgBB selama 14 hari. Tikus dibedah pada hari ke-42 untuk pengambilan organ ovarium kanan dan kiri dan pengambilan darah jantung untuk pemeriksaan kadar insulin.

Hasil: Hasil menunjukkan bahwa pemberian ekstrak teh hijau pada K5 dapat menurunkan kadar insulin dan meningkatkan folikulogenesis secara signifikan (signifikansi $p < 0,05$).

Simpulan: ekstrak teh hijau mampu menurunkan kadar insulin dan meningkatkan folikulogenesis pada tikus SOPK – Resistensi Insulin pada dosis 800 mg/kgBB.

Kata kunci: green tea; insulin levels; folliculogenesis; polycystic ovary syndrome

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INTRODUCTION

In Indonesia, infertility in women is found as much as 15% at the age of 30-34 years, increases of to 30% at the age of 35-39 years, and 55% at the age of 40-44 years. The survey results about pregnancy failure in couples who have been married for 12 months show that 40% is due to infertility in males, 40% due to infertility in females, 10% of males and females, and 10% of unknown causes.¹ One of the most common cases of infertility in the age group of infertile women is Polycystic Ovary Syndrome (PCOS).² Polycystic ovary syndrome is the most common endocrine disorder that occurs in women, comprising 6-10% of the cases based on the criteria of the US National Institutes of Health (NIH).³

Polycystic ovary syndrome (PCOS) is a reproductive endocrinological problem that often occurs and is still a matter of controversy. The clinical symptoms vary, but usually include oligo-ovulation or anovulation, hyperandrogenism (both clinical or biochemical), and the presence of polycystic ovaries. PCOS is not only the most common cause of ovulatory disorders and hirsutism, but is also associated with metabolic disorders that have an important influence on women's health.^{3,4}

The exact etiology and pathogenesis of this disorder are unclear. However, several subsequent studies agree that this disorder is genetically influenced through autosomal dominant mode of inheritance (especially from paternal origin). Pathogenesis of PCOS includes abnormal gonadotropin secretion, abnormal steroidogenesis, insulin resistance, p450 c 17 dysregulation, and genetics. Insulin resistance is one of the greatest factor in influencing pathogenesis of PCOS (69%).^{4,5}

Insulin resistance is defined as the body's inability to adapt to normal glucose intake or the inability of insulin to produce adequate physiological metabolic effect for the body. This results in the body's insulin requirement increase so that it becomes hyperinsulinemia to maintain plasma glucose levels to remain within normal limits.⁶ Insulin is equivalent to Insulin-Like Growth Factor (IGF). Insulin and IGF-1 are responsible for interfering with ovulation. Insulin works directly, both alone and together with LH, in increasing androgen production in the ovaries. Insulin can also indirectly increase androgen levels by reducing the production of SHBG (Sex Hormone Binding Globulin) in the liver and IGFBP-1 (Insulin Like Growth Factor Binding Protein-1) and, thus, increasing free testosterone and IGF-I, IGF-II levels.

This excess androgen disrupts folliculogenesis, causing menstrual disorders and the development of several cysts in the ovary. Androgen causes insulin resistance by reducing the amount and effectiveness of glucose-carrying proteins, specifically glucose transporter type 4 (GLUT-4) which is responsible for the transport of glucose in muscles and fat.^{7,8}

Herbal therapy is one issue in alternative medicines that is widely developing in the community. One of the plants used in traditional medicine is green tea. Tea is one of the most popular drinks consumed by two-thirds of the world's population. Health practitioners use green tea for stimulants, diuretics, astringents, and to improve heart health. Other traditional treatments use green tea to treat flatulence, regulate body temperature, lower blood sugar levels, help digestion and improve mind processes.⁹

The largest content in the buds of green tea leaves is catechins or Epigallocatechin-3-Gallate (EGCG) which is a polyphenol compound that has 15 carbon atoms in its core which are arranged in a C6-C3-C6 configuration. Consumption of green tea Epigallocatechin Gallate (EGCG) is reported to have many benefits in efforts to improve health, such as fat burning, preventing obesity and insulin sensitivity. Green tea (*Camelia sinensis*) can be developed as a potential therapeutic agent for obesity and insulin resistance. It is expected that EGCG action can inhibit insulin resistance by inhibiting the decrease in PI3K and inhibiting the increase in MAPK that results in an increase in GLUT-4 translocation.^{11,12}

A previous study¹¹ aimed at evaluating the effect of green tea extract on serum glucose levels showed that green tea extract at a dose of 200 mg/kgBW was significantly able to reduce serum glucose levels. Green tea administration is expected to reduce insulin levels in blood so that it is followed by a decrease in androgens. Decreased androgen level allows the process of aromatization from the hormone androgen into estrogen, so that folliculogenesis (the number of primary, secondary, tertiary, and de Graaf follicles) can occur again.

The purpose of this study was to examine the effect of various doses of green tea extract on insulin levels and folliculogenesis (primary, secondary, tertiary and de Graaf follicles) of insulin resistant PCOS rats.

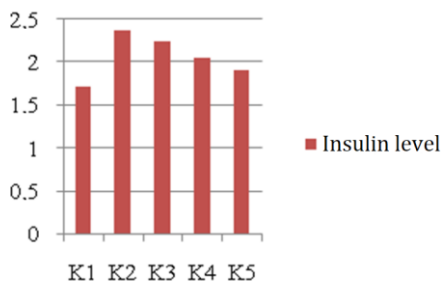
MATERIALS AND METHODS

This study was a true experimental study using complete random design. The samples in this study

were female white rats aged 3 months with a body weight of 100-110 grams, healthy, and not pregnant. The sample size was 7 rats in each group so that the total sample amounted to 35 rats. This study was conducted at the Experimental Animal Cage Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga. The study was conducted in May - July 2015. Data were analyzed with descriptive analysis to determine the frequency distribution and analytic analysis to determine differences between variables using Kruskal-Wallis test. Significant results of Kruskal-Wallis test were followed by a post-Kruskal-Wallis/post-hoc test to determine the most effective dose.

RESULTS AND DISCUSSION

The results of insulin levels measurement using ELISA examination in intracardial of *Rattus norvegicus* rats in control and treatment groups can be seen in Figure 1.



K1 : control (-)
 K2 : control (+)
 K3 : treatment 1
 K4 : treatment 2
 K5 : treatment 3

Figure 1. Average insulin levels

The average insulin levels in Figure 1 shows that the administration of green tea extract of 200 mg/kgBW, 400 mg/kgBW, and 800mg/kgBW 1x/day for 14 days in insulin-resistant PCOS rats can reduce average insulin levels compared to that in K2 (positive control) group, especially the administration of green tea extract of 800mg/kg 1x/day for 14 days. These results indicate that the administration of green tea extract can reduce average insulin levels in SOPK-insulin resistance mice.

Data on insulin levels were not normally distributed and were not homogeneous, so the analytical test used was the Kruskal-Wallis non-parametric test as a variance analysis test. The test results showed that insulin levels had p values of <0.05, indicating that there were significant differences in insulin levels between groups (K1, K2, K3, K4, K5).

Table 1. Bonferroni post-hoc test results of insulin levels

Groups	K ₁	K ₂	K ₃	K ₄
K ₂	0,006*	-	-	-
K ₃	0,006*	1,000	-	-
K ₄	0,048*	0,550	1,000	-
K ₅	0,069	0,023*	0,069	1,000

*Significantly different (significance p <0.05)

The Bonferroni test as a post-hoc test can be seen in Table 2. The average number of follicles (primary, secondary, tertiary, and de Graaf) in both ovaries of each rat in each group K1, K2, K3, K4, K5 can be seen in Figure 2

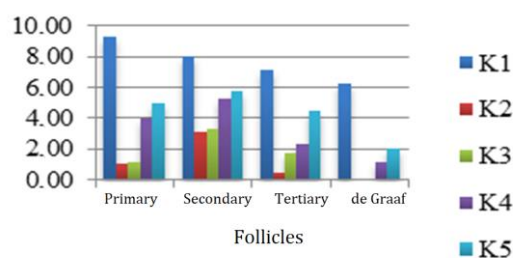


Figure 2. Average number of follicles in each group

The average number of follicles (primary, secondary, tertiary, and de Graaf) in Figure 2 shows that the administration of green tea extract of 200 mg/kgBW 1x/day, 400 mg/kgBW 1x/day, and 800 mg/kgBW 1x/day for 14 days in insulin-resistant PCOS rats model can increase the average number of follicles (primary, secondary, tertiary, and de Graaf) compared to those in positive control group (K2). This shows that the administration of green tea extract in insulin-resistant PCOS rats can increase the average number of follicles (primary, secondary, tertiary, and de Graaf).

Table 2. Kruskal-Wallis test of primordial, primary, secondary, tertiary, and de Graaf follicles

Variables	P values
Primary follicles	0.000*
Secondary follicles	0.000*
Tertiary follicles	0.000*
de Graaf follicles	0.000*

*significantly different (significance p <0.05)

Table 2 shows that the folliculogenesis variable has a significance value of p <0.05, which shows a significant difference in folliculogenesis between groups (K1, K2, K3, K4, and K5). Bonferroni's post-hoc test was carried out after the Kruskal-Wallis test which aimed to determine folliculogenesis between different groups.

Bonferroni's post-hoc test results can be seen in Tables 3 to Table 6.

Table 3. Results of post-hoc Bonferroni primary follicle test

Groups	K ₁	K ₂	K ₃	K ₄
K ₂	0.019*	-	-	-
K ₃	0.019*	1.00	-	-
K ₄	0.020*	0.042*	0.059	-
K ₅	0.019*	0.019*	0.019*	1.000

*significantly different (significance p <0.05)

Table 4. Results of post-hoc Bonferroni secondary follicle test

Groups	K ₁	K ₂	K ₃	K ₄
K ₂	0.020*	-	-	-
K ₃	0.020*	1.000	-	-
K ₄	0.281	1.000	0.757	-
K ₅	0.229	0.090	0.051	1.000

*significantly different (significance p <0.05)

Table 5. Results of post-hoc Bonferroni tertiary follicle test

Groups	K ₁	K ₂	K ₃	K ₄
K ₂	0.018*	-	-	-
K ₃	0.019*	0.083	-	-
K ₄	0.018*	0.029*	1.000	-
K ₅	0.270	0.017*	0.028*	0.105

*significantly different (significance p <0.05)

Table 6. Results of post-hoc Bonferroni de Graaf follicle test

Groups	K ₁	K ₂	K ₃	K ₄
K ₂	0.009*	-	-	-
K ₃	0.009*	-	-	-
K ₄	0.018*	0.108	0.108	-
K ₅	0.018*	0.009*	0.009*	1.000

*significantly different (significance p <0.05)

A previous study,¹² aimed at evaluating the effects of green tea extract on serum glucose levels, showed that green tea extract at a dose of 200 mg/kgBW was significantly able to reduce serum glucose levels. The results of Haidari's study and this study showed a result that green tea extract significantly reduced blood glucose and insulin levels.

In another study,¹⁰ Epigallocatechin gallate (EGCG), which is the result of the isolation and purification of green tea, was administered with the aim of proving the effect of these substances in inhibiting insulin resistance in mice. The administration was carried out in a dose of 8 mg/kgBW/day for 60 days and showed results that EGCG isolates were able to inhibit insulin resistance as indicated by the inhibition of an increase in p38 MAPK and a decrease in p110 PI3K.

Green tea extract of 800 mg/kg for 14 days in insulin-resistant PCOS rats can significantly reduce insulin levels and increase the average number of primordial follicles, primary follicles, secondary follicles, tertiary follicles, and de Graaf follicles significantly. Decreased insulin resistance is thought to improve the condition so that the follicle is stimulated again by FSH and not depressed by LH. The decrease in insulin is thought to occur due to catechin which is the main flavonol, which mainly consists of Epigallocatechin gallate (EGCG), Epigallocatechin (EGC), Epicatechin gallate (ECG), and Epicatechin (EC), which can be absorbed by cells through GLUT-4 as the biggest effector of homeostasis glycaemic insulin mediated expressed in adipocyte cells and muscle cells. The action of EGCG is expected to inhibit insulin resistance by inhibiting the decrease in PI3K and inhibiting the increase in MAPK so that GLUT-4 translocation increases.

CONCLUSION

Green tea extract can reduce insulin levels and increase folliculogenesis in insulin resistant PCOS in a dose of 800 mg/kgBW.

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