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RESEARCH ARTICLE

Letrozole and HFD_HG on Insulin and GDP

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ABSTRACT

The pathogenesis of Polycystic ovary syndrome (PCOS) involves several mechanisms, that include the disorders of carbohydrate and lipid metabolism, oxidative stress, and insulin resistance. That condition makes PCOS have a great impact on long-term health problems such as cardiovascular disease, atherosclerosis, and diabetes. What is the potential for increased diabetic in a PCOS model mouse? This study aims to determine the potential for diabetes cases in PCOS patients through observations in PCOS model mice. Research method: using Postest Only Control Group Design method. Samples included 24 female, 3 months aged rats Rattus norvegicus strain Wistar and weighing around 100–130 grams. After observing the estrous cycle, 16 rats were randomly selected to make a model PCOS with letrozol induction 1 mg/kg BW/day orally in combination with a high-fat high-fructose diet. The treatments were given for 18, 21, 24 and 27 days. Furthermore, after fasting for 12 hours, fasting sugar levels and insulin levels were measured using the ELISA method. The results were analyzed by One Way Anova test with a significance level of $\alpha = 0.05$. Data analysis was conducted with the SPSS program version 24.0 for Windows. Results: There was no significant difference in fasting insulin levels between the control group and the PCOS group ($p > 0.05$), but fasting blood sugar levels increased significantly ($p < 0.05$). Conclusion: There was an increase in the diabetic potency of PCOS mice-induced by the combination of testosterone propionate and a high-fat, high-fructose diet even when insulin levels are not significantly increased.

Keywords: Blood Sugar, High fat high fructose diet, Insulin, Letrozole.

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1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder with a global prevalence of 5%–10% and is an important cause of chronic anovulation in young women. PCOS accounts for approximately three-quarters of all cases of anovulatory infertility [1]. Several studies have reported an increase in prevalence recently due to a high-calorie and high-carbohydrate diet, lack of exercise, and a tendency to increase obesity which further increases insulin resistance [2]–[5]. PCOS is characterized by menstrual irregularities, and signs of hyperandrogenism such as acne, excess body hair, male pattern baldness, and infertility. In addition, PCOS is linked to many long-term health problems such as cardiovascular disease and diabetes. PCOS has wide-ranging health implications including adverse metabolic (obesity, type two diabetes, cardiovascular disease), reproductive (infertility, miscarriage, pregnancy, and neonatal complications), and psychological risks (anxiety, depression, and stress). The etiology of PCOS is not clearly understood, but lipid imbalance, oxidative stress, insulin resistance, and genetics are some of the contributing factors [6].

Several studies have proven that insulin resistance and compensatory hyperinsulinemia play a role in the pathogenesis of Polycystic Ovary Syndrome (PCOS). Women with PCOS have an increased rate of insulin resistance compared to controls, with absolute insulin resistance rates as high as 65%–75% in normal weight women and 95% in obese women. Insulin resistance as well as hyperandrogenism in women with PCOS have been implicated in dysfunction of the hypothalamic-pituitary-ovarian axis, leading to anovulation and menstrual irregularities [1]. Impaired insulin action on peripheral target tissues and inhibition of insulin secretion is thought to play a role in the mechanism of insulin resistance. Insulin-sensitizing agents, such as metformin and thiazolidinediones, have long been used in the treatment of PCOS. These agents have been shown to significantly reduce insulin resistance and androgen levels as well as several inflammatory markers, improve menstrual regularity and improve ovulatory

function in women with PCOS. However, thiazolidinediones have some safety concerns while metformin is often not tolerated due to side effects of nausea (61%), vomiting (30%), and diarrhea (65%) [1].

More research on the pathophysiology and management of PCOS still needs to be done. Research related to the discovery of new drugs for PCOS cases is important to do. Many studies must be carried out first on animals before being tested on humans so research is needed regarding the development of representative experimental animal models, especially with simple techniques, with low costs, and in a short time. Many studies have been conducted to create a mouse model of polycystic ovary syndrome with the advantages and disadvantages of each. In Indonesia alone, there is only one institution that has succeeded in making PCOS-Insulin-Resistant mice, but the PCOS model itself has never been done [7]. In this study, an aromatase inhibitor, letrozole, was used in combination with a high-fat diet and a high-glucose diet. As parameters for the occurrence of insulin resistance, insulin levels, and fasting blood sugar levels were measured. What is the effect of letrozole and a high-fat, high-glucose diet on insulin levels and blood glucose levels in Wistar rats? This research is important to develop a PCOS-Insulin Resistance mouse model as a support in research to find natural insulin sensitizer ingredients that are commonly found in Indonesia and have the potential for PCOS therapy.

2. Materials and Method

25 female Rattus norvegicus Wistar strains were used, aged 3 months and weighing 100–130 grams. Before the study began, an adaptation period was carried out for a week. Used female rats in healthy condition, in normal behavior and normal vaginal swab results, and are not pregnant and have no anatomical abnormalities. All mice were kept in the same environment in plastic cages measuring 40 cm × 30 cm × 10 cm covered with woven wire. Mice were kept under the same conditions of feed, water, and lighting. On day 8, 25 animals were randomly selected for the treatment group. Next, the groups were divided randomly as follows (n = 5 mice):

1. Normal Group
2. Letrozol + High Fat High Glucose orally for 18 days group
3. Letrozol + High Fat High Glucose orally for 21 days group,
4. Letrozol + High Fat High Glucose orally for 24 days group,
5. Letrozol + High Fat High Glucose orally for 27 days group,

Starting on day 8, letrozole treatment in 0.5% CMC was given at a dose of 1 mg/kbBW intragastrically combined with ad libitum administration of a high-fat and high-glucose diet. After treatment, vaginal smear tests were carried out every day, evaluated by Giemsa staining. PCOS is established when there is a series of changes in the estrus cycle until it reaches the stage of persistent vaginal cornification. After treatment according to groups, mice were allowed to fast for 12 hours, then anesthetized under mild ether anesthesia, and blood was collected from the retro-orbital venous plexus of the eye using a heparinized capillary tube. Blood samples were collected in tubes for serum separation and then centrifuged for 10 minutes. Serum was separated and stored at −20 °C and ready for fasting blood insulin levels by ELISA method. Blood glucose levels were determined by the glucose oxidase biosensor method, using the "One Touch Ultra" tool. Blood was taken from the tail of the rat, by cleaning the tail of the rat and then massaged or massaged slowly, then pierced the tip with a needle (lancet). The blood that comes out is then attached to the glucometer strip. Blood glucose levels will be measured and appear on the glucometer screen after 5 seconds, expressed in mg/dl [7], [8].

3. Result

This study used 5 groups of rats with different treatments on the duration of exposure to letrozole and a high fat and glucose diet orally. The results of measuring fasting blood sugar levels and fasting insulin levels are shown in Table I.

The average control fasting blood sugar level was 107.00 while in the letrozole group and the high-fat diet, the average was 169.00. Regression analysis using One Way Anova found there was a significant relationship ($p < 0.05$) on the average fasting blood sugar level, while on the average fasting insulin level there was no significant relationship ($p > 0.05$).

4. Discussion

It is addressed that PCOS is a heterogeneous gynaecological syndrome associated with a wide range of endocrine and metabolic abnormalities, including hyperinsulinaemia, hyperglycaemia, glucose intolerance, dyslipidaemia, and obesity, which are regarded as the hallmark components of metabolic syndrome (MetS) [8]. Insulin resistance (IR) is considered the common cause of other aberrations in this disorder which affects the long-term health of PCOS patients. The present study aimed to investigate the associations of Letrozol induced PCOS rats with the marker of insulince resistance (fasting plasma glucose and fasting insulin level).

The mean of fasting plasma glucose was higher in PCOS group compared to the control group ($p < 0,05$). This finding is similar to the previous larger study with 400 subjects. 14.3% of PCOS women had elevated Fasting plasma glucose. This study also found that glucose intolerance has even higher result (24%) [9]. The pathophysiology of PCOS is largely unknown but has been attributed to

TABLE I: Result

Group	Fasting Glucose Level	Fasting Insulin Level
	Mean ± Std Deviation	
Control	107,0000 ± 14,00000	1,7060 ± 0,014502
L+HFHGD 27 days	169,0000 ± 132,28002	1,7998 ± 0,08257
L+HFHGD 24 days	138,0000 ± 10,21029	1,8350 ± 0,07208
L+HFHGD 21 days	154,2500 ± 1,25831	1,7860 ± 0,11025
L+HFHG D 18 days	110,7500 ± 24,08838	1,7885 ± 0,07889

defects in various organ systems. Uncontrolled ovarian steroidogenesis with a thickened thecal layer that secretes excessive androgen is thought to be a primary abnormality of PCOS. PCOS is combined with defects in insulin action and insulin resistance (IR) finally leading to diabetes, and it also displays neuroendocrine dysfunction with exaggerated LH pulsatility, and altered production of adrenal androgen [5], [10]. Once a diagnosis of PCOS is confirmed, it is imperative to assess the woman for diabetes mellitus (DM) risk factors.

Fasting plasma insulin levels were also evaluated. The insulin levels were increased in PCOS groups, but there was no significant difference between the groups. These findings were similar to the study conducted by Forrester-Dumont *et al*. [11] that there were no statistical differences found in Diastolic Blood Pressure, fasting blood glucose, lipid panel, LH, FSH, or Prolactin based on hyperandrogenisme in a patient with PCOS. The difference with our study is that the author used testosterone levels for grouping the PCOS model. Almost similar results were also found by several studies [12], concluding that hyperinsulinemia and Insulin resistance are seen only in obese women with PCOS. Our study did not evaluate the Body Mass Index as the study criteria, so this might interfere with the result. These results are contrary provided by another study [13] in which andronate and High Fat Diet, Fasting Blood glucose levels were similar and not significantly different, but The HOMA-IR was markedly elevated. Our study reveals opposite findings. The difference between that study and our study was a longer study duration–8 weeks. This study duration might not be long enough to demonstrate the increase in insulin levels. Up to 70% of women with PCOS are also insulin resistant (IR), and the prevalence of DM in women with PCOS is 10%. An increased level of Fasting blood Glucose may show a disturbance of glucose metabolism, which leads to glucose intolerance problems, but there has been no increase in the fasting insulin plasma yet. As the result of the study that was conducted to analyze fasting plasma glucose and peak fasting insulin level between newly onset diabetes mellitus and known diabetes mellitus [9], [13], concluded that fasting plasma glucose was lower in newly onset diabetes, and peak fasting insulin was also lower in newly onset diabetes. This may explain why the study duration might have impacted this study result.

A study [14] showed that metabolic syndrome and its components are common in PCOS, especially among women with the highest BMIs and insulin levels. While obesity is regarded as one of the putative factors leading to MetS, IR seems to contribute mainly to the link between PCOS and MetS. In addition, accumulating evidence indicates that women with MetS also exhibit hyperandrogenism, a well-established contributor to PCOS aetiology [15]. Androgen in excess appears to affect independently, which further exacerbates the cardiometabolic aberrations in PCOS women [16].

5. Conclusion

Based on the results, Fasting blood glucose was significantly greater in Letrozol and HFD induced PCOS rats compared to control (107,0000 ± 14,00 vs. 169,0000 ± 132,28, $p < 0,05$). On the opposite, although there is an increase in insulin levels in the experiment group, there is no statistical difference in fasting plasma insulin levels between the two groups.

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Conflict of Interest

Authors declare no conflicts of interest.

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