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The Effect of 96% Ethanol Extract of Moringa Leaf (*Moringa oleifera*) on Insulin Level in Wistar Rats Model(*Rattus norvegicus*) with PCOS (Polycystic Ovarian Syndrome)

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A B S T R A C T

Purpose: Polycystic Ovary Syndrome (PCOS) is a complex endocrine disorder which cause hyperandrogenism, anovulation, and also polycystic ovaries. The effect of PCOS can reach several organs such as cardiovascular, immune system, reproductive, metabolic, and psychological. This study investigates the effect of moringa leaves on insulin level within rats model with PCOS. ***Methods***: Data were collected through Post Test Only Control Group Design with Wistar rats (*Rattus norvegicus*) model from the insulin level in the body. The rats are injected with testosterone and then given Moringa leaf extract. ***Results***: The analysis revealed that the PCOS rats have an increased insulin levels than the normal rats and there isa significant decrease in insulin levels in rats given Moringa leaf extract with the PCOS rats. Normal rats and rats given Moringa leaf extract have almost the same Insulin level. ***Conclusions***: These findings suggest that Moringa leaf extract decreased the insulin levels with PCOS rats. Further research needed to find more about the impact of Moringa leaf extract on rats insulin levels.

Keywords: Moringa oleifera, Insulin, PCOS, Effect.

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is a complex, common and heterogeneous endocrine disorder that characterized by menstrual irregularities, infertility, and excessive androgen levels. It often has long-term metabolic implications, ranking as most common metabolic disorder and also leading cause of female infertility (Vassalou*et al*., 2019). Key symptoms include anovulation, hyperandrogenism, and polycystic ovaries, accompanied by additional characteristics such as hormonal imbalances, insulin resistance, and various metabolic complications (Deswal *et al*., 2020). Polycystic Ovarian Syndrome (PCOS) can be diagnosed if there is two out of three criteria: Oligo-Anovulation, Hiperandrogenism, Policystic Ovaries(≥ 12 folicle with diameter 2-9 mm or ovarian volume > 10mL) (Smet *et al*., 2018). Environment and heredity play an important role in PCOS. The effect of PCOS can reach several organs such as cardiovascular, immune system, reproductive, metabolic, and psychological (Deswal *et al*., 2020). Various factors can

cause this disorder, ranging from intrinsic factors to extrinsic factors. Such as epigenetic changes, lifestyle, obesity, insulin resistance, and inflammation (Sadeghi *et al*., 2022).

One study said, in patients with PCOS, there was an increase in insulin resistance than in patients without PCOS (Dahan *et al*., 2019). Insulin resistance is a compensatory mechanism that increases plasma insulin levels as a metabolic response or basal condition to regulate glycemic values to normal (Genazzani*et al*., 2019).

According to Deswal *et al* (2020) the prevalence of PCOS in South China, Iran, USA reached 2.2%,3% and 4%. While in Beijing, Palestine, Brazil, Sri Lanka it reached around 5-10%. The highest prevalence of PCOS are in Australia, Turkey, and Denmark, which was 15-20%5%-10%.

Rats with PCOS models have similar clinical and metabolic manifestation to women with PCOS, including changes in the estrous cycle, ovarian cysts, hyperandrogenic, weight gain, and glucose intolerance (Noroozzadeh*et al*., 2019).

Moringa has a lot of pharmacological activities such as anti inflammatory, analgesic, antibiotic, neuroprotective, and cytotoxic. Moringa extract also has strong antioxidant activity, so it can prevent and protect pancreatic cells from oxidative stress caused by hyperglycemia (Siahaan *et al*., 2022).

Polycystic Ovary Syndrome is a complex endocrine disorder, so there is still a lot of research that needs to be done to understand this disease. Even in Indonesia itself, it is still rarely studied and most people are still unfamiliar with PCOS and it symptoms. The pathophysiology of PCOS is really complex and unclear, so this study is expected to help explain the effects of Moringa leaves on PCOS.

Research Methods/Methodology

Collection of Plant: Moringa oleifera leaves are obtained from PT. Moringa Organik Indonesia from central java.

Extraction Procedure

Moringa leaves calibrated on watch glass, then the porcelain cup and ointment pot calibrated. Next, weigh 50g of Moringa oleifera simplicia on analytical glass and then put the leaves into a beaker. Then add 96% Ethanol with ratio 50g:500ml and carry out the ultrasonication process of Moringa leaves while stirring for 3x2 minutes. Then the filtrate and residue are filtered and collect the filtrate result. Carry out the process of separating the solution from the extract using a rotary evaporator and put the extract into an oven at a temperature of 64°C to carry out the evaporation process so that the remaining ethanol liquid evaporates.

Rats Grouping

The population of this study was female wistar rat (Rattus norvegicus) aged 2-3 months with a body weight of 150-170. The rats are sorted into 3 groups, each group of rats will be put randomly in the amount of 9 each. The grouping is Control group (Only given standard feed), PCOS group (Given testosterone), and Moringa group (Given testosterone, and then given extract of moringa leaves.

PCOS Procedure

To change the rat into PCOS-IR, testosterone was given by injecting testosterone propionate 100mg/gram. Give 0.03-0,06cc each injection for 21 days, the injection was given when the rat is in pre-estrus. Then on the 22nd day, a vaginal smear examination was performed by inserting a cotton bud into the vagina and rotating it. Next the cotton bud was then rubbed on a glass object and fixed using 70% alcohol for 5 minutes. Furthermore, it was stained with giemsa and left for 2-3 minutes. After that wash with water and let it dry then examine under the microscope to determine is the rat already enter diestrus cycle.

Experiment Procedure

Ethanol extract of 96% moringa leaves was given as much as 500 mg/KgBW, which is 60mg and then dissolved with distilled water. Then the extract given orally using a probe to the mouth for 21 days.

RESULTS

Insulin Levels in Rats

From the experiment, it is found that the highest mean insulin level is rats with PCOS with 11.836µIU/ml and the lowest mean insulin level is rats given ethanol extract of 96% moringa leaves with 0.199µIU/ml.

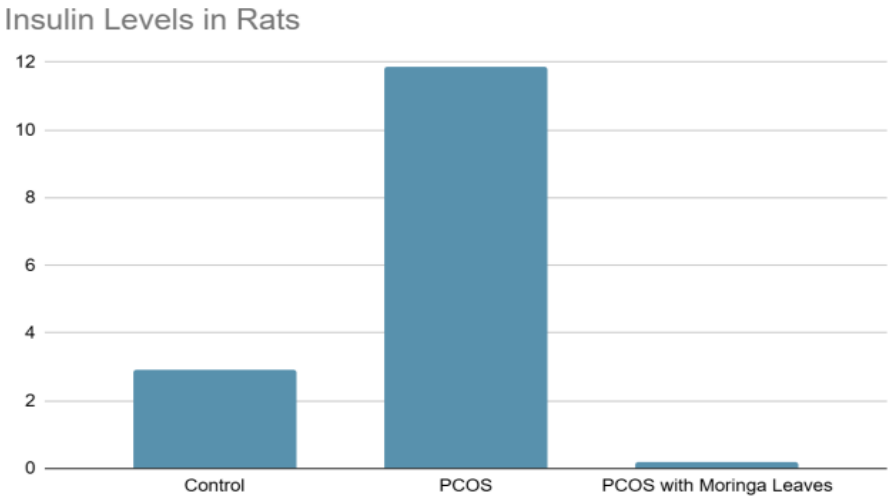

Figure 1: Insulin levels

Normality Test

The result of the normality test can be found from the data of the first group (P1), the second group (P2), and the third group (P3). In Table 1 based on the results of the normality test with the Shapiro Wilk Test, the results can be seen as under 0.05 then the insulin data in all groups is declared abnormal.

Table 1: Normality Test

Sample Group	Statistic	df	Sig.
Normal (P1)	0.511	10	0.000
PCOS (P2)	0.817	10	0.023
Given Moringa Extract (P3)	-	10	-

Normality Test Value
df: Degrees of freedom; Sig: Significance level

Homogeneity Test

After conducting the normality test, Next step is to conduct homogeneity test using the Levene Test. As seen in Table 2, the p value is 0.000 where p value under 0.05 is considered as not homogeneous.

Table 2: Homogeneity Test with Levene Test

Sample Group	Levene	df1	df2	Sig.
Based on Mean	26.798	2	27	0
Based on Median	8.081	2	27	0.002
Based on Median and with adjusted df	8.081	2	17.761	0.003
Based on trimmed mean	24.630	2	27	0

Normality Test Value
df: Degrees of freedom; Sig: Significance level

Effect of Moringa Leaves with Insulin Levels

In the results of the study, it is found that the normality test was not normal and the results of the homogeneity test were found to be non-homogeneous. Data analysis conducted from this study using the Kruskal-Wallis Test obtained a p value of 0,005 where <0,05 which means there is a significant difference in insulin levels between the 3 test groups. To identify which groups differ from the other, Post-Hoc Analysis is done using the Mann-Whitney Test, the result can be concluded as in Table 3. In which, if the p value is more than 0,05 it is considered that the group does not have significant difference.

Table 3: Post Hoc with Mann-Whitney Test

Sample Group	Mann-Whitney
P1 and P2	0.027
P1 and P3	0.147
P2 and P3	0.005

DISCUSSION

Insulin level in normal rats and rats with PCOS

Based on the results obtained using the Mann Whitney test, the p value of P1 and P2 is 0.027, which means that there is significant difference in insulin levels between the normal rats and the PCOS rats. When looking at the insulin data for each group, it was found that the average insulin level in normal rats was 2.895µIU/ml while the average insulin level in PCOS rats was 11.863µIU/ml. The results showed there was a difference in insulin levels between normal rats and PCOS rats, so that insulin in PCOS rats increased from normal levels. This study is in line with the results of the study (Amelia *et al*., 2018) where in PCOS rats, there was an increase in insulin levels compared to normal rats. There is another study that also says that there is an increase in insulin levels in PCOS rats compared to normal rats (Tohma *et al*., 2019).

Insulin level in normal rats and with given Moringa leaves

Using the Kruskall-Wallis test, the p value can be obtained as 0,005 which means there is a significant difference in the two sample groups. In accordance with the data, the average insulin levels in normal rats are 2.895µIU/ml and the average insulin levels in rats given extract moringa leaf are 0.199µIU/ml. There is a slight difference in the average between two groups and then post-hoc test was conducted to determine whether there is a difference between the two groups, a p value of 0,147 was found. Which means there is no significant difference between the two test groups. These results indicate that rats given moringa leaf extract will have insulin levels similar to normal rats.

Insulin level in rats with PCOS and with given Moringa leaves

Using Mann Whitney test, the result of the p value P2 and P3 is 0.005 meaning that there is a significant difference between the insulin levels between P2 and P3. The average insulin level in P2 was 11.863µIU/ml while the average Insulin level in P3 was 0.199µIU/ml. This study shows that when PCOS rats given Moringa leaf extract (Moringa oleifera) experienced a decrease in Insulin levels in the body, which means that there is an effect of Moringa oleifera leaves on Insulin level in PCOS rats. Other studies say that Moringa oleifera improves metabolic health which helps to modulate insulin signals so that it can reduce insulin levels (Mohamed *et al*., 2019). Meanwhile, there is a research by Sanchez *et al* (2019) which states that evidence of changes in insulin levels due to Moringa oleifera is not significant enough because many research results fail to show optimal results.

CONCLUSION

There is increased Insulin level within rats after being injected with testosterone. The effect of Moringa leaves on PCOS rats has decreased the insulin levels after giving Moringa leaves extract for 21 days. These findings suggest that antioxidants in Moringa leaves work to decrease the insulin level in the body and also help to increase the protection of the pancreatic cell against oxidative stress.

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