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HbA1c Levels in PCOS-IR Rats (*Rattus Norvegicus*) Models After Administration of *Moringa oleifera*Extract

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A B S T R A C T

Abstract: Polycystic Ovary Syndrome (PCOS) is a hormonal disorder characterized by irregular ovulation and the presence of small cysts on the ovaries. This condition is frequently associated with obesity, insulin resistance, and diabetes. ***Aim:*** This research aims to investigate the effects of *Moringa oleifera* leaves extract on HbA1c levels in white rats (*Rattus norvegicus*) that have been induced to model insulin-resistant PCOS. *Moringa oleifera* has various bioactive compounds such as flavonoids, that function as insulin sensitizers, which help improve glucose uptake. ***Method:*** This research employs a laboratory experimental design using female rats as subjects. The rats were divided into three groups (n=10): normal rats, PCOS-IR rats (received an intraperitoneal injection of testosterone at a dose of 1 mg/100 g BW for 21 days), and PCOS-IR rats with *Moringa oleifera* (received the same testosterone injection for 21 days followed by an administration of 500mg/kg *Moringa oleifera*leaves extract for an additional 21 days).Blood samples are subsequently collected and analyzed for HbA1c, and the gathered data will undergo statistical processing. ***Results:*** The PCOS-IR group exhibited higher HbA1c levels than the negative control group, while the group treated with *Moringa oleifera* demonstrated significantly lower HbA1c levels than the PCOS-IR group. The HbA1c levels in the *Moringa oleifera* treated group approached those of the control group. ***Conclusion:*** This research indicates that the 96% ethanol extract of moringa leaves may provide an effective alternative treatment for managing metabolic issues associated with PCOS-IR, particularly in relation to HbA1c levels.
Keywords: PCOS, *Moringa oleifera extract*, HbA1c, *Rattus norvegicus.*

INTRODUCTION

One of the hormonal disorders that affects women of reproductive age is called PCOS (Polycystic Ovary Syndrome). PCOS affects around 116 million women worldwide, about 3.4% of the female population, according to the World Health Organization (WHO) (Jabeen *et al*., 2022). This hormonal disorder impacts around 5-10% women of reproductive age in Indonesia (Sari *et al*., 2023).

PCOS or Polycystic Ovary Syndrome is a hormonal disorder in women of reproductive age. It is characterized by anovulation, high androgen hormones, and the presence of small cysts in the ovaries. There are four types of PCOS. The most common type of PCOS is PCOS with insulin resistance (PCOS-IR). If a woman has PCOS-IR, they are more prone to get Type 2 diabetes and metabolic problems. Insulin resistance leads to high insulin levels and high blood sugar, raising HbA1c levels. Long-term blood sugar control is shown by HbA1c numbers (Hoffman *et al*., 2020).

Hemoglobin A1c (HbA1c) is one form of hemoglobin (Hb) that binds to glucose via non-enzymatic. HbA1c can be used for diagnose, predictor of type 2 diabetes. For those with PCOS-IR HbA1c is used for evaluate glucose metabolism(Sacca *et al*., 2024). Some of serious health issues, such as type 2 diabetes, and cardiovascular disorders can develop in PCOS-IR women (Krisnadi, 2015).

PCOS-IR is increasing worldwide, and the need for therapy with little or minimal side effects is increasing. Common drugs used for treating PCOS-IR, such as metformin, and other insulin sensitizer medications, have been studied to evaluate their effectiveness for treating PCOS-IR. However, the side effects of the medications such as

gastrointestinal problems, and potential long-term health risks, are safety concerns for patients. There's an increasing interest in alternative therapies, specifically herbal treatment.

Moringa oleifera is a herbal treatment that is gaining popularity because it has many benefits. It can be used as an anti-hyperglycemic agent, anti-inflammation agent, and anti-oxidant. *Moringa oleifera* is known to have various bioactive compounds, such as flavonoids. Flavonoids are well-known as insulin sensitizers. Rutin and quercetin are flavonoids that have been shown to increase the activation of insulin receptors and activate the PI3K/ AKT pathway, improving glucose absorption via GLUT-4 translocation. HbA1c levels in serum are a good indicator for long-term glucose management and may be used to assess *Moringa oleifera* efficacy as an insulin sensitizer (Siahaan *et al*., 2022).

This research aims to determine whether there is a correlation between insulin resistance polycystic ovary syndrome (PCOS-IR) in rats and how the administration of *Moringa oleifera* extract affects HbA1c levels.

MATERIALS & METHODS

Research was carried out from June to August 2024 at the Animal Housing and Research Laboratory of the Faculty of Medicine, Ciputra University in Surabaya, Indonesia, using a laboratory experimental design with a post-test-only control group approach. *KomisiEtikPenelitian KesehatanFakultasKedokteranUniversitas Ciputra* gave its approval for this research, with ethical clearance number 126/EC/KEPK-FKUC/VI/2024.

Moringa oleifera leaves were sourced from *Moringa oleifera* tree and obtained from PT. MoringaOrganik, located in Blora, Central Java. The extraction of *Moringa oleifera* leaves was performed using a maceration method with 96% ethanol at Materia Medica Laboratory in Batu.

The subjects for this research were three-month-old female white rats from Wistar strain (*Rattus norvegicus*), 2-3 months old and weighing between 150-200 grams. *Rattus norvegicus* underwent a 7-day acclimatization period to adapt to the laboratory setting. Animals that died or became unwell during this adaptation phase were removed from the research. After the adaptation phase the rats were randomly divided into three groups, each consisting of 10 rats, namely group X0, X1, and X2.

Group X0 (Negative control) was given standard feed *ad libitum*, mineral water, and 0,5% CMCNa. Group X1 (Positive control) was given standard feed *ad libitum*, mineral water, and 0,5% CMCNa followed by intraperitoneal injections of testosterone propionate 1mg/100grams body weight for 21 days to induce PCOS insulin resistance (PCOS-IR). Group X2 (Treatment) was given standard feed *ad libitum*, mineral water, and 0,5% CMCNa followed by intraperitoneal injections of testosterone propionate 1mg/100grBW for 21 days, after that they received *Moringa oleifera* leaf extract 500mg/kg body weight via gavage for 21 days.

On day 22, to ensure successful PCOS induction in X1 and X2 groups, a vaginal swab examination was performed. Rats with Polycystic Ovary Syndrome (PCOS) show a*diestrus* stage during vaginal examination, indicating an ovulatory disorder (Wilcox, 2005)., (Y. Wang *et al*., 2021). Blood collecting was done on day 43. Blood samples are evaluated using ELISA to determine serum HbA1c levels. This method is used for assessing glycatedhemoglobin quantitatively, offering valuable information regarding long-term glucose management. The data collected were analyzed using SPSS version 27. One-Way ANOVA test was used to compares the differences in HbA1c levels among the groups.

STATISTICAL ANALYSIS

HbA1c levels of each group is recorded and a normality test is performed using the Shapiro-Wilk test. If the data distribution is found to be normal, One-way ANOVA test will be conducted. Further analysis is carried out using a One-way ANOVA test to assess the impact of *Moringa oleifera* extract administration on HbA1c levels across the different groups. Statistical analysis is performed using SPSS Windows version 27.

RESULT & DISCUSSION

The HbA1c levels are recorded and Shapiro Wilk test is done to find out the normality. The test shows that the HbA1c data from all groups were normally distributed ($p>0.05$). The groups are compared using the One-Way ANOVA test. Significant differences are found, and each group will subsequently be compared to the others using the Tukey HSD test. There was a significant difference in HbA1c levels in the three groups ($p < 0.001$). Since there is a significant difference, each group will be compared to the others using the Tukey HSD test. The results of the Tukey HSD test indicate that there is no significant difference between the control group and the *Moringa oleifera* treated group ($p > 0.05$). However, the PCOS-IR group showed a significant difference compared to the *Moringa oleifera* treated group ($p < 0.05$).

Table 1: Results of Differences in HbA1c levels between groups

Groups	n	Med ± Std. Deviation	p-value
X0	10	109,90 ± 19,785[a]	< 0.001
X1	10	163,90 ± 24,297[b]	
X2	10	110,70 ± 17,480[a]	

Notes: Different superscripts indicate significant differences according to the Tukey HSD test. X0: Negative control group. X1: Positive control group (PCOS-IR). X2: PCOS treated with *Moringa oleifera* group.

The findings demonstrate that the administration of *Moringa oleifera* extract to PCOS-IR models significantly reduces HbA1c levels, bringing them closer to those observed in normal rats. In comparison to the PCOS-IR group, the group treated with *Moringa oleifera* exhibited lower HbA1c levels, suggesting a notable decrease in insulin resistance. In rats with PCOS-IR, there is an increase in HbA1c levels, indicating the presence of insulin resistance. The body's capacity to control blood sugar levels is compromised by chronic insulin resistance, which subsequently influences HbA1c results (Wilcox, 2005).

Moringa oleifera leaves are abundant in flavonoids, which function as insulin sensitizers, leading to a reduction in blood sugar levels and, consequently, decreasing HbA1c levels. Flavonoids such as rutin and quercetin have been shown to mitigate insulin resistance in hepatocytes by phosphorylating AKT. The activation of the PI3K-AKT pathway facilitates GLUT4 translocation (Russo *et al.*, 2019). Alongside its role in facilitating GLUT4 translocation, the PI3K-AKT pathway inhibits gluconeogenesis, the metabolic process by which glucose is produced in the liver from a non-carbohydrate source. Suppressing gluconeogenesis prevents the overproduction of glucose. PI3K-AKT pathway also enhances glycogen synthesis (W. Wang *et al*., 2022). Enhanced GLUT4 translocation in muscle and adipose tissues results in increased glucose uptake (Russo *et al*., 2019). This shows the effectiveness of the *Moringa oleifera* extract as an insulin sensitizer for PCOS-IR with metabolic disease.

CONCLUSION

Research on the utilization of 96% ethanol extract from moringa leaves (*Moringa oleifera*) as an alternative therapy for PCOS-IR and the risk of metabolic syndrome, particularly type 2 diabetes, demonstrates significant potential. The administration of moringa extract can aid in lowering HbA1c levels in patients with PCOS-IR, contributing to the management of this condition and the prevention of future metabolic complications. This finding suggests that further research is needed to explore the potential benefits of *Moringa oleifera* consumption as an insulin sensitizer for individuals with PCOS.

Conflict of interest: The authors declare no conflicts of interest.

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