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CRP Levels in PCOS-IR Rat Models (*Rattus norvegicus*) After Administration of *Moringa oleifera* Extract

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A B S T R A C T

Polycystic Ovarian Syndrome (PCOS) is a metabolic syndrome affecting women's reproductive health. PCOS is diagnosed using Rotterdam Criteria, and is often associated with insulin resistance. PCOS with insulin resistance (PCOS-IR) can both induce inflammation, in which the inflammation itself can worsen PCOS-IR. *Moringa oleifera* is a plant tested for its anti-inflammatory and anti-oxidant properties from pytochemicals that can be found in it. ***Aim:*** This research aims to study the effect of *Moringa oleifera* leaf extract administration to PCOS-IR rat models towards levels of C-reactive protein (CRP), a widely known biomarker of inflammation. ***Method:*** Female Wistar rats were divided into 3 groups (n=10): normal rats, PCOS-IR rats and PCOS-IR rats administered with 500 mg/kg *Moringa oleifera* leaf extract. PCOS rat model is created by injecting 1 mg / 100 gr testosterone proprionate for 21 days, and another 21 days for *Moringa oleifera* leaf extract administration. Blood is then collected and tested for CRP, and data collected will be statistically processed. ***Results:*** The PCOS-IR group showed increase in CRP level compared to control group, and the *Moringa oleifera* treated group showed significantly lower CRP level compared to PCOS group. The CRP level in *Moringa oleifera* treated group comes near to that of control group. ***Conclusion:*** Consumption of *Moringa oleifera* leaves can reduce inflammation in PCOS-IR shown by significant decrease of CRP levels.

Keywords: Polycystic ovary syndrome, C-reactive protein, *Moringa oleifera*, inflammation.

INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is a well-known and prevalent metabolic disease in women of reproductive age, which is defined as a metabolic syndrome that is diagnosed using 3 main objectives from the Rotterdam Criteria 2003: Hyperandrogenism, problems of ovulation and/or positive findings of Polycystic Ovary Morphology (PCOM) [1]. PCOS is also related to insulin resistance, in which hyperinsulinemia due to the resistance causes enhanced production of androgen by theca cell in the ovary [2]. Both hyperandrogenism and hyperglycemia (caused by insulin resistance) causes inflammation due to activation of M1 macrophage that releases proinflammatory cytokines and oxidative stress respectively [3, 4].

Inflammation in PCOS with insulin resistance (PCOS-IR) can worsen the condition itself by causing chronic anovulation and increased androgen production by the actions of proinflammatory cytokines on theca cells [5, 6]. A way to detect inflammation is by checking for a known biomarker called C-reactive protein (CRP). CRP is produced by the liver from proinflammatory cytokine stimulation [7].

Research for the topic of inflammation in PCOS-IR is increasing, highlighting its role in the pathophysiology of PCOS-IR and how it complicates the condition in patients. Many indicators of inflammation have been investigated and many drugs have undergone trials to treat the chronic inflammation in PCOS-IR. However, the side effects of said drugs have compromised the safety of patients, which results in the need of an alternative – herbal therapy with anti-inflammatory properties that is safe for consumption. *Moringa oleifera* is an increasingly known plant due to its anti-inflammatory and anti-oxidant properties and has been used in traditional medicine since thousands of years ago by the Romans, Greeks, Indians and Egyptians [8]. The plant contains flavonoids such as rutin, quercetin and kaempferol that

could decrease proinflammatory cytokines production by inhibition of transcription factor NF-κB [9]. The anti-inflammatory effect of the chosen therapy can be investigated using CRP level in serum as the indicator of inflammation.

This research aims to find the effects of *Moringa oleifera* extract administration to the CRP serum level in PCOS-IR induced rats.

RESEARCH METHOD

This experimental research uses the post-test only control group design and was conducted in School of Medicine, Universitas Ciputra located in Surabaya, Indonesia from June to August 2024.This research received ethical clearance from *Komisi Etik Penelitian Kesehatan* Universitas Ciputra with clearance number No. 127/EC/KEPK-FKUC/VI/2024.

Inclusion criteria for this research is female Wistar rats aged 2-3 months which are healthy and active, while the criteria for exclusion is sick rats.

The research was done on 30 female Wistar rats (*Rattus norvegicus*) by injecting testosterone propionate of dose 1 mg / 100 gr subcutaneously for 21 days. The rats were acclimated first before the procedure for 7 days, and then divided into 3 groups of 10 rats (K0, K1, K2). K1 and K2 groups were injected with the testosterone proprionate for 21 days to create PCOS-IR rat models, while K0 acts as a control group. Vaginal swab is done on the 22nd day to determine the rat's estrous cycle, in which a diestrous stage shows ovarian problems [10]. K2 group was then given 500 mg / kg *Moringa oleifera* leaf extract for 21 days.

Moringa oleifera leaves were extracted by ultrasonication using 96% ethanol solvent. 50 gr of Moringa oleifera were put in a beaker with 500 ml of 96% ethanol and went through the ultrasonic process. The filtrate would then be processed using the rotary evaporator to let the solvent evaporate.

Blood is collected and tested using Biotime CRP Rapid Quantitative Test Kit with the Fluorescence Immunoassay (FIA) technique.

STATISTICAL ANALYSIS

CRP levels of each group is recorded and normality test is conducted using the Shapiro-Wilk test. If distribution of data is normal, ANOVA test can proceed. On the contrary, Kruskall Wallis test will be conducted if the distribution is not normal. Data is further analyzed using Mann-Whitney test to determine the effect of *Moringa oleifera* extract administration towards CRP levels in the respective groups. SPSS Windows version 16 is used to conduct the statistical analysis.

RESULTS AND DISCUSSION

The CRP levels are recorded and Shapiro Wilk test is done to find out the normality. The test shows that PCOS group is normally distributed ($p > 0.092$), but the *Moringa oleifera* treated group was not normally distributed ($p < 0.001$). Thus, Kruskal Wallis test is used. There was a significant difference in CRP levels in the three groups ($p < 0.001$), therefore required additional test to understand which groups are different. Mann Whitney test result shows that control group and *Moringa oleifera* treated group have no significant difference ($p > 0.05$), while PCOS-IR group depicted significant difference when compared to *Moringa oleifera* treated group ($p < 0.05$).

Table 1: Results of Differences in CRP levels between groups

Groups	n	Med (min-max)	p-value
K0	10	0,46(0,46-0,46)^{a}	< 0.001
K1	10	0,53(0,46-0,74)^{b}	
K2	10	0,49(0,46-0,59) ^{a}	

Notes: Different superscripts show significant differences. K0: Control group. K1: PCOS group. K2: PCOS treated with *Moringa oleifera* group.

The results shows that the administration of *Moringa oleifera* leaf extract causes a decrease in CRP levels, even closing in to the CRP level in the control group. When compared to the PCOS-IR group, PCOS-IR treated with *Moringa oleifera* extract group has a lower level of CRP in serum which suggests a decrease in inflammation due to the extract administration. Kuang *et al.,* [11] stated that findings show high levels of proinflammatory cytokines IL-1 and IL-6 in PCOS patients which causes inflammation. This condition is caused by hyperandrogenism and insulin resistance by various methods. Shabir *et al.,* [12] proposed that hyperandrogenism causes increased activation of M1 macrophages which produces IL-1 and IL-6 cytokines. IL-1 cytokine can worsen condition of inflammation by stimulating the ovary to increase androgen production which contributes to the worsening of hyperandrogenism (Shabbir *et al*., 2023). Oxidative

stress due to insulin resistance also causes inflammation by activation of transcription factor NF-κB due to high levels of glucose, which activation of said transcription factor causes production of proinflammatory cytokines [6]. The cytokines produced by mechanisms explained induces liver production of an acute phase reactant, CRP, namely the IL-1, IL-6 and TNF- αcytokines [7]. CRP is a common biomarker used to detect inflammation, and Kelly *et al*., [13] was the first to find increased CRP in PCOS which showed inflammation.

Moringa leaf extract was able to decrease CRP levels in PCOS-IR rat models due to one of its most powerful component, flavonoids. Pareek *et al*., [14] studied that Moringa contains flavonoids such as rutin, quercetin and kaempferol, known for their anti-inflammatory qualities.Another study shows that those flavonoids are able to decrease levels of IL-1, IL-6 and TNF- α cytokines, also Cyclooxygenase-2 (COX-2) which is important in the inflammation pathway [9].Transcription factor NF-κB is also inhibited by flavonoids, thus blocks the production of inflammatory cytokines [9]. The Moringa extract was able to decrease the CRP level of PCOS-IR rat models closing to that of the control group, shown in Table 1. This shows the effectiveness of the extract as an anti-inflammatory herbal for PCOS.

CONCLUSION

This study on female Wistar rats turned PCOS-IR model by injection of 1 mg / 100 gr testosterone proprionate for 21 days then given Moringa leaf extract for another 21 days shows that the extract administration reduces CRP levels, which shows a decrease in inflammation in PCOS. This finding can be further explored for the future potential usage of anti-inflammatory benefits from Moringa consumption by PCOS patients.

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

REFERENCES

1. Smet, M. & McLennan, A. (2018). Rotterdam criteria, the end. *Australasian Journal of Ultrasound Medicine, 21*(2), 59-60.
2. Lewis, V. (2007). *Reproductive endocrinology and infertility*. U.S.A: Landes Bioscience.
3. Hu, C., Pang, B., Ma, Z., & Yi, H. (2020). Immunophenotypic profiles in polycystic ovary syndrome. *Mediators Inflamm.* 2020, pp. 1-10.
4. Sun, Q., Li, J., & Gao, F. (2014). New insights into insulin: the anti-inflammatory effect and its clinical relevance. *World J Diabetes*, *5*(2), 89-96.
5. Fox, C. W., Zhang, L., Sohni, A., Doblado, M., Wilkinson, M. F., Chang, R. J., & Duleba, A. J. (2019). Inflammatory Stimuli Trigger Increased Androgen Production and Shifts in Gene Expression in Theca-Interstitial Cells. *Endocrinology*, *160*(12), 2946-2958.
6. Gonzalez, F., Mather, K. J., Considine, R. V., Abdelhadi, O. A., & Acton, A. J. (2020). Salicylate administration suppresses the inflammatory response to nutrients and improves ovarian function in polycystic ovary syndrome. *Translational Physiology, 319*(4), E744-E752.
7. Boras, E., Slevin, M., Alexander, M. Y., Aljohi, A., Gilmore, W., Ashworth, J., Krupinski, J., Potempa, L. A., Abdulkareem, I. A., Elobeid, A., & Matou-Nasri, S. (2014). Monomeric C-reactive protein and Notch-3 co-operativelyincrease angiogenesis through PI3K signalling pathway. *Cytokine*, *69*(2), 165–179.
8. Rani K. C., Ekajayani, N. I., Darmasetiawan, N. K., & Dewi, A. D. R. (2019). *Modul PelatihanKandunganNutrisiTanaman Kelor*. Surabaya: Fakultas Farmasi Universitas Surabaya.
9. Muvhulawa, N., Dludla, P. V., Ziqubu, K., Mthembu, S. X., Mthiyane, F., Nkambule, B. B., & Mazibuko-Mbeje, S. E. (2022). Rutin ameliorates inflammation and improvesmetabolic function: a comprehensive analysis ofscientific literature. *Pharmacological Research*, 178, pp. 106-163.
10. Siahaan, S. C. P., Santoso, B., & Widjiati. (2022). Effectiveness of *Moringa oleifera* leaves on TNF-α expression, insulin levels, glucose levels and follicle count in *Rattus norvegicus* PCOS model.*Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 15, 3255-3270.
11. Kuang, H., Duan, Y., Li, D., Xu, Y., Ai, W., Li, W., Wang, Y., Liu, S., Li, M., Liu, X., & Shao, M. (2020). The role of serum inflammatory cytokines and berberine in the insulin signaling pathway among women with polycystic ovary syndrome. *PLoS ONE, 15*(8), e0235404.
12. Shabbir, S., Khurram, E., Moorthi, V.S., Eissa, Y. T. H., Kamal, M. A., & Butler, A. E. (2023). The interplay between androgens and the immune response in polycystic ovary syndrome. *Journal of Translational Medicine*, (21)259, pp. 1-26.
13. Kelly, C. C. J., Lyall, H., Petrie, J. R., Gould, G. W., Connell, J. M. C., & Sattar, N. (2001). Low grade chronic inflammation in women with polycystic ovarian syndrome. *J Clin Endocrinol Metab,* 86, pp. 2453–2455.
14. Pareek, A., Pant, M., Gupta, M. M., Kashania, P., Ratan, Y., Jain, V., Pareek, A., & Chuturgoon, A. A. (2023). *Moringa oleifera*: an updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects. *International Journal of Molecular Sciences*, *24*(3), pp. 2098.