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PRELIMINARY FINDINGS ON THE INFLUENCE OF WILD GARLIC EXTRACT ON CARDIOVASCULAR ISSUES IN PCOS RATS

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Abstract: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive-age women, linked to cardiometabolic issues. Conventional treatments often fail to address PCOS's complexity, prompting interest in alternatives like Allium ursinum L. (wild garlic), known for its therapeutic properties. This study evaluated the effects of wild garlic extract (500 mg/kg) on hemodynamic and echocardiographic parameters in a rat model of PCOS induced by letrozole and a high-fat diet. Results showed significant reductions in blood pressure, suggesting wild garlic may improve reproductive health and reduce cardiovascular risks associated with PCOS.

Keywords: *Allium ursinum*, PCOS, cardiovascular, rat

Introduction

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder affecting women of reproductive age, often associated with various metabolic complications. Conventional treatments frequently fall short in addressing the multifaceted nature of PCOS, leading to increased interest in alternative therapeutic options (Mimouni and Giacobini, 2024). Among potential therapeutic agents, Allium ursinum L. (wild garlic) has garnered attention due to its historical use in traditional medicine and its rich composition of bioactive

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compounds, including sulfur and phenolic compounds, which are believed to contribute to its health benefits (Kovačević et al., 2023). Despite its promising profile, the specific effects of wild garlic on PCOS and associated metabolic parameters remain inadequately explored. This study aims to investigate the impact of wild garlic extract on reproductive, but also hemodynamic and echocardiographic parameters in a rat model of PCOS, providing insights into its potential therapeutic role in managing this complex condition.

Materials and methods

Plant Material and Extract Preparation

The wild garlic plant (A. ursinnum) was collected on Mount Rudnik during May. After collection, the plant material was cleaned of impurities and admixtures of other plants. The extract was prepared by cold pressing of fresh plant material (slow juicer Hurom HH Elite, South Korea, strength 150 W) (Khaksar et al., 2019). After pressing, the resulting juice was centrifuged (6000 rpm, 10 minutes, at 4°C) in order to remove parts of the plant material. The final product was a liquid, dark green in color, with a characteristic garlic odor. The resulting extract was stored in dark bottle, at 4°C until use.

Animals and Experimental Design

The present study was carried out on 18 male *Wistar albino* rats. 6 rats served as control group, and 12 rats were submitted to letrozole at a concentration of 1 mg/kg p.o. dissolved in 0.5% CMC solutions once daily and fed with the high-fat diet to induce PCOS (Begum et al., 2022). After 21 day, PCOS rats were randomly divided into two groups.

1. PCOS, rats treated distilled water by oral gavage for 28 days;
2. PCOS + WG, rats treated with 500 mg/kg of extract of *A. ursinum* by oral gavage for 28 days.

Arterial blood pressure values determination

A non-invasive method for the determination of systolic and diastolic arterial BP and heart rate in rats was performed using BP system (Rat Tail Cuff Method Blood pressure Systems (MRBP-R), IITC Life Science Inc., Los Angeles, CA, USA). Heart rate was determined simultaneously (Feng et al. 2008).

Cardiac echocardiography

Transthoracic echocardiography was performed day after blood pressure assessment. The animals were anesthetized intraperitoneally using a mixture of ketamine (75 mg/kg) and xylazine (5 mg/kg). Echocardiograms were obtained using a Hewlett-Packard Sonos 5500 scanner equipped with a 15.0 MHz

phased-array transducer. From the parasternal long-axis view in 2-dimensional mode, M-mode images were captured by positioning the cursor perpendicularly to the interventricular septum and posterior wall of the left ventricle at the papillary muscle level. Key parameters measured included interventricular septal wall thickness, left ventricular internal dimensions, and wall thicknesses at both end-diastole and end-systole. Fractional shortening percentage (FS%) was calculated using the formula [(LVIDd− LVIDs)/LVIDd] × 100%, and ejection fraction (EF%) was calculated according to the Teichholz formula, utilizing left ventricular end-diastolic and end-systolic volumes (Fox et al., 2002).

Results and discussion

The results of this study demonstrate significant differences in estrous cycle patterns and hemodynamic parameters among the control (CTRL), polycystic ovary syndrome (PCOS), and wild garlic-treated (PCOS+WG) groups.

Estrous Cyclicity

The CTRL group exhibited regular cyclicity, characterized by a well-distributed presence of four estrous phases. In stark contrast, the PCOS group showed a complete cessation of the estrous cycle, with 100% of the observed days spent in diestrus. This finding indicates a substantial disruption of normal reproductive function, consistent with established pathophysiological mechanisms associated with PCOS (Wen et al., 2020). Notably, the introduction of wild garlic in the PCOS+WG group led to a partial restoration of cyclicity. This suggests that wild garlic may possess bioactive properties that ameliorate some reproductive dysfunctions linked to PCOS, potentially through its influence on hormonal balance or ovarian function.

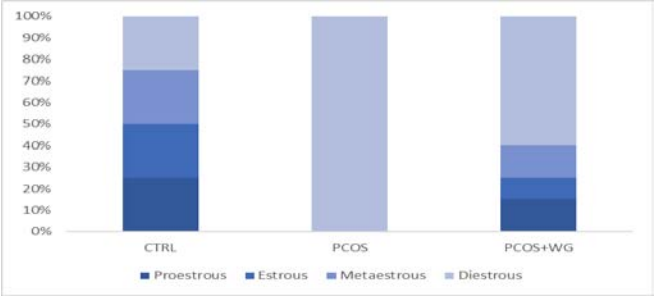

Figure 1. Percentage of estrous cycle phases distribution in different groups.

Hemodynamic Parameters

The hemodynamic analysis revealed significant differences among the groups. The PCOS group exhibited elevated systolic blood pressure compared to the CTRL group, a concerning finding given the well-documented association between hypertension and PCOS (Kintoye et al., 2023). The addition of wild garlic in the PCOS+WG group resulted in a marked decrease in systolic blood pressure, aligning it with CTRL values. This suggests a potential cardiovascular protective effect of wild garlic, which may be beneficial for managing hypertension associated with PCOS (Preuss et al., 2001).

Diastolic blood pressure results further corroborate these findings, as the PCOS group exhibited increased values compared to both the CTRL and PCOS+WG groups. The significant decrease in diastolic blood pressure in the PCOS+WG group indicates that wild garlic may enhance overall cardiovascular health by mitigating some of the hypertension observed in the PCOS group.

Additionally, heart rate was significantly reduced in the PCOS+WG group compared to both CTRL and PCOS groups. This decrease may reflect an improved cardiovascular status associated with dietary inclusion of wild garlic, although further investigation is needed to elucidate the underlying mechanisms.

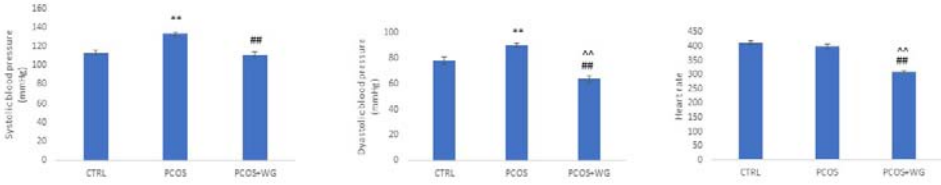

Figure 2. Hemodynamic parameters: systolic blood pressure, diastolic blood pressure, heart rate. ** Statistical significance at level of 0.01 between CTRL and PCOS group; ## Statistical significance at level of 0.01 between PCOS and PCOS+WG group; ^^ Statistical significance at level of 0.01 between CTRL and PCOS+WG group (n=6 rats per group).

Despite the significant changes observed in hemodynamic parameters, echocardiographic measures did not reveal substantial alterations among the groups (Table 1). This model of PCOS did not affect cardiac function, which could be related to short period of time for observation. Moreover, while wild garlic may influence systemic blood pressure and heart rate, it may not have a

pronounced effect on cardiac structure or function within the timeframe of this study.

Table 1. Results or echocardiographic parameters

	CTRL	PCOS	P+SREMUŠ
IVSD	0,16±0,02	0,16±0,02	0,16±0,01
LViDd	0,8±0,06	0,84±0,04	0,79±0,08
LVPWd	0,17±0,02	0,15±0,03	0,17±0,03
IVSs	0,24±0,03	0,22±0,02	0,2±0,01
LViDs	0,54±0,08	0,56±0,04	0,54±0,1
LVPWs	0,2±0,03	0,21±0,02	0,41±0,28
FS	33,12±7,97	33,94±5,31	29,06±10,82
LVEDV	1,14±0,22	1,31±0,16	1,11±0,32
LVESV	0,39±0,18	0,41±0,08	0,44±0,24
EF	66,44±11,38	68,14±6,96	60,12±14,69

IVSd—end-diastolic interventricular septal thickness (cm); LVIDd—left ventricular internal diameter end diastole (cm); PWd—left ventricular end-diastolic posterior wall thickness (cm); IVSs—end-systolic interventricular septal thickness (cm); LVIDs—left ventricular internal diameter end systole; PWs—left ventricular posterior wall thickness (cm); FS—fractional shortening (%); EF—ejection fraction (%). Values are shown as mean ± SD.

Conclusion

Overall, the findings of this study underscore the potential benefits of wild garlic in restoring estrous cyclicity and improving hemodynamic parameters in a PCOS model. These results warrant further exploration into the mechanisms underlying these effects and the potential therapeutic applications of wild garlic in the management of PCOS and its associated complications.

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References

Mimouni N. E. H., Giacobini P. (2024). Polycystic ovary syndrome (PCOS): progress towards a better understanding and treatment of the syndrome. Comptes Rendus Biologies, 347, 19–25.

Kovačević T. K., Major N., Sivec M., Horvat D., Krpan M., Hruškar M., et al. (2023). Phenolic content, amino acids, volatile compounds, antioxidant capacity, and their relationship in wild garlic (Allium ursinum L.). Foods (Basel, Switzerland), 12(11), 2110.

Khaksar G., Assatarakul K., Sirikantaramas S. (2019). Effect of cold-pressed and normal centrifugal juicing on quality attributes of fresh juices: do cold-pressed juices harbor a superior nutritional quality and antioxidant capacity? Heliyon, 5(6), e01917.

Begum N., Manipriya K., Veeresh B. (2022). Role of high-fat diet on letrozole-induced polycystic ovarian syndrome in rats. European Journal of Pharmacology, 917, 174746.

Feng M., Whitesall S., Zhang Y., Beibel M., D'Alecy L., DiPetrillo K. (2008). Validation of volume-pressure recording tail-cuff blood pressure measurements. American Journal of Hypertension, 21(12), 1288–1291.

Fox J. G., Anderson L. C., Loew F. M., Quimby F. W. (eds.) (2002). Laboratory Animal Medicine, 2nd ed. London, UK: Academic Press.

Wen L., Lin W., Li Q., Chen G., Wen J. (2020). Effect of sleeve gastrectomy on kisspeptin expression in the hypothalamus of rats with polycystic ovary syndrome. Obesity, 28, 1117–1128. Erratum in: Obesity, 28, 2242.

Kintoye O. O., Ajibare A. J., Oriyomi I. A., Olofinbiyi B. A., Yusuf G. O., Afuye D. C., et al. (2023). Synergistic action of carvedilol and clomiphene in mitigating the behavioral phenotypes of letrozole-model of PCOS rats by modulating the NRF2/NFKB pathway. Life Sciences, 324, 121737.

Preuss H. G., Clouatre D., Mohamadi A., Jarrell S. T. (2001). Wild garlic has a greater effect than regular garlic on blood pressure and blood chemistries of rats. International Urology and Nephrology, 32(4), 525–530.