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"SYNERGISTIC EFFECTS OF ZINC AND CHOLINE FROM DIETARY SOURCES ON ALLEVIATING EXPERIMENTAL POLYCYSTIC OVARIAN SYNDROME IN RAT MODELS"

Samra Faisal[1], Tabeen Irfan[2]*, Noor ul ain Shah[3], Mahnoor Naseer Ahmed[4], Saad Shafi[5], Smeea Fatima[6], Rimsha Sattar[7], Safa Sahar[8]

[1]Samra Faisal (samra.faisal@riphah.edu.pk) Department of Human Nutrition and Dietetics, Riphah International University, Islamabad, Pakistan
[2]*Tabeen Irfan (corresponding author) (tabeen.irfan@superior.edu.pk) Department of Human Nutrition and Food Technology, Faculty of Allied Health Sciences, Superior University Lahore, Pakistan
[3]Noor ul ain Shah (Noorulain.shah@superior.edu.pk) Department of Human Nutrition and Food Technology, Faculty of Allied Health Sciences, Superior University Lahore, Pakistan
[4]Mahnoor Naseer Ahmed (Mahnoornaseer077@gmail.com) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan
[5]Saad Shafi (Saadimehar125@gmail.com) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan
[6]Smeea Fatima (smeeafatima21c@gmail.com) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan
[7]Rimsha Sattar (rimshasattar953@gmail.com) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan
[8]Safa Sahar (Saharsafa005@gmail.com) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan

***Corresponding Author:** Tabeen Irfan
*Email: tabeen.irfan@superior.edu.pk

Abstract

This study investigates the synergistic effects of zinc and choline on PCOS. PCOS is a complex endocrine disorder with reproductive and metabolic implications. Different dietary interventions were administered through sources including eggs, almonds, poppy seeds, and sesame seeds to female Wistar albino rats, induced with PCOS. Forty-five rats were randomly divided into five groups and were given with iso-caloric and iso-nitrogenous diet. Those, without induced PCOS and basal diet were under NC, with induced PCOS and basal diet under in PC and remaining were under three treatment groups named ZC1, ZC2, and ZC3; Induced PCOS and tested diets with varying zinc and choline concentrations, as per Recommended Dietary Allowances (RDA). Daily water and feed intake, weekly weight changes, total collection methods and blood samples were used to determine results. The ZC1 treatment group, adhering to RDA for zinc and choline, demonstrated significant decrease ($p<0.05$) in estrogen, progesterone, LH, FSH, TG, TC, LDL, insulin and weight. Low levels of testosterone were found in group ZC3 and mature follicular count increased with fewer cyst compared to the PC group. Research findings concludes the potential of zinc and choline synergist effect in alleviating hormonal imbalances, insulin resistance, lipid abnormalities, and nutrient digestibility in

PCOS induced female Wistar albino rats offering insights into dietary interventions for PCOS management.

Keywords: Animal Models, Choline, Dietary Sources, Hormonal Regulation, Ovarian Health, Polycystic Ovarian Syndrome (PCOS), Reproductive Health, Zinc

Introduction

Polycystic Ovarian Syndrome (PCOS) is the most prevalent endocrine disorder in women globally, affecting 5-10% (Zaeemzadeh *et al*., 2021). PCOS poses high risks of miscarriages, repeated abortions, premature neonatal births, implantation failures, and endometrial cancers (Xu *et al*., 2021). micronutrients rich dietary interventions, particularly zinc and choline, have shown promising results in alleviating PCOS symptoms. Insufficient zinc levels in body correlate with hormonal imbalances, irregular menstrual cycles, and pregnancy complications like prolonged gestation, abortions, and stillbirths (Nasiadek *et al*., 2020). Zinc plays a vital role in metabolizing estrogens, progesterone, and androgens, impacting ovulation and embryo development. Zinc is important for ovulation and embryo development as zinc metabolizing estrogens, progesterone, and androgens (Szczuko *et al*., 2016). In addition to that, zinc plays a role in formation, storage, and release of insulin, effecting the prognosis of type 2 diabetes (Olechnowicz *et al*., 2018), influencing insulin secretion pathways relevant to PCOS.

On the other hand, Insufficient choline in plasma and serum leads to ovarian follicular fluid imbalance, affecting fertility by influencing ovarian growth and immature follicle formation (Pokorska-Niewiada *et al.*, 2021). Choline is beneficial for fertility, embryonic development, and endocrine conditions like PCOS (Zhan *et al*., 2021). Estrogen regulates choline's production via estrogen response elements, Studies suggest that a combination of high endogenous estrogen-induced choline production and dietary choline intake is crucial for a normal pregnancy (Jia *et al*., 2016).

Previous research indicates that zinc and choline have positive effects on female reproductive health. This study investigates how does this synergic effect of zinc and choline derived from different food sources eggs, almonds, poppy seeds, and sesame seeds on induced PCOS in female wistar albino rats (Abbott *et al*., 2019). How tested groups, ZC1 (zinc 38 mg and choline 1000 mg as per RDA), ZC2 (choline 1050 mg and zinc 38 mg) and ZC3 (39.5mg and choline 1000mg) impact hormonal profile compared to the control groups and see without induced PCOS and basal diet PC induced PCOS with basal diet? Additionally, how does in nutrient digestibility vary among the treatment groups and what are the overall implications for combating PCOS through dietary interventions? (Cruz *et al*., 2018).

Methods

Design and participants

Forty-five female Wistar albino rats (120 days old, 130±5 g) were maintained under specific conditions (12hr light-dark cycle, 25-28℃) for 75 days, they had ad libitum access to food and water. PCOS was induced over 45 days using estradiol valerate via gavage method (Manzoor *et al*., 2020). The subsequent 30 days dedicated for treatment and sample collection. On day 40th PCOS were confirmation through vaginal smear test, observing irregular estrus cycles, hair loss, and weight gain. All procedures adhered to the guidelines for the Care and Management of Laboratory Animals (National Research Council, 1995).

Material and procedure

A completely randomized design was made to create five groups of rats, each with three replicates. After confirming PCOS on the 45th day, all 45 rats were divided in randomized manner to five equal groups (n=9). Each group was provided with varying levels of zinc and choline; ZC1 (Zinc 38 mg and Choline 1000 mg), ZC2 (Choline 1050 mg & Zinc 38 mg), ZC3 (Zinc 39.5 mg & Choline 1000 mg), NC (normal control with basal diet), and PC (positive control with induced PCOS and basal diet). Treatment diets were made of zinc and choline from food sources like sesame seeds, poppy seeds,

eggs, and almonds, were mixed with the basal diet to create a 1000 g diet and provided ad libitum. Soft pallets were formulated following Nutrient Requirements of Laboratory Animals (Fourth revised edition, 1995) and given to the rats (Nimalaratne *et al*., 2015).

Data collection and analysis

During the final seven days of the trial, feces were gathered and preserved at -20°C for digestibility assessment (Zhan et al., 2021). The proximate analysis of both feed and feces was conducted following the protocols of Official Method of Analysis Chemists (Association of Official Analytical Chemists, 2000). Digestibility was assessed using the total collection method, and the formula was utilized for measurement (Biswas *et al*., 2015).

$$\text{Nutrient Digestibility (\%)} = \text{Nutrient Intake (g/kg)} - \text{Nutrient in feces (g/kg)} / \text{Nutrient intake (g/kg)} * 100$$

At the conclusion of the trial, the rats underwent an overnight fast and were sacrificed in the morning. Blood samples of 5cc were drawn and left to clot. Following centrifugation at 5000 rpm for 20 minutes, serum was obtained and stored at -20°C for biochemical analysis (Okafor *et al*., 2021). Afterwards, the rats were decapitated, and their right and left ovaries were separated and preserved in a 10% formalin solution for histological examination (Allahbadia *et al*., 2011). Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), prolactin, testosterone, progesterone, and estrogen levels in serum were assessed using Enzyme-Linked Immunosorbent Assay (ELISA) kits from Bio-check, Inc, Foster City, CA 94404, USA. Serum insulin levels were examined utilizing ELISA microwells from Calbiotech, Inc., USA, purchased from a scientific store in Faisalabad. Serum cholesterol (mg/dl) and triglyceride (mg/dl) levels were spectrometrically analyzed using the Enzymatic Calorimetric Method with lipid clearing factor (LCF) after enzymatic hydrolysis and oxidations, following the procedure outlined by Car et al. (Takacs *et al*., 2020). Additionally, HDL, LDL, and VLDL levels were measured using the DiaSys lipid profile kit from (DiaSys Diagnostic System GmbH, Germany). The ovarian tissues were excised and preserved in a 10% formalin solution, utilizing neural buffered formalin (10%) as the fixing agent. Dehydration was achieved through alcohol at various intervals, followed by tissue clarification using xylene. Subsequently, the tissues were embedded in liquid paraffin. Slices, 5 microns thick, were obtained and affixed to slides using hematoxylin and eosin stain (Takacs et al., 2020). Analysis of ovarian tissues was conducted using the XSZ 107BN optical microscope from Zeith lab, Inc., China, equipped with an optima B1 digital camera (Optika microscope, Italy). All images were observed at 40X and 100X magnifications (Kamil *et al*., 2012).

Hormonal and histological outcomes from the rat studies across all groups were statistically compared using Statistix 8.1 software and one-way ANOVA. The results were presented as mean ± standard deviation (Mean ± SD), with significance set at $p<0.05$. Hormonal and histological findings from the studies involving the rats in all groups were compared using Statistix 8.1 software and one-way ANOVA. Results were presented as mean ± standard deviation (Mean ± SD), with significance determined at $p<0.05$ (Nimalaratne *et al*., 2015).

Results

In the current study, feed intake among rats fed different levels of zinc and choline showed non-significant differences ($p>0.05$). However, the maximum feed intake was observed in the last week of treatment. The impact of varying levels of zinc and choline on water intake in PCOS-induced rats, revealing non-significant changes ($p>0.05$) over the four weeks of the trial. Significant increases ($p<0.05$) in Dry matter, Crude Fiber, Ash, and Nitrogen Free Extract of nutrient digestibility were noted in all treatment groups compared to PC, while a reduction trend was observed in Crude protein and ether extract compared to the PC group. Weekly measurements of weight changes, showed a significant ($p<0.05$) increase in the weight of the PC-induced PCOS group compared to the healthy control group was observed. Notably, the treatment group ZC1, receiving zinc and choline as per the Recommended Dietary Allowance (RDA), exhibited the most significant ($p<0.05$) decrease in weight compared to ZC2 and ZC3.

Discussion

This study aimed to assess the synergistic impact of zinc and choline on PCOS-induced rats. A significant increase ($p \leq 0.05$) in the digestibility of dry matter (DM), crude fiber (CF), ash, and nitrogen-free extract (NFE) was observed. (Olechnowicz *et al*., 2018) found a similar increase in nutrient digestibility for crude protein when pigs were fed 80mg Zinc oxide. Our findings align with Cheng and Hardy's (2004) results, suggesting that increased phytic acid ingestion might interfere with food and mineral digestion.

PCOS-induced rats exhibited weight gain, typical of PCOS symptoms, which can be mitigated through lifestyle modifications, including dietary changes (Olechnowicz *et al*., 2018). Treatment with zinc and choline resulted in a significant decrease in body weight, aligned with findings by Fazel Torshizi et al. (2020), who reported weight loss in PCOS rats with 75mg/kg zinc supplementation. In contrast, Foroozanfard *et al*. (2015) found no significant effect on body weight with 50mg zinc supplementation in PCOS patients.

Hormonal profile analysis revealed a significant decrease ($p<0.05$) in serum levels of estrogen, progesterone, LH, FSH, testosterone, and prolactin, as well as insulin. Elevated estradiol levels in PCOS-induced rats may result from androgen conversion to estrogens. The decrease in FSH and LH levels with the zinc and choline diet aligns with studies suggesting improved follicular growth and reduced testosterone levels with choline supplementation. Insulin status improvement in rats fed with ZC1 diet is consistent with Foroozanfard *et al*. (2015) findings with zinc sulfate supplementation in PCOS patients.

Conclusion

Our study highlighted the significant impact and potential of zinc and choline on reproductive hormones, lipid profile, and insulin levels. A notable decrease in weight was observed across all treatment groups. Administering Zinc and choline according to the Recommended Dietary Allowance (RDA) resulted in reduced levels of LH, estrogen, testosterone, and progesterone. Furthermore, there was no significant effect observed on cholesterol, LDL, HDL, triglycerides, and VLDL. Primary and secondary follicles were significantly increased in the ZC1 and ZC3 treatment groups. Importantly, no toxicity was observed throughout or at the conclusion of the trial, indicating that safe levels of zinc safe utilization of zinc and choline in this study. At the whole, group ZC1 demonstrated the most favorable outcomes in enhancing hormonal, lipid, and insulin profiles and choline was maintained. Overall, group ZC1 exhibited the most promising results in improving hormonal, lipid, and insulin profiles.

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