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Original Article

Chamomile Extract as a Natural Hepatoprotective Agent against PCOS-Induced Liver Dysfunction: Antioxidant and Anti-Apoptotic Mechanisms

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ABSTRACT

Objectives: Polycystic ovary syndrome (PCOS) is a common endocrine disorder associated with metabolic dysfunctions, oxidative stress (OXS), and liver abnormalities, including an increased risk of non-alcoholic steatotic liver. Chamomile (CHAM) (*Matricaria chamomilla* L.) is known for its antioxidant, anti-inflammatory, and hepatoprotective properties. This study investigates the hepatoprotective effects of CHAM extract in a PCOS-induced rat model. Methods: PCOS was induced in female Wistar rats, which were then treated with CHAM extract or metformin (METF) for 12 weeks. Serum levels of liver function markers (ALT, AST, LDH), OXS markers (MDA, GPx, CAT), and sex hormones (testosterone, estrogen) were analyzed. Histopathological and immunohistochemical assessments were performed to evaluate liver architecture and apoptosis markers (Bax and Bcl-2). Results: The induction of PCOS significantly increased serum testosterone and estrogen ($p \leq 0.05$), while CHAM administration significantly reduced them ($p \leq 0.001$ and $p \leq 0.01$, respectively). PCOS induction led to significant liver damage, as evidenced by increased levels of ALT ($p<0.001$), AST ($p<0.01$), and LDH ($p<0.001$), elevated MDA ($p<0.001$), and reduced activity of both GPx ($p<0.01$) and CAT ($p<0.001$). Histological examination revealed central vein dilation, hepatocyte apoptosis, and inflammatory infiltration. CHAM treatment reduced OXS (decreased MDA ($p<0.001$) and increased the activity of GPx ($p<0.05$) and CAT ($p<0.01$)), improved liver function markers, and restored hepatic architecture. Furthermore, CHAM decreased hepatic Bax expression while enhancing Bcl-2 expression, indicating an anti-apoptotic effect. Conclusion: CHAM extract exerts significant hepatoprotective effects in a PCOS model by mitigating OXS and reducing apoptosis. These findings support its potential as an adjunct therapy for managing PCOS-related liver dysfunction.

Keywords: PCOS, *Matricaria chamomilla*, Oxidative Stress, Apoptosis, Liver.

1. INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting the female reproductive system, often leading to health issues such as irregular menstruation, hyperandrogenism, and infertility. It is prevalent among

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women of reproductive age. Recent studies indicate a strong association between PCOS and liver abnormalities, including non-alcoholic steatotic liver disease (NASLD), fibrosis, and enzyme imbalances, raising concerns about liver health in affected individuals [1–3].

The exact causes of these coexisting conditions affecting liver and reproductive functions remain unclear. However, potential contributing factors include androgen imbalance, insulin resistance, and lipid peroxidation (LPO). Notably, LPO is linked to elevated oxidative stress (OXS), a growing concern due to modern lifestyle habits among women [4–6]. Therefore, several studies have utilized antioxidants to manage oxidative stress linked to the development of diseases in the female reproductive system, including infertility, endometriosis, and PCOS [1,7].

Estradiol valerate (ESV) is a long-acting estrogen used to induce ovulation in cases of anovulatory PCOS. This compound disrupts the release of hypothalamic gonadotropin-releasing hormone, thereby inhibiting both the secretion and storage of luteinizing hormone (LH). The physiological and histological characteristics of the ovary in the ESV-induced PCOS model closely resemble those observed in humans [8–10].

Matricaria chamomilla L., belonging to the Asteraceae family and commonly referred to as chamomile (CHAM), is one of the most widely used herbal medicines in Southern and Eastern Europe [11]. CHAM is rich in various bioactive compounds, including flavonoids, coumarins, volatile oils, terpenes, sterols, organic acids, and polysaccharides. These compounds play a key role in its pharmacological properties, primarily contributing to its anti-inflammatory and antioxidant effects [12]. CHAM has been traditionally used to treat various ailments, including digestive disorders, common colds, liver conditions, psychological and neurological issues, and respiratory ailments. It is also commonly applied for pain relief, infections, and diseases affecting the eyes, skin, and mouth [13]. CHAM flower extract has been shown to enhance ovarian histology in rats with PCOS and support improved thyroid function [8,14]. It may influence the secretion of hormones such as LH and follicle-stimulating hormone (FSH), potentially aiding in ovulatory function. CHAM extract has been also shown to alleviate kidney damage linked to PCOS [3].

Given its antioxidant and hepatoprotective properties, CHAM extract may offer a natural therapeutic alternative for managing PCOS-related liver dysfunction. This study aims to (1) investigate PCOS-induced liver abnormalities in a rat model and (2) evaluate the antioxidant and anti-apoptotic effects of *M. chamomilla* extract compared to metformin (METF), a standard treatment for metabolic dysfunction in PCOS.

2. METHODS

2.1. Animals and chemicals

In this study, the following chemicals were utilized: ESV “Abcam Inc, USA”, METF “Sigma-Aldrich Co, USA”, and CHAM “World of Herbs, Egypt”. To prepare the CHAM extract, the dried flowers were finely crushed and subjected to repeated extraction using 70% ethyl alcohol. The resulting filtrate was then vacuum dried to obtain a powdered form. A total of 20 mature, virgin female Wistar rats were obtained from the King Fahad Research Centre, King Abdulaziz University, Jeddah, SA. The rats had an average body weight of 200 g. One week later, the research was conducted following the controlled environmental conditions, including a standard temperature and humidity range and a 12:12-hour light/dark cycle. Food and water were provided ad libitum, with no restrictions. The research received approval (168–19) from the Biomedical Ethics Research Council of the Faculty of Medicine at King Abdulaziz University, Jeddah, Saudi Arabia.

Induction of PCOS

To induce PCOS, two doses of ESV (0.2 mg each) were administered six weeks apart. This induction method was originally established by Farideh et al. (2010) and further validated in studies investigating PCOS associated with hypothyroidism and kidney dysfunction [3,8,14].

2.2. Experimental design

The 20 rats involved in this study were randomly distributed into four groups (n=5) **(Figure 1)**:

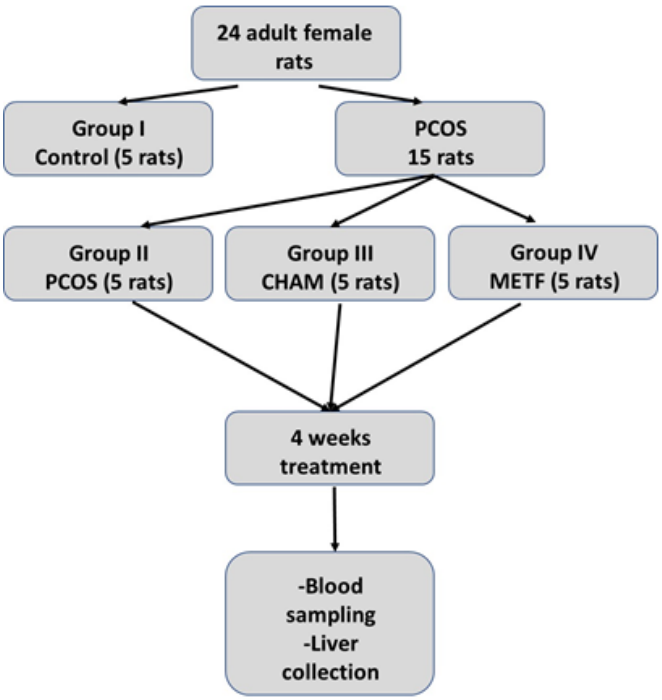

Figure 1. A diagram illustrating the research process

Table 1. Summary of experimental groups and interventions.

Groups	Intervention
Group I	Healthy rats with no medication
Group II	Rats induced with PCOS
Group III	PCOS-induced animals medicated with CHAM
Group IV	PCOS-induced animals medicated with METF

Table 1 showed the distribution of experimental groups and their corresponding interventions in the study. Following PCOS induction, Group 3 received CHAM flower extract (75 mg/kg/day), while Group 4 was treated with METF (500 mg/kg/day). Both medicines were administered each day over the course of a month to evaluate their potential therapeutic effects [14].

2.3. Collection of serum and liver samples

The collection of serum samples and liver specimens is described in **Table 2**.

Table 2. Collection and processing of serum and liver samples.

Procedure	Details
Blood sample collection	Collected via heart puncture
Serum processing	Serum was separated by centrifugation at 3,000 rpm for 10 minutes and stored at −80°C for analysis.
Analyzed parameters in serum	Liver function markers, OXS, and antioxidant markers
Liver collection	Liver samples were extracted via abdominal dissection
Liver samples preservation	Liver samples were fixed in 10% neutral buffered formalin solution for further automatic paraffin processing
Purpose of liver samples analysis	Assess hepatic histopathological changes, and evaluate apoptotic/anti-apoptotic markers using immunohistochemistry

2.4. Measurement of serum testosterone and estrogen levels

Serum testosterone and estrogen levels were assessed at El-Safwa Laboratory, Tanta, Egypt, using the ADVIA Centaur automated competitive chemiluminescence immunoassay (Bayer HealthCare).

2.5. Measurement of liver function markers

Serum levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were measured using commercial detection kits from Biodiagnostic, Egypt [15].

2.6. Histopathological analysis

Liver specimens, fixed in formalin, were processed, encased in paraffin and cut into 4 μm sections. The liver sections were treated with hematoxylin and eosin (H&E) stain and examined under a light microscope, where images were captured for analysis. Histological processing followed the previously described method [16].

2.7. Measurement of serum OXS markers

The levels of oxidative lipid degradation product (MDA), glutathione peroxidase (GPx), and catalase (CAT) in serum were assessed by the kits BioDiagnostic, Egypt.

2.8. Immunohistochemical analysis of apoptotic/antiapoptotic markers

The immunoperoxidase (peroxidase-anti-peroxidase) technique was employed. The liver sections were treated to examine the Bax and Bcl-2 gene expression. Antibodies from Lab Vision (Fremont, CA) (1:200 dilution). The stained sections were then examined and photographed.

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2.9. Statistics

The findings were analyzed through analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Scatter plots were generated to represent the mean ± standard error (SE) (n=5) using GraphPad Prism (version 5). Statistical significance was set at $p \leq 0.05$.

3. RESULTS

3.1. Impact of CHAM and METF treatment on serum testosterone and estrogen levels

Rats with induced PCOS revealed a significant increase in both blood testosterone and estrogen concentrations relative to the control rats ($p \leq 0.05$). However, treatment with either CHAM flower extract or METF led to a notable reduction in both blood testosterone ($p \leq 0.001$) and estrogen ($p \leq 0.01$) relative to untreated PCOS group **(Table 3)**.

Table 3. Impact of CHAM and METF treatment on serum testosterone and estrogen levels measured in PCOS-induced rats.

Group		Testosterone (ng/ml)	Estrogen (ng/ml)
Group I	**Control**	3.21±0.19	90.45±1.13
Group II	**PCOS**	4.74±0.18[a]	300.39±20.33[a]
Group III	**CHAM**	2.48±0.17[b]	100.24±2.98[b]
Group IV	**METF**	2.86±0.39[b]	98.48±2.35[b]

Results were presented as mean ± SE (n=5). [a] denoted a significant variation relative to the control rats ($p \leq 0.05$), while [b] denoted a significant variation relative to the untreated PCOS rats ($p \leq 0.05$).

3.2. Impact of PCOS, CHAM, and METF on hepatic function indicators

The findings showed significant elevation in blood ALT ($p<0.001$), AST ($p<0.01$), and LDH ($p<0.001$) 12 weeks after PCOS induction **(Figure 2A, 2B, and 2C, respectively)**. Conversely, treatment with CHAM flower extract significantly reduced serum ALT, AST, and LDH levels in PCOS-induced rats compared to their untreated counterparts ($p<0.001$) **(Figure 2A, 2B, and 2C, respectively)**.

Additionally, treatment with METF significantly reduced serum ALT ($p<0.001$), AST ($p<0.01$), and LDH ($p<0.001$) levels in PCOS-induced rats compared to their untreated counterparts **(Figure 2A, 2B, and 2C, respectively)**.

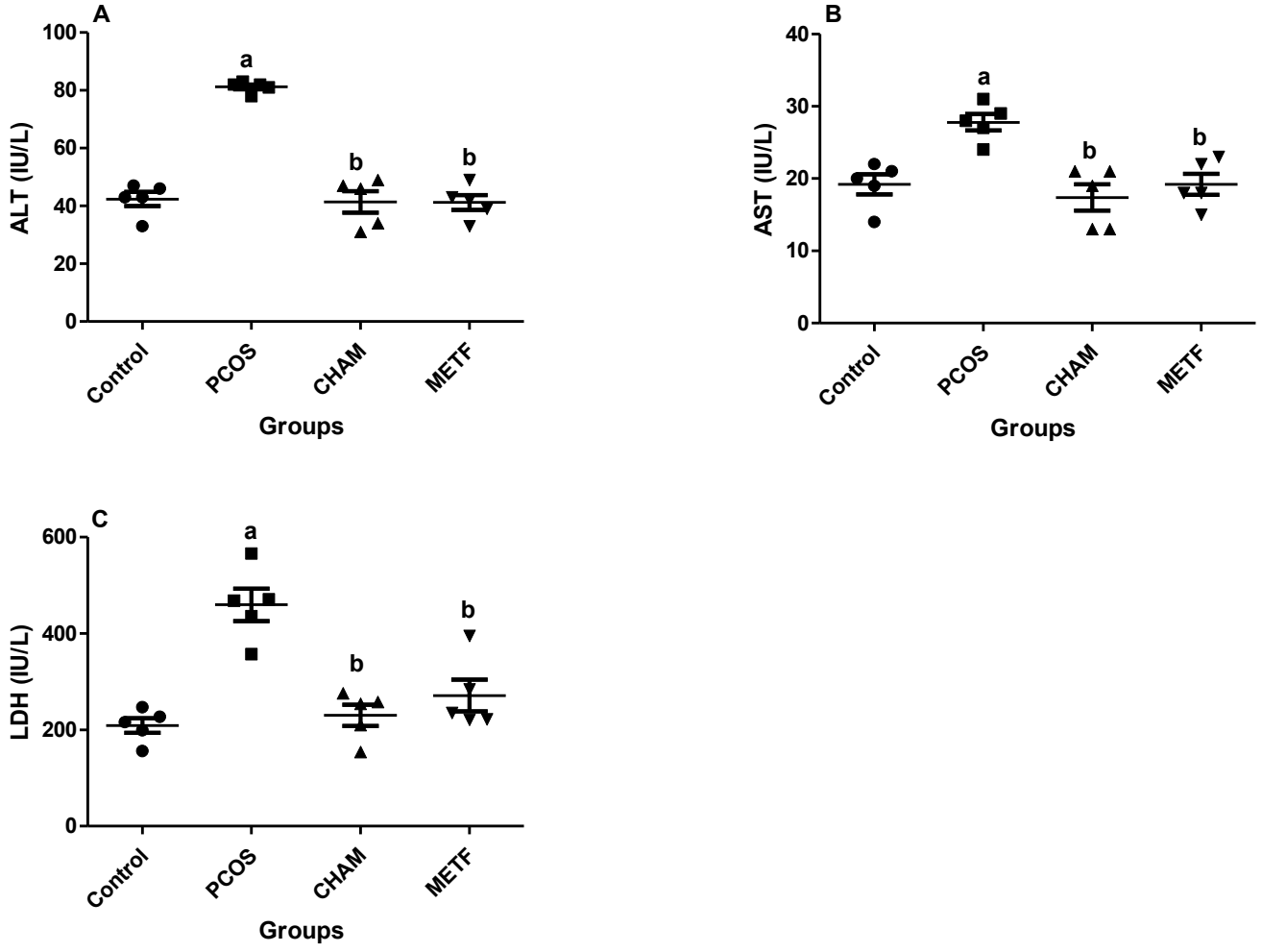

Figure 2. Impact of CHAM and METF treatment on serum liver function markers measured in PCOS-induced rats. Results were presented as a Scatter plot vertical representing mean ± SE (n=5). [a] denoted a significant variation relative to the control rats ($p \leq 0.05$), while [b] denoted a significant variation relative to the untreated PCOS rats ($p \leq 0.05$).

3.3. Impact of PCOS, CHAM, and METF on liver histology

Hepatic sections from the normal rats exhibited normal histological structure, with well-organized hepatocyte cords surrounding the central vein (CV) and intact blood sinusoids. The portal vein region (PVR) showed hepatocytes with active

vesicular nuclei and normal portal structures, including the bile duct (BD) and hepatic artery **(Figure 3)**.

In contrast, the PCOS group displayed significant histopathological alterations, including marked central vein dilation, congestion, and focal hepatocyte necrosis. Many hepatocytes exhibited dark, degenerated nuclei indicative of apoptosis. The PVR contained hepatocytes with nuclear size variations, dark-stained apoptotic nuclei, and prominent Kupffer cells lining the blood sinusoids **(Figure 3)**.

The CHAM-treated group demonstrated notable histological improvement, with a normal central vein and hepatocytes displaying active vesicular nuclei. Blood sinusoids and portal elements remained structurally intact, resembling the control group **(Figure 3)**.

The METF-treated group showed partial restoration of hepatocyte morphology, with hepatocytes exhibiting active vesicular nuclei. However, central vein dilation and focal sinusoidal congestion persisted. The PVR displayed mild congestion in the portal vein and bile duct, with epithelial cells maintaining active nuclei **(Figure 3)**.

3.4. Impact of PCOS, CHAM, and METF on serum OXS indicators

The findings showed that the induction of PCOS significantly increased the blood level of the OXS indicators, MDA ($p<0.001$), 12 weeks after PCOS induction **(Figure 4A)**. Conversely, the induction of PCOS significantly decreased the serum level of the antioxidant enzymes, GPx and CAT, 12 weeks after PCOS induction ($p< 0.01$ and $p<0.001$, respectively) **(Figure 4B and 4C, respectively)**. PCOS-induced rats subjected to both CHAM and METF showed a significant reduction in blood MDA relative to the PCOS rats ($p<0.001$) **(Figure 4A)**.

Additionally, treatment with both CHAM flower extract and METF significantly increased serum levels of GPx ($p<0.05$) and CAT ($p<0.01$ and $p<0.001$, respectively) in PCOS-induced rats compared to their untreated PCOS group (Figure 4B and 4C, respectively).

3.5. Impact of PCOS, CHAM, and METF on liver bax immunoexpression

Sections from rat liver immunostained for Bax (a marker for cell apoptosis) showed increased expression in the PCOS group. However, treatment with CHAM and METF reduced Bax immunoexpression expression compared to untreated PCOS-induced rats **(Figure 5)**.

3.6. Impact of PCOS, CHAM, and METF on Liver Bcl-2 Immunoexpression

Sections from rat liver immunostained for Bcl-2 (a marker for cell protection) showed nearly negative expression in the PCOS group. However, treatment with CHAM and METF moderately enhanced Bcl-2 immunoexpression expression compared to untreated PCOS-induced rats **(Figure 6)**.

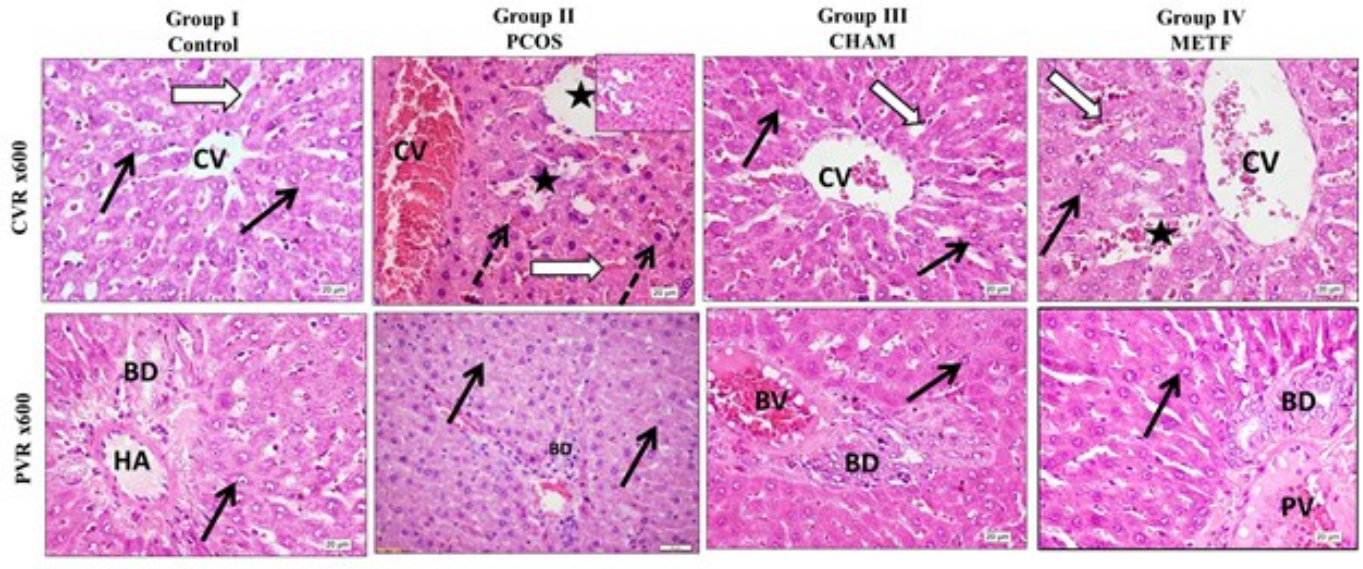

Figure 3. Impact of CHAM and METF treatment on liver histopathology examined in PCOS-induced rats. Sections of rat liver stained by H&E. Group I: The Control group showed central vein region (CVR) with normal hepatocyte cell cords (black arrows) separated by normal blood sinusoids (white arrows) & portal vein region (PVR) also showed normal hepatocytes with active vesicular nuclei (black arrows) with normal portal elements (bile duct) (BD) and hepatic artery. Group II: The PCOS group showed CVR with marked central vein (CV) dilation, congestion, and focal hepatocyte necrosis (stars). The rest of the hepatocytes (dotted arrows) showed dark small degenerated nuclei (features of apoptosis). PVR showed areas of dark stained hepatocytes with small dark nuclei that can be considered features of hepatocyte apoptosis, nuclear size variation could be seen

in hepatocytes. Prominent nuclei of Kupffer cells lining hepatic blood sinusoids were evident. Group III: The CHAM group showed CVR with normal CV, and hepatocytes showed active vesicular nuclei (black arrows) with normal blood sinusoids (white arrows). PVR showed hepatocytes with normal vesicular nuclei (black arrows) separated by normal blood sinusoids (white arrows). Group IV: The METF group showed CVR with potential restoration of hepatocyte (black arrow) with normal appearance (active vesicular nuclei), still dilated CV, focal dilation, and congestion of blood sinusoids (white arrow). PVR Showed slight congestion of PV, BD with active nuclei of lining epithelium

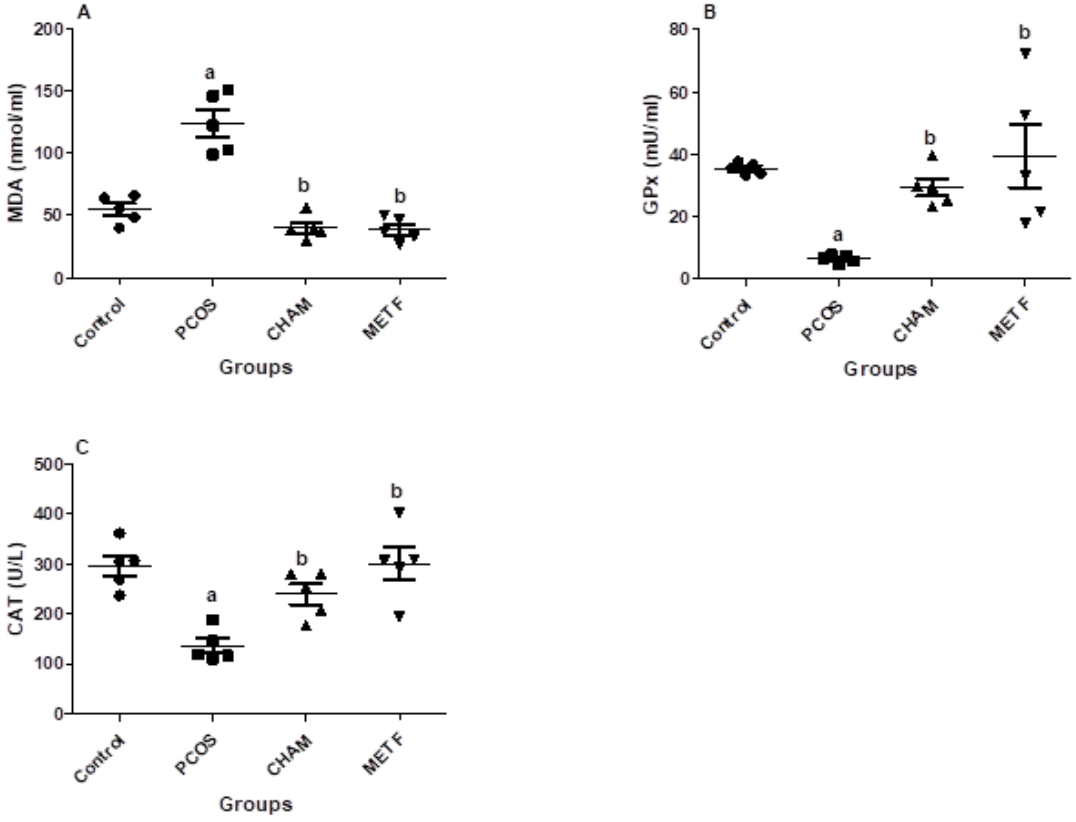

Figure 4. Impact of CHAM and METF treatment on serum OXS indicators measured in PCOS-induced rats. Results were presented as a Scatter plot vertical representing mean ± SE (n=5). [a] denoted a significant variation relative to the control rats ($p \leq 0.05$), while [b] denoted a significant variation relative to the untreated PCOS rats ($p \leq 0.05$).

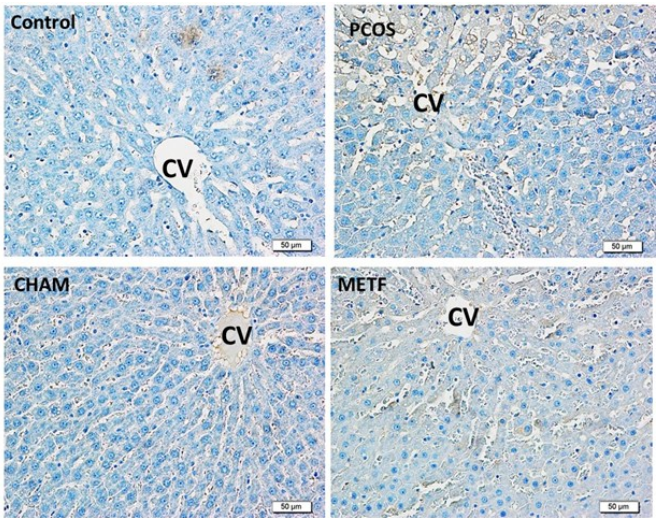

Figure 5. Impact of CHAM and METF treatment on hepatic Bax gene expression examined in PCOS-induced rats. Moderate immuno-positive reaction was observed in necrotic regions of the PCOS group compared to the control group. Decreased such areas were observed in the METF group and nearly the absence of any reaction in the group treated by CHAM, which looked similar to the control group.

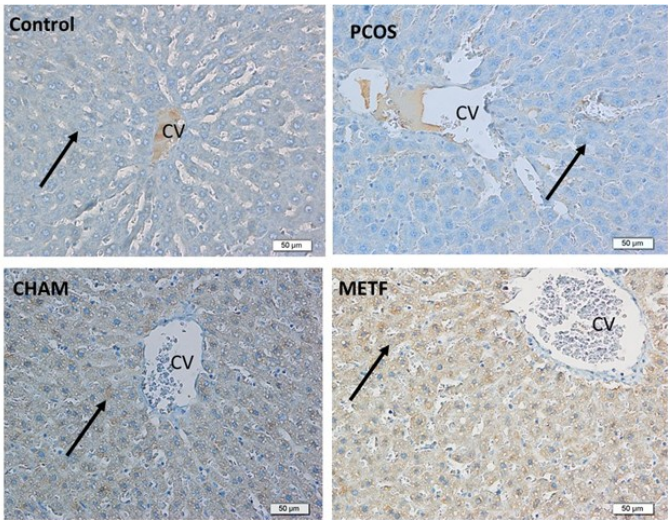

Figure 6. Impact of CHAM and METF treatment on hepatic Bcl-2 gene expression examined in PCOS-induced rats. Sections from rat liver immunostained for Bcl-2 (a marker for cell protection) showed nearly negative expression in the PCOS group and a moderate diffuse increase in CHAM and METF-treated groups.

4. DISCUSSION

The present study highlights the significant impact of CHAM flower extract and METF on hormonal balance, liver function, oxidative stress, and histopathological changes in a rat model of PCOS. The findings indicate that both treatments effectively mitigate PCOS-induced alterations, suggesting potential therapeutic benefits.

PCOS is often associated with hormonal imbalances, including elevated testosterone and estrogen levels. In the current study, PCOS-induced rats exhibited significantly increased serum testosterone and estrogen levels compared to the control group. These hormonal changes contribute to the pathophysiology of PCOS, including metabolic and reproductive disturbances [17–19]. Treatment with either CHAM or METF significantly reduced both hormone levels, indicating their potential role in restoring hormonal balance [3,14].

Notably, previous studies have demonstrated that metformin at a dose of 1500 mg/day significantly reduced free testosterone levels by 29% and estradiol levels by 38%, suggesting its role in modulating sex hormone bioavailability, primarily through the reduction of testosterone [20]. CHAM has also been identified as a phytoestrogen with antiestrogenic properties. In a study by Johari et al., investigating the effects of hydroalcoholic CHAM extract on the hypothalamus-pituitary-ovary axis in rats, CHAM significantly reduced estrogen levels while increasing progesterone levels [21] . This suggests that CHAM may act as a selective estrogen receptor modulator, helping to regulate endogenous estrogen levels in individuals with PCOS who typically exhibit elevated estrogen concentrations. These properties reinforce CHAM's potential as a natural alternative for managing hormonal imbalances in PCOS. The observed reduction in testosterone and estrogen levels suggests that CHAM and METF may regulate ovarian steroidogenesis, possibly through modulation of androgen synthesis and estrogen metabolism [22,23].

Liver dysfunction is commonly reported in PCOS, often manifesting as increased levels of liver enzymes [24,25]. The results of this research showed a significant elevation in serum ALT, AST, and LDH levels in PCOS-induced rats, reflecting hepatic damage. Histopathological examination further confirmed this damage, with PCOS rats exhibiting central vein dilation, congestion, hepatocyte necrosis, and increased apoptosis, as indicated by dark, shrunken nuclei [26]. Treatment with CHAM and METF significantly reduced serum ALT, AST, and LDH levels, suggesting hepatoprotective effects. Additionally, liver histology revealed notable improvement, with CHAM-treated rats displaying nearly normal hepatic architecture, while METF-treated rats showed partial restoration of hepatocyte morphology despite some persistent sinusoidal congestion.

These results indicate that CHAM and METF may alleviate PCOS-induced hepatic damage by modulating liver enzyme activity and improving histological integrity. The findings of the present work align with previous research indicating the protective role of CHAM extract in liver toxicity. Methotrexate (MTX) has been shown to induce OXS and liver damage, primarily through increased oxidative biomarkers and histopathological alterations. A study investigating the impact of CHAM on MTX-produced liver toxicity demonstrated significant improvements in liver biomarkers, including aminotransferases, alkaline phosphatase (ALP), and albumin levels [27]. CHAM extract exhibits hepatoprotective effects by mitigating OXS and structural liver damage induced by toxins like 2, 4-dichlorophenoxy acetic acid. The study show that CHAM improves the hepatic ultrathin structure changes, highlighting its potential in protecting against hepatotoxicity [28].

OXS plays a crucial role in the pathogenesis of PCOS, contributing to metabolic and reproductive dysfunctions. The results of this study showed that PCOS induction significantly increased serum MDA levels, a marker of LPO, while significantly decreasing antioxidant enzyme levels (GPx and CAT). These findings confirm the OXS burden in PCOS [29,30]. Treatment with both CHAM and METF significantly reduced serum MDA levels while enhancing GPx and CAT activity, suggesting their potent antioxidant properties [3,14]. Beyond hormonal regulation, the current findings highlight the preventive impact of CHAM and METF against liver dysfunction and induction of OXS in PCOS-induced rats. These findings align with prior research demonstrating CHAM's ability to mitigate liver injury and oxidative stress (MTX-induced liver toxicity study).

CHAM enhances antioxidant enzyme activity, such as GPx and CAT, while reducing LPO markers like MDA, thereby protecting against oxidative damage [27]. The ability of CHAM to modulate OXS may be attributed to its bioactive

compounds, which enhance antioxidant defense mechanisms and reduce LPO, ultimately protecting cellular integrity [13,31]. CHAM extract is rich in various antioxidant compounds that contribute to its protective effects against OXS. Among these, flavonoids and hydroxycinnamic acids play a crucial role in scavenging free radicals and reducing cellular damage [32,33]. Additionally, CHAM contains phenolic acids, including caffeic acid and chlorogenic acid, which further enhance its antioxidant capacity [34]. Studies have shown that the antioxidant properties of CHAM contribute to its hepatoprotective, making it a valuable natural remedy for the liver protection [27,35].

Apoptosis dysregulation is another hallmark of PCOS, particularly in hepatic and ovarian tissues. Immunohistochemical analysis revealed increased hepatic Bax (a pro-apoptotic marker) and reduced hepatic Bcl-2 (an anti-apoptotic marker) expression in PCOS-induced rats, indicating enhanced apoptotic activity. Increased androgen levels in PCOS have been linked to hepatic apoptosis, partly through the upregulation of Bax (a pro-apoptotic protein) and the downregulation of Bcl-2 (an anti-apoptotic protein). Treatment with CHAM and METF significantly reduced Bax expression while increasing Bcl-2 expression, suggesting their ability to counteract androgen-induced apoptosis in hepatocytes. This further supports their role in protecting against PCOS-related liver dysfunction.

5. CONCLUSION

The present study highlights the hepatoprotective effects of CHAM extract in a PCOS-induced rat model. PCOS is often associated with hyperandrogenism and OXS, which contribute to liver dysfunction and structural damage. The significant reduction in serum ALT, AST, and LDH levels following CHAM treatment suggests improved liver function and reduced hepatic injury. The histopathological analysis further confirmed the protective role of CHAM, as it mitigated liver structural damage, preserved hepatocyte integrity, and reduced apoptotic markers. The antioxidant properties of CHAM, attributed to its flavonoid content, played a key role in reducing OXS, restoring antioxidant enzyme levels, and suppressing apoptotic pathways. These results suggest that CHAM extract may serve as a promising natural hepatoprotective agent in managing liver complications associated with PCOS-induced metabolic disturbances. Further research is warranted to explore its potential for clinical applications.

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CONFLICT OF INTEREST

The author declares that there are no conflicts of interest related to the submitted work.

6. REFERENCES

1. Lemos A, Peixoto C, Teixeira A, et al. Effect of the combination of metformin hydrochloride and melatonin on oxidative stress before and during pregnancy, and biochemical and histopathological analysis of the livers of rats after treatment for polycystic ovary syndrome. *Toxicol Appl Pharmacol*. 2014;280(1):159-168. doi:10.1016/J.TAAP.2014.05.015
2. Cooney L, Dokras A. Beyond fertility: polycystic ovary syndrome and long-term health. *Fertil Steril*. 2018;110(5):794-809. doi:10.1016/J.FERTNSTERT.2018.08.021
3. Alahmadi A, Alahmadi B, Wahman L, El-Shitany N. Chamomile flower extract ameliorates biochemical and histological kidney dysfunction associated with polycystic ovary syndrome. *Saudi J Biol Sci*. 2021;28(11):6158-6166. doi:10.1016/J.SJBS.2021.06.066
4. Manna P, Jain S. Obesity, Oxidative Stress, Adipose Tissue Dysfunction, and the Associated Health Risks: Causes and Therapeutic Strategies. *Metab Syndr Relat Disord*. 2015;13(10):423-444. doi:10.1089/MET.2015.0095
5. Hutchison A, Tavaglione F, Romeo S, Charlton M. Endocrine aspects of metabolic dysfunction-associated steatotic liver disease (MASLD): Beyond insulin resistance. *J Hepatol*. 2023;79(6):1524-1541. doi:10.1016/J.JHEP.2023.08.030
6. Vassilatou E. Nonalcoholic fatty liver disease and polycystic ovary syndrome. *World J Gastroenterol*. 2014;20(26):8351–8363. doi:10.3748/WJG.V20.I26.8351
7. Shao Y, Ma L, Zhou J, Wu K, Tang X. Impact of dietary antioxidants on female infertility risk: evidence from NHANES. *Sci Reports 2024 141*. 2024;14(1):1-10. doi:10.1038/s41598-024-72434-8
8. Alzahrani A, Alahmadi A, Ali S, et al. Biochemical and histological evidence of thyroid gland dysfunction in estradiol valerate model of the polycystic ovary in Wistar rats. *Biochem Biophys Res Commun*. 2019;514(1):194-199. doi:10.1016/J.BBRC.2019.04.126
9. Jelodar G, Masoomi S, Rahmanifar F. Hydroalcoholic extract of flaxseed improves polycystic ovary syndrome in a rat model. *Iran J Basic Med Sci*. 2018;21(6):645-650. doi:10.22038/IJBMS.2018.25778.6349
10. Singh K. Rat Models of Polycystic Ovary Syndrome. *Source B Model Biomed Res*. Published online 2008:405-410. doi:10.1007/978-1-59745-285-4_43

11. Mihyaoui A, Esteves Da Silva JCG, Charfi S, Castillo M, Lamarti A, Arnao M. Chamomile (Matricaria chamomilla L.): A Review of Ethnomedicinal Use, Phytochemistry and Pharmacological Uses. *Life*. 2022;12(4):479. doi:10.3390/LIFE12040479
12. Dai Y, Li Y, Wang Q, et al. Chamomile: A Review of Its Traditional Uses, Chemical Constituents, Pharmacological Activities and Quality Control Studies. *Molecules*. 2022;28(1):133. doi:10.3390/MOLECULES28010133
13. EL Mihyaoui A, Esteves Da Silva JCG, Charfi S, Castillo M, Lamarti A, Arnao M. Chamomile (Matricaria chamomilla L.): A Review of Ethnomedicinal Use, Phytochemistry and Pharmacological Uses. *Life*. 2022;12(4):479. doi:10.3390/life12040479
14. Alahmadi AA, Alzahrani AA, Ali SS, Alahmadi BA, Arab RA, El-Shitany NAEA. Both Matricaria chamomilla and Metformin Extract Improved the Function and Histological Structure of Thyroid Gland in Polycystic Ovary Syndrome Rats through Antioxidant Mechanism. *Biomol 2020, Vol 10, Page 88*. 2020;10(1):88. doi:10.3390/BIOM10010088
15. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol*. 1957;28(1):56-63. doi:10.1093/AJCP/28.1.56
16. Bancroft J, Layton C. The Hematoxylin and Eosin. In: Suvarna, S.K., Layton, C. and Bancroft, J.D., Eds.,. *Theory Pract Histol Tech 7th Ed Churchill Livingstone El Sevier, Philadelphia, Ch 10 11*. 2013;(10, 11):179–220.
17. Rosenfield RL, Ehrmann DA. The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited. *Endocr Rev*. 2016;37(5):467-520. doi:10.1210/ER.2015-1104
18. Singh S, Pal N, Shubham S, et al. Polycystic Ovary Syndrome: Etiology, Current Management, and Future Therapeutics. *J Clin Med*. 2023;12(4):1454. doi:10.3390/JCM12041454
19. Hajam Y, Rather H, Neelam, Kumar R, Basheer M, Reshi M. A review on critical appraisal and pathogenesis of polycystic ovarian syndrome. *Endocr Metab Sci*. 2024;14:100162. doi:10.1016/J.ENDMTS.2024.100162
20. Campagnoli C, Berrino F, Venturelli E, et al. Metformin decreases circulating androgen and estrogen levels in nondiabetic women with breast cancer. *Clin Breast Cancer*. 2013;13(6):433-438. doi:10.1016/J.CLBC.2013.08.012
21. Johari H, Sharifi E, Mardan M, et al. The effects of a hydroalcoholic extract of Matricaria chamomilla flower on the pituitary-gonadal axis and ovaries of rats. *Int J Endocrinol Metab*. 2011;9(2):330-334. doi:10.5812/kowsar.1726913X.1822
22. Rani R, Hajam Y, Kumar R, Bhat R, Rai S, Rather M. A landscape analysis of the potential role of polyphenols for the treatment of Polycystic Ovarian Syndrome (PCOS). *Phytomedicine Plus*. 2022;2(1):100161. doi:10.1016/J.PHYPLU.2021.100161
23. Attia GR, Rainey WE, Carr BR. Metformin directly inhibits androgen production in human thecal cells. *Fertil Steril*. 2001;76(3):517-524. doi:10.1016/S0015-0282(01)01975-6
24. Minato S, Sakane N, Kotani K, et al. Prevalence and Risk Factors of Elevated Liver Enzymes in Japanese Women With Polycystic Ovary Syndrome. *J Clin Med Res*. 2018;10(12):904. doi:10.14740/JOCMR3639
25. Won Y, Seo S, Yun B, Cho S, Choi Y, Lee B. Non-alcoholic fatty liver disease in polycystic ovary syndrome women. *Sci Reports 2021 111*. 2021;11(1):1-11. doi:10.1038/s41598-021-86697-y
26. Abulfadle K, Edrees HM, Hassan N, Mohammed H. Spexin and Metformin Comparative Ameliorated Ovarian and Liver Function Changes in Letrozole-Induced Polycystic Ovary SyndromeinRats(Histological,Biochemical,Immunohistochemical and Morphometric Study). *Egypt J Histol*. 2022;45(3):774-790. doi:10.21608/ejh.2021.76972.1486
27. Soltani Afarani M, Mohammadi M, Monsef Shokri M, Mohammadzadeh S. Investigation of Protective Effect of Matricaria Chamomilla L. Extract on Methotrexate-Induced Hepatotoxicity in Wistar Rat. *Brazilian Arch Biol Technol*. 2020;63:2020. doi:10.1590/1678-4324-2020180626
28. Baroudi DA Al, Kholy T El, Arafat R, Ali A. The Potential Protective Role of Chamomile Extract on Rat Liver Ultrastructural Changes Induced by 2, 4-Dichlorophenoxyacetic Acid. *J Diagnostics*. 2016;3(1):11-20. doi:10.18488/JOURNAL.98/2016.3.1/98.1.11.20
29. Mahmud AA, Anu UH, Foysal KA, et al. Elevated serum malondialdehyde (MDA), insulin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH), and reduced antioxidant vitamins in polycystic ovarian syndrome patients. *Narra J*. 2022;2(1):e56. doi:10.52225/NARRA.V2I1.56
30. Enechukwu C, Onuegbu A, Olisekodiaka M, et al. Oxidative stress markers and lipid profiles of patients with polycystic ovary syndrome in a Nigerian tertiary hospital. *Obstet Gynecol Sci*. 2019;62(5):335-343. doi:10.5468/OGS.2019.62.5.335
31. Hussein M, Fajer A. In vivo Evaluation of Antioxidant Activity of Chamomile Extract against Procyclidine-Induced Oxidative Stress: Potential Application in Cancer Prevention. *Asian Pac J Cancer Prev*. 2024;25(8):2919-2928. doi:10.31557/APJCP.2024.25.8.2919
32. de Angelis M, Amicucci C, Banchelli M, et al. Rapid determination of phenolic composition in chamomile (Matricaria recutita L.) using surface-enhanced Raman spectroscopy. *Food Chem*. 2025;463:141084. doi:10.1016/J.FOODCHEM.2024.141084
33. Foss K, Przybyłowicz KE, Sawicki T. Antioxidant Activity and Profile of Phenolic Compounds in Selected Herbal Plants. *Plant Foods Hum Nutr*. 2022;77(3):383-389. doi:10.1007/S11130-022-00989-W/FIGURES/2

34. Nováková L, Vildová A, Mateus J, Gonalves T, Solich P. Development and application of UHPLC–MS/MS method for the determination of phenolic compounds in Chamomile flowers and Chamomile tea extracts. *Talanta*. 2010;82(4):1271-1280. doi:10.1016/J.TALANTA.2010.06.057
35. Alzahrani M. The effect of different levels of matricaria chamomilla on functional status of the liver in rats injected with carbon tetrachloride. *Pak J Zool*. 2020;52(6):2111-2119. doi:10.17582/JOURNAL.PJZ/20190309080304
36. Song M, Choi J. Androgen dysfunction in non-alcoholic fatty liver disease: Role of sex hormone binding globulin. *Front Endocrinol (Lausanne)*. 2022;13:1053709. doi:10.3389/FENDO.2022.1053709
37. Kelley C, Brown A, Diehl A, Setji T. Review of nonalcoholic fatty liver disease in women with polycystic ovary syndrome. *World J Gastroenterol*. 2014;20(39):14172. doi:10.3748/WJG.V20.I39.14172