

Biomedical Communication

Biochemical and Histological Remodeling of Adrenal Glands Associated with Estradiol Valerate-Induced Polycystic Ovary in Rats: Protective Effects of *Matricaria chamomilla*

Ahlam Abdulaziz Alahmadi

Department of Biological Sciences, College of Science, King Abdulaziz University, Jeddah, Saudi Arabia

ABSTRACT

Polycystic ovary (PCO) is a condition in which the secretion of specific hormones is disrupted. The adrenal glands are essential for the manufacture of certain hormones and the monitoring of steroid concentration, and they may be impacted in PCO situations. In a PCO-induced rat model, the researcher was aimed to investigate how administering exogenous estrogen, estradiol valerate affected on adrenal gland secretions and histology. The effects of *Matricaria chamomilla* flowers extract, and metformin will also be evaluated. 24 rats were divided into four equal groups: 1- control group, 2- PCO group, 3- metformin group, 4- *Matricaria chamomilla* group. PCO was induced by injecting 2 doses of estradiol valerate. The findings revealed a considerable increase in estrogen and ACTH levels in the PCO rats. The concentrations of cortisol and aldosterone were likewise higher in the PCO rats. These findings were corroborated by histological investigation of adrenal sections (both H&E and PCNA staining). *Matricaria chamomilla* flowers extract reduced all the hormonal alterations linked to PCO, including estrogen, ACTH, cortisol, and aldosterone. Metformin similarly reduced estrogen and ACTH. On the other hand, Metformin did not affect the hormonal changes associated with this model in terms of adrenal gland hormones (cortisol and aldosterone). In conclusion both treatments are improved that the histological investigation of adrenal secretions (both H&E and PCNA staining). The antiestrogen activity of *Matricaria chamomilla* could clarify that all the hormonal modulation occurs in this PCO model.

KEY WORDS: POLYCYSTIC OVARY; ADRENAL GLANDS; CORTISOL; ALDOSTERONE, ESTROGEN; ACTH; PCNA.

INTRODUCTION

Polycystic ovary (PCO) is a condition in which the secretion of specific hormones is disrupted, which causing reproductive issues in women. Irregular menstruation, the development of ovarian cysts, and insulin intolerance are all common PCO problems. Estrogen is a hormone generated chiefly by the ovaries but also in minor amounts by the adrenal glands. It's in charge of the formation of feminine sex traits. Estrogen is necessary for the normal functioning of sexual organs, skeletal system preservation, menstrual cycle control, and pregnancy maintenance (Reed and Carr, 2000; Clarke and Khosla, 2010; Ndefo et al., 2013; Witchel et al., 2019; Delgado and Lopez-Ojeda, 2021).

Although estrogen levels vary naturally throughout the monthly cycle throughout a woman's life, several women may experience estrogen dominance, in which their estrogen levels are more significant than average. Estrogen dominance, or raised estrogen concentrations, can occur in PCO ladies. PCO is the reasonable explanation of oligo-ovulation, and it's supposed that estrogen dominance exerts a role. The absence of ovulation results in perpetual extraordinary estrogen levels and lacking progesterone (Hambridge et al., 2013; Dennett and Simon, 2015; Leon et al., 2021).

The adrenal glands are essential for the manufacture of certain hormones as well as the monitoring of steroid concentration in the body, and they may be impacted in PCO patients. Recently, a PCO model was established in our lab via injecting estradiol valerate in rats. The model was characterized by an increase in blood estrogen levels. Women with PCO have widespread hypersecretion of adrenal gland cortical hormones, including pregnenolone,

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17-hydroxypregnenolone, DHEA, androstenedione, 11-deoxycortisol, and cortisol both at basically and after stimulation with adrenocorticotrophic hormone (ACTH) (Azziz et al., 1998; Dumitrescu et al., 2015; Rosenfield and Ehrmann, 2016; Alahmadi et al., 2020).

There are also subtle systemic steroid metabolic problems in PCO women, such as tendencies favoring increased estrogen and cortisol output. Oral estrogen replacement medication has already been demonstrated to raise total cortisol levels in women in various trials. Some reports, on the other hand, did not reveal similar disparities. Most studies haven't looked at cortisol levels after estrogen replacement medication (Tsilchorozidou et al., 2003; Tock et al., 2014). It's uncertain whether the use of exogenous estrogen therapy will affect the levels of various adrenal hormones. Chamomile (*Matricaria chamomilla* L.) is a widespread herbal medicine in Southern and Eastern Europe. It is a member of the Asteraceae family. Chamomile's active components include flavonoids responsible for the pharmacological attributes of chamomile, mainly the anti-inflammatory and antioxidant benefits. *Matricaria chamomilla* extract has recently been shown to lower PCO-related estrogen increase (Heidary et al., 2018; Alahmadi et al., 2020; Sotiropoulou et al., 2020).

The purpose of this study was to look at the effects of injecting exogenous estrogen, estradiol valerate, on adrenal gland secretions and histology in a PCO-induced rat model. Furthermore, the impact of *Matricaria chamomilla* flowers extract, and metformin were assessed.

MATERIALS AND METHODS

Chemicals: In this research, *Matricaria chamomilla* flowers was purchased from World of Herbs, Egypt, estradiol valerate purchased from Abcam Inc, USA, and metformin purchased from Sigma-Aldrich Co, USA.

Preparation of *Matricaria chamomilla* flowers extract: The extract was made by extracting the crushed *Matricaria chamomilla* flowers with ethyl alcohol (70 percent), then drying the extract under vacuum.

Animals: In the current study, twenty-four adult, virgin female Wistar rats were used. They obtained from the King Fahad Research Centre at King Abdullah University in Jeddah, Saudi Arabia. Their body weight varies between 186 and 208 grams. The research was conducted out under a conventional laboratory environment of temperature, humidity, and a 12:12 h light/dark cycle after a one-week acclimatization period. The animals were not subjected to any water or food restrictions. The research procedures were accepted by the Biomedical Ethics Research Council, Faculty of Medicine, KAU, Jeddah, SA, acceptance number, 168-19.

Induction of PCO: PCO was induced by administering two doses of estradiol valerate (0.2 mg each), one dose at the start and the other after six weeks of the experiment (Alzahrani et al., 2019; Farideh et al., 2010).

Experimental groups: The rats were divided into four equal groups (n = 6). Control group: corn oil-injected group, PCO group: estradiol valerate injected group, Metformin group: PCO rats treated with metformin (500 mg/kg) (Alahmadi et al., 2020), *Matricaria chamomilla*: PCO rats treated with *Matricaria chamomilla* flower extract (75 mg/kg) (Alahmadi et al., 2020). Corn oil, metformin, and *Matricaria chamomilla* were injected every day into the animals starting at the beginning of the experiment and remained for one month following PCO initiation.

Collection of serum and adrenal glands samples: A cardiac puncture was used to gather blood samples. Adrenal hormones (cortisol and aldosterone), anterior pituitary hormone (ACTH), and estrogen levels were measured after the serum was extracted and kept frozen at -80°C. Both adrenal glands were removed and preserved in 10% buffered formalin for histopathological evaluations and immunohistochemical proliferating cell nuclear antigen (PCNA) expression.

Measurement of serum cortisol, aldosterone, ACTH, and estrogen: Serum cortisol, aldosterone, ACTH, and estrogen were quantified in El-Safwa Laboratory, Tanta, Egypt, utilizing ADVIA Centaur automated competitive chemiluminescence immunoassay (Bayer HealthCare).

Evaluation of histopathological changes (light microscope): Adrenal glands were preserved in formalin, fixed in paraffin wax, sectioned at 3–5 mm, and stained with hematoxylin and eosin (H&E).

Evaluation of PCNA immune expression (light microscope): Immunohistochemical staining was performed employing the immune peroxidase (PAP, peroxidase/anti peroxidase) procedure utilizing anti PCNA antibodies from Lab Vision (Fremont, CA. USA) diluted at 1/100.

Table 1. Effect of *Matricaria chamomilla* flowers extract on serum ACTH and estrogen levels measured in estradiol valerate-induced PCO in rats.

Experimental group	ACTH (pmol/L)	Estrogen (ng/mL)
Control	55.33 ± 1.15	99.47 ± 1.13
PCO	89.83 ± 4.29 ^a	339.27 ± 20.33 ^a
Metformin	70.50 ± 3.55 ^b	92.42 ± 2.98 ^b
<i>Matricaria chamomilla</i>	58.83 ± 2.41 ^b	95.80 ± 2.36 ^b

Results are expressed as mean ± SE (n=6). ^asignificant difference compared to the control group. ^bsignificant difference compared to the PCO group.

Statistical interpretation of findings: GraphPad Prism version 5 was used to analyse the results, which included an ANOVA test and a Tukey's multiple comparison test. p

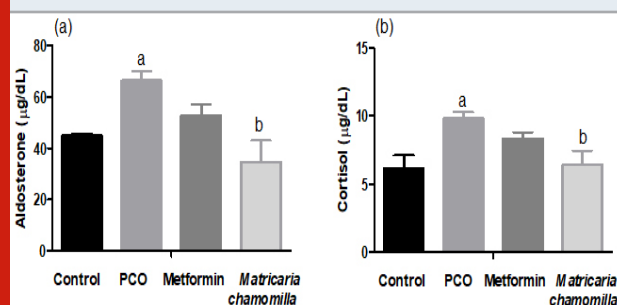
less than 0.05 or at $p < 0.05$, the results were considered statistically significant.

RESULTS AND DISCUSSION

Effect of *Matricaria chamomilla* flowers extract on serum ACTH and estrogen levels measured in estradiol valerate-induced PCO in rats: PCO-induced rats showed significantly increased serum ACTH and estrogen levels compared to the control group. Treatment of PCO-induced rats with *Matricaria chamomilla* and metformin significantly decreased serum ACTH and estrogen levels compared to the PCO group. *Matricaria chamomilla* significantly decreased serum ACTH level compared to the metformin group (Table 1).

Effect of *Matricaria chamomilla* flowers extract on serum aldosterone and cortisol levels measured in estradiol valerate-induced PCO in rats: PCO-induced rats showed significantly increased serum aldosterone and cortisol levels compared to the control group. Treatment of PCO-induced rats with *Matricaria chamomilla* significantly decreased serum aldosterone and cortisol levels compared to the PCO group. There was no difference between the metformin group and PCO group concerning serum aldosterone and cortisol levels (Figure 1).

Figure 1. Effect of *Matricaria chamomilla* flowers extract on serum aldosterone and cortisol levels measured in estradiol valerate-induced PCO in rats.



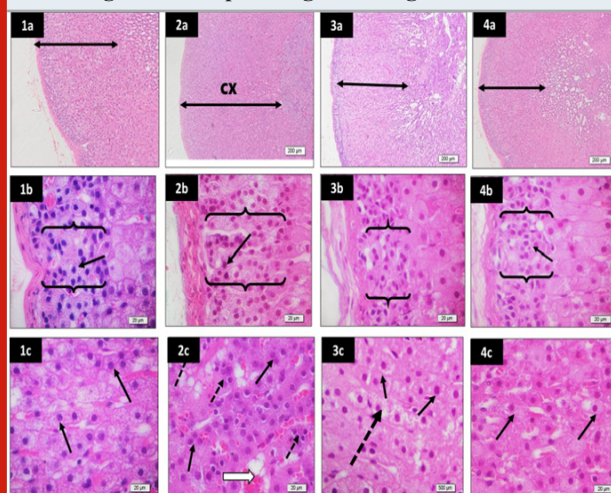
Results are expressed as mean \pm SE (n=6). ^asignificant difference compared to the control group. ^bsignificant difference compared to the PCO group.

Effect of *Matricaria chamomilla* flowers extract on adrenal glands histopathological changes: The examination of H & E-stained adrenal glands sections of PCO-induced rats revealed a potential increase in cortical zone thickness, an increase in thickness of zona glomerulosa, increase in cellularity of both zona glomerulosa and zona fasciculata. Treatment of PCO group with either metformin or *Matricaria chamomilla* decreased cortical zone and zona glomerulosa thickness, besides normal cellular features of zona fasciculata (Figure 2).

Effect of *Matricaria chamomilla* flowers extract on adrenal glands zona glomerulosa PCNA immune expression: Sections of PCO-induced rats were showed relative increase in zona glomerulosa thickness, besides, increased number of nuclei expressing PCNA. A mild or

moderate decrease in nuclei expressing PCNA is observed in the metformin and *Matricaria chamomilla* treated rats (Figure 3).

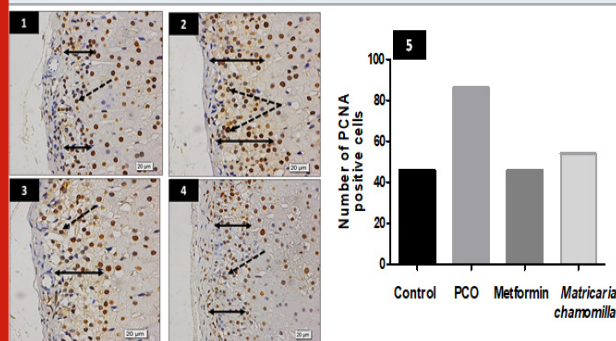
Figure 2. Effect of *Matricaria chamomilla* flowers extract on adrenal glands histopathological changes.



Sections from rat adrenal glands (low power x40) and higher magnification (x400) of zona glomerulosa and zona fasciculata stained by H&E to show a potential increase in cortical zone (cx) thickness in PCO group (2a) compared to control (1a). Besides, an increase in thickness of zona glomerulosa of PCO group (brackets) (2b) compared to control (1b) is observed. The cellularity of both zona glomerulosa (2b) and zona fasciculata (2c) of the PCO group is increased compared to control (1b, and 1c). Mild dilation of sinusoids (white arrow) and presence of lymphocytes (dotted arrows) are also evident in the zona fasciculata of the PCO group (2c). The metformin group showed decreased cortical zone (3a) and zona glomerulosa (3b) thickness compared to the PCO (2a and 2b) and the control groups (1a and 1b). Besides, moderate return to normal cellular features (black arrows) of zona fasciculata is observed in the metformin group (3c), although frequent cells showed unstained cytoplasm (dotted arrow). The *Matricaria chamomilla* group show relatively normal cortical zone thickness (4a), zona glomerulosa thickness (4b), and cellular features of zona fasciculata (4c) compared to the PCO (2a and 2b) and the control groups (1a and 1b).

Effect of *Matricaria chamomilla* flowers extract on adrenal glands zona fasciculata PCNA immune expression: Sections of PCO-induced rats show relative increase in zona fasciculata, the number of nuclei expressing PCNA. A mild or moderate decrease in nuclei expressing PCNA is observed in the metformin and *Matricaria chamomilla* treated rats (Figure 4). The current study results showed a significant increase in the level of estrogen in the PCO model developed by exogenous estrogen injection. There was also a significant increase in the level of the ACTH in the PCO rats. It was also clear that the adrenal gland cortisol and aldosterone concentrations were increased in the PCO group. The histological examination (both H&E and PCNA staining) of adrenal sections confirmed these findings. Similar to this study findings, previous research showed an overproduction of various adrenal gland hormones, including cortisol, in PCO women (Azziz et al., 1998).

Figure 3: Effect of *Matricaria chamomilla* flowers extract on adrenal glands zona glomerulosa PCNA immune expression.

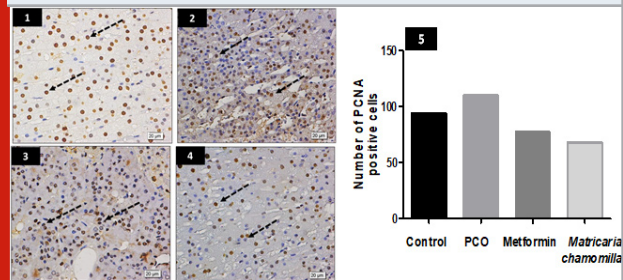


Sections from rat adrenal gland zona glomerulosa stained for PCNA show relative increase in zona thickness (double head arrows) in the PCO group (2) compared to the control group (1). Besides, the number of nuclei expressing PCNA looked to be more numerous (dotted arrows) (2). A mild or moderate decrease in nuclei expressing PCNA is observed in the metformin and *Matricaria chamomilla* groups (dotted arrows) (3 and 4). (5) A bar chart shows the number of PCNA positive cells in all groups.

The researchers attributed this to the increased sensitivity of adrenal glands to the ACTH, 7 α -hydroxylase, and increased cytochrome P450c17 α . According to previous research, ladies with PCO were observed to have enhanced peripheral cortisol metabolism, which was linked to raised ACTH production in order to maintain optimal cortisol levels. The possible causes for elevated adrenal hormones levels in PCO ladies incorporate changes in adrenocortical biosynthesis, increased responsiveness of the adrenal to ACTH stimulation, or abnormalities in adrenal product metabolism (Stewart et al., 1990; Bhatena, 2005; Yildiz and Azziz, 2007; Baskind and Balen, 2016; Alahmadi et al., 2020). Numerous probable theories can describe how ACTH and cortisol levels were raised after estradiol valerate therapy. One probability is that estrogen treatment directly or indirectly impacted ACTH production from the pituitary gland, which encouraged cortisol release from the adrenal gland. In pregnant ladies, it is affirmed that the hypothalamic-pituitary-adrenal axis is activated, and hypercortisolism is recognized. Different probability is that high estradiol suppressed transcription of 11- β hydroxysteroid dehydrogenase (11- β HSD), which is identified to decrease cortisol synthesis. It is likely that if local cortisol concentration is reduced in the pituitary and/or hypothalamus, ACTH concentration is raised by the low cortisol concentration (Carr et al., 1981; Ho et al., 2007; Chapman et al., 2013; Anno et al., 2019; Sotiropoulou et al., 2020).

Previously reported data indicated that estrogen impacts 11 β -HSD, however, it persists questionable how ACTH and cortisol concentrations were raised following estradiol valerate therapy. This study results showed that *Matricaria chamomilla* flowers extract modified all the hormonal changes associated with this PCO model

Figure 4: Effect of *Matricaria chamomilla* flowers extract on adrenal glands zona fasciculata PCNA immune expression.



Sections from rat adrenal gland zona fasciculata stained for PCNA show increasing nuclei expressing the marker in the PCO group (2) compared to the control group (1) (dotted arrows). PCNA immuno-expression is still high in the metformin group (3) (dotted arrows). PCNA immuno-expression is nearly normal in *Matricaria chamomilla* group (4). (5) A bar chart shows the number of PCNA positive cells in all groups.

including, estrogen, ACTH, cortisol, and aldosterone. Similarly, metformin significantly decreased estrogen and ACTH in PCO rats. On the other hand, metformin did not affect the hormonal changes associated with this model concerning the hormones of the adrenal glands (cortisol and aldosterone) (Low et al., 1993; Pepe et al., 2001; Anno et al., 2019; Sotiropoulou et al., 2020).

The histological examination (both H&E and PCNA staining) of adrenal sections confirmed these findings. The active phytoestrogen ingredients of *Matricaria chamomilla* extract are coumarins, which may inhibit estrogen production. Moreover, phytoestrogens interfere with the action of cytochrome P450 enzymes, preventing cholesterol from being converted to pregnenolone and reducing estrogen synthesis. The chamomile flower, *Matricaria chamomilla*, has traditionally been used to relieve tension (Brueggemeier et al., 2001; Löfgren et al., 2004; Ronis, 2016; Witchel et al., 2019; Delgado and Lopez- Ojeda, 2021).

Furthermore, apigenin, the main ingredient in chamomile, lowers cortisol levels in the blood. An earlier study found that breathing chamomile oil vapor alleviated restriction stress in ovariectomized rodents by lowering plasma ACTH levels. In bovines, *Matricaria chamomilla* CH12 reduced stress by inhibiting cortisol secretion. According to the current study's findings, *Matricaria chamomilla* extract may be responsible for the modification of adrenal gland hormones by lowering the pituitary hormone ACTH by restoring estrogen levels to their normal levels. However, it appears that there is a mechanism behind the impact of *Matricaria chamomilla* other than regulating estrogen and ACTH, as metformin changes estrogen and ACTH but does not reduce cortisol and aldosterone levels, implying another mechanism that has to be investigated further (Yamada et al., 1996; Reis et al., 2006; Sotiropoulou et al., 2020; Leon et al., 2021).

CONCLUSION

The PCO model produced by estradiol valerate was linked to higher serum ACTH and estrogen levels. The PCO model also showed that cortisol and aldosterone levels of the adrenal glands were also increased. The hormonal alterations associated with this PCO model were altered by *Matricaria chamomilla* flowers extract, including estrogen, ACTH, cortisol, and aldosterone. These findings were corroborated by histological investigation of adrenal sections (both H&E and PCNA staining).

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