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## Evaluation of renal disturbance in animal models of polycystic ovary syndrome

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### Abstract

**Background:** Polycystic ovary syndrome (PCOS) is an endocrine disease characterized by hyperandrogenism and hyperinsulinemia, followed by luteinizing hormone and follicle-stimulating hormone deficiency. PCOS conditions cause metabolic disorders that increase uric acid levels and malondialdehyde (MDA) levels. Animal models of PCOS have been used extensively in research to study the pathogenesis, clinical characteristics, and treatment of PCOS.

**Aim:** This study aimed to identify the pathological mechanisms underlying renal dysfunction in PCOS by observing several parameters, including blood urea nitrogen (BUN), creatinine, uric acid, and renal MDA levels.

**Methods:** This research was an experimentally designed study using a Wistar rat (*Rattus norvegicus*) as an animal model of PCOS which were divided into three groups: negative control group ( $n = 6$ ), Testosterone propionate (TP) induction group ( $n = 6$ ), and estradiol valerate (EV) induction group ( $n = 6$ ).

**Results:** According to statistical analysis it indicated that induction of TP and EV can increase blood uric acid levels in PCOS model rats ( $p < 0.05$ ), TP induction can increase kidney BUN and MDA levels significantly ( $p < 0.05$ ). However, the observation of creatinine levels did not show significant differences in all treatment groups ( $p > 0.05$ ).

**Conclusion:** Based on the results of this study, it can be concluded that the induction of animal models with TP can trigger significant renal damage compared to EV.

**Keywords:** PCOS, Animal model, Renal disturbance, Testosterone propionate, Estradiol valerate.

### Introduction

Polycystic ovary syndrome (PCOS) affects 1 in 10 women of reproductive age worldwide. The World Health Organization states that 116 million women, or 3.4% of women worldwide, suffer from PCOS (Jabeen *et al.*, 2022). In Indonesia, 44.8% of PCOS patients have both ovulatory disorders and polycystic ovarian phenotype, and the incidence of PCOS patients is 20–30 years, for example, 45.7% (Pangastuti and Sumapradja, 2011). The symptoms of women with PCOS are variable. In general, women with PCOS have menstrual irregularity, androgen excess (such as hirsutism and acne). 40% of women with PCOS have infertility due to anovulation (Sirmans and Pate, 2013). PCOS is also present in animals, especially cows. Cystic ovarian disease is a very common condition with a reported prevalence of 5%–10%. Cystic ovarian disease is a common cause of infertility in cattle, and ovarian cysts resemble atretic follicles (Jeengar *et al.*, 2014). The effects of cysts on the ovaries can cause infertility in cows, repeat breeders, poor egg quality, and infertility (Gümüşay *et al.*, 2018). In addition, the decrease in livestock production has caused economic losses for farmers.

Animal models of PCOS have been used extensively in research to study the pathogenesis, clinical characteristics, and treatment of PCOS (Walters *et al.*, 2018). In addition, using PCOS animal models allows us to understand the impact of PCOS on various organs, including the kidney. By observing renal organ parameters in PCOS animal models, we can gain insight into the physiological and pathological changes in the kidney due to PCOS.

The stimulating hormones used in this study are testosterone propionate (TP) as an androgen and estradiol valerate (EV) as an estrogen. TP induction can cause hyperandrogenism, abnormal reproductive morphology, hyperinsulinemia, promote atretic follicular changes, and produce luteinizing theca cells (Corrie *et al.*, 2021). Stimulation of EV causes a decrease in follicle-stimulating hormone hormones and their transformation into large follicles in ovarian morphology, such as cysts with atresia. While estradiol is the main female sex hormone controlling female reproduction during estrus and menstruation, high estrogen levels in the body can cause PCOS (Oyebanji *et al.*, 2018).

Cardiovascular risk factors, obesity, glucose intolerance, dyslipidemia, and increased inflammation

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are disorders that are often seen in PCOS patients (Can *et al.*, 2020). Increased production of excess androgens in the ovaries is a cause of PCOS which is called hyperandrogenism. Hyperandrogenism causes disturbances in the cardiovascular system such as hypertension through the activation of the androgen vasoconstrictor, namely Endothelin-1 (Kataoka *et al.*, 2022). Vasoconstriction of blood vessels results in oxidative stress (Dalmaso *et al.*, 2016) which initiates the membrane lipid peroxidase process and triggers the increase in free radicals.

Lipid peroxide is unstable, so it breaks down easily into complex compounds, one of which is malondialdehyde (MDA). Increased MDA production catalyzed by nicotinamide adenine dinucleotide phosphate (NADPH) oxidation, insulin, and xanthine oxidase (Anita, 2015). Increased uric acid is strongly associated with metabolic disorders in PCOS, including insulin resistance, obesity, and metabolic and dyslipidemia. Dyslipidemia can increase androgen levels, which can worsen PCOS (Anisya and Graharti, 2019). In addition, dyslipidemia is one of the conditions that affect the increase in the level of uric acid in the body. Uric acid is the end product of purine (adenine and guanine) metabolism. In terms of PCOS, gout is considered a different metabolic disease and is one of the risk factors for PCOS exacerbation (Toda *et al.*, 2018). The evaluation of uric acid levels can be used as a simple diagnostic marker to determine the risk of metabolic syndrome in PCOS patients.

Studies using animal models of PCOS are used to examine the etiology, long-term health, pathophysiology, physiology, and treatment of PCOS (Koçak, 2021). An animal model of PCOS can be established by inducing androgens, estrogens, and aromatase inhibitors (Walters *et al.*, 2018). It is crucial to understand the urgency of observing renal organ parameters in animal models of PCOS as this provides insight into the impact of PCOS on renal health. This study may help identify pathological mechanisms underlying renal dysfunction in PCOS and provide a better understanding of the relationship between PCOS and renal disease. In addition, research in animal models of PCOS may provide a foundation for developing effective prevention and treatment strategies to protect and restore kidney health in individuals with PCOS.

Observing renal organ parameters in PCOS animal models involves various analytical techniques and methods. Frequently observed parameters include renal function, histological structure, molecular expression, and enzyme activity associated with renal function. Focusing on these parameters, we can identify changes in the kidney due to PCOS manifestations, including inflammation, fibrosis, and renal dysfunction.

### Materials and Methods

This research is an experimental laboratory study using white rats (*Rattus norvegicus*) as an animal model experiment weighing between 130–150 g aged 6–8 months. The animals were then acclimatized for 14 days and maintained at the Animal Laboratory of the Faculty of Veterinary Medicine, Universitas Brawijaya. Eighteen female rats were divided into three groups (Table 1): the negative control (CN) group, the TP induction group, and the EV induction group.

#### Animal preparation

There were 18 female Wistar strain rats (*R. norvegicus*) with body weights between 130–180 g and 6–8 months old. Before treatment, acclimatization was carried out for approximately 7 days, so the experimental animals adapted to the new environment. During the study, rats (*R. norvegicus*) were given feed and drinking water ad libitum. Rats were fed twice a day in the morning and evening. Animals were housed based on the treatment group.

#### Administration of TP and EV

TP was given at 100 mg/kg BW with a volume of 0.13 ml/tail for 12 days. EV was given with 2 mg/kg BW with a volume of 0.13 ml/head for 2 days. EV was dissolved with virgin coconut oil and induced intraperitoneally. The CN group was given physiological NaCl orally with a gastric sonde. TP and EV were given as liquid preparations. EV was dissolved in virgin coconut oil and administered by intraperitoneal injection. TP was administered at 100 mg/kg BW in group P1 for 12 days. EV was administered at 2 mg/kg BW in group P2 for 2 days. EV is used as an induction of PCOS model animals by modifying the technique of Venegas *et al.* (2019), which uses EV of 2 mg diluted with sesame oil for 60 days. TP used as PCOS induction is also a modification of Siahaan *et al.* (2022), which uses 100 mg/kg BW TP for 21 days via the intraperitoneal route.

Table 1. Table of group distribution and treatment in the study.

| Name of groups (Abbreviation) | Treatments  |
|-------------------------------|---|
| CN                            | Group of normal rats ( <i>R. norvegicus</i> ) with NaCl administration (0.5 ml)                         |
| TP induction group            | Group of PCOS-induced rats ( <i>R. norvegicus</i> ) with TP 100 mg/kg BW intraperitoneally for 12 days. |
| EV induction group            | Group of PCOS-induced rats ( <i>R. norvegicus</i> ) with EV 2 mg/kg BW intraperitoneally for 2 days.    |

### Vaginal swab

The examination of the estrous cycle using the vaginal swab method was carried out twice a day. Vaginal swab cytological sampling uses a cotton swab moistened with NaCl 0.9%. Then insert into the vulva up to the part of the vagina adjacent to the cervix, then make a circular motion to get epithelial cells. Then the cotton swab was applied to the glass object. After drying, the slides were stained with methylene blue dye and observed under a microscope at 400×—1,000× magnification to identify the various stages of the estrous cycle.

### Euthanasia method

Euthanasia and kidney organ collection in experimental animals were divided into days 4 and 14. The euthanasia method was carried out by injecting ketamine-xylazine anesthetic drugs with ten times the dose of anesthesia intramuscularly, followed by cervical dislocation.

### Sample collection

Serum samples can be used to identify changes in protein levels associated with PCOS pathomechanisms (Rheza *et al.*, 2023). Before the necropsy, blood samples are taken first through the intracardiac as much as 3–5 ml. Then, the necropsy of the experimental animals was started by making an incision on the abdomen to the thorax so that all organs could be seen clearly. After that, one of the kidneys was collected and washed with 0.9% physiological NaCl. Then the kidneys were wrapped in aluminum foil, put into a plastic zip-lock, and stored at –20°C to examine MDA levels.

### Examination of uric acid levels

The procedure for checking uric acid levels using the point of care testing stick method with three and one blood tests (Nesco Multicheck, India) using blood samples. Rat blood samples were taken through the intracardiac as much as 0.3 ml. Examination of uric acid levels is carried out by inserting the chip and strip into the device with the same code. The device is ready for use if “UA” and a picture of blood drops appear. The blood sample is inserted through the strip gap until the end of the strip and beeps; wait 10 seconds, then the tool reads a few seconds, and the results will appear on the screen.

### Examination of blood urea nitrogen (BUN) and creatinine levels in blood serum

BUN and creatinine levels in blood serum were examined by biochemical blood tests using a spectrophotometer (RIELE Photometer 5010 Version 5).

### Examination of kidney MDA level

In the procedure for examining kidney MDA levels using the thiobarbituric acid method, the kidney organs were cut, then weighed to 0.1 g, crushed with a cooled mortar (mortar placed on ice gel), and 0.9% physiological NaCl was added. The kidney homogenate was transferred into a microtube and centrifuged at 8,000 rpm for 20 minutes. Afterward, 100 µl of supernatant was transferred to a new microtube, and 550 µl of distilled water, 100 µl of 10% TCA, 250 µl of 1 N HCl, and 100 µl of 1% Na-Thio were added

and homogenized with a vortex. The solution was centrifuged at 500 rpm for 10 minutes. The resulting supernatant was heated on a water bath/water bath at 100°C for 20 minutes, and the absorbance was measured with a spectrophotometer at  $\lambda = 532$  nm. The resulting absorbance value was entered into the equation to determine the MDA level.

### Data analysis

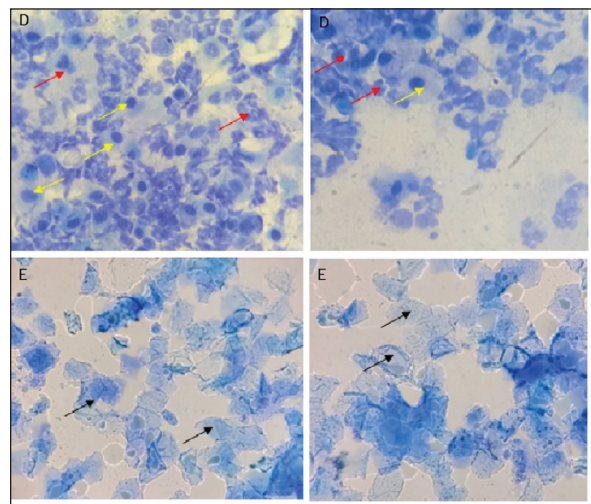
The parameters used in this study, namely uric acid levels, kidney organ MDA levels, and serum creatinine BUN levels, were analyzed quantitatively using One-way Analysis of Variance data analysis with a confidence level of 95% ( $\alpha = 0.05$ ) to determine differences between treatment groups and continued with *post hoc* Test using the least real difference to determine which treatment gave the best results. Software used for quantitative data analysis is Statistical Program for Social Science 016 Version 2.9 for Windows.

### Ethical approval

The Research Ethics Committee (Animal Care and Use Committee) of Brawijaya University approved the research with an ethical certificate number No. 090-KEP-UB-2022.

## Results

The method used to ensure animal models have been induced PCOS is by examining vaginal swabs and checking testosterone levels by the ELISA method. The vaginal swab determines changes in cells found in the vaginal mucosa during one estrous cycle (Foeh *et al.*, 2020). Animals with PCOS conditions will show a dominant reproductive cycle in the estrus phase through vaginal swab examination results (Haslan *et al.*, 2021). In this study, both TP and EV induction



**Fig. 1.** Histological features of vaginal swabs in TP and EV groups indicated estrous dan diestrus phase. (D): Diestrus; (E): Estrus; (Yellow arrows): nucleated epithelial cells; (Red arrows): leukocytes cells; (Black arrows): cornified epithelial cells.

groups experienced irregularity of the estrous cycle characterized by the presence of persistent estrus and or persistent diestrus phases followed by abnormal estrous cycles (Fig. 1). The results of measuring testosterone levels by ELISA method showed that TP-induced rats experienced an increase from normal levels of 423.08 ng/l. In contrast, the testosterone levels of EV-induced rats were 482.32 ng/l. Fernanda *et al.* (2019) stated that the limit of normal levels of testosterone levels in rats is 200–400 ng/l and so these conditions indicate an increase in testosterone levels, indicating a biochemical hyperandrogenism condition which is one of the diagnoses of PCOS.

**Uric acid measurement**

Uric acid is the end product of exogenous and endogenous purine metabolism (Chaudhary *et al.*, 2013). Data from the calculation of uric acid levels in the blood of rats in the CN group, the TP induction group at a dose of 100 mg/kg BW, and the EV induction group at a dose of 2 mg/kg BW are presented in Table 3. The results of uric acid measurements in PCOS animal models show that acid levels in the TP induction group at a dose of 100 mg/kg BW and the EV induction group at a dose of 2 mg/kg BW are significantly different ( $p < 0.05$ ) from the CN. The CN group (K-) has an average uric acid level of 3.33 mg/dl which is still considered normal. This follows Umboh *et al.* (2019), the standard level of blood uric acid in rats is 1.2–5.0 mg/dl. These measurements indicate that induction of TP and EV can increase blood uric acid levels in PCOS model rats.

**BUN and creatinine levels**

BUN is a marker parameter for acute kidney damage (Fitria *et al.*, 2019). This is because the urea in

the blood is the main element produced from the process of decomposing proteins and other chemical compounds containing nitrogen which will typically be excreted through the kidneys, so an increase in blood serum levels can indicate a failure of kidney function (Suryawan, 2016). The results of the measurement of BUN and creatinine levels are presented in Table 4.

According to the data in Table 2, it can be stated that the TP group obtained the highest BUN levels and were significantly different ( $p < 0.05$ ) compared to the CN group and EV. This indicates that the induction of TP in PCOS animal models caused a significant increase in BUN levels. In contrast, the induction of PCOS model animals with EV did not increase BUN levels. Statistical results of serum creatinine levels in all treatment groups showed no significant differences ( $p > 0.05$ ), so in this study, it can be concluded that induction of TP and EV did not trigger an increase in creatinine levels.

**Kidney MDA**

MDA level was measured to determine oxidative damage to the kidney organs, which is thought to be caused by increased free radicals in PCOS animal models. The statistical analysis results of the calculation of kidney MDA levels can be seen in Figure 2.

Examination of MDA levels is used as an indicator of oxidative stress (Judiono *et al.*, 2014), considering that MDA is the end product of excess lipid peroxidation due to oxidative stress (Morales and Munne, 2019). Statistically, TP induction can increase kidney MDA levels significantly different from the other two groups ( $p < 0.05$ ).

**Table 2.** The difference between the induction method in this research and the reference research.

| Induction | In this research   | Reference  |
|-----------|--|--|
| TP        | Dose: 100 mg/kg BB<br>Duration: 12 days<br>Route of administration: Intraperitoneal                                  | Dose: 100 mg/kg BB<br>Duration: 21 days<br>Route of administration: Intraperitoneal<br>Results:<br>TP causes insulin resistance by reducing the amount and effectiveness of glucose transport proteins, especially GLUT-4 which plays a critical role in glucose transport in muscle and fat (Siahaan <i>et al.</i> , 2022). |
|           | Dose: 2 mg/kg BB<br>Duration: 2 days<br>Route of administration: Intraperitoneal<br>Diluted with: virgin coconut oil | Dose: 2 mg/kg BB<br>Duration: 60 days<br>Route of administration: Intraperitoneal<br>Diluted with: sesame oil<br>Results:<br>The ovaries of rats injected with EV presented follicular cysts, and only the ovaries of a single rat had CL (Venegas <i>et al.</i> , 2019).  |

**Table 3.** Uric acid level values of various treatment groups.

| Groups | Uric acid (mg/dl)        |
|--------|--------------------------|
| CN     | 3.33 ± 0.34 <sup>a</sup> |
| TP     | 6.65 ± 1.42 <sup>b</sup> |
| EV     | 7.76 ± 2.44 <sup>b</sup> |

Various notations indicate significant treatment differences ( $p < 0.05$ ).

**Table 4.** BUN and creatinine levels in various treatment groups.

| Groups     | Mean ± SD (mg/dl) |                           |
|------------|-------------------|---------------------------|
| BUN        | CN                | 14.62 ± 4.43 <sup>a</sup> |
|            | TP                | 30.90 ± 6.93 <sup>b</sup> |
|            | EV                | 16.28 ± 5.65 <sup>a</sup> |
| Creatinine | CN                | 1.27 ± 0.55 <sup>a</sup>  |
|            | TP                | 1.05 ± 0.30 <sup>a</sup>  |
|            | EV                | 1.55 ± 0.44 <sup>a</sup>  |

Various notations indicate significant treatment differences ( $p < 0.05$ ).

### Discussion

Under normal circumstances, uric acid, as an end product of purine metabolism, describes the body's metabolic state and can maintain the level of oxidation in the body's physiological conditions (Chaudhary *et al.*, 2013). High levels of testosterone in the blood are associated with increased uric acid levels and the prevalence of hyperinsulinemia (Hu *et al.*, 2021). The testosterone hormone can induce the hepatic metabolism of purine nucleotides. High concentrations of uric acid levels affect lipid synthesis and distortion of lipid oxidation. Abnormal lipid metabolism in PCOS results in lipotoxicity that affects cells produces large amounts of reactive oxygen species (ROS), and induces damage to intracellular organelles, leading to cell death (Lima *et al.*, 2015).

The increased testosterone level in PCOS can contribute to uric acid levels. This is in line with the study's results, which found that induction of TP and EV can increase blood uric acid levels in PCOS model rats. According to Liu *et al.* (2021), hyperandrogenism, insulin resistance, abnormal lipid metabolism, and complications in PCOS can increase uric acid. In cases of PCOS in humans, it also shows an increase in uric acid due to androgens and insulin encouraging reabsorption of uric acid in the proximal tubules of the kidney and reducing uric acid secretion, thereby accelerating the occurrence of hyperuricemia (Nasrul and Sofitri, 2012).

The mechanism of this condition involves the role of testosterone, which can increase the activation of the xanthine oxidase enzyme, which plays a role in converting xanthine into uric acid. In addition, testosterone causes insulin resistance which can interfere with uric acid excretion in the kidneys. Increased blood uric acid levels in patients with PCOS are also associated with hyperinsulinemia, resulting in low uric acid excretion. In conditions of

insulin resistance, the kidneys cannot excrete uric acid effectively, resulting in increased blood uric acid levels (Battelli *et al.*, 2018; Paleva, 2019).

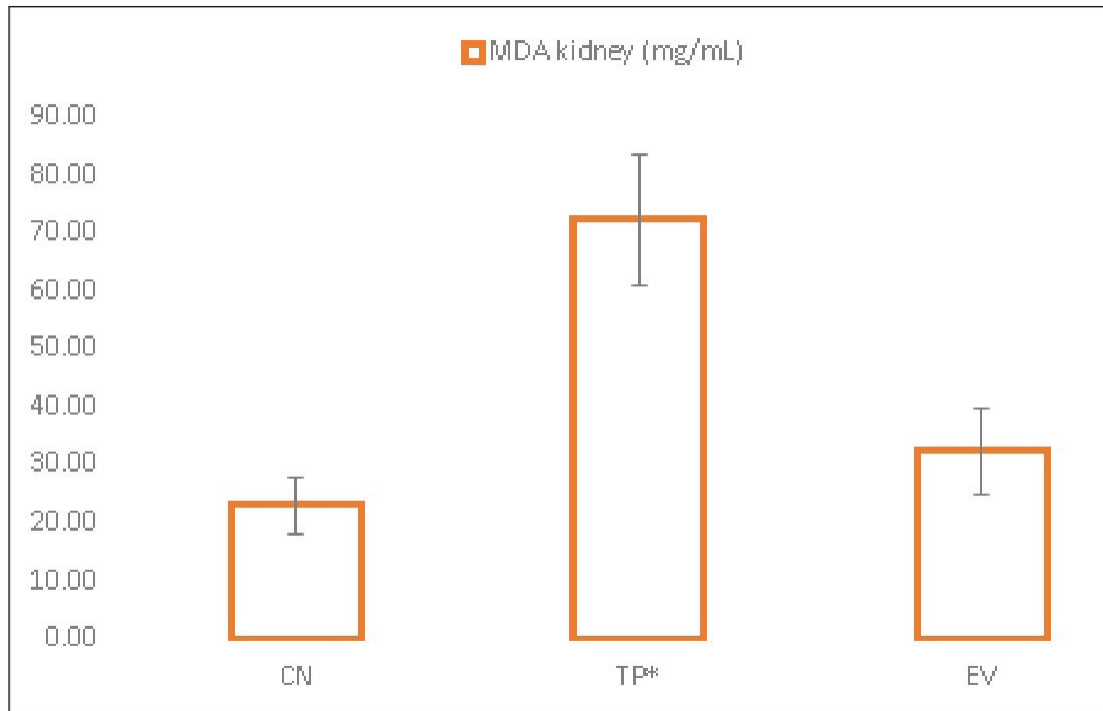
In addition, according to Gill (2013), elevated uric acid levels in PCOS are associated with increased insulin resistance. EV induction increases purine biosynthesis due to the activity of the hexose monophosphate pathway, which is characterized by hyperinsulinemia. Increased glucose-6-phosphate flux caused by hexose monophosphate is characterized by glycolytic damage and increased lipogenesis. Uric acid is formed from purines via hypoxanthine. Uric acid is excreted from the body through urine as a reflection of purine catabolism in the body. Excessive accumulation of uric acid can cause metabolic disorders in the body (Mu *et al.*, 2018). PCOS cases in humans also show increased uric acid because androgens and insulin encourage uric acid reabsorption in the proximal tubules of the kidneys and reduce uric acid secretion, thus accelerating hyperuricemia (Nasrul and Sofitri, 2012).

BUN is the end product of protein metabolism and amino acid catabolism produced by the liver and distributed throughout intracellular and extracellular fluids and excreted through the urine. Elevated BUN associated with kidney disease or renal failure, obstruction of the urinary tract by kidney stones, congestive heart failure, dehydration, fever, shock, bleeding in the gastrointestinal tract, and rapid muscle damage can also cause a transient increase in serum urea concentration (Gowda *et al.*, 2010).

TP induction (TP group) can cause BUN levels to increase significantly; the following research by Fitria *et al.* (2019) indicates acute renal impairment in PCOS cases. However, this increase did not occur in the EV induction group (EV group). This is thought to be because, in this study, EV induction only lasted for 2 days, while based on the literature, EV is most effective in causing PCOS at a level of 4.5 mg/kg BW for 60 days resulting in effects on changes in rat BUN levels (Ramadoss *et al.*, 2019). Therefore, EV at 2 mg/kg BW for 2 days still cannot significantly affect BUN levels.

In this study, the value of the CN group (K-) is the standard value of rat MDA levels (*R. norvegicus*) in normal conditions, where rats are not given TP and EV induction. This is under the statement of Valko *et al.* (2006) that in normal rats, it is known that MDA remains due to biochemical processes in the body that produce free radicals. Free radicals are beneficial for biological functions such as phagocytosis. Endogenous antioxidants in the body can neutralize free radicals in low amounts; if the amount of free radical compounds exceeds the number of antioxidants in the body, free radicals will damage lipid components resulting in oxidative stress (Srivastava and Kumar, 2015).

Hyperandrogenism in PCOS has been shown to cause decreased kidney function (Dalmasso *et al.*, 2016). The kidneys play a role in various homeostatic functions of the body, namely the excretion of metabolic waste



**Fig. 2.** Kidney MDA levels in treatment groups. (CN): Negative control; (TP): Testosterone propionate; (EV): Estradiol valerate; (\*): indicate significant differences ( $p < 0.05$ ).

and foreign chemicals, both produced by the body and entering the body (Guyton and Hall, 2016). According to Zhang *et al.* (2018), TP induction can escalate MDA levels by inducing lipid peroxidation and reducing antioxidant activity. A significant elevation in MDA levels occurred in the P1 group due to oxidative stress caused by exposure to TP. The results of this study are also in line with Serrano *et al.* (2018), which state that TP induction at a dose of 100 mg/kg BW causes glucose intolerance, dyslipidemia, and increased levels of oxidative stress which potentially causes lipid peroxidation, ROS toxicity and decreased antioxidant function. In addition, the half-life of TP is about 33 hours and can stay in the body for 5 days. This is because the extended structure of the ester group in TP correlates with a long half-life so that TP will remain in the bloodstream for a long time (Turza *et al.*, 2022). EV is used to create animal models of PCOS by inducing hormonal changes from normal conditions. Exposure to EV induction in rats causes reproductive cycle irregularities, anovulation, and polycystic ovaries showing increased atresia follicles. The PCOS condition in mice due to EV induction is similar to human PCOS cases (Walters *et al.*, 2018). It is also evidenced that EV induction has a reproductive cycle with a consistent, persistent estrus phase and higher testosterone, uric acid, and renal MDA levels as metabolic disorders in PCOS. EV also has a half-life of about 11–14 hours and provides a faster effect on the appearance of PCOS conditions (Ndefo and Mosely, 2010).

The role of oxidative stress and renal tissue damage response in the pathogenesis of PCOS shows the relationship of renal MDA levels as a result of lipid peroxidation in metabolic syndrome conditions that trigger increased apoptosis and decreased mitochondrial function (Olatunji *et al.*, 2021)—the formation of ROS in the mitochondrial respiration chain as second messengers for NF- $\kappa$ B activation. Increased MDA production is catalyzed through NADPH oxidation, insulin, and xanthine oxidase (Anita, 2015). Several studies have shown a correlation between high levels of androgen hormones and the development of kidney damage (Gozukara *et al.*, 2015; Valdivielso *et al.*, 2019; Lau *et al.*, 2022). However, the exact pathomechanisms associated with this still need to be fully understood. Androgens are known to influence the renin-angiotensin-aldosterone system (RAAS) profoundly. Hyperandrogenism due to PCOS syndrome may affect RAAS regulation by increasing the production of angiotensinogen and angiotensin II. This can lead to vasoconstriction of the renal arteries and increased reabsorption of salt and water, which can trigger blood pressure disorders and kidney damage. In addition, hyperandrogenism may also contribute to inflammation and oxidative stress (Dalmasso *et al.*, 2016), which are factors also associated with kidney damage. Androgen hormones can influence immune system activity and stimulate the production of inflammatory mediators such as pro-inflammatory cytokines (Traish *et al.*, 2018). This can trigger an

inflammatory response and oxidative stress in the kidney, which can cause structural damage to kidney function.

### Conclusion

Based on the results of this study, it can be concluded that the induction of animal models with TP can trigger more significant renal damage compared to EV. Although both compounds can induce PCOS conditions in animal models with similar clinical symptoms, researchers still need to pay attention to the side effects caused, especially those related to kidney function. This is because there are many variations in the duration of induction of both drugs that can potentially trigger other disorders/complications in PCOS animal models.

### Acknowledgments

None.

### Conflict of interest

The Authors declare that there is no conflict of interest.

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### Data availability

The data used to support the findings of this study are included within the article and no additional data sources are required.

### Authors contributions

Y, Oktanella: devised the project, drafted the manuscript, the main conceptual ideas, and the proof outline. H, Untari, DK, and Wuragil were involved in planning and supervising the work. H, Ismiawati and NA, Hasanah processed the experimental data and performed the analysis. GC, Agustina and DAO, Pratama were performed in interpreting the results and worked on the revised manuscript.

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