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Original Article

Effect of Formalin Exposure on Antral Follicle Development Disorders through Decreased Gonadotropin Hormone Regulation

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ABSTRACT

An excessive amount of formalin exposure might result in reactive oxygen species (ROS) and oxidative stress in the body, which lowers the body's ability to regulate the gonadotropin hormone and prevents ovulation from happening. The purpose of this work is to clarify how exposure to formalin alters the levels of GnRH, LHR, estradiol, and antral follicle growth in the ovaries of female mice (Mus musculus). This experimental research used a posttest only control group design. The research sample was 30 mice (Mus musculus). The samples were randomly allocated into three groups. Group 1 (K0) as control group without formalin administration, Group 2 (P1) as treatment 1, which was given formalin at a dose of 140 mg/kg body weight given in 0.1 ml/10 g body weight/day for 12 days, and Group 3 (P2) as tretment 2, which was given formalin at a dose of 210 mg/kg body weight given in 0.1 ml/10 g body weight/day for 12 days. Each group assessed GnRH levels using the Enzyme Linked Immunosorbent Assay (ELISA) method, examined LHR expression and estradiol expression using immunohistochemistry (IHC), and counted the number of antral follicles using hematoxylin-eosin (HE). The results of the statistical tests demonstrated a decrease in GnRH levels (p=0.001), a decrease in LHR expression (p=0.001), a decrease in Estradiol expression (0.001), and a reduction in the total number of antral follicles, specifically tertiary and de Graaf follicles (p=0.001). The findings demonstrate that female rats exposed to formalin have lower levels of GnRH, LHR, and antral ovarian follicles.

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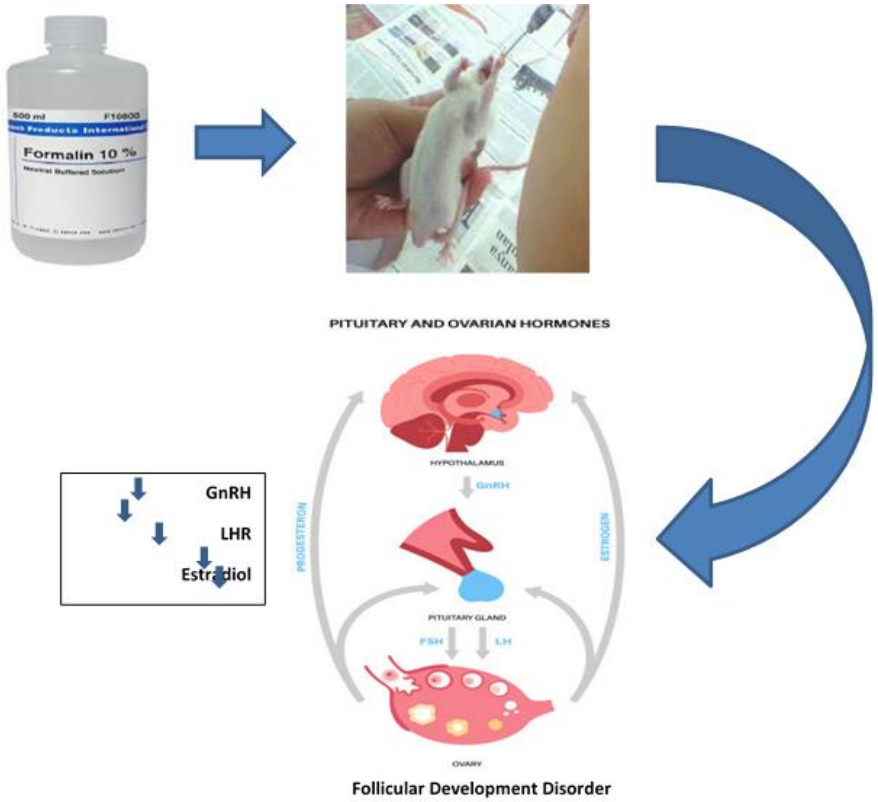
GRAPHICAL ABSTRACT



Introduction

Formalin is a xenobiotic that is currently used extensively in the food industry as a preservative. According to BPOM (2019) [1], formaldehyde is the third most common food additive in Indonesia, behind rhodamine B and borax. Formalin is a colorless, strong liquid that has been stabilized with methanol and contains 30 to 50% formaldehyde [2]. Formaldehyde is converted into formic acid by the enzymes Aldehyde Dehydrogenase 2 (ALDH2) found in cell mitochondria and Alcohol Dehydrogenase 3 (ADH3) located in the cytoplasm [3]. The enzyme formalin dehydrogenase quickly converts formalin to formic acid. Formic acid, on the other hand, has a slower metabolism and accumulates in the stomach [4]. When too many formic acid products are generated, ROS are formed [5].

ROS (reactive oxygen species) that trigger oxidative stress can be regulated by cellular defense mechanisms, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) [6, 7]. The brain contains unsaturated fatty acids, which are easily oxidized and require a lot of oxygen per weight. Moreover, the brain has higher amounts of oxidative metabolic activity and lower levels of antioxidant enzyme activity. As a result, toxic or ischemic CNS events are more likely to occur, according to Wardaya *et al.* [8-10]. According to a study conducted by Mei *et al.*, the exposure to formaldehyde 3.0 mg/m^3 for eight hours a day for seven days increased the brain's ROS levels [11].

The cerebrum's Gonadotropin Releasing Hormone (GnRH) stimulates the anterior pituitary to release the luteinizing and follicle-stimulating hormones (FSH and LH), which are essential for follicular development [12]. FSH deficiency can result in follicular atresia through apoptosis and is the most crucial survival factor for follicular development in the pre-ovulatory phase. Follicle growth and rupture are induced by LH. The physiological effects of LH mediated in the late stages of follicular development, final oocyte maturation, ovulation, and follicular wall luteinization depend on the luteinizing hormone receptor (LHR) on follicular theca cells [13-15].

If formalin has the ability to change GnRH levels in the blood, it will also change the levels of FSH and LH, which will reduce the generation of

estradiol and stop follicular development [16, 17] and prevent ovulation [18].

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Since that exposure to formaldehyde cannot be prevented, scientists are interested in learning more about the impacts of exposure and the harm this xenobiotic does to the body's systems, particularly the reproductive system. This knowledge can be used to build new development methods that lower the danger of FA exposure.

Materials and Methods

This experimental research used a posttest only control group design. The research sample was 30 mice (Mus musculus). The samples were randomly allocated into three groups. Group 1 (K0) as control group without formalin administration, Group 2 (P1) as treatment 1, which was given formalin at a dose of 140 mg/kg body weight given in 0.1 ml/10 g body weight/day for 12 days, and Group 3 (P2) as tretment 2, which was given formalin at a dose of 210 mg/kg body weight given in 0.1 ml/10 g body weight/day for 12 days. Each group assessed GnRH levels using the Enzyme Linked Immunosorbent Assay (ELISA) method, examined LHR expression and estradiol expression using immunohistochemistry (IHC), and counted the number of antral follicles using hematoxylin-eosin (HE).

This study was carried out at the Airlanggga University Faculty of Medicine's Embryology Division. The Faculty of Veterinary Medicine at Airlangga University in Surabaya, Indonesia's Institutional Animal Ethics Committee approved the study protocol (Ethical Clearance No. 2.KE.090.07.2021), and the Committee's standards for the supervision and control of animal experimentation were followed when treating the animals (Ethical Clearance No. 2.KE.090.07.2021). If the data were regularly distributed, the one-way ANOVA test was used. Otherwise, the Kruskal-Wallis test was applied.

Results and Discussion

The ELISA test used to measure GnRH levels in blood serum revealed that the formalin exposure group had significantly lower GnRH levels (p=0.001), as presented in Table 1.

The expression of LHR (p=0.009) in theca cells and estradiol (p=0.001) in granulosa cells were significantly decreased after immunohistochemical labeling, as listed in Tables 2 and 3.

In Table 4, the count of number of antral follicles, namely tertiary follicles (p=0.001) and de Graaf follicles (p=0.001) in the ovary shows a significant decrease.

There were significant changes between the control group and the treatment group on GnRH levels, estradiol expression, and the number of tertiary follicles, but no significant differences between treatment groups 1 and 2.

Table 1: GnRH level in blood serum

Variable	Group	Mean±SD	P Anova	Different Test		
				KO	P1	P2
GnRH	K0	19.61±2.93	0.001*	-	0.017	0.001*
	P1	16.70±2.37		0.017	-	0.064
	P2	14.47±2.34		0.001*	0.064	-

Description: (*) significant at α<0.05.

Table 2: LHR expression in theca cells

Variable	Group	Mean±SD	P Kruskal-Walis
LHR	K0	4.65±1.04	0.009*
	P1	3.60±2.19	
	P2	2.84±0.87	

Description: (*) significant at α<0.05.

Table 3: Estradiol expression in granulosa cells

Variable	Group	Mean±SD	P Anova	Different Test		
				KO	P1	P2
Estradiol	K0	5.77±1.04	0.001*	-	0.001*	0.001*
	P1	4.13±0.81		0.001*	-	0.029
	P2	3.23±0.72		0.001*	0.029	-

Description: (*) significant at α<0.05.

Table 4: Tertiary follicle in the ovary

Variable	Group	Mean±SD	P Anova	Different Test		
				KO	P1	P2
Tertiary folicle	K0	10.10±3.34	0.001*	-	0.001*	0.001*
	P1	6.30±1.41		0.073	-	0.689
	P2	5.90±1.19		0.001*	0.689	-

Description: (*) significant at α<0.05.

Table 5: Graafian follicles in the ovaries

Variable	Group	Mean±SD	P Krusskal-Wallis
Graafian Follicles	K0	1.60±0.51	0.001*
	P1	1.10±0.73	
	P2	0.60±0.51	

Description: (*) significant at α<0.05.

Figures 1 and 2 display that when chromogen brown hue was present, more cells in the treatment group expressed LHR in theca cells and estradiol in granulosa cells. Figure 3 depicts that compared to the P1 and P2 treatment groups, the control group exhibited more tertiary and de Graaf follicles.

The histological image (Figure 3) indicates that the histopathological picture of ovaries in the control group (K0), i.e. mice who did not receive formalin, is normal. The number of follicles in the control group was substantially higher than in the experimental group. The treatment P1 (formalin dose 140 mg/kg BB) and P2 (formalin dose 210 mg/kg BB) in the experimental group caused considerable tissue damage, as evidenced by the decrease in the number of follicles compared to the control group.

The findings demonstrated that formalin exposure caused a significant variation in the blood serum GnRH levels of mice. The results of the investigation demonstrated that formaldehyde gas not only can significantly reduce gonadotropin hormone in rats, but also has no effect on testicular cell death, in agreement with the study of the effects of formaldehyde gas on gonadotropins and testicular germ cell apoptosis in adult rats [19].

Formalin is swiftly converted to formic acid by formaldehyde dehydrogenase (FDH). Formic acid, on the other hand, has a slow metabolism and accumulates in the blood [20]. Super Oxide Dismutase (SOD) transforms the radical Super Oxide (O2), which is released by formic acid, into H_2O_2, a ROS that can pass across the blood-brain barrier. The start phase of protein translation can be interfered with by this ROS, which inhibits mRNA from being transcribed into the series of polypeptides needed to make GnRH, lowering GnRH levels [21, 22]. Moreover, ROS can change GnR protein into GnR carbonyl protein, which then enters the proteasome and stops serum GnRH from being released. The outcome is a drop in both FSH and LH secretion [23, 24], which leads to a reduction in LH receptors in theca cells, the termination of follicular development [18], a lack of LH secretion, and failure ovulation [19].

LH receptors are only present in theca cells at the beginning of the cycle, but FSH receptors are only present in granulosa cells.

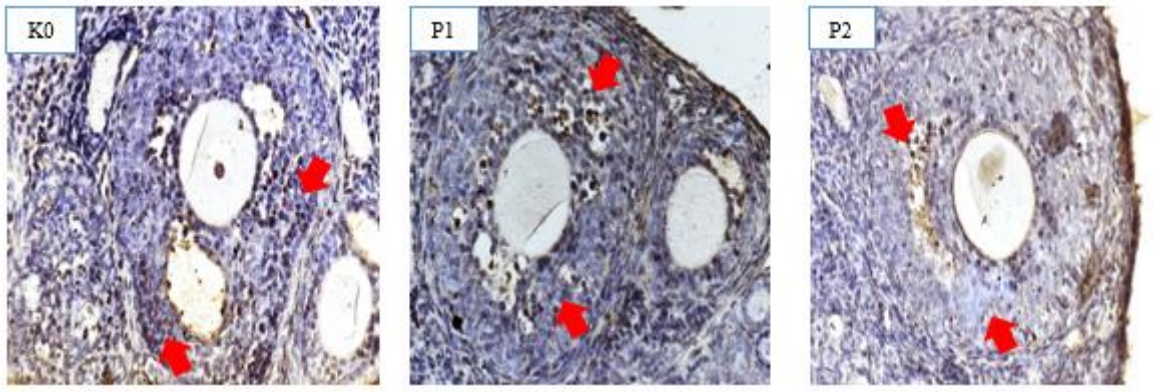

Figure 1: The control group (K0), treatment groups P1 and P2 shows LHR expression in theca cells which is indicated by the presence of brown color (arrows). (Immunohistokimia: Magnification 400x)

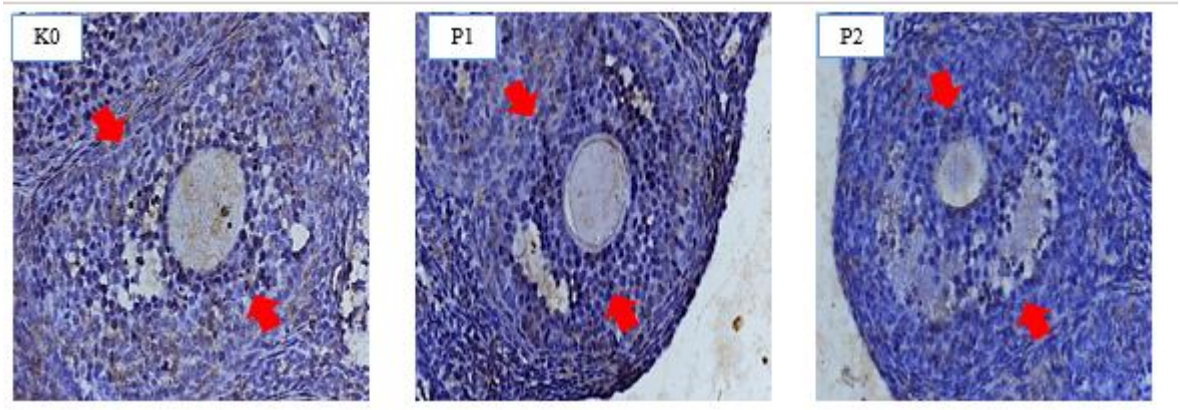

Figure 2: The contol group (K0), treatment groups P1 and P2 shows estradiol expression in granulosa cell which is indicated by the presence of brown color (arrows). (Immunohistokimia: manification 400x)

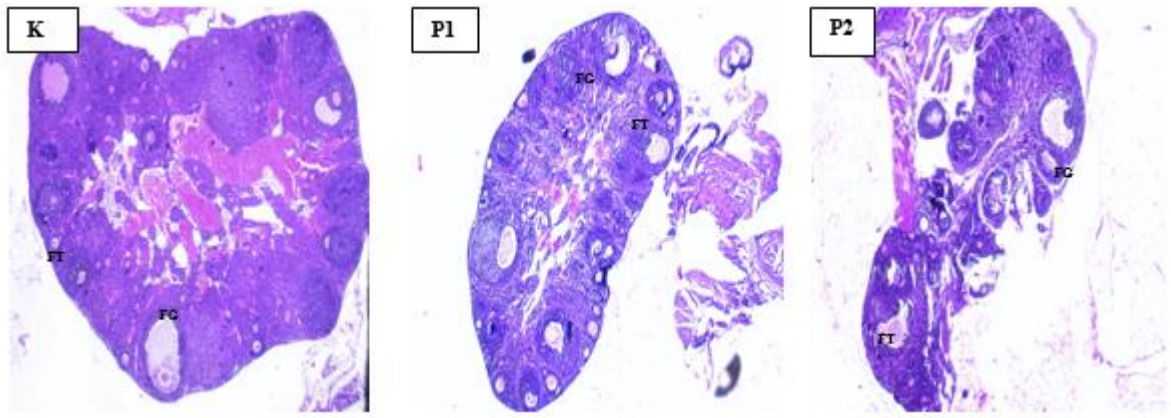

Figure 3: The description of ovarian follicle development in each therapy group was compared. FP; primary follicle, FS; Secondary Follicle, FT; Tertiary Follicle, FG; Follicle de Graaf. (*Hematoxylin – Eosin:* magnification 400x)

LH receptors have an impact on the cAMP stimulation, which is necessary for theca cells to convert cholesterol into progesterone.

Theca cells will transform progesterone into endostenedion, which will then be released into the granulosa cells via an aromatization process. The intrafollicular network within the follicle may get damaged due to the oxidative stress brought on by formalin exposure. As a result, granulosa cells produce less estradiol because LH

receptors are unable to stimulate cAMP to cause cholesterol to be converted into andostenedione. Cells with FSH receptor defects may produce dominant androgens, undergo apoptosis or atresia, and have an impact on follicular development [25, 26]. LHR expression in theca cells was much lower after exposure to formalin compared to the control group, which is consistent with the findings of this investigation.

Estradiol is crucial for maintaining ovarian function because it controls follicle formation and ovarian atresia, prevents granulosa cell death, and promotes granulosa cell division and expansion [27]. The FSH binding to its receptors on the granulosa cell membrane, which activates the aromatase enzyme and causes it to convert testosterone into estradiol, stimulates the estrogen synthesis [28]. The findings demonstrated that formalin exposure caused estradiol expression in ovarian granulosa cells to be considerably lower than in the control group. Similar research on female rats treated to peritoneal injections of formaldehyde at doses of 0.2 mg/kg, 2.0 mg/kg, and 20 mg/kg for 14 days revealed lower levels of estradiol (E2) and inhibin than the controls [29]. In the Askaripour *et al.* (2015) study, female mice who were not exposed to formaldehyde had lower levels of hormones estrogen and progesterone than those who were [30].

The results of the study showed that exposure to formalin significantly decreased the number of tertiary and de Graaf follicles during antral follicle development (Tables 4 and 5). Although the tertiary and de Graaf follicle phases of the follicle require gonadotropin for development, disruption of the hormonal system will stop the creation of these follicles. This is in line with research by Wang *et al.* [31], which shown that the quantity and size of mature ovarian follicles significantly decreased after exposure to inhaled formaldehyde for 60 days. Because the oxidative stress caused by formalin exposure, granulosa cells suffer severe damage, which disturbs the follicle's regular activity and prevents ovulation from occurring.

Conclusion

The key finding of this study is that the hormones that regulate antral folicle development, notably GnRH secretion, LHR expression, and estradiol expression can be inhibited by formalin, which can then lead to issues with antral follicles growth. The formalin infusion doses of 140 mg/kg BB and 210 mg/kg BB do not differ statistically significantly, and these levels are effective for halting the formation of ovarian folicles.

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No potential conflict of interest was reported by the authors.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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