ISRG Journal of Multidisciplinary Studies (ISRGJMS)

ISRG PUBLISHERS
Abbreviated Key Title: isrg j. multidiscip. Stud.
ISSN: 2584-0452 (Online)
Journal homepage: https://isrgpublishers.com/isrgjms/
Volume – II Issue - IX (September) 2024
Frequency: Monthly

Rice Bran Oil Treatment in Controlling Folliculogenesis as the Effect of Transfluthrin Exposure

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| **Received:** 19.07.2024 | **Accepted:** 25.07.2024 | **Published:** 3.09.2024

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Abstract

The high use of mosquito repellent has become the norm in preventing the spread of diseases caused by mosquitoes. Meanwhile, mosquito spray contains active ingredients such as Endocrine Disruptive Chemicals. This chemical has an impact on the stimulation of FSH and LH excretion and has a direct effect on the disruption of follicular development (folliculogenesis) in the ovary. Rice bran oil (RBO) has potential as a folliculogenesis treatment as it contains antioxidants that can reduce the effects of damage to plasma lipids in mitochondria and can block chronic inflammation. Aims: To analyze the effect of subacute administration of RBO (for 28 days) as an anti-inflammatory in controlling folliculogenesis due to transfluthrin.

Methods: *Rats were divided into four groups, namely negative controls (without treatment), positive controls (groups given RBO), a group with one-push exposure, and a group with one-push and RBO. One-push exposure contains a 21.3% transfluthrin active ingredient. RBO treatment was done for 28 days. Rats were then put under an inhaled sub-chronic toxicity test for 6 hours. The samples were analyzed using a post-test only control group design, with a completely randomized design and MANOVA statistical analysis in IBM SPSS Statistics 25 software.*

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DOI: 10.5281/zenodo.13637534

Results: *There were significant differences in ovarian weight (p = 0.022) and the number of pathological follicles (p = 0.009) in all groups.*

Conclusion: *RBO as a source of antioxidants can reduce the number of follicular abnormalities in the ovary caused by exposure to one-push aerosols containing transfluthrin.*

Keywords: *follicle, one-push, ovarium, rice bran oil, transfluthrin*

1. Introduction

The high use of mosquito repellent has become the norm in preventing the spread of diseases caused by mosquitoes. Meanwhile, a mosquito repellent spray (one-push aerosol) contains active ingredients such as Endocrine Disruptive Chemicals (EDCs) [1].

One type of active ingredient in mosquito repellents that can cause tissue damage or oxidative stress is the pyrethroid synthetic compound (PS) contained in the one-push aerosol. PS toxicity can affect the reproductive organs through two mechanisms: hormonal and cellular. PS can reduce the secretion of the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH) and induce gonadotropin disorders by apoptosis that is characterized by follicular growth inhibition. The cellular impact includes increased damage in the mitochondria. This condition results in an x-linked factor not being able to inhibit ovarian damage [2,3]. Transfluthrin, an active compound of PS derivatives, is highly reactive, so it is widely used in mosquito repellents including one-push aerosol [4]

PS toxicity to ovum cells during folliculogenesis includes interruption of extra-ovarian and intrafollicular signal integration [5], endocrinology [6], and gonadal development [7]. Thus, the effectiveness of mosquito repellents in preventing the spread of diseases caused by mosquitoes, such as DHF and malaria, is in contrast with the EDC's properties that have a negative impact on human health, especially the reproductive system. Extensive research on hormonal regulation for the development of ovarian follicles (folliculogenesis) has been widely carried out, with most studies focusing on the development of antral follicles from the initial to the re-ovulation stage. The intraovarian mechanism through exogenous FSH stimulation has also been observed. This stimulation has only been given to patients diagnosed with primary ovarian insufficiency and PCOS and to infertile (secondary) women in the reproductive age group [8]. This condition is a long-term effect of damage/disruption of follicular growth, and a healthy lifestyle is one of the preventive measures to control folliculogenesis.

The current lifestyle is accompanied by the development of the food industry that offers a variety of natural potential, such as antioxidants that play a role in preventing oxidative stress due to exogenous factors. One of the antioxidant sources in food is rice bran oil (RBO).

RBO is a by-product of rice milling. The role of rice bran as a functional food source can be seen from its bioactive components and dietary fiber [9]. The bioactive content of tocopherol and γ-oryzanol in crude bran oil (i.e., unrefined rice bran oil) is higher than that in other vegetable oils [10]. RBO can be consumed directly, used as a substitute for cooking oil or food supplements, and has the benefit of lowering cholesterol, inhibiting platelet aggregation and antioxidant. Besides, the antioxidant potential of RBO can be used as a radioprotective, in treatment for cancer, and in chemotherapy [11].

RBO contains bioactive compounds such as ferulic acid, tricin, beta-sitosterol, γ-oryzanol, tocotrienol, tocopherol, and phytic acid, which can prevent oxidative stress by reducing damage to plasma lipids and blocking the chronic inflammatory response. Phytochemicals in RBO can activate anti-cancer immune responses by inducing apoptosis, inhibiting cell proliferation, altering the development of the cell cycle, and affecting the micro-tumor environment [12]. There are several studies on RBO and its bioactive compounds as chemopreventive agents of colon cancer through the mechanism of signal inhibition in TLR2 and TLR4 [13]. Other studies of RBO as a chemopreventive agent have also been proven through the utilization of the bioactive component γ-tocotrienol which can stimulate apoptosis in gastric cancer cells through caspase 3 activation and inhibition of phosphatidylinositol 3-kinase AKT signaling [14,15].

This research was conducted to observe the potential of RBO bioactive and phytochemical contents in the cellular and humoral level that provide opportunities for the development of RBO research as a preventive and curative agent in maintaining reproductive function (ovaries), especially in controlling folliculogenesis. Preliminary tests conducted in this study were done by in a silico test through two stages, with the first result showing that the γ-oryzanol content in RBO has potential as an anti-inflammatory, and no Scavenger was higher than the antioxidant compounds. The γ-oryzanol can stimulate Foxo3 expression that is associated with follicular growth signaling in the ovary [16]. Based on those explanations, RBO is a candidate for multifunctional processed-food ingredients that have the benefits for the reproductive system.

The second test showed that γ-oryzanol can interact with PS, so it is predicted to be used as a therapy for insecticides exposure. Based on the two results of the insilico test, this study aims to analyze the effects of RBO administration in controlling folliculogenesis due to transfluthrin exposure.

2. Methods

2.1 Experimental Design

This study was experimental research with a post-test only control group and a completely randomized design (CRD). Female Wistar rats were divided into four groups based on the Federer formula, namely negative control (no intervention), positive control (given RBO), a group treated with transfluthrin, and a group with transfluthrin and RBO. Rats were obtained from the Animal Laboratory of Bandung Institute of Technology, which was declared healthy with veterinary certificate no. 524.3/3947-Dispangtan/2019. Following OECD 412 guidelines on subacute inhalation procedures, experimental animals used were 8 weeks old

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and weighed 150–200 grams; the acclimation process was carried out for 7 days before the research process began.

Animal maintenance procedures. Rat cages were made of plastic boxes covered with perforated wire (1.6 cm^2), with a base of 148.4 cm^2, and a height of 17.8 cm. The cage pads were replaced and cleaned every 3 days. The room temperature was set to 22 ± 3°C, with relative humidity of 30%–70% and lighting 14 hours on and 10 hours off. Rats were fed wit COMFEED, or calf starter PAP MILK was given 5–10 grams per 100 g/BW/day and drink of 10 ml per 100 g/BW/day. Monitoring was carried out by observing the activity of rats before, during (6 hours in a glass box), and after exposure was given.

The type of RBO used was Oryzanol Gamma (OG), which is a type of RBO production that was purified through filtering using "extra cold." The determination of the oral dose of OG-RBO was based on the content of oryzanol (as the highest bioactive element contained in RBO) multiplied by the need per kg of rat's body weight. Weight observations were done every week in the morning (09:00 AM) before giving any treatment, to determine the need for OG-RBO doses.

Inhalation of one-push aerosol to measure subacute toxicity refers to the OECD 412 guidelines for the testing of the chemical's 28-day (subacute) inhalation toxicity study. The one-push exposure was using brand V aerosol with the active ingredient of 21.3% transfluthrin. The exposure was given once a day by inhalation in one spray and left in a glass box for 6 hours and repeated for 28 days. The research was following and approved by the ethical committee as no. 38/EC/KEPK-S3/02/2019, Faculty of Medicine, Brawijaya University.

2.2. Analysis of Antioxidant Content in RBO

Analysis of antioxidant content was carried out using the DPPH method. The Duplo measurement process used six test tubes with varying concentrations (0, 30, 60, 90, 120, and 150 ppm) mixed with methanol as the standard: The sample was made in a stock solution and diluted to a certain concentration with a volume of 1 mL. The standard and the sample solution were incubated for 30 minutes and then measured with a UV-Vis spectrophotometer at a wavelength of 517 nm. The absorbance value of each concentration was recorded, and the IC_{50} value was calculated [17].

2.3. Follicle Extraction and Analysis

The analysis was carried out by single-blinded methods, without knowing which group was the exposure and control, using quantitative observations, namely counting primary, secondary, tertiary, and de Graaf follicles.

2.4. Ovarian Histology Analysis

The ovarian assessment was done qualitatively by observing cellular or ovarian structure damage caused by transfluthrin exposure. The ovarium sample was stained by hematoxylin and eosin (HE) and observed with an Olympus CX-31 microscope.

2.5. Statistical Analysis

Statistical analysis was done using IBM SPSS software version 25. The analysis was carried out using the MANOVA (Multivariate Analysis Variance) method, to analyze the effect of each independent variable (RBO, one-push) on a categorical scale on each dependent variable (ovarian weight and number of follicles, separately).

3. Results and Discussion

Table 1
The results of RBO analysis using DPPH

No	Concentration	Absorbance		% Inhibition	
	(ppm)	1st repetition	2nd repetition	1st repetition	2nd repetition
1	0	0.8360	0.8255	0.0000	0.0000
2	500	0.7136	0.7207	14.8449	12.6953
3	1,000	0.5771	0.5767	31.1337	30.1393
4	1,500	0.4233	0.4280	49.4869	48.1526
5	2,000	0.2964	0.2837	64.6301	65.6330
6	2,500	0.1638	0.1328	80.4535	83.9128

Table 1 shows that the highest RBO antioxidant activity was found at a concentration of 2,500 ppm, with the least absorbance of 0.14 ppm and the inhibition of 82.18315 ppm. This occurs due to the reduction of DPPH radicals by antioxidants, where a higher concentration means more antioxidant particles contained with greater antioxidant activity that causes the absorbance to decrease [18].

The results show that the IC_{50} value on OG-RBO was 82 ppm. IC_{50} values that are between 50 and 100 ppm indicate a strong antioxidant activity [17,19]. The antioxidant content of RBO is largely determined by the rice varieties used and the type of solvent used in the extraction process. Suitable solvents for the RBO extraction process must be polar. Among the types of polar solvents, ethanol gives higher concentrations of oryzanol compared to other solvents (such as ethyl acetate, n-hexane, isopropanol, and methanol) [20]. The g-oryzanol compounds have the potential to contribute to high antioxidant activity in RBO. Besides containing g-oryzanol, every 100 grams of RBO contains 19–46 mg a-tocopherol, 1–3 mg b-tocopherol, 1–10 mg g-tocopherol, 4–9 mg d-tocopherol, 14–33 mg a-tocotrienol, and 9–69 mg g-tocotrienol [21]. The bioactive component of γ-oryzanol as a source of antioxidants contained in RBO can be seen in Table 2.

Table 2
Oryzanol levels in RBO

Sample ID	Sample Mass	Absorbance	Tools Concentration	Final Concentration
	(g)		(ppm)	(mg/kg)
RBO 1	0.1005	1.1955	30.9611	3,080.7105
RBO 2	0.1005	1.2393	32.0959	3,193.6174

* The sample was dissolved in 10 ml.

The results of the study (Table 2) show that 100 mL OG-RBO contains 314 mg γ-oryzanol; then, the RBO dose given to rats was 0.3 mL/BW/kg.

According to [22], the dose of oryzanol needed by humans is 57.6 mg. This dose was then converted to rats by the following formula: Oryzanol dose × conversion factor, 57.6 mg × 0.018 = 1.04 mg (~1 mg γ-oryzanol). Thus, if 100 mL RBO contains 314 mg γ-oryzanol, then the conversion result was 1 mg; next, the RBO dose

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for rats was calculated using this formula = (100 mL × 1)/314 mg = 0.32 mL = 0.3 mL RBO. The RBO administration meets the requirements because the maximum fluid volume per administration is 5 ml/200 g body weight [23].

3.1. One-push particle measurement

The average number of one-push particles (V brand) in three sprays, calculated with P-Track UPC 852, was as follows:

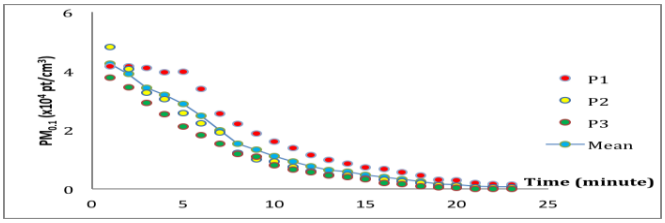

Figure 1. Fine Particle Concentrations

Information:

P1: First spray, P2: second spray, and P3: third spray.
The concentration was obtained from the results of 3× one-push aerosol spray with P-Track UPC 852 and has an average number of particle concentrations of 12.392 ± 1.356 mg/m^3.

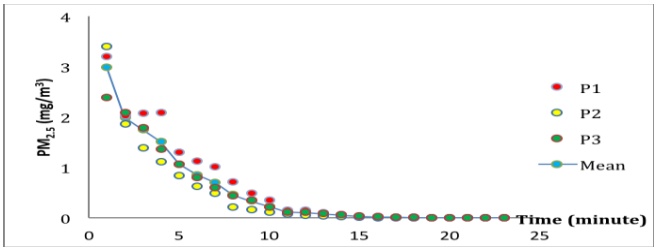

Figure 2. Ultrafine Particle Concentrations

Information: P1: First spray, P2: second spray, and P3: third spray.

The concentration was obtained from the results of 3× one-push aerosol spray with P-Track UPC 852 and has an average number of particle concentrations of 311.500 ± 46.755 particles/cm^3.

Table 3
Analysis of one-push aerosol particle concentration with P-Track UPC 852

Control Var				Result			
RH	69.60 %	66.20 %	65.10 %	$PM_{0.1}$	311500 ± 46755	Particles /cm^3	0.1–2.5 µm
Temp	24.1°C	23.9°C	23.8°C	PM $_{2.5}$	12.392 ± 1.356	Mg/m^3	0.01–0.1 µm
Gas	0.1 ppm	0.1 ppm	0,1 ppm				

Based on the data (Table 3), the concentration of ultrafine particles (<0.1 micrometers) in each spray was higher compared with fine particles (<2.5 micrometers).

The nature of transfluthrin compounds, which are reactive and contain more ultrafine particles than fine particles, has a more dangerous impact through inhalation exposure, including precipitation in the organs and flows through the bloodstream. Inhalation of ultrafine particles (UFPs) will be precipitated in the lungs [24]. UFPs' very small size enables them to penetrate tissue and be absorbed directly into the bloodstream, and they are not easily removed from the body [25]. The UFP exposure can cause oxidative stress [26]. Other research stated that UFP exposure causes the release of inflammatory mediators that can cause heart, lung, and other systemic diseases [27,28,29,30].

In the reproductive system, especially in the ovaries, transfluthrin exposure has an impact on follicular growth disorders. This is evidenced by the results of the insilico experiment, where transfluthrin affects the signaling pathways of several proteins in folliculogenesis such as GDF-9 and Foxo3a [16].

GDF-9 in folliculogenesis prevents apoptosis of granulosa cells and follicular atresia and stimulation of the expression of FSH receptors (FSHR). An adequate level of FSHR in granulosa cells is required for dependent FSH for antral follicle growth; thus, a decrease in GDF-9 expression will alter follicular growth [31]. Foxo3a in oocytes has a negative effect in regulating the process of oocyte growth and the initial stages of follicular development, by stopping the growth of primordial follicles, and plays an important role in AKT signaling for cell cycle regulation [32].

The nature of EDC owned by transfluthrin disrupts the secretion of FSH-LH and impaired AKT signaling, which is characterized by overexpression from Foxo3, decreased mutational function in PTEN, and activation in PIK3CA (p110a). This condition increases the activation of AKT, thus encouraging tumor formation and development into cancer [1,32].

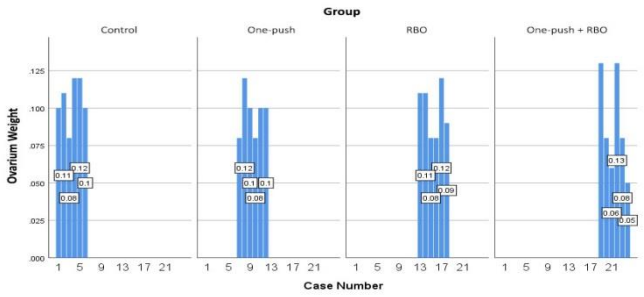

Figure 3. Comparison of Ovarian Weights in Each Group

The average ovarian weight for RBO + one-push group was the smallest compared with that for other groups, with 0.05–0.013 g, which have the same average ovarian weight (0.08–0.12 g).

These results are consistent with the results of multivariate tests used to test major hypotheses.

Table 4
Multivariate tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.961	234.087[b]	2.000	19.000	.000
	Wilks' Lambda	.039	234.087[b]	2.000	19.000	.000
	Hotelling's Trace	24.641	234.087[b]	2.000	19.000	.000
	Roy's Largest Root	24.641	234.087[b]	2.000	19.000	.000
A	Pillai's Trace	.541	2.475	6.000	40.000	.039
	Wilks' Lambda	.476	2.851[b]	6.000	38.000	.022
	Hotelling's Trace	1.067	3.201	6.000	36.000	.013
	Roy's Largest Root	1.032	6.883[c]	3.000	20.000	.002

a. Design: Intercept + A
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.

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DOI: 10.5281/zenodo.13637534

The results of the stimulatory analysis found significant differences from all groups tested for the ovarian weight ($F = 2.851$; $p < 0.05$). Ovarian weight has an eta squared of 0.99 (99 percent), which showed an effective contribution in explaining the four variables. Thus, the major hypothesis of the research was "accepted." The data were then analyzed descriptively by comparing the observations of data on rat body weight, ovarian weight, and estrous cycle in each sample.

Table 5
Analysis of body weight, estrous phase, and ovarian weight in Wistar rats

N	Control				One-push				RBO			
	Ac	**BW**	**Ec**	**Ov**	**Ac**	**BW**	**Ec**	**Ov**	**Ac**	**BW**	**Ec**	**Ov**
T1	172	193	E	0.10	205	223	E	0.08	162	207	E	0.11
T2	185	209	D	0.11	188	206	D	0.12	186	236	E	0.11
T3	202	228	Ea	0.08	224	251	Pk	0.10	203	211	E	0.08
T4	191	210	E	0.12	194	224	E	0.08	198	228	Ea	0.08
T5	229	210	D	0.12	156	180	M	0.10	182	223	E	0.12
T6	192	218	E	0.10	185	203	M	0.10	185	221	M	0.09
Means (×) Ov				**0.11**	**(×)**			**0.10**	**(×)**			**0.10**

*** Ac:** rats body weight in acclimatization, **BW:** rats body weight at day-28**, Es:** estrous cycle, **Ov:** ovarian weight, E: Estrous, Ea: Late Estrous, Ma: Late Metestrus, D: Diestrus, Pk: Late Proestrus, Ek: final estrous

The average body weight of rats during the estrous phase is greater (>216 g) that those in other phases (metestrus and diestrus) but smaller than that in the proestrus phase (Table 5). In normal conditions, the proestrus and estrous phase is the stage of growth and development of the follicle in preparation for pre-ovulation and ovulation. Follicular growth and development are influenced by anterior pituitary stimulation to increase FSH and LH secretion followed by estrogen secretion [33,34]. The existence of estrogen activity in the proestrus phase is followed by the growth and development of the reproductive organs, such as increased ovarian weight, endometrial proliferation, and increased epithelial wall thickness of the fallopian and vaginal walls [35]. This study's results were consistent with those of previous research.

The data indicate that the control group and the group with RBO treatment had the same ovarian weight of 0.1 g and were larger, compared to the other two groups. The enlargement of ovarian in this phase was due to the follicle growing optimally (mature) in preparation for the ovulation of the estrous phase, which lasts 9–15 hours, marked by the peak level of estrogen. The estrous phase is related to the pre-ovulation and ovulation phases. In the pre-ovulatory and ovulatory phases, cells that release are mainly epithelial cells; thus, the phase is known as the epithelial phase [33,34].

In the estrous phase, mature and enlarged de Graaf follicles will form, and estradiol produced by de Graff follicles will cause changes in the reproductive tract [36]. An increase, growth, and maturation of the ovarian follicles causes an increase in ovarian weight in the estrous phase, making it greater than that in the other phases [37,38]. However, if there is an enlargement of the ovary outside the estrous phase, it is necessary for one to be aware of the overexpression of Foxo3a or FKHRL1, which acts to inhibit follicular development [39].

The enlargement of the uterus (0.1 g) outside the estrous phase (metestrus phase) was observed in the group with transfluthrin. The transfluthrin group enlargement was greater compared to those in the other groups (0.09 g and 0.08 g) in the same phase (metestrus phase).

The metestrus phase is largely influenced by progesterone, which inhibits FSH secretion; thus, follicular maturation will not occur in this phase [35]. In this phase, the reproductive tracts gradually return to normal size. From the data, the ovarian weight in the group with one-push treatment has a greater value compared to those in other groups, whereas in the metestrus phase, the ovarian weight will be decreased because of follicular degeneration due to decreased estrogen [40].

Conditions that affect uterus enlargement in the transfluthrin group were related to the EDC's properties of PS, wherein the post estrus phase (metestrus and diestrus) gonadotropin activity in secreting FSH and LH will be decreased, thus decreasing the x-linked factor. This indicates the occurrence of mRNA overexpression that is supported by increased expression of P53, thereby increasing follicular atresia [5]. Other studies have shown that repeated administration of PS in female rats results in high follicular atresia [40]. Ovarian enlargement can be caused by the abnormal release of LH, which then interferes with follicular maturation and ovulation and increases the number of atretic follicles [42]. In the absence of peak release of LH, the follicle theca cells actively proliferate and are densely distributed. Theca cells contain smooth endoplasmic reticulum and tubular mitochondria, where the increased theca cell production suggests the presence of steroid hormone synthesis may be active in the rat ovary. The intended steroid hormone is related to the active synthesis of androgen hormones [41].

The large number of atretic follicles and enlargement of the ovaries can cause a formation of follicular cysts in the ovaries. Follicular cysts form when the follicle does not release an oocyte during ovulation. Instead, these cells will grow and turn into cysts. Follicular cysts typically have no symptoms and do not require intervention because they will disappear eventually [41]. This condition is more likely to occur in the elongation of the leukocyte phase or the post estrus phase (metestrus-diestrus) than that of the epithelial phase. This indicates an interruption of the estrous cycle in the transfluthrin group; there was a prolongation of the leukocyte phase compared to the epithelial phase due to impaired LH and FSH stimulation disorders for follicular maturation at the next estrous cycle stage [5,43]

The RBO group body weight was greater ($x = 221$ g) compared to the transfluthrin + RBO groups ($x = 190$ g). The presence of nutritional intake influences the growth and development of follicles [44,45]. This is in line with research on the benefits of using RBO to improve body conditions including changes in body weight [46]. The RBO and control groups showed that the bodyweight of rats with ovarian weight during the estrous cycle was relatively normal. This condition indicates the antioxidant activity of bioactive contained in RBO to protect against ovarian tissue damage through free radical scavenging and blocking of the chronic inflammatory response [12].

The estrous cycle takes place simultaneously with the development of follicles (folliculogenesis). This condition is influenced by the

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presence of growth factor stimulation in oocytes (SCF) and externally through the pituitary gland with hormonal stimulation (FSH and LH). Table 6 illustrates the follicular development of each treatment group.

Table 6
Frequency distribution of follicular development

K	n	Fr	%	P	%	S	%	G	%	Ab	%
1	84	7	8	35	22	13	12	6	24	23	9
2	228	25	31	34	21	44	41	12	48	112	46
3	122	22	26	48	29	32	30	4	16	16	7
4	188	29	35	45	28	18	17	3	12	93	38
total	622	83		162		107		25		244	

* **Pr: Primordial, P: Primary, S: Secondary, G: De Graaf, Ab: Abnormality**

The difference in follicular growth and follicular development in each group shows that each treatment has a different effect. This was consistent with the results of the MANOVA test using the test of between-subject effect that shows a significant level (Sig) of 0.009, <0.05. This shows that there were differences in the number of follicular abnormalities caused by one-push, control, RBO, and RBO + one-push treatment.

In Table 6, the largest number of follicles was in the one-push group with 228 follicles. Rats in the one-push group were divided into several estrous cycles, namely proestrus, estrous, metestrus, and diestrus. Thus, the most likely contribution of follicles comes from rats with the proestrus and estrus cycle. The proestrus and estrus phase is the growth and development of the follicle in preparation for pre-ovulation and ovulation [33,34]. Based on the distribution of follicular development, the number of preantral follicles (primordial and primary) was higher than the antral (secondary, tertiary, de Graaf). The highest number of follicles with abnormalities was in the one-push group with 112 follicles. The condition where the number of preantral follicles increases was followed by a high number of follicular atresia, indicating the follicles become atretic before developing into antral follicles [41].

The results of the HE examination (Fig. 4) showed that follicular abnormalities such as karyolisis (KL), ruptured granulosa (GP), and karyorrhexis (KR)

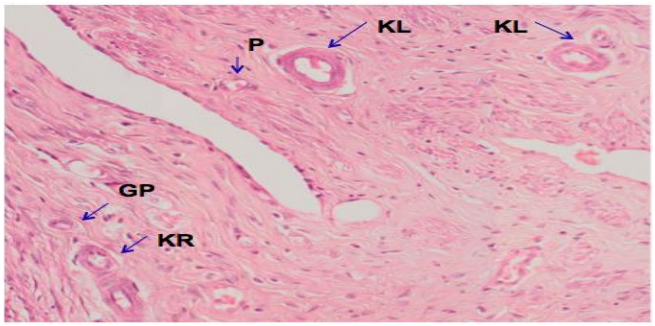

Figure 4. HE Examination of One-push Group

were found in the ovarium of the one-push group. These results show the presence of folliculogenesis (follicular growth), which was disrupted by the high number of follicular abnormalities that affect the follicle's preparation for the ovulation process.

The presence of one-push inhalation made from active PS can have a toxic effect on the reproductive organs [5]. These toxicity effects occur through two mechanisms, namely direct influence on cells and biochemical reactions on cell metabolism [7]. In this study, the condition of toxicity that gives a direct influence on cells was indicated by the presence of follicular growth disturbance (folliculogenesis). The disturbance includes the absence of primordial follicle development into secondary primary, the presence of de Graaf follicles (estrous phase), and alarge number of follicles with abnormalities (such as karyorrhexis, karyolysis, and pyknosis).

Types of ovarian karyolysis abnormalities are the loss or fading of the cell nucleus, whereas karyorrhexis is where the nucleus was divided into several fragment pieces. Karyolysis and karyorrhexis is a condition of granulose cells that undergo apoptosis [42]. Atrestic follicles or atresia occur as aresult of degenerative follicles that are characterized by pyknosis, reduction of granulosa cells due to proliferation, and damage to the basal membrane or have altered glucose [47].

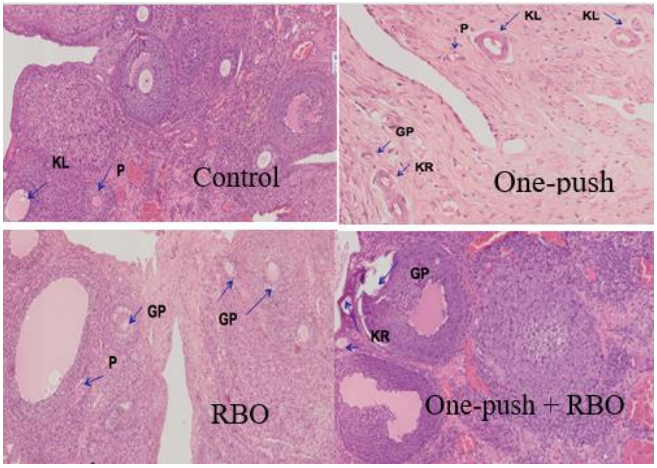

Figure 5. HE Examination Results in Each Group

GP: altered granulosa cells, KL: Karyolysis, KR: karyorrhexis, and P: Pyknosis

The RBO + one-push group had the largest number of follicles after the one-push group. Based on the estrous cycle phase (Table 5), the RBO + one-push group consisted of the proestrus, estrus, metestrus, and diestrus phases, with 188 follicles, and 93 (38%) of it had abnormalities. This condition shows the existence of follicular defense during folliculogenesis through RBO administration, so that the percentage of follicular abnormalities in the RBO + one-push group was lower than that in the one-push group. This occurs because the bioactive content of RBO as a source of antioxidants can repair follicular tissue damage from oxidative stress due to transfluthrin and block chronic inflammation [12]. Repaired follicles that were damaged can be seen from the distribution and number of follicles where preantral follicles were more (63%) than antral ones (29%), with a follicular abnormality of 38%. The data shows that RBO as a source of antioxidants has not been seen yet effective; this can be because the length of time given or the dose given was not enough to neutralize oxidative stress so that the number of follicular abnormalities was only partially neutralized [44,45].

The RBO group had smaller abnormal follicles (7% of total follicles) compared to the control group (9% of total follicles). These results provide an opportunity for RBO as an alternative treatment to repair and prevent follicular damage in the ovary.

Based on multiple comparison test results, there were follicular abnormalities that vary between groups. Aerosol (one-push)

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DOI: 10.5281/zenodo.13637534

exposed group has the highest follicle damage compared to other groups; the RBO-only group had less follicle damage compared with the control; and the aerosol (on-push) + RBO group had the least follicle damage compared with other groups.

Based on the above discussion, all the experimental groups showed vary conditions in follicular development in each estrous cycle. This condition is controlled by genetics and modified by external factors such as exogenous hormones, steroid hormones, weather, and nutrition [39,43], so nutrient intake exerts an influence on follicular growth and development [44,45]. Another important role of RBO is that it is effective in preventing toxic effects and controlling sex hormones when considering the length of time given and the dose requirements [46].

4. Conclusion

The use of RBO as a source of antioxidants can reduce the number of follicular abnormalities in the ovary caused by exposure of one-push aerosols containing transfluthrin compounds.

5. Suggestion

It is necessary to study further about the use of RBO as a preventive and curative agent in controlling folliculogenesis in the ovary by applying several RBO doses so that it can measure antioxidant and anti-inflammatory activity in acute and chronic toxicity.

Conflict of interest declaration

The researcher has no conflict of interest regarding the publication of this research article.

Funding

The research was funded by the Domestic Postgraduate Education Scholarships from the Director-General of the Ministry of Research and Technology of the Republic of Indonesia

Acknowledgments

Researchers would like to thank all those who have helped the process of conducting this research, Promoters, and Co-promoters at Faculty of Medicine of Brawijaya University; Laboratory of Applied Science, Padjajaran University; Laboratory of Physics in Faculty of Science, Brawijaya University; laboratory of Parasite, Biomedicine, Pathology, and Animal House in Faculty of Medicine, Brawijaya University; and Saiful Anwar Hospital Malang.

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DOI: 10.5281/zenodo.13637534