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Effect of *Curcuma longa* maceration treatment on ovarian follicular development, serum oestradiol, uterine growth and vascularisation in female albino rats

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Abstract

Introduction: *Curcuma longa* is a well-known medicinal plant with various health benefits. This study was designed to evaluate the administration of Indonesian *C. longa* maceration for its effect on promoting growth and development of the ovary and uterus before mating in female albino rats. **Material and Methods:** A total of 15 female Sprague Dawley rats in their dioestrous phase were assigned into three different groups: the Control group (mineral water); the Cur-Low group (mineral water with 1% *C. longa* maceration) and the Cur-High group (mineral water with 5% *C. longa* maceration). The treatments were given for 20 days. Serum concentrations of follicle-stimulating hormone, oestradiol and progesterone were determined. After the sacrifice of the rats, ovary and uterine relative weight, uterine cornua diameter and length, uterine gland diameter (by histology), the number of primary, secondary, tertiary, and Graafian follicles, the number of corpora lutea and vascular endothelial growth factor (VEGF) expression in the ovary were measured. Uterine vascularisation was also evaluated. **Results:** Administration of *C. longa* maceration significantly improved the relative weights of the uterus and ovary; uterine cornua diameter, length and vascularisation; uterine gland diameter; and expression of VEGF in the ovary. It also increased the number of tertiary follicles and corpora lutea, albeit not significantly. Follicle-stimulating hormone serum concentrations were lower in the administered rats. **Conclusion:** Oestradiol and progesterone levels rose with *C. longa* maceration treatment. The maceration improved the reproductive organs of unmated rats and had potential to optimise the uterine environment for supporting pregnancy in order to produce high-quality offspring.

Keywords: follicle number, uterine morphometric analysis, sex, steroids, reproductive system.

Introduction

Curcuma longa, a plant producing turmeric, has long been recognised for its medicinal properties. The benefits of *C. longa*'s rhizome to health are largely attributed to its most prominent biologically-active compound, curcumin, found in turmeric (12). The multiple pharmacological benefits of herbs including curcumin are seen in antioxidant, anti-inflammatory, antimicrobial and anti-cancer activity (8, 10, 16). The beneficial properties of curcumin were further demonstrated in female animal studies. Curcumin treatments were reported to improve the ovulation and corpus luteum of rats with polycystic ovary syndrome by inhibiting the expression of tumour necrosis factor alpha (TNF-α), interleukin 6, and C-reactive protein. *Curcuma longa* and curcumin could inhibit the production of TNF-α and prostaglandin E2 as well as suppress the production of vascular endothelial growth factor (VEGF) in ovarian cancer. Curcumin also attenuated ovarian failure and improved embryonic development in aging mice (5, 13).

However, studies on the effects of *C. longa* and its active components on the reproductive function of female animals are limited. Administration of ethanol extract of *C. longa* in female rats was reported to increase the weight of the ovary, uterus and body (1). Another study reported that dietary *C. longa* in female rabbits improved folliculogenesis and ovarian viability by increasing progesterone synthesis and secretion and

modulating luteinising hormone receptors and the release of leptin by the ovary (7). Phytooestrogenic effects of curcumin have been described, but there are very few studies investigating the action of *C. longa* on the regulation of endocrine functions in female reproduction and how it affects the reproductive organs. Therefore, the objective of the study was to evaluate the improving effect of *C. longa* on the uterus, uterine and ovarian development and vascularisation, and hormone synthesis and secretions in female Sprague Dawley rats.

Material and Methods

Preparation of macerated *C. longa*. A 100 g mass of fresh *C. longa* was coarsely chopped and then added into 100 mL of boiling water. The resulting maceration was chilled at room temperature for 1 h and filtered using 100 µm wire mesh to be a stock solution. By diluting this with mineral water, 1% and 5% drinking water solutions were prepared. The stock solutions were prepared freshly every three days and kept in a refrigerator at 4°C.

Experimental procedure. The experimental rats were divided into three treatment groups, each group consisting of five females (according to the Federer formula). The Control group was only given mineral water without *C. longa* content, the Cur-Low group was given mineral water containing 1% *C. longa* maceration, and the Cur-High group was given mineral water containing 5% *C. longa* maceration.

Follicle stimulating hormone, oestradiol and progesterone assays. Intracardiac blood was collected into centrifuge tubes and serum was prepared by centrifugation (1,500 × *g* for 20 min at 4°C). Serum was then stored at −20°C until it was assayed. The hormone, oestradiol and progesterone concentrations were measured using a Rat FSH (Follicle Stimulating Hormone) ELISA Kit, Rat/Porcine E2(Estradiol) ELISA Kit and QuicKey Pro Rat Pg(Progesterone) ELISA Kit (all products of Elabscience, Houston, TX, USA) according to the manufacturer's instructions.

Uterine and ovarian relative weight. The relative weight of the uterus and the ovary was determined by dividing each organ's weight by the rat's body weight.

Macroscopic examination of the uterus. Uterus images were captured during euthanasia to represent the organ under ideal vascular conditions. The diameter and length of the left and right uterine cornua and the proportion of uterine blood vessels were measured using ImageJ image analysis (23).

Uterine gland measurement and ovarian follicle counting. Formalin-fixed blocks of the ovary and uterus tissue embedded in paraffin were cut into sections of 4 µm using a Leica RM2135 Microtome (Leica Biosystems, Nussloch, Germany). Tissue sections were then placed onto a glass slide, deparaffinised in xylene, rehydrated in graded alcohols, stained with haematoxylin and eosin (H&E) and sealed with Entellan (Merck, Darmstadt, Germany). All experimental groups' tissue samples were evaluated on H&E-stained slides. The diameter of the uterine gland, the number of primary, secondary, tertiary and Graafian follicles, and the number of corpora lutea were determined using image analysis methods in ImageJ.

Immunohistochemical staining of ovarian VEGF. Immunoperoxidase staining was performed to investigate changes in VEGF. Glass slides were coated with poly-l-lysine (Biogear, Indonesia). Then, slide sections of the ovary were dewaxed, rehydrated and submitted for endogenous peroxidase quenching. Antigen epitope retrieval was enhanced with heat-induced epitope retrieval solution (ScyTek, Logan, UT, USA) for 5 min at 121°C. Tissue slide sections were washed with 10 mM PBS with pH of 7.4 for 5 min, then incubated with a mouse monoclonal antibody to VEGF at a dilution of 1:50 (C-1 clone raised against amino acids of VEGF-A of human origin, sc-7269; Santa Cruz Biotechnology, Dallas, TX, USA), immersed in wash buffer for 5 min and visualised with EnVision FLEX polymer (Dako Omnis, Agilent, Santa Clara, CA, USA) for 20 min.

Statistical analyses. Analyses were performed using R software 3.5.1 version (21). The data expressed as mean ± standard deviation were analysed by one-way analysis of variance followed by Duncan's post-hoc test. A P-value < 0.05 was considered statistically significant.

Results

The relative weights of the uterus and of the ovary of the experimental rats are shown in Fig. 1.

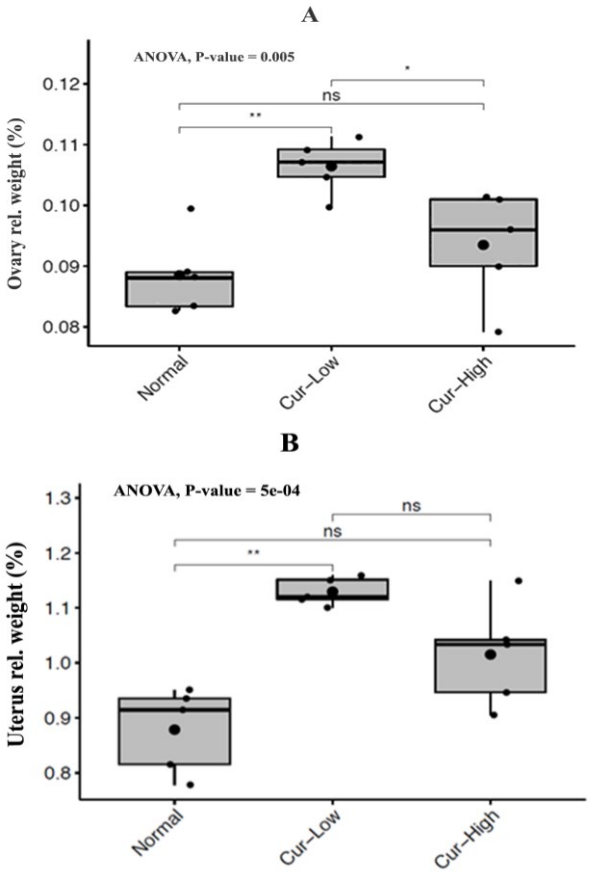

Fig. 1. The uterine (A) and ovary (B) relative weight of experimental Sprague Dawley rats after 20 days' treatment with *Curcuma longa* maceration at doses of 0% (Control), 1% (Cur-Low) or 5% (Cur-High). ANOVA – one-way analysis of variance; * – significant difference compared to the control group (P-value < 0.05); ns – non significant

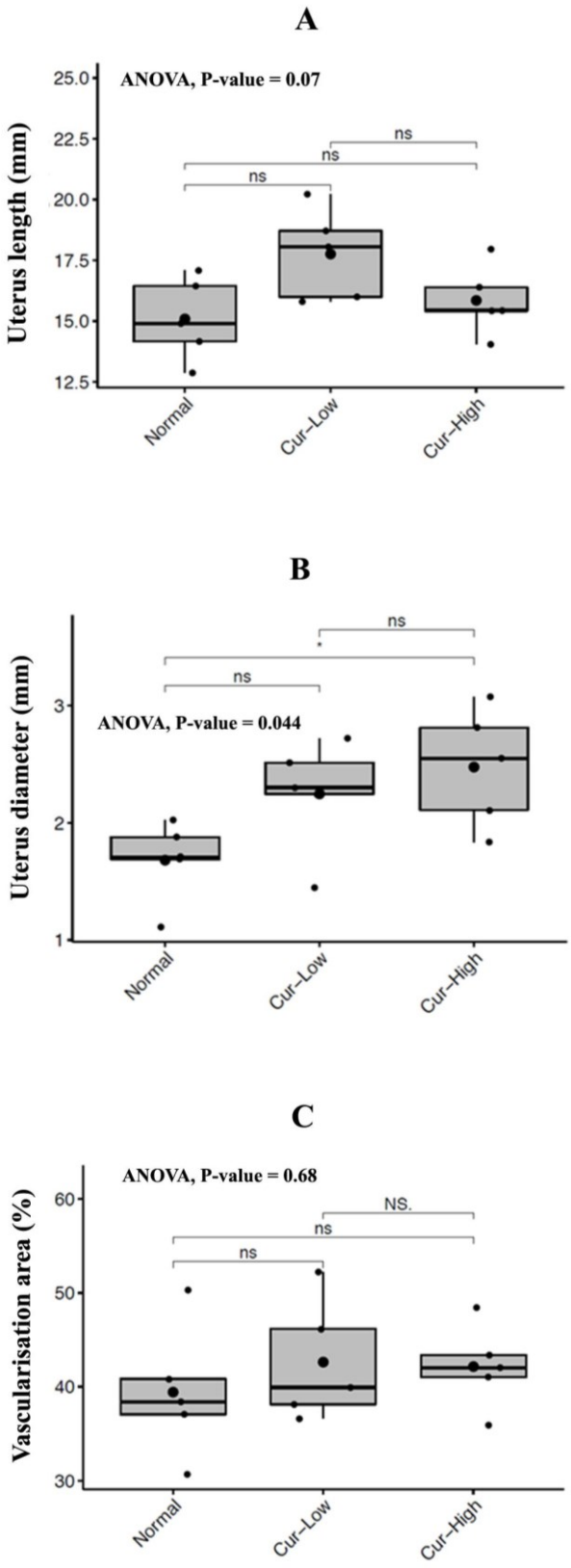

Fig. 2. Diameter (A), length (B), and vascularisation (C) of uterus in experimental Sprague Dawley rats after 20 days' treatment with *Curcuma longa* maceration at doses of 0% (Control), 1% (Cur-Low) or 5% (Cur-High). ANOVA – one-way analysis of variance; NS/ns – non significant

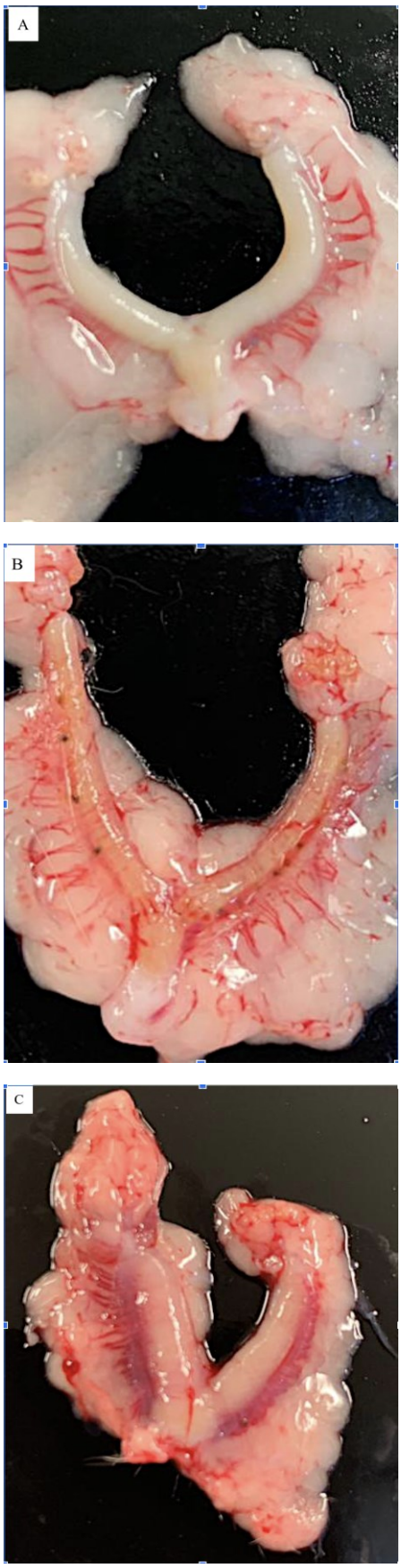

Fig. 3. Gross pathology of the uterus and its vascularisation in experimental Sprague Dawley rats after 20 days' treatment with *Curcuma longa* maceration at doses of 0% (A – Control), 1% (B – Cur-Low) or 5% (C – Cur-High)

The relative weight of the uterus was significantly higher in rats treated with *C. longa* maceration at doses of 1% (Cur-Low) and 5% (Cur-High) (P-value < 0.05) compared to that in control rats without administration of *C. longa* maceration. However, there was no significant difference in the relative weights of the uterus between the Cur-Low and Cur-High groups. The variation of the relative weights of the uterus and ovary was quite high (Fig. 1). The results also show significantly larger uterine cornua diameters in experimental rats compared to the control rats (P-value < 0.05) (Fig. 2).

The macroscopic evaluation of uterine vascularisation in the experimental Sprague Dawley rats treated with different doses of *C. longa* maceration is presented in Fig. 3. Stronger uterine vascularisation was observed in the uteri of experimental rats treated with 1% (Cur-Low) and 5% (Cur-High) *C. longa* maceration compared to the uteri of control rats. In accordance with the macroscopic observations, histological evaluation of the uterus in the experimental rats treated with *C. longa* maceration showed an improvement in gland activity, which was indicated by the larger sizes of the uterine glands. The size of the lumen of the uterine glands in the experimental rats treated with *C. longa* maceration at both doses was observed to be larger than that in the Control group (P-value < 0.05) (Fig. 4). Histological examination was also performed to count the number of ovarian follicles. The results showed that treatment with 1% and 5% *C. longa* maceration significantly increased the number of tertiary follicles and corpora lutea in the experimental rats compared to the control animals (P-value < 0.05) (Fig. 5). Vascular endothelial growth factor expression in the interstitial tissue of the ovary indicates the stimulated progression of neovascularisation, which is important for ovarian function and particularly for follicular development. Expression of VEGF revealed by immunostaining in both the Cur-Low and Cur-High groups was statistically significantly higher than that in the Control group (Fig. 6).

The overall serum concentrations of FSH in experimental rats treated with *C. longa* at a dose of 1% (the Cur-Low group) were significantly lower than those in the control rats without *C. longa* treatment, while in the experimental rats treated with *C. longa* maceration at a dose of 5% (the Cur-High group), only three out of five serum samples' concentrations were significantly lower (Fig. 7A). Serum oestradiol concentrations were increased in the experimental rats treated with 1% and 5% *C. longa* maceration, with four out of five samples from the Cur-Low group showing a significant difference (P-value < 0.05) compared to the control rats (Fig. 7B). There was no significant difference observed in the serum progesterone concentration between control rats and rats treated with *C. longa* maceration at doses of 1% and 5%, and only three out of five samples' concentrations from the Cur-Low group and two out of five samples' concentrations from the Cur-High group were higher than those in the control group (Fig. 7C).

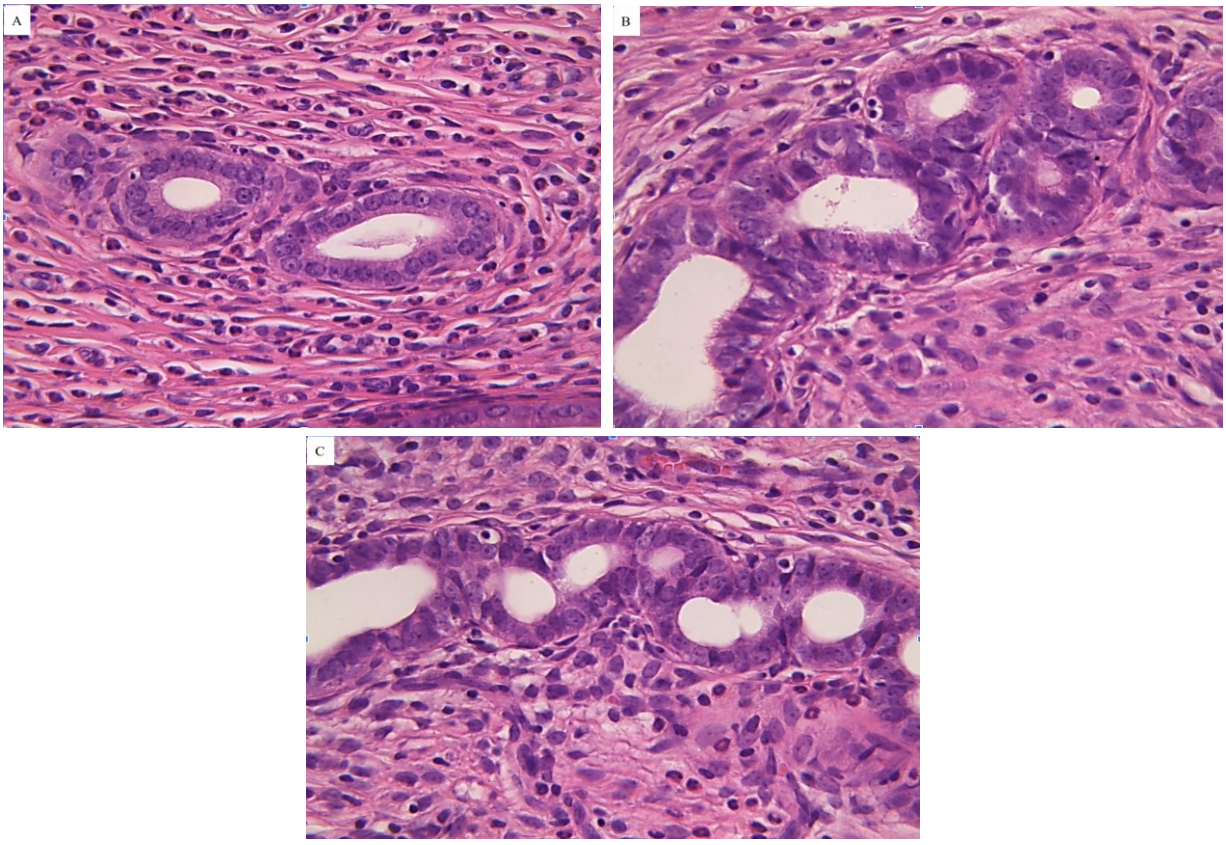

Fig. 4. Histopathological visualisation showing the effect of 20 days' *Curcuma longa* maceration administration on the uterine gland of experimental Sprague Dawley rats. The maceration was administered at doses of 0% (A – Control), 1% (B – Cur-Low) or 5% (C – Cur-High)

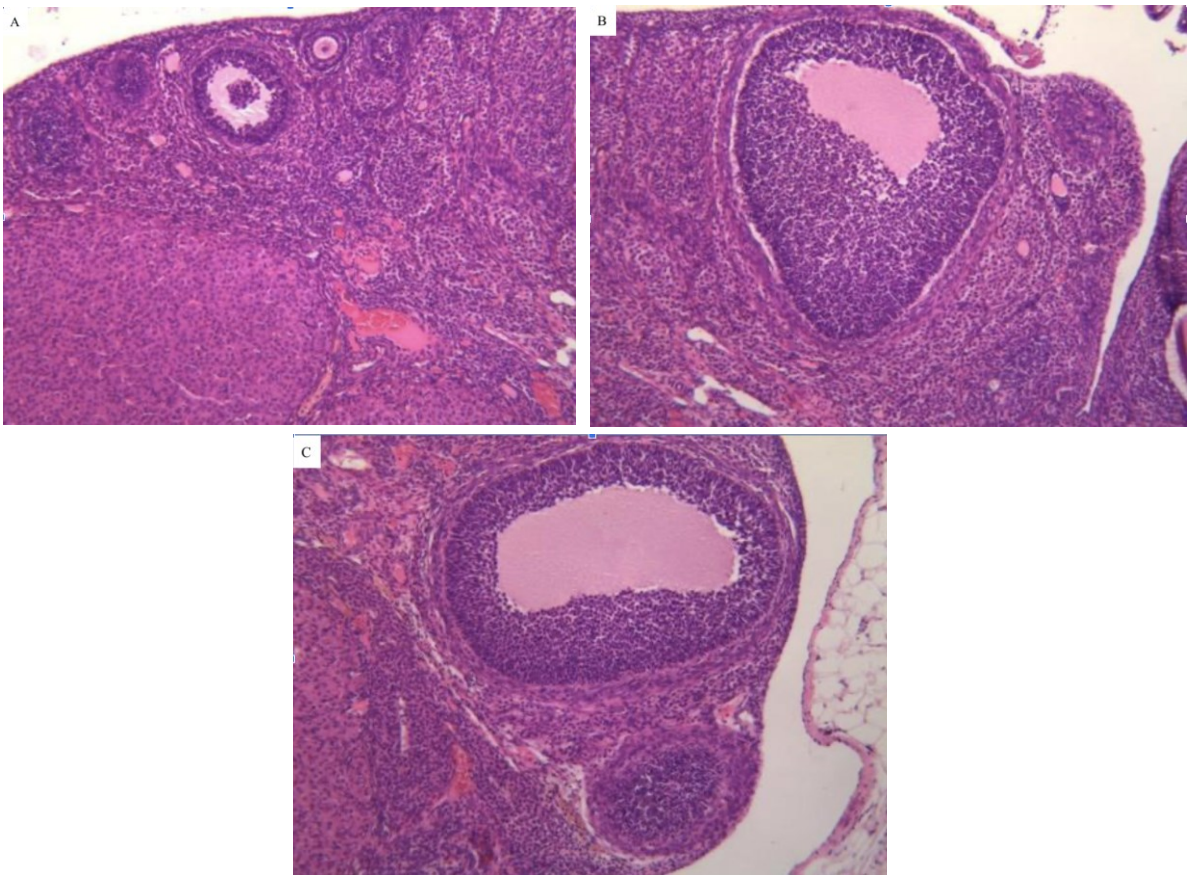

Fig. 5. Histopathological visualisation showing the effect of 20 days' *Curcuma longa* maceration administration on the follicles of experimental Sprague Dawley rats. The maceration was administered at doses of 0% (A – Control), 1% (B – Cur-Low) or 5% (C – Cur-High)

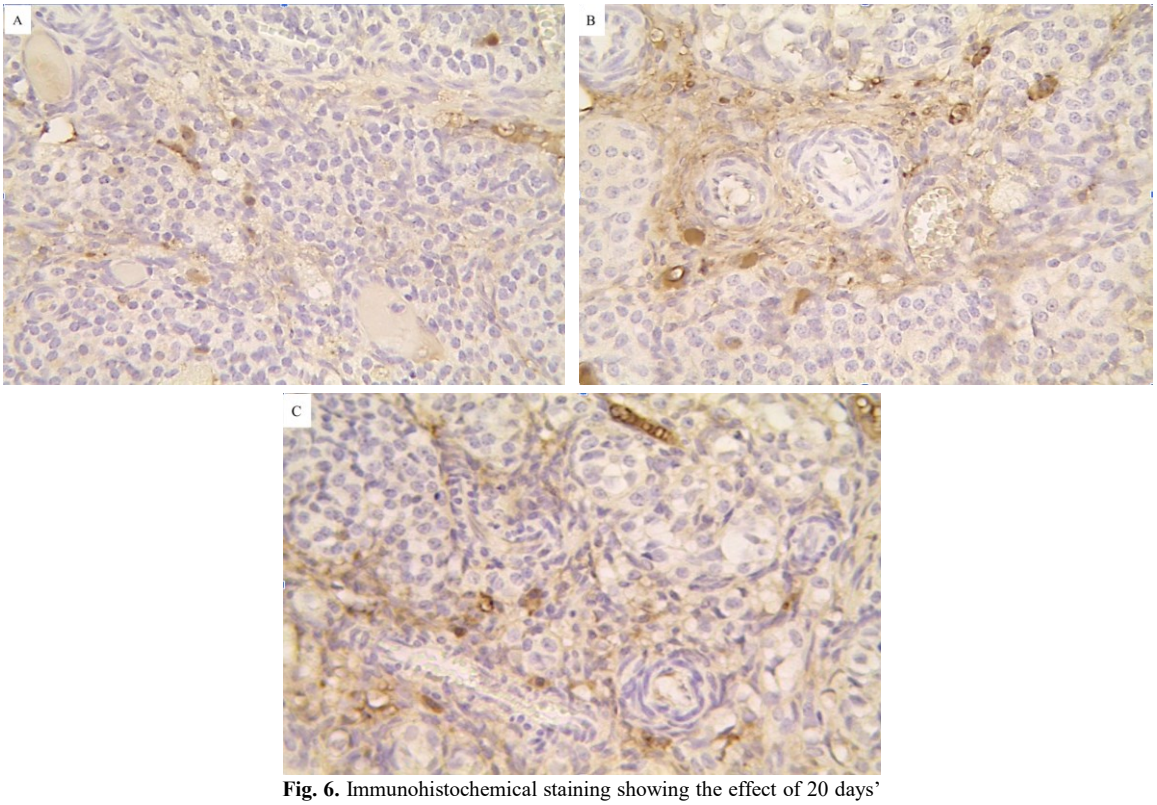

Fig. 6. Immunohistochemical staining showing the effect of 20 days' *Curcuma longa* maceration administration on the expression of ovarian vascular endothelial growth factor by experimental Sprague Dawley rats. The maceration was administered at doses of 0% (A – Control), 1% (B – Cur-Low) or 5% (C – Cur-High)

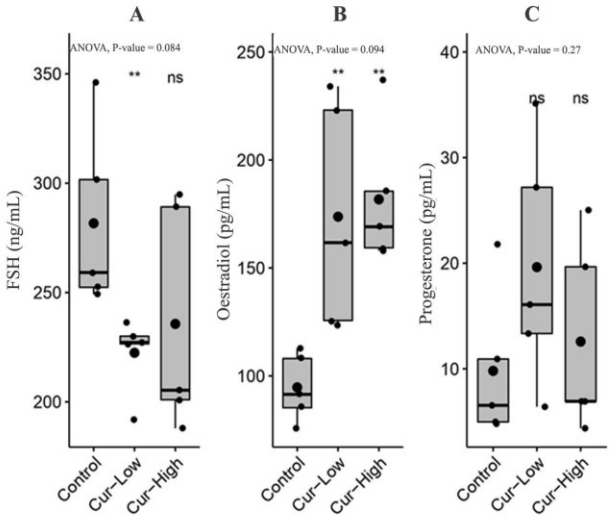

Fig. 7. Serum follicle-stimulating hormone (FSH) (A), oestradiol (B) and progesterone (C) concentrations in experimental female Sprague Dawley rats after 20 days' treatment with *Curcuma longa* maceration at doses of 0% (Control), 1% (Cur-Low) or 5% (Cur-High). ANOVA – one-way analysis of variance; * – significant difference compared to the control group (P-value < 0.05); ns – non significant

Discussion

The administration of *C. longa* maceration for 20 days to mature female Sprague Dawley rats in their dioestrous phase showed great potential as an alternative method for improving the gonadal growth and development of female animals. The findings of the higher relative weight of the ovary and the significantly higher number of tertiary follicles and corpora lutea point to these reproductive health benefits being imparted by *C. longa* administration. The extract of this plant may reduce ovarian cell death and boost folliculogenesis through the anti-oxidative properties of curcumin, the primary active component of turmeric. In studies conducted previously to evaluate the *C. longa* effect on female rabbit reproduction, dietary turmeric increased the number of primary follicles, as well as the diameter of primary, secondary and tertiary follicles, which suggests that turmeric promotes the production of primary ovarian follicles and stimulates follicle growth throughout folliculogenesis (2, 6).

In addition to the direct mechanism of action by which *C. longa* produces its pharmacological effect of strengthening the development of the ovary and its function, another suggested pathway along which the reproductive health–enhancing effect of *C. longa* is exerted is the augmentation of VEGF expression. Rats treated with *C. longa* maceration had higher expression of VEGF in the ovary. Ovarian function is dependent on the establishment and continual remodelling of a complex vascular system, and VEGF is an important factor in this mechanism. The growth factor can increase vascular permeability and stimulate angiogenesis, and it particularly functions as a stimulator for endothelial proliferation and migration (3, 20).

The follicle-stimulating hormone serum concentration indicates how much FSH is circulating in the blood. In this study, most rats given *C. longa* treatment had lower serum FSH concentrations than control rats. These data indicated that FSH was metabolised for reproductive activity, and particularly to stimulate the growth and development of follicles in the ovary; it was observed to interact with membrane receptors in the ovary to stimulate the signalling pathway of folliculogenesis during follicle growth and development (25, 27).

Oestradiol was more highly concentrated with *C. longa* maceration treatment, and the greater concentration consequently raised the relative weight and the size of the uteri over those of control rats. In previous studies, the administration of oestradiol to rodents induced uterine growth particularly in the endometrium, and that growth was affected by the bioavailability of oestradiol in serum (17, 18). In our present study, the level of endogenous oestradiol was exceptionally elevated. Curcumin's phytooestrogenic properties and stimulatory effect upon oestradiol secretion contributed to the increased relative weight of the uterus. Furthermore, the upregulation of oestradiol caused by *C. longa* administration also improved the vascularisation of the uterine cornua. Stronger vascularisation also directly contributes to the higher uterine relative weight and is one of the important indications of better-developed uteri (15, 19).

Elevated oestradiol and progesterone concentrations further improve the development of the uterus and particularly of the uterine glands. Rats supplemented with *C. longa* maceration benefitted from better gland development. It was reported in previous studies that uterine function is primarily regulated by ovarian oestrogen and progesterone (4, 14). These hormones influenced the development of luminal and glandular

epithelia and induced the stroma to express genes that are important for uterine receptivity and blastocyst implantation, including cytokines, growth factors and lipid mediators (9, 11). As described in the previous study by Kelleher *et al.* (14), the uterine gland is essential for fertility and pregnancy, as its secretions and products affected embryo survival and implantation in humans and rodents, which also mediated stromal cell decidualisation and placental development in mice.

The 20-day experiment was performed in the dioestrous phase, which has the longest duration but which also varied among individuals (around 48–72 h in rats), and this variability implied that the experimental rats were not in exactly the same stage of the oestrous cycle. Nevertheless, exceptional effects of the supplementation of *C. longa* maceration on sex steroid hormone concentrations and gonad development were still highly manifest in the parameters measured in the present study. Therefore, *C. longa* has the potential to be developed and used to improve reproduction performance and produce better quality and performance of the offspring (22, 24).

Conclusion

Application of *Curcuma longa* maceration in female albino rats made their reproductive organs better able to support gestation by improving their morphological profile, quality and functions. *Curcuma longa* maceration is also a potential supplement to refine the signalling pathway of the reproductive system to promote a healthy prenatal environment for the optimum growth and development of embryos and foetuses of animals.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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Animal Rights Statement: The authors declare that experiments on animals were approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University (No. 094/KEH/SKE/VIII/2023).

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