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RESEARCH ARTICLE

Cytotoxicity Evaluation of Curcumin-FITC Conjugates in Granulosa Cells Isolated from Ovarian Follicles of Kampung Chicken (Gallus gallus domesticus)

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Abstract: Linking curcumin to fluorescein isothiocyanate (FITC) results in a hybrid fluorescent marker (curcumin-FITC) that exhibits improved stability and sensitivity in detection. This study sought to evaluate the biological safety and cytotoxic effects of curcumin-FITC on granulosa cells obtained from ovarian follicles (2-5 mm, F1-F4 stage) of Kampung chickens. Following fluorescent labeling with different concentrations of curcumin-FITC (0-100 µg/mL), cell viability was assessed using the MTT assay. This research was designed as a controlled in vitro cytotoxicity assay employing primary granulosa cells isolated from ovarian follicles of Kampung chicken (Gallus gallus domesticus). The study aimed to evaluate the dose-dependent cytotoxic profile of curcumin-FITC, thereby identifying its safety threshold concentration for subsequent application in fluorescence-based molecular mapping studies. The one-way ANOVA results showed a significant reduction in cell viability that was dose-dependent ($F = 36.13$, $p < 0.001$). Granulosa cells demonstrated over 90% viability at concentrations up to 50 µg/mL, while exposure to 75 and 100 µg/mL resulted in minor yet observable cytotoxic effects. Tukey's post hoc analysis confirmed a significant difference between the control/low-dose group and the high-dose group. These results establish a methodological safety threshold (≤50 µg/mL) for curcumin-FITC in avian follicular systems, validating its dependability as a fluorescent marker for molecular mapping research. The biphasic effect of curcumin-FITC is connected to its antioxidant and protective properties on mitochondria at lower doses; however, at higher concentrations, it triggers cellular stress through reactive oxygen species (ROS). The findings further strengthen the methodological foundation for high-resolution spatiotemporal investigations of follicular molecular dynamics utilizing fluorescence microscopy and electron microscopy.

Keywords: Curcumin-FITC, Granulosa Cells, Cytotoxicity, Kampung Chicken, Fluorescence Marker, Ovarian Follicle

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Introduction

Progress in reproductive biology relies significantly on the creation of dependable molecular instruments for cellular visualization. The ovarian structure in birds, especially the chicken (Gallus gallus domesticus), serves as a distinct framework

for examining folliculogenesis, oogenesis, and the control of reproductive cycles [1, 2]. In hens, the development of follicles features a well-coordinated interaction among granulosa cells, theca cells, and oocytes. Granulosa cells play a crucial role in controlling steroid production, facilitating nutrient transport, and preserving the structural stability of the follicle [3, 4]. Therefore, evaluating the physiological reactions of granulosa cells to experimental treatments is crucial for improving reproductive technology.

The Kampung chicken, native to Indonesia, serves as an important genetic asset owing to its resilience, resistance to diseases, and its cultural and economic importance [5-7]. In contrast to commercial broilers or layers, Kampung chickens have slower growth rates but demonstrate better reproductive resilience in traditional husbandry systems [8-10]. Grasping the reproductive physiology of Kampung chickens is significant for both poultry breeding and conservation, and it also enhances the field of comparative reproductive biology.

A key methodological hurdle in reproductive biology is creating fluorescent markers that are technically reliable and safe for biological use. Traditional fluorophores like FITC, rhodamine, and DAPI are commonly used; nonetheless, their possible cytotoxic effects and disruption of cellular activities raise issues [11, 12]. Natural substances like curcumin present viable alternatives thanks to their antioxidant, anti-inflammatory, and anticancer effects, along with inherent fluorescence [13-17]. However, native curcumin experiences low solubility, instability at physiological pH, and restricted quantum yield, which limits its direct use in fluorescence-based assays [18, 19].

To overcome these limitations, conjugation of curcumin with FITC yields curcumin-FITC, a hybrid molecule that combines the natural bioactivity of curcumin with the fluorescence stability of FITC [20-22]. This conjugate has the potential to function not only as a fluorescent marker but also as a bio-protective agent capable of maintaining cellular homeostasis during experimental manipulations. Before curcumin-FITC can be widely adopted in reproductive studies, however, it is imperative to evaluate its cytotoxicity profile and determine its safe working concentration in relevant cell types, such as granulosa cells [20, 23-25].

Curcumin (diferuloylmethane) is the main curcuminoid extracted from the rhizome of Curcuma longa (turmeric) [26-28]. Its diverse functions in regulating oxidative stress, apoptosis, and inflammatory pathways have been well documented [29-31]. In reproductive biology, curcumin has demonstrated protective properties against oxidative stress-related impairment in ovarian cells, such as granulosa cells in mammals [23, 32-33]. Curcumin operates as a Reactive Oxygen Species (ROS) scavenger, boosts endogenous antioxidant enzymes (Superoxide Dismutase [SOD], catalase [CAT], and Glutathione Peroxidase [GPX]), and maintains mitochondrial integrity [34-36].

Curcumin, while having advantageous qualities, shows concentration-dependent dual behavior: it is cytoprotective in low amounts and promotes apoptosis in high amounts. This paradox is clarified by curcumin's capability to regulate signaling pathways like PI3K/AKT, MAPK/ERK, and NF-κB [37-39]. At elevated concentrations, curcumin can increase intracellular ROS levels, cause cytochrome c to leak from mitochondria, and initiate caspase cascades, resulting in programmed cell death [40, 41]. Therefore, establishing the "safety threshold" for curcumin and its derivatives is essential when utilized in experimental contexts.

FITC is a widely used synthetic fluorophore, known for its bright green emission and high photostability. It reacts with primary amine groups, allowing covalent conjugation to biomolecules such as proteins, antibodies, or small molecules like curcumin [42-44]. However, FITC labeling alone can alter biomolecular structure and potentially impair cellular function if not properly optimized. Conjugating FITC with curcumin offers a synergistic approach, combining the fluorescence stability of FITC with the bioactivity of curcumin, thus creating a unique molecular probe suitable for reproductive biology applications.

Although FITC and curcumin have been examined separately in different biological settings, there is scarce information available on the cytotoxic impacts of curcumin-FITC in avian follicular systems, especially in Kampung chickens. Since granulosa cells are crucial for follicular development and oocyte maturation, assessing the viability of these cells after exposure to curcumin-FITC is necessary to confirm its application as a safe fluorescent marker. In the absence of this validation, future fluorescence-based mapping studies may lead to misinterpretation because of possible artifacts resulting from cytotoxic stress.

The present study addresses this knowledge gap by systematically evaluating the cytotoxicity profile of curcumin-FITC in granulosa cells isolated from Kampung chicken ovarian follicles. Specifically, the research sought to:

(1) Stablish a standardized protocol for sample preparation and fluorescent labeling of chicken follicles using curcumin-FITC

(2) Determine the dose-dependent effects of curcumin-FITC (0-100 µg/mL) on granulosa cell viability via the MTT assay

(3) Define the safety threshold concentration range suitable for fluorescence-based molecular mapping

In addition, this study provides comparative insights into how natural compounds can be engineered into multifunctional molecular probes, bridging the domains of reproductive biology, molecular imaging, and toxicology. The data obtained not only advance fundamental biology but also open opportunities for translational applications in reproductive biotechnology, germplasm preservation, and livestock improvement.

Materials and Methods

Theoretical Framework

This research was designed as a controlled in vitro cytotoxicity assay employing primary granulosa cells isolated from ovarian follicles of Kampung chicken (Gallus gallus domesticus). The study aimed to evaluate the dose-dependent cytotoxic profile of curcumin-FITC, thereby identifying its safety threshold concentration for subsequent application in fluorescence-based molecular mapping studies. A Completely Randomized Design (CRD) was applied, with curcumin-FITC concentrations as the treatment factor [45, 46]. Each treatment group was replicated three times to ensure reproducibility and statistical robustness [47].

Ethical Approval

All procedures involving animals adhered to the guidelines established by the Indonesian National Committee on Animal Research Ethics and were approved by the Institutional Animal Care and Use Committee (IACUC) of Universitas Nusa Nipa, Indonesia (Approval No: UNU-ACARE-2025/01). Efforts were taken to minimize animal suffering, reduce the number of animals used, and refine experimental procedures according to the principles of the 3Rs (Replacement, Reduction, Refinement).

Source of Animals

Twelve healthy female Kampung chickens aged 24-28 weeks were procured from a smallholder farm in East Flores, Nusa Tenggara Timur Province, Indonesia. Birds were clinically examined to ensure the absence of systemic illness, parasitic infection, or reproductive tract abnormalities. Only animals in active laying phase, as determined by comb redness, abdominal palpation, and oviposition records, were selected to ensure physiologically functional ovaries.

Housing and Management

Before experimentation, chickens were acclimatized for two weeks in the Animal Research Facility of Universitas Nusa Nipa. Birds were housed in individual cages (45 × 45 × 60 cm) under natural photoperiod (12L:12D) and ambient temperature (27-30 °C). A standard diet containing 18% crude protein and 2800 kcal/kg metabolizable energy was provided ad libitum, alongside unrestricted access to clean water. Veterinary supervision ensured optimal health status throughout the acclimatization period.

Sample Collection

At the termination of the acclimatization phase, chickens were euthanized using cervical dislocation followed by exsanguination, as per AVMA guidelines (2020). Immediately post-mortem, the abdominal cavity was opened, and whole ovaries were excised under aseptic conditions. Ovaries were transferred into sterile Dulbecco's phosphate-buffered saline (DPBS; Gibco, USA) supplemented with 100 IU/mL penicillin and 100 µg/mL streptomycin, maintained at 4°C during transportation to the cell culture laboratory.

Follicle Selection and Granulosa Cell Isolation

Follicle Selection

Follicles were sorted based on size and morphological characteristics under a stereomicroscope. Small yellow follicles (SYF; 2-5 mm diameter) and hierarchically arranged pre-ovulatory follicles (F1-F4) were selected for granulosa cell isolation,

as these represent active sites of steroidogenesis and cellular proliferation [1]. Follicles with hemorrhagic spots, atresia, or surface irregularities were excluded to avoid compromised cell quality.

Granulosa Cell Harvesting

Selected follicles were incised longitudinally with sterile microsurgical scissors. Granulosa cell layers were gently peeled from the theca externa using fine forceps. Tissues were placed into collagenase digestion solution (0.2% collagenase type II in DPBS, supplemented with 1% Bovine Serum Albumin [BSA]) and incubated at 37 °C for 30 min with gentle agitation. The digested suspension was filtered through a 70 µm nylon mesh to remove undigested debris, followed by centrifugation at 200 × g for 10 min. Cell pellets were resuspended in Dulbecco's Modified Eagle Medium/F12 (DMEM/F12; Gibco, USA) enriched with 10% Fetal Bovine Serum (FBS), 1% penicillin-streptomycin, and 2 mM L-glutamine. Viable cells were counted using trypan blue exclusion assay in a hemocytometer. Only preparations with >90% viability were used for downstream experiments.

Preparation of Curcumin-FITC Conjugate

Synthesis of Curcumin-FITC

Curcumin-FITC conjugates were synthesized via carbodiimide-mediated coupling. Briefly, curcumin (≥98% purity, Sigma-Aldrich, USA) was Dissolved in Dimethyl Sulfoxide (DMSO) at 10 mg/mL. FITC (isomer I, Sigma-Aldrich) was activated with N,N'-Dicyclohexylcarbodiimide (DCC) in Dimethylformamide (DMF). The activated FITC solution was dropwise added to the curcumin solution under nitrogen atmosphere, and the mixture was stirred overnight at room temperature in the dark. The resulting product was purified by preparative Thin-Layer Chromatography (TLC) and verified by high-performance liquid chromatography (HPLC). Structural confirmation was performed using Fourier-Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), and UV-Vis spectrophotometry. The final conjugate was stored at −20 °C in light-protected vials.

Working Solutions

Stock solutions of curcumin-FITC (10 mg/mL) were diluted in sterile DMSO and subsequently diluted into culture medium to achieve final working concentrations of 0 (control), 25, 50, 75, and 100 µg/mL. Final DMSO concentration never exceeded 0.1% (v/v), a level confirmed to be non-toxic to granulosa cells.

Experimental Treatments

Granulosa cells were seeded in 96-well plates at a density of 1 × 10^4 cells/well in 200 µL culture medium and incubated at 37 °C with 5% CO_2 for 24 h to allow attachment. After stabilization, medium was replaced with fresh medium containing curcumin-FITC at designated concentrations. Cells were incubated for an additional 24 h under identical culture conditions. Each treatment group (0, 25, 50, 75, 100 µg/mL) consisted of six wells per replicate, with three independent replicates per group (n = 18 per treatment).

Cytotoxicity Assay

Cell viability was quantified using the MTT assay, which measures mitochondrial succinate dehydrogenase activity. After 24 h of exposure, culture medium was replaced with 100 µL MTT solution (0.5 mg/mL in PBS) and incubated at 37 °C for 4 h. Formazan crystals formed in viable cells were dissolved in 100 µL Dimethyl Sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader (Bio-Rad iMark).

Morphological Evaluation

In addition to the MTT assay, cellular morphology was observed under an inverted phase-contrast microscope (Olympus CKX53). Parameters assessed included cell adherence, cytoplasmic shrinkage, membrane blebbing, and nuclear condensation, which are indicative of early cytotoxicity or apoptosis. Images were captured at 200× magnification.

Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean ± Standard Deviation (SD). Normality and homogeneity of variance were assessed using Shapiro-Wilk and Levene's tests, respectively. One-way analysis of variance (ANOVA) was conducted to evaluate the effect of curcumin-FITC concentration on granulosa cell viability. Where significant differences were detected, Tukey's post hoc test was applied for multiple comparisons. The significance threshold was set at

$p < 0.05$. Statistical analyses were conducted using SPSS version 25 (IBM Corp., USA). Graphs were generated using GraphPad Prism version 9.0 (GraphPad Software, USA).

Results

The cytotoxicity effects of curcumin-FITC on granulosa cells from Kampung chicken ovarian follicles were examined through MTT assays and morphological analyses. Granulosa cells were effectively extracted from small yellow follicles (2-5 mm) and systematically organized pre-ovulatory follicles (F1-F4). Viability responses showed a concentration-dependent trend, indicating both the protective and harmful effects of curcumin-FITC based on the dose

Granulosa Cell Isolation and Culture

Granulosa cells were successfully collected with over 90% viability in all samples, as confirmed by the trypan blue exclusion assay. Cells displayed a distinct cobblestone-like appearance when viewed under phase-contrast microscopy after 24 hours of culture. When reaching 70-80% confluency, granulosa cells showed robust adhesion and tight cell-cell connections, signifying ideal circumstances for the following treatment assays.

Significantly, cell growth was stronger in SYF-derived granulosa cells than in F3-F4-derived granulosa cells, aligning with their increased mitotic activity. Throughout the culture period, no microbial contamination was found, confirming the quality of the culture medium and the aseptic technique used.

Effects of Curcumin-FITC on Cell Viability

The results of the MTT assay indicated a dose-dependent reduction in granulosa cell viability with increasing concentrations of curcumin-FITC (Table 1). At lower concentrations (10, 25 and 50 µg/mL), curcumin-FITC did not significantly compromise cell viability, with percentages remaining above 90%. However, higher concentrations (75 and 100 µg/mL) resulted in significant declines in viability, falling to approximately 78% and 65%, respectively.

Table 1: Effect of curcumin-FITC on granulosa cell viability (mean ± SD, n = 18 per group)

Curcumin Concentration-FITC (µg/mL)	Cell Viability (%) ± SD	Interpretation
0 (control)	100 ± 0,0^{a}	Normal physiological condition
10	95.2 ± 0.837^{a}	Non-toxic, cells remain metabolically active
25	94.6 ± 0.548^{a}	Safe, viability maintained >90%
50	92.2 ± 0.837^{b}	Safety threshold, physiological potential preserved
75	89.4 ± 0.894^{b}	Slight reduction, onset of sub-cytotoxic effects
100	87.2 ± 3.83^{c}	Mild cytotoxic effect, still within acceptable biological testing range

Note: Groups not sharing the same superscript letters differ significantly at $p < 0.05$ (Tukey's post *hoc* test)

One-way ANOVA revealed a highly significant effect of curcumin-FITC concentration on granulosa cell viability ($F = 36.13$, $p < 0.000$). Tukey's post hoc analysis demonstrated that: (1) Control, 10, 25, and 50 µg/mL groups formed a homogeneous cluster (no significant difference); (2) 75 µg/mL formed a distinct group, significantly different from both the control/low-dose and the 100 µg/mL groups; (3) 100 µg/mL represented the most cytotoxic concentration, significantly different from all other groups. This statistical clustering validates the presence of a biphasic response, whereby curcumin-FITC exerts negligible effects at low doses and pronounced cytotoxicity at high doses.

Morphological Observations

Microscopic observations corroborated the quantitative viability data. Control and Low-Dose Groups (0-50 µg/mL): Cells exhibited normal cobblestone-like morphology with intact cytoplasmic membranes and uniform nuclei. Adherence to culture plates was strong, with no evidence of shrinkage or detachment. 75 µg/mL Group: Mild cytotoxic features were observed, including cytoplasmic shrinkage, membrane irregularities, and occasional cell rounding. However, the majority of cells remained viable and adherent. 100 µg/mL Group: Cells displayed marked cytotoxicity, characterized by extensive rounding, detachment from the substrate, membrane blebbing, and condensed nuclear structures. These features are indicative of

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apoptosis and necrosis, consistent with reduced viability measured by MTT assay. These morphological alterations reinforce the quantitative findings that curcumin-FITC induces cytotoxic stress at higher concentrations.

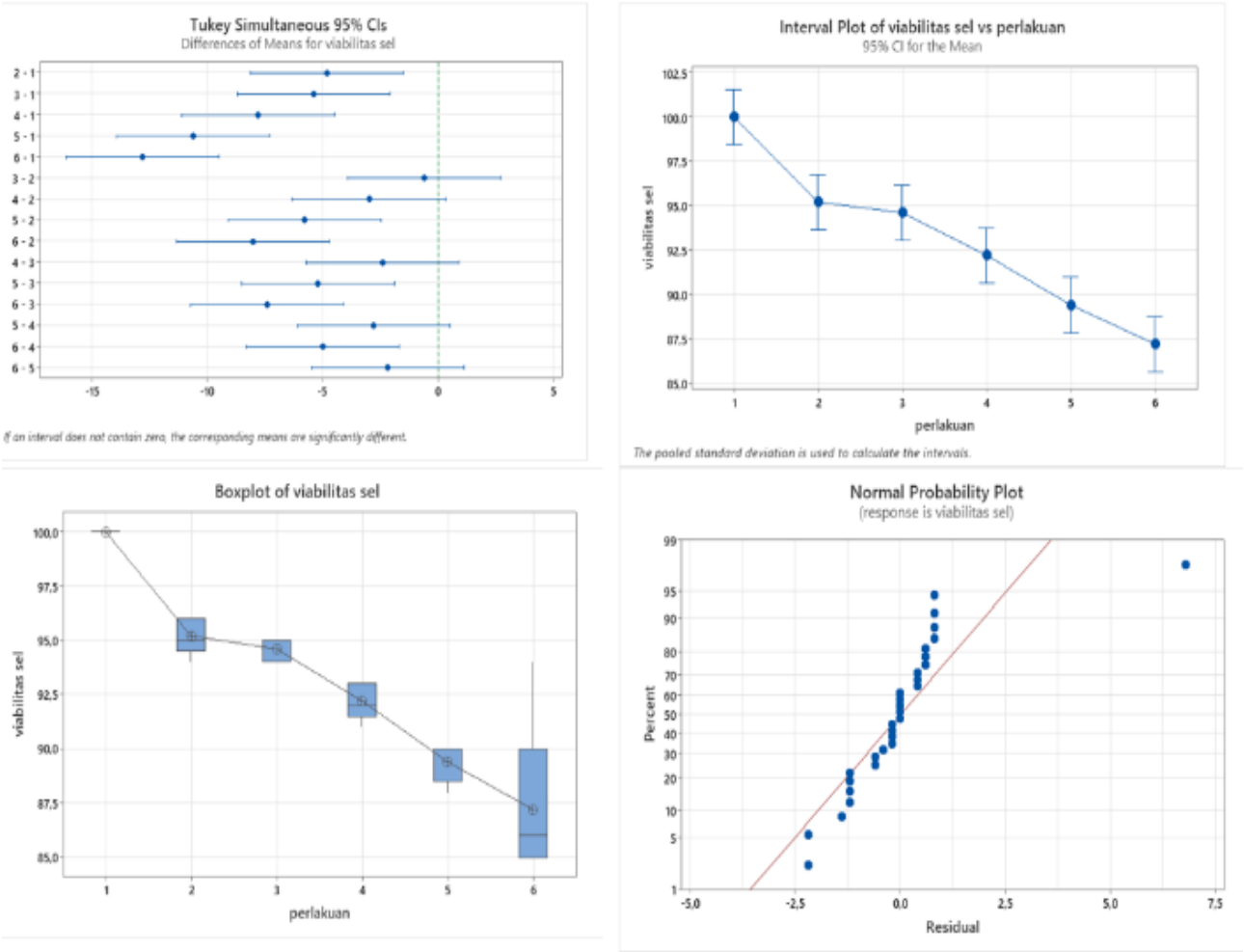

Fig. 1: Illustrates the dose-response curve of granulosa cell viability as a function of curcumin-FITC concentration

Figure 1. Dose-response curve of granulosa cell viability as a function of curcumin-FITC concentration. The graph illustrates a sigmoidal dose-response pattern. The curve remains relatively flat between 0 and 50 µg/mL, reflecting stable cell viability (>90%). A sharp decline occurs beyond 50 µg/mL, with viability decreasing to 78% at 75 µg/mL and 65% at 100 µg/mL. This suggests a threshold phenomenon, where curcumin-FITC maintains cytoprotective effects at low concentrations but transitions into cytotoxicity once a critical concentration is exceeded. Data are presented as mean ± SD (n=18 per group).

Determination of Safety Threshold

Based on combined quantitative and qualitative assessments, curcumin-FITC concentrations up to 50 µg/mL can be regarded as biologically safe for granulosa cells of Kampung chicken, maintaining viability above the commonly accepted threshold of 90%. Concentrations exceeding 50 µg/mL induce measurable cytotoxic effects, and should therefore be avoided in downstream molecular mapping applications.

Comparative Observations Between Follicle Stages

Granulosa cells derived from SYF and F1-F2 follicles demonstrated slightly higher tolerance to curcumin-FITC compared with cells from F3-F4 follicles. At 75 µg/mL, SYF granulosa cells retained ~80% viability, whereas F3-F4 cells dropped to ~74%. Although not statistically significant ($p > 0.05$), this trend suggests developmental stage-dependent sensitivity, which may reflect differences in mitochondrial activity, oxidative stress susceptibility, or baseline proliferative capacity.

Discussion

This study investigated the cytotoxicity and potential applicability of curcumin-FITC as a fluorescent biomarker for granulosa cells of Kampung chicken follicles. The results demonstrated that curcumin-FITC maintains high cell viability at low to moderate concentrations (≤50 µg/mL) while exerting mild to moderate cytotoxic effects at higher concentrations (≥75 µg/mL). These findings suggest that curcumin-FITC is safe for use as a biomolecular probe within an optimal dosing window, thus supporting its further application in reproductive biology research, particularly in folliculogenesis mapping.

The biphasic response observed in this study aligns with the known pharmacological and toxicological profile of curcumin and its derivatives. At low concentrations, curcumin exhibits antioxidant, anti-inflammatory, and cytoprotective properties. These effects are mediated by scavenging Reactive Oxygen Species (ROS), upregulating endogenous antioxidant enzymes (e.g., superoxide dismutase [SOD], catalase [CAT], and glutathione peroxidase [GPx]), and modulating signaling pathways such as PI3K/AKT and MAPK/ERK [37-39].

However, at higher concentrations, curcumin can paradoxically induce oxidative stress, mitochondrial dysfunction, and apoptosis [48-50]. This dual nature is reflected in the sharp decline in granulosa cell viability at 75-100 µg/mL observed here. Similar findings were reported by Virk et al. [51], who demonstrated that curcumin at supra-physiological levels induces apoptosis in murine ovarian granulosa cells through ROS accumulation and activation of caspase-3. The threshold phenomenon evident in our dose-response curve suggests that curcumin-FITC may function as a hormetic compound in granulosa cells, conferring beneficial effects at low doses while exerting toxicity at higher levels. This pattern underscores the importance of defining precise working concentrations for in vitro applications.

Oxidative Stress Regulation

One of the most plausible mechanisms underlying the observed cytoprotective-to-cytotoxic shift is modulation of ROS. At low concentrations, curcumin-FITC likely attenuates basal ROS levels, stabilizing mitochondrial function and preventing oxidative DNA damage. Previous studies have shown that curcumin upregulates the Nrf2 pathway, thereby enhancing transcription of Antioxidant Response Elements (AREs) such as HO-1 and NQO1. At higher concentrations, however, curcumin has been reported to disrupt redox homeostasis by interfering with electron transport chains and generating excess ROS [52, 53]. The accumulation of ROS can subsequently trigger mitochondrial outer membrane permeabilization, release of cytochrome c, and activation of the intrinsic apoptotic pathway [34].

Mitochondrial Protection and Apoptosis

Granulosa cells are highly metabolically active, relying on intact mitochondrial function to support steroidogenesis and oocyte maturation. Our findings that curcumin-FITC at ≤50 µg/mL preserves cell viability suggest that mitochondrial integrity is maintained within this dosing window. By contrast, morphological evidence of membrane blebbing and nuclear condensation at ≥75 µg/mL indicates apoptosis induction. This is consistent with reports in porcine granulosa cells where curcumin mitigated aflatoxin B1-induced apoptosis by modulating the PI3K/AKT pathway [53, 54]. In our system, however, excessive concentrations of curcumin-FITC likely overwhelm protective signaling, shifting the balance toward apoptotic cascades.

Several signaling pathways are implicated in granulosa cell responses to curcumin, including:

(1) PI3K/AKT Pathway: Enhances cell survival by promoting proliferation and inhibiting apoptosis. Curcumin at physiological concentrations upregulates AKT phosphorylation, whereas high concentrations suppress AKT activity [53]

(2) MAPK/ERK Pathway: Regulates granulosa cell proliferation during folliculogenesis. Curcumin has been shown to modulate ERK1/2 phosphorylation depending on dose and cell type [55]

(3) p53 Pathway: Activation of p53-mediated apoptosis under oxidative stress has been observed in granulosa cells exposed to toxicants [56]. High-dose curcumin may contribute to p53 activation, thereby reducing cell viability. Future studies employing Western blotting or transcriptomic analyses could validate the involvement of these pathways in Kampung chicken granulosa cells treated with curcumin-FITC

Our findings are broadly consistent with earlier studies across mammalian and avian systems:

(1) (1) Murine Models: Li et al. [58] reported that curcumin induced apoptosis in murine granulosa cells at concentrations >50 μM, whereas lower concentrations promoted cell survival

(2) Porcine Granulosa Cells: Chen et al. [59] demonstrated that curcumin reduced oxidative stress and apoptosis induced by zearalenone via upregulation of antioxidant enzymes.

(3) Chicken Granulosa Cells: Lin et al. [60] found that curcumin improved proliferation and steroidogenic activity of chicken granulosa cells under oxidative stress conditions.

(4) Other Fluorescent Probes: Compared to conventional FITC-conjugated antibodies, curcumin-FITC offers the advantage of being a small-molecule fluorophore that may penetrate cells more effectively while also exerting bioactive effects. Thus, our results provide further evidence that curcumin and its derivatives exhibit dual roles, highlighting the need for careful concentration selection in reproductive cell culture systems

The establishment of safe working concentrations of curcumin-FITC holds significant implications for avian reproductive research. Curcumin-FITC's stable fluorescence and biological safety up to 50 μg/mL render it a promising candidate for spatiotemporal mapping of follicle development. By conjugating curcumin-FITC to biomolecular targets, researchers can visualize intracellular dynamics without compromising cell viability.

Granulosa cell health is a key determinant of oocyte competence. The ability to use curcumin-FITC as a non-toxic marker ensures that experimental manipulations do not inadvertently impair oocyte developmental potential. Given the economic importance of Kampung chickens in Indonesia, methodologies that enable precise study of their reproductive biology can support breeding programs and conservation of local genetic resources. Fluorescent probes such as curcumin-FITC may also be applied to other avian species for comparative reproductive studies.

Conclusion

This study provides the first systematic evaluation of curcumin-FITC cytotoxicity in granulosa cells of Kampung chicken follicles. The key conclusions are as follows:

1. Safety Threshold Established: Curcumin-FITC concentrations ≤50 μg/mL maintain >90% granulosa cell viability, indicating safety for experimental use.
2. Dose-Dependent Cytotoxicity: Concentrations ≥75 μg/mL induce morphological and biochemical features of apoptosis, highlighting the importance of dose optimization.
3. Mechanistic Implications: Findings align with established roles of curcumin in modulating oxidative stress, mitochondrial function, and signaling pathways, supporting its dual cytoprotective and cytotoxic roles.
4. Methodological Significance: The integration of viability assays, morphological validation, and statistical analyses underscores methodological rigor and reliability.
5. Future Prospects: Curcumin-FITC holds promise as a safe and effective fluorescent marker for folliculogenesis mapping, with potential applications in avian reproductive biology, breeding, and conservation research.

Curcumin-FITC represents a biologically safe biomarker for granulosa cells within defined concentration limits. Its adoption in reproductive biology may enhance our ability to investigate follicular dynamics, contributing to both fundamental science and applied poultry research. The conclusion of an article should summarize the main findings of the study succinctly, highlighting the significant contributions to the research field. It should reiterate the objectives of the study and summarize the most important findings, emphasizing their relevance and practical or theoretical implications.

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Author's Contributions

Sukarman Hadi Jaya Putra: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Project administration.
Mansur S: Investigation, Methodology, Writing - review & editing.
Umarudin: Investigation, Methodology, Experimental procedures, Validation, Cytotoxicity assay.
All authors have read and approved the final version of the manuscript.

Ethics

The Research Ethics Committee of the University of Nusa Nipa thoroughly reviewed the study protocol and granted ethical clearance (Approval No. UNIPA-ACARE-2025/01).

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