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Research article

In vitro evaluation of oral contraceptives on long-tailed macaque (*Macaca fascicularis*) primary ovarian cells

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ABSTRACT

Hormonal contraception has been advocated as an alternative population control method for the long-tailed macaque population, which has increased exponentially due to anthropogenic changes and incidental food subsidies from human food waste. Risks of increased zoonosis and conflict are imminent if the population growth of long-tailed macaques is unchecked. However, there's a gap in the literature about the effect of hormonal contraceptives on long-tailed macaque reproductive tissues cell line. The present study aims to investigate the effect of oral contraceptives (Nordette, Noriday, and Ella) on long-tailed macaque ovarian cells. We determine the cell viability and cytotoxicity as well as the morphological changes of the drugs on long-tailed macaque ovarian cells using the MTT assay, Acridine orange/propidium iodide double staining method, morphological examination, and the 4, 6-diamidino-2-phenylindole (DAPI) staining method. For the MTT assay, The drugs were dissolved in culture media before use to have a concentration ranging from 0.5 μg/mL, 2.5 μg/mL, 0.125 μg/mL, 0.0625 μg/mL, and 0.0315 μg/mL to have three replicates for each treatment.

In contrast, the concentration of 0.0315 μg/mL was used for the morphological and histopathological analysis. The result of the study indicates that human oral contraceptives (Nordette, Noriday, and Ella) inhibit the growth of long-tailed macaque ovarian cells and induce apoptosis in a concentration- and time-dependent manner (at a concentration of 0.0315 μg/mL and an IC_{50} lower than 10 μg/mL), With a statistically significant value of ****$P < 0.001$ for each drug compared to the negative control. The result of the present study contributes toward addressing the gap in the literature on the effect of oral contraceptives in long-tailed macaque ovarian cells. Hence, we conclude that human oral contraceptives (Nordette, Noriday, and Ella) are safe and effective in long-tailed macaque ovarian cells as such could be used to develop non-invasive oral

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contraceptives for controlling the population of long-tailed macaques as an alternative population control method.

1. Introduction

The Habitat of long-tailed macaque in Peninsular Malaysia, especially in states such as Selangor, Johor, and Kedah, is threatened due to anthropogenic activities [1,2], as a result of which they utilized the fragmented forest within human settlements as their Habitat. Long-tailed macaque adapt well by consuming human food waste, referred to as "subsidized food [3] and reproduce in anthropogenic environments [4]. Long-tailed macaque are regarded as a pest primates in Malaysia and most of the natural home range of Long-tailed macaque in Southeast Asia the natural home range of long-tailed macaques, they are regarded as Pest Primates [5,6]. As a pest primates Long-tailed macaque exhibit nuisance and aggressive behavior towards tourists at various tourist attraction sites as well as recreational areas, raid agricultural areas, human settlements, and human waste bins, thereby littering the environment [7,8].

However, it's important to note that, there is a contradiction about the classification of Long-tailed Macaque as Endangered by the International Union for Conservation of Nature (IUCN) Red List [1,6]. In Malaysia, the Long-tailed Macaque population are reported to be hyperabundant [9]due to available food subsidies as they are dietary generalist, have high fecundity, can exploit resources within the shortest time as well as withstand hunting pressure as they are rarely hunted in the region [9] due to Islam restriction which makes the meat of macaque as non-halal [9].

Notwithstanding, the hyperabundant population of long-tailed macaques is unchecked. In that case, the implications of the hyperabundance include risks of zoonotic disease transmission to humans and other domestic animals from a macaque near the human settlement. For instance, there was an outbreak of human malaria where macaques act as carriers in Malaysian Borneo [10] and monkeypox was reported in Denmark [11]. As such, there is a need for an alternative to the culling method of population control in Long-tailed macaques, even though an alternative approach to the measures above is the use of contraceptives which studies indicate the effectiveness of hormonal contraception as alternative method of population control [4]. However, there is a literature gap about the effect of oral contraceptives on long-tailed macaque ovarian cells.

Human oral contraceptives such Progestin-only pills, e.g, Noriday, exert their action by inhibiting the emergence of new dominant follicles through their intraovarian action. This results in ovulation inhibition [12], affecting cervical mucus, fallopian tube motility, and the endometrium [13]. Moreover, its contraceptive effect occurs in a dose-dependent manner [14]. Similarly, Human combined oral contraceptives, e. g, Nordette, inhibit follicular development, the corpus luteum, and ovulation, which is the crucial mechanism of contraceptive action for combined oral contraceptives [15]. Ella (Ulipristal acetate), used as an emergency contraceptive, is a selective progesterone receptor modulator that exerts its contraceptive effect by binding to the progesterone receptors in the target tissues, namely the uterus, cervix, ovaries, and hypothalamus [16,17] and this effect varies according to the time of administration [18].

The current study aims to investigate the effects of commercially available human formulations of combined oral contraceptives (COC) Nordette (0.03 mg ethyl estradiol and 0.15 mg levonorgestrel), progestin-only contraceptives (POP), Noriday (0.35 mg norethisterone), and a non-hormonal oral contraceptive, Ella (Ulipristal acetate, 30 mg), on the primary ovarian cells of the long-tailed macaque. Furthermore, the central nervous and reproductive functions, gonadotropin and steroid hormone secretion pattern, and the average 28-day menstrual cycle of long-tailed macaques are similar to those of women [19,20]. As such, we hypothesized that human oral contraceptives have potent drug activity on long-tailed macaque ovarian cells.

2. Materials and methods

Ethical statement

The study and sampling were approved and permitted by the Department of Wildlife and National Parks (DWNP) in Kuala Lumpur, Peninsular Malaysia, with a permit no **JPHL&TN(I.P.):100-34/1.24 Jld 19(34)** and the Institutional animal care and use committee of the Universiti Putra Malaysia with **AUP no UPM/IACUC/AUP-R031/2023.**The study complied with all the rules and regulations concerning the Malaysian government regarding wildlife. It used only animal tissues provided by the Department of Wildlife and National Parks, Peninsular Malaysia.

2.1. Primary ovarian cell isolation and culture

Primary ovarian cells were isolated using the collagenase dissociation method. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) low glucose with 20 % Foetal bovine serum (FBS) and 100 μg/mL each of penicillin, streptomycin, and Amphotericin at 37 °C in a 5 % CO_2 incubator.

2.2. Cell viability assay and IC50 determination

The cells were cultured in a T25 flask containing Dulbecco's Modified Eagle Medium DMEM low glucose (Thermo Fisher Scientific, USA)™ supplemented with 20 % Foetal bovine serum (FBS) (Thermo Fisher Scientific, USA)™ 1 % of antibiotics (Penicillin, streptomycin & Amphotericin) (Thermo Fisher Scientific, USA)™, and incubated in a humidified incubator supplemented with 5 % CO_2, at

a temperature of 37 °C. Upon reaching 80–90 % confluency, cultured cells were trypsinized, counted, and seeded in a 96-well plate at a density of 1×10^5 cells/well and incubated overnight. The ovarian cells were treated with combined oral contraceptives (Nordette), Progestin-only contraceptives (Noriday), and Non-hormonal contraceptives (Ella) for 24, 48, and 72h [21]. The contraceptive drugs were dissolved in culture media before use in a serial dilution to have a concentration ranging from 0.5 μg/mL, 2.5 μg/mL, 0.125 μg/mL, 0.0625 μg/mL, and 0.0315 μg/mL respectively as described by Refs. [22,23]. Untreated cells served as control(Positive), while 10 wells without seeded cells acted as the blank control(Negative). For each drug concentration, three replicates were performed for each concentration. After the completion of the treatment, the cells were washed with PBS. Subsequently, 100 μl of fresh media and 10 μl of MTT solution (5 mg/mL) were added to the wells and incubated in a humidified incubator at 37 °C for 4 h. The MTT medium was discarded, and 200 μl DMSO was added to dissolve the dark blue formazan crystals. Then the absorbance was measured at 570 nm using a Microplate reader. The cell viability was calculated using the following equation according to Ref. [23].

$$\text{Cell viability } (\%) = \frac{\text{OD treated} - \text{OD blank}}{\text{OD control} - \text{OD blank}} \text{ X } 100$$

OD treated is the absorbance of the cells incubated with different contraceptive drugs concentrations, and OD control is the absorbance of the cells incubated with medium only. At the same time, O.D. blank is the absorbance of the medium in the wells without cell seeding. The IC_{50} values, which indicate the contraceptive drug concentration that inhibits 50 % of the tested cells, were calculated by the use of online resources Quest Graph™ IC50 Calculator [24].

2.3. Cell apoptosis evaluation using Acridine Orange and Propidium Iodide (AO/PI) staining

Cell apoptosis evaluations were performed using Acridine orange and propidium iodide double-staining methods. Ovarian cells were seeded at a density of 5 × 105 cells in coverslips inside a 6-well plate and incubated for 48 h. Following the incubation, the media was discarded and replaced with a complete growth medium. For 24 h, cells were treated with concentrations of 0.0315 μg/mL (IC_{50}) of the Nordette, Noriday, and Ella.

After 24 h, the medium was removed, and the cells were rinsed three times with 1 X PBS. A volume of 20 μl (1:1) of Acridine orange and propidium iodide (10 mg/mL of each) was added to the well-containing coverslip and incubated at room temperature for 15 min. This process was carried out under dark conditions since both Acridine Orange and Propidium Iodide are light-sensitive. The coverslip was mounted on a microscopic slide and viewed under the fluorescence microscope (Nikon ECLIPSE Ti S Japan).

2.4. Assessment of nuclei morphology using DNA dye, 4′,6-diamidino-2-phenylindole (DAPI) staining

Cells were seeded onto a coverslip, placed in a 6-well plate at a density of 5×10^5 cells/well, and incubated overnight. The media was discarded, and the cells were treated for 24 h with Nordette, Noriday, and Ella at a concentration of 0.0315 μg/mL (IC_{50}). After treatment, the media was discarded, and cells were rinsed three times with 1X PBS. Fixation was done using 400 μl of 4 % paraformaldehyde at room temperature for 5 min on each coverslip placed on a 6-well plate. Following fixation, paraformaldehyde was removed, and cells were rinsed three times with 1X PBS. Then 400 μl of 0.2 % Triton X-100 was added and incubated at room temperature for 10 min. Triton X-100 was removed after permeabilization of the cells, and the cells were rinsed twice with 1X PBS. Finally, the cells were stained with 300 μl of 2.5 mg/mL DNA dye, 4′,6-diamidino-2-phenylindole (DAPI) for 10 min at room temperature, and the stain was removed and rinsed with 1X PBS. The coverslip was mounted on a microscopic slide to visualize the nuclei morphology under a fluorescence microscope.

2.5. Morphological evaluation using inverted light microscopy

Ovarian cells were seeded onto a coverslip, placed in a 6-well plate at a density of 5×10^5 cells/well, and incubated overnight at 37 °C in the 5 % CO_2 incubator. The media was discarded, and the cells were incubated at 37 °C in the 5 % CO_2 incubator for 24 h with concentrations of 0.0315 μg/mL (IC_{50}) Nordette, Noriday, and Ella. After treatment, the medium was discarded, and cells were rinsed three times with 1X PBS. The cells were later fixed with 400 μl 4 % paraformaldehyde for 30 min on each coverslip placed on a 6-well plate. Using an inverted light microscope, the coverslip was mounted on a microscopic slide to visualize the cellular morphology.

2.6. Statistical analysis

All Statistical analysis was performed using GraphPad Prism 9.4.1 (GraphPad Software, San Diego, CA, USA). All experiments were performed at least three times. The result was expressed as mean ± S.E. Data from the result were subjected to one-way analysis of variance (ANOVA) followed by Tukey's post-test for comparison between pairs of treatment concentration. A value of $^*P < 0.05$; $^{**}P < 0.01$; $^{****}P < 0.001$ is set as statistically significant, while $P > 0.05$ denote ns is not statistically significant.

3. Results

3.1. Nordette, Noriday, and Ella induce cytotoxicity in long-tailed macaque primary ovarian cells

This study determined the viability of ovarian cells exposed to Nordette, Noriday, and Ella. Using MTT Assays at 24, 48, and 72 h,

Fig. 1 shows the cell viability of ovarian cells after 24, 48, and 72 h of exposure to Nordette, Noriday, and Ella at different evaluated concentrations (0.0315 μg/mL to 0.5 μg/mL). The cell viability decreased as the drug concentration and exposure time increased. Nordette, Noriday, and Ella demonstrated concentration and time-dependent cytotoxicity against ovarian cells as shown by IC_{50} value (Table 1)

3.2. Morphological effect of Nordette, Noriday, and Ella in long-tailed macaque ovarian cells

The morphological observation of macaque ovarian cells treated with Nordette, Noriday, and Ella after 48 h revealed that both the treated and untreated cells showed attached round ovarian cell aggregation (Fig. 3A, B, C, D). However, in the cells treated with Nordette (Fig. 3B), Noriday (Fig. 3C), and Ella (Fig. 3D), cells shrinkage, swollen cells, condensed and disorganized chromatin, and ruptured cell membranes were seen, which are the clear morphology of apoptotic and necrotic cells.

3.3. Nordette, Noriday, and Ella induce nuclei damage and chromatin condensation in long-tailed macaque ovarian cells

To determine the changes in nuclear morphology upon treatment with Nordette, Noriday, and Ella, we stained the ovarian cells with a DNA dye, 4′,6-diamidino-2-phenylindole (DAPI) and observed them under a fluorescent microscope. The nuclei of untreated cells were normal in size and shape (Fig. 4A). However, the Nordette, Noriday, and Ella-treated cells showed condensed chromatin and/or fragmented nuclei (Fig. 4B, C, and D). Notwithstanding, these results indicate that the observed nuclei damages could trigger cell death by apoptosis, implicating the contraceptive mechanisms of action by promoting apoptosis which can results in the inhibition of ovulation in long-tailed macaques.

3.4. Nordette, Noriday, and Ella promote cell dead in long-tailed macaque ovarian cells

Following treatment with Nordette, Noriday, and Ella, the viable and dead cells were determined by using the Acridine Orange and Propidium Iodide double staining method. The stained cells were visualized under a fluorescence microscope in Acridine Orange and Propidium Iodide staining. The untreated cells (Fig. 5A) showed bright, intact green fluorescence, which is a characteristic of viable cells, indicating no signs of apoptosis. However, the compromised membrane and dead cells produced yellow-orange and red fluorescence, which is characteristic of apoptosis and was evident upon 48-h treatment with Nordette (Fig. 5B), Noriday (Fig. 5C), and Ella (Fig. 5D). However, these results suggest that Nordette, Noriday, and Ella could trigger cell dead in long-tailed macaque ovarian cells, which can results in the inhibition of ovulation in Long-tailed macaques.

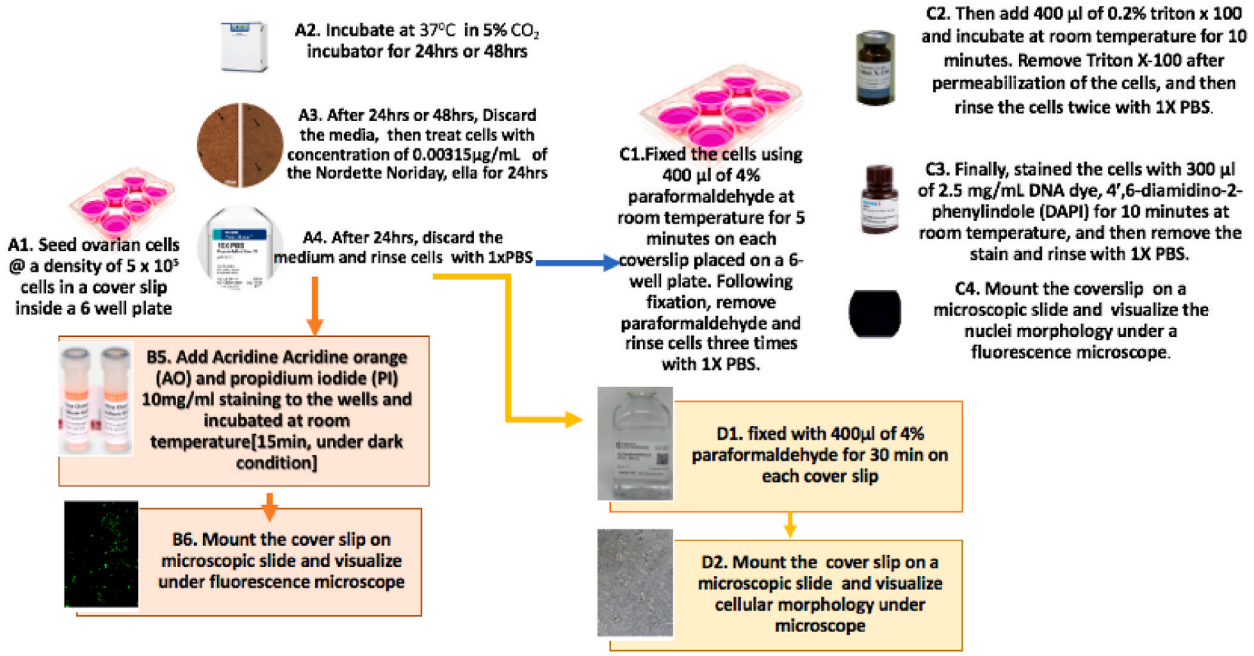
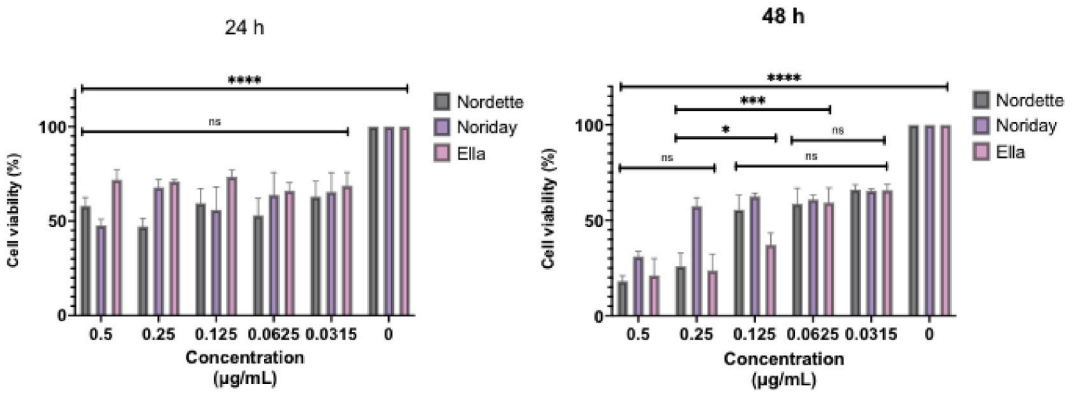


Fig. 1. Illustration of the procedures for Apoptotic evaluation using Acridine orange/Propidium Iodide (AO/PI), DAPI staining method (Fluorescence microscopy) and morphological evaluation (inverted microscopy). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



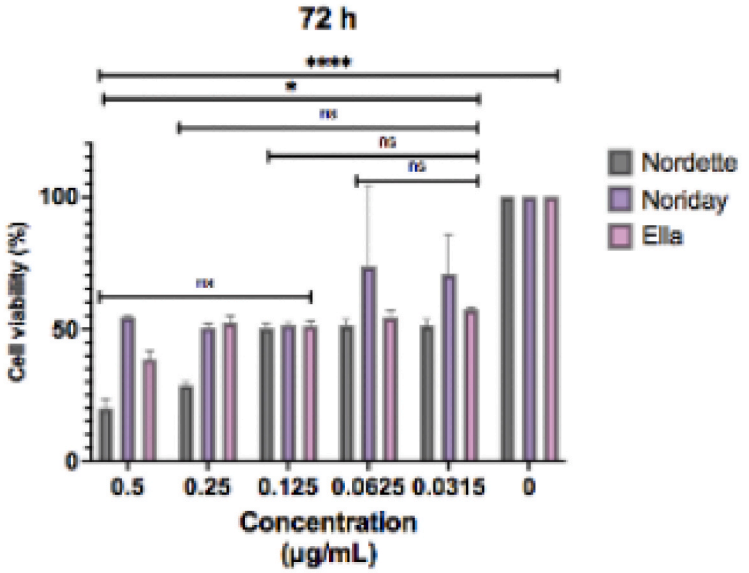


Fig. 2. (24h): In vitro cytotoxicity study of long-tailed macaque ovarian cells after 24 h of treatment with Nordette, Noriday, and Ella. Note A value of *P < 0.05; **P < 0.01; ****P < 0.001 is set as statistically significant for each drug compared with the negative control, while ns is not significant when compared within various concentrations of each drug.
Fig. 2(48h): In vitro cytotoxicity study of long-tailed macaque ovarian cells after 48 h of treatment with Nordette, Noriday, and Ella. Note A value of *P < 0.05; **P < 0.01; ****P < 0.001 is set as statistically significant for each drug compared with the negative control, while ns is not significant when compared within various concentrations of each drug.
Fig. 2(72h): In vitro cytotoxicity study of long-tailed macaque ovarian cells after 72 h of treatment with Nordette, Noriday, and Ella. Note A value of *P < 0.05; **P < 0.01; ****P < 0.001 is set as statistically significant for each drug compared with the negative control, while ns is not significant when compared within various concentrations of each drug.

4. Discussion

The present study represents the first in vitro investigation of the effects of hormonal (Nordette and Noriday) and non-hormonal (Ella) human oral contraceptive drugs on the wild-captured long-tailed macaque ovarian cells. Even though, there is similarities in reproductive physiology, specifically the menstrual cycle, between female long-tailed macaques and women [25,26], in vitro studies are regarded as one of the most critical steps in drug development. In vitro, studies offer insight into the tested compound's safety and efficacy. Furthermore, in vitro studies often characterize the metabolism of the tested drug or agent and the physiochemical properties of the compound, aid in predicting its toxic response in vivo, and play a role in understanding the mechanism of action of the tested

Table 1
Cytotoxicity (IC_{50}) of Nordette, Noriday, and Ella on long-tailed macaque ovarian cells following incubation at 24, 48, and 72 h using MTT assay. Data are expressed as three independent experiments' mean ± standard deviation (SD).

Drugs	IC_{50}		
	24 h	48 h	72 h
Nordette	0.044 μM (0.027 μg/mL)	2.38 μM (1.4524 μg/mL)	3.424 μM (2.085 μg/mL)
Noriday	0.045 μM (0.0137 μg/mL)	0.0046 μM (0.0014 μg/mL)	0.096 μM (0.0289 μg/mL)
Ella	4.8 μM (2.2992 μg/mL)	0.126 μM (0.0603 μg/mL)	1.600 μM (0.761 μg/mL)

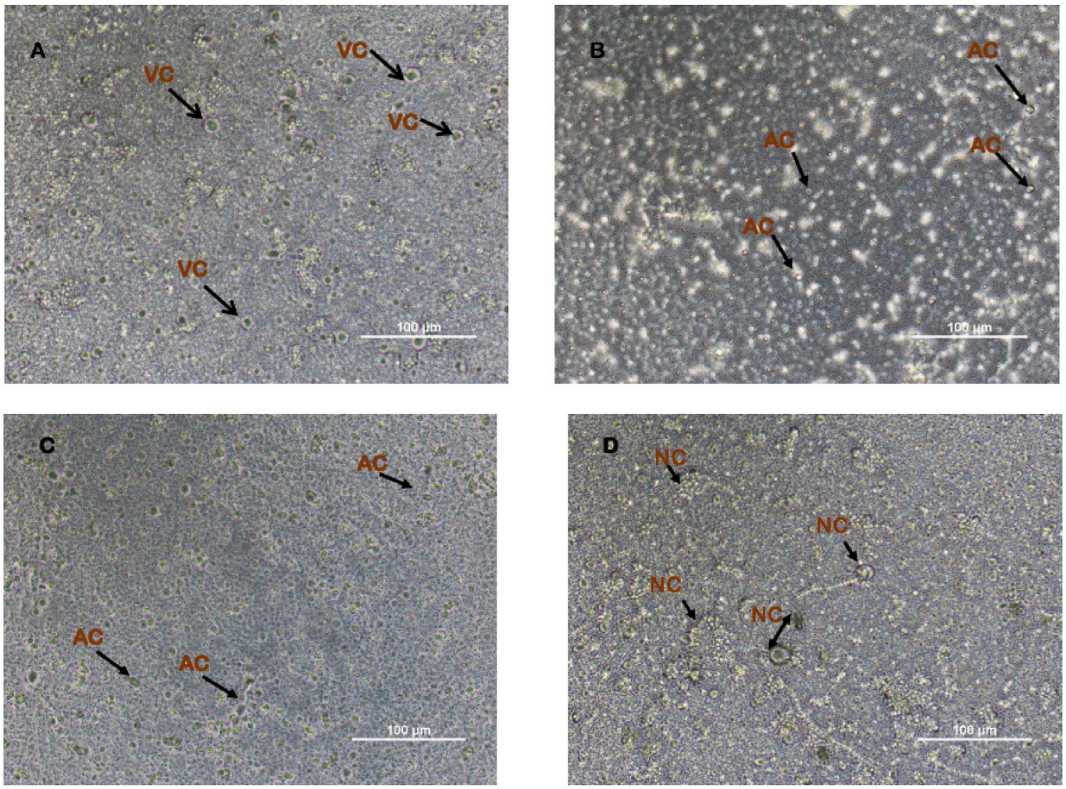


Fig. 3. Inverted light microscopy images of long-tailed macaque ovarian cells treated for 48 h with 0.03125 μg/mL (IC_{50}) concentration of human oral contraceptives (A) Control cell(untreated); (B) cells treated with Nordette(Combined oral contraceptives); (C) Cells treated with Noriday (Progestin-only pills); (D) Cells treated with Ella (Progesterone antagonist). Note V.C. arrow denotes viable cells, A.C. arrow denotes apoptotic cells, and N.C. arrow denotes necrotic cells.

compound or drug and its metabolites [27]. As such, the evaluation of synthetic hormones is a step to consider prior to their application as a non-invasive method of population control for long-tailed macaques.

The results of the MTT assay indicated that human oral contraceptives (Nordette, Noriday, Ella) exhibit concentration- and time-dependent cytotoxicity against long-tailed macaque ovarian cell lines. The cell viability decreases with increasing concentration and or time of incubation. At 24 h, both drugs induce toxicity in the ovarian cells from a low to a high concentration. Furthermore, the result indicates significance upon comparing the various concentrations with the control (**P < 0.05; **P < 0.01; ****P < 0.001) and statistical non-significance upon comparing within the various concentrations (P ns).

The IC_{50} values of Norddette at 24 h, 48 h, and 72 h are 0.027 μg/mL (0.044 μM), 1.4524 μg/mL (2.38 μM), and 2.085 μg/mL (3.424 μM), respectively. These values indicate that Nordette exhibits potent activity on long-tailed ovarian cells at 24 h and very strong cytotoxicity at 48 h and 72 h. For Noriday, the IC_{50} values at 24 h, 48 h, and 72 h are 0.0137 μg/mL (0.045 μM), 0.0014 μg/mL (0.0046 μM), and 0.0289 μg/mL(0.096 μM), respectively. However, the IC_{50} values indicate that Noriday has a potent activity on long-tailed macaque ovarian cells [28,29], across different times. Notwithstanding, the IC_{50} values of Ella at 24 h, 48 h, and 72 h are 2.2992 μg/mL (4.8 μM), 0.0603 μg/mL (0.126 μM), and 0.761 μg/mL (1.600 μM), respectively. The values indicate that Ella at 24 h has very strong cytotoxicity on long-tailed macaque ovarian cells. In contrast, Ella exhibited potent activity at 48 h and strong cytotoxicity at 72 h [30,31].

Apoptosis was determined using the Acridine Orange and Propidium Iodide double staining method, DAPI Staining, followed by a

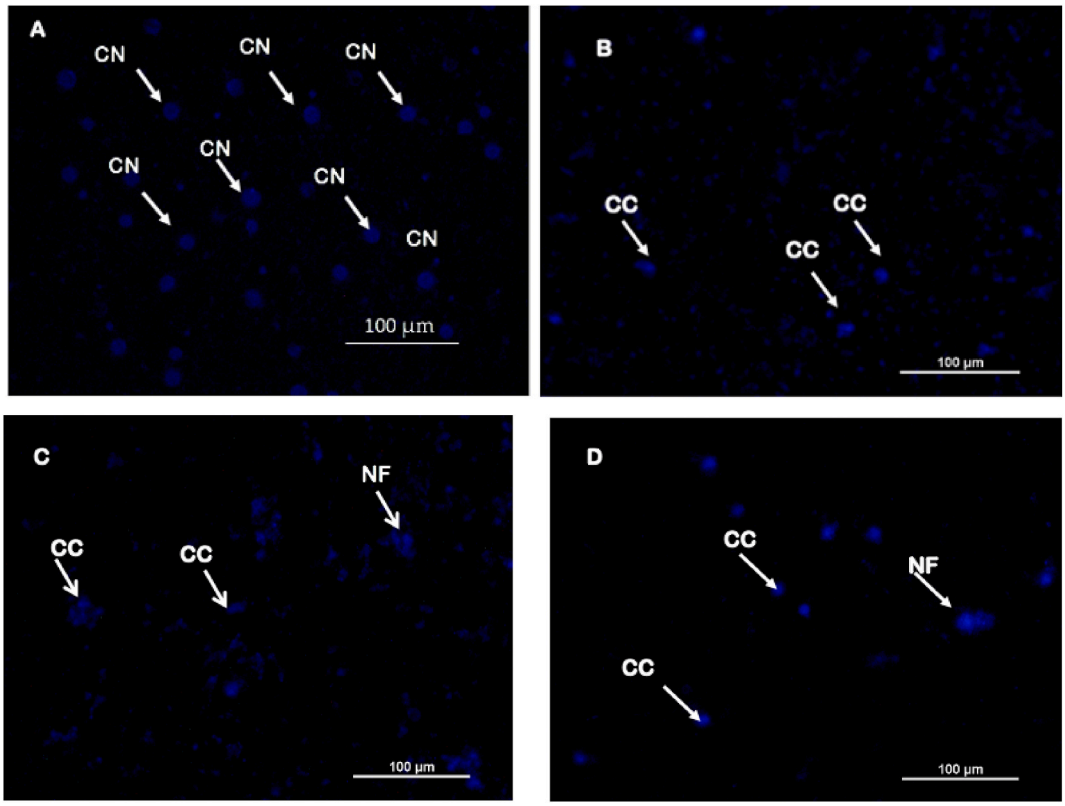


Fig. 4. The fluorescence photomicrographs showing the apoptotic effect of human oral contraceptives on the nuclei morphology of long-tailed macaque ovarian cells, using DAPI Staining. Ovarian cells were treated for 48hrs with 0.03125 µg/mL (IC_{50}) concentration of human oral contraceptives. (A) Control cells (untreated cells); (B) cells treated with Nordette (combined oral contraceptives), (C) cells treated with Noriday (Progestin-only pills), (D) cells treated with Ella (Progesterone antagonist). Note CC arrow denotes chromatin condensation; the N.F. arrow denotes Nuclei fragmentation.

morphological examination. Nordette, Noriday, and Ella demonstrated apoptosis in all the evaluation methods, evident by nuclei fragmentation, chromatin condensation, and cell shrinkage. These findings indicate that Nordette, Noriday, and Ella induced apoptosis in the long-tailed macaque ovarian cells.

Apoptosis refers to the process of programmed cell death. Nevertheless, apoptotic cells are characterized morphologically by cell shrinkage, chromatin condensation (nuclei pyknosis), and membrane blebbing [32,33]. Furthermore, apoptosis is differentiated from necrosis, where cells undergoing apoptosis shrink while necrotic cells rupture. The nuclei condense and later become fragmented in apoptosis, while the necrotic cells have swollen cells with ruptured nuclei, and the cell content bursts out [28].

It is important to note that both the hormonal and non-hormonal contraceptive drugs tested in these studies exhibited cell growth inhibition and induced apoptosis in the long-tailed macaque ovarian cell. Furthermore, the result of the present study of Combined oral contraceptives (Nordette) and progestin-only pills(Noriday) on ovarian cells of long-tailed macaques agrees with the previous finding that Progestin-only pills exert their contraceptive effects through a direct action on the ovary [29,34,35] and the findings that estrogens in contraceptives, inhibit the growth of preantral and medium-sized antral follicles of primates, specifically through a direct effect at the ovary [36,37]. Furthermore, the result of the study about Ella (Non-hormonal contraceptive) also agrees with the previous study that Ella, a progesterone agonist or antagonist, exerts its direct effect on the ovary where it suppresses or delays ovulation and on the endometrium, where it decreases the endometrial thickness by binding to the body's progesterone receptors to produce an anti-progesterone contraceptive effect [38].

Studies indicate that administering exogenous hormones to nonhuman primates has extended action beyond reduced reproductive function. Furthermore, oral contraceptives affect sexual behavior directly by reducing female attractiveness and receptivity or indirectly through reduced female agonistic behavior [39]. Changes reported in the breeding behavior of female nonhuman primates were in those treated with gonadotropin-releasing hormone and progestin analogs [40,41], and males were attracted to areas with sterilized females, thereby increasing male mortality [42]. However, most of the observed behavioral effect is reported in drugs with more progesterone due to the effect of progesterone on the estradiol receptor, which affects the tactile stimulus effect associated with copulation [25,43,44] although reports indicate the effect is less detrimental when combined with estrogen than progesterone alone [45].

However, the progestin-only pill drug Noriday used in these present in-vitro studies has only 0.35 mg of progestin, while the

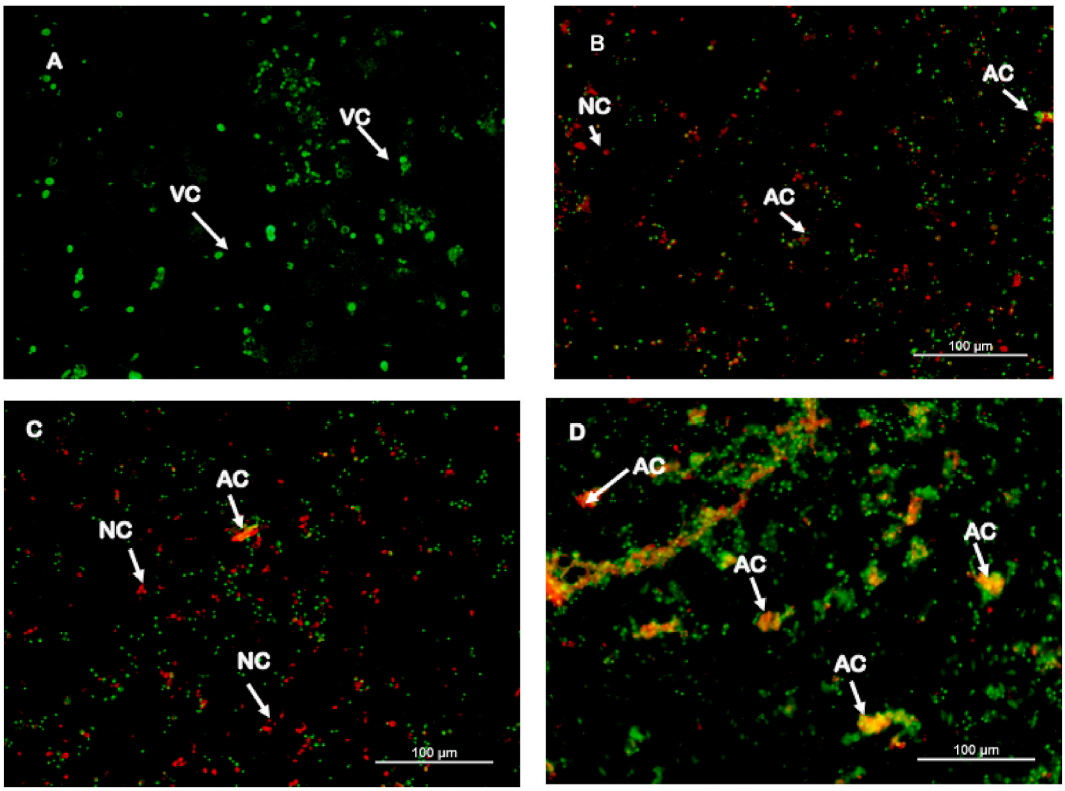


Fig. 5. The fluorescent photomicrographs showing the human oral contraceptives induced apoptosis on the long-tailed macaque ovarian cells, assessed by Acridine Orange/Propidium iodide (AO/PI) double staining methods. Ovarian cells were treated for 48hrs with 0.03125 µg/mL (IC_{50}) concentration of human oral contraceptives. (A) Control cells (untreated cells); (B) cells treated with Nordette (combined oral contraceptives), (C) cells treated with Noriday (Progestin-only pills), (D) cells treated with Ella (Progesterone antagonist). Note V.C. arrow denotes viable cells, the N.C. arrow denotes Necrotic cells, and the A.C. arrow denotes apoptotic cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

combined oral contraceptive Nordette has only 0.15 mg of progestin plus 30 mcg of estrogen. Notwithstanding these, human oral contraceptives are reported to have minimal non-contraceptive effects in women, such as a decrease in libido [46], reduced ejaculation [25], an increase in sexual desire, vaginal lubrication enjoyment, and frequency of orgasm [44]. Notwithstanding, some of the natural diets of nonhuman primates may contain estrogen-like substances and other phytochemicals that can also influence sexual behavior and provide contraceptive benefits [47–49].

It is important to note that, the results of the present in vitro studies adequately addressed a few characteristics of ideal oral contraceptives for field application, such as availability, cost, minimal or no side effects, and inhibition of female reproduction. Furthermore, the results contribute toward addressing the gap in the literature about the effect of oral contraceptives on Long-tailed macaque ovarian cells.

However, a non-invasive approach should be considered for developing the long-tailed macaques contraceptive [50–53]. Hence, using the once-a-month enhanced delivery method, the drug can be administered as an oral bait or a pelleted diet formulation [54], and a conditional behavioural approach in the reported macaque conflict hotspots, developed oral contraceptives can be administered to the long-tailed macaques.

Furthermore, considering the burden of the direct and indirect effects of long-tailed macaque overpopulation, such as the harassment of tourists, the raiding of farmland and people's homes, and the potential risk of zoonotic disease transmission, notwithstanding the cost of culling, such as personnel wages, training, and other logistics. However, long-tailed macaque non-invasive oral contraceptives development, production, and administration costs are nominal and the administration can be without personnel training or expertise.

5. Conclusion

The commercial availability and less cost of Nordette, Noriday, and Ella informed us to investigate their potential for use in long-tailed macaque contraception as an alternative to population control. It is pertinent to point out that the tested contraceptive drugs in our study showed a concentration (at a concentration of 0.0315 µg/ml) and time-dependent cytotoxicity (at IC_{50} lower than 10 µg/mL). Which is an indication of excellent potent activity of the drugs on macaque ovarian cell line, As such, they are both safe and

effective, and they could be used as a potential contraceptive agent for controlling the population of long-tailed macaques as an alternative to methods for addressing human-long-tailed macaque conflicts.

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Data availability

The dataset used and analyzed during the current study is available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Muhammed Mikail: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Tengku Rinalfi Putra Bin Tengku Azizan:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Mohd Hezmee Mohd Noor:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Hasliza Abu Hassim:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Azlan Che'Amat:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Annas Bin Saleh:** Writing – review & editing, Visualization, Validation, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Mark Hiew Wen Han:** Writing – review & editing, Visualization, Validation, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Mohd Qayyum Ab Latip:** Writing – review & editing, Visualization, Validation, Resources, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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