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Melatonin defends mouse oocyte quality from benzo[ghi] perylene-induced deterioration

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Abstract

Benzo[ghi]perylene (B[ghi]P) is a polycyclic aromatic hydrocarbon widely found in haze. Long-term exposure to humans or animals can cause serious damage to the respiratory system. Melatonin is an endogenous natural hormone synthesized and released by the pineal gland. In this study, we investigated the effects of melatonin on in vitro cultured B[ghi]P-exposed mouse oocytes and the protective roles of melatonin. Our data indicate that B[ghi]P exposure leads to meiotic maturation arrest and reduced ability of sperm binding and parthenogenetic activation. Also, B[ghi]P exposure disrupts actin filament dynamics, spindle assembly, and kinetochore–microtubule attachment stability, which results in oocyte aneuploidy. Simultaneously, B[ghi]P exposure disturbs the distribution of mitochondria, increases the level of oxidative stress, and induces apoptosis of oocytes. Whereas all of these toxic effects of B[ghi]P can be restored after melatonin supplement. In conclusion, our findings validate that melatonin has a certain protective effect on preventing the reduced oocyte quality caused by B[ghi]P exposure during meiotic maturation in mouse oocytes.

KEYWORDS
benzo[ghi]perylene (B[ghi]P), melatonin, oocyte, cytoskeleton, mitochondrion

1 | INTRODUCTION

Environment pollution has attracted considerable attention in recent years (B. Zhang & Hughes, 2017). Human activities, including life and production, produce all kinds of toxic substances, such as polycyclic aromatic hydrocarbons (PAHs), which are the main pollutants of haze because of its toxicity and persistence (Ewa & Danuta, 2017; Mukherjee & Agrawal, 2018). The human being can be exposed to PAHs through various ways, such as inhalation, ingestion, and skin contact. PAHs are not only an endocrine disruptor, but also a potential environmental estrogen, which can perturb hormone homeostasis (Fertuck, Kumar, Sikka, Matthews, & Zacharewski, 2001; Santodonato, 1997). Therefore, PAHs have reproductive toxicity and can cause reproductive defects (Madeen & Williams, 2017).

Benzo[ghi]perylene (B[ghi]P) is a cyclized six-ring aromatic hydrocarbon with a molecular weight of 276, and it is mostly produced by the incomplete combustion of organics (Cherng, Lin, Yang, Hsu, & Lee, 2001). At the same time, B[ghi]P is the main component of PAHs that are controlled by the United States Environmental Protection Agency (Ewa & Danuta, 2017; Platt & Grupe, 2005). Because it has the highest exhaust in a light-duty vehicle and it is not detected in heavy-duty diesel exhaust, so it can be used as a mark for gasoline-powered vehicle activities (Platt & Grupe, 2005). B[ghi]P is the most abundant PAHs in Mexico City with an average content of 1.46 ng/m^3 (AmadorAmador-Munoz-Munoz et al., 2013). In Guangzhou, China, B[ghi]P is distributed fairly in urban and rural areas with the average concentration of 1.55 ng/m^3 (Gao et al., 2012). B[ghi]P is rarely studied because the 1983

International Agency for Research on Cancer's first study showed that it was noncarcinogenic to animals and humans. Since then, research on B[ghi]P has been almost static. However, the genotoxicity of B[ghi]P has been controversial. In recent years, many studies have shown that B[ghi]P not only form adducts with DNA to cause lung tumors, but also synergize with benzo(a)pyrene to increase carcinogenic effects (Cherng et al., 2001; Deutsch-Wenzel, Brune, Grimmer, Dettbarn, & Misfeld, 1983; Hughes & Phillips, 1993; Van Duuren & Goldschmidt, 1976). Facts show that although B[ghi]P lacks the "typical" bay area, it still has genotoxicity. The latest research reports show that B[ghi]P can damage the cell membrane, stimulate the transposition of aryl hydrocarbon receptor (AhR) induce gene transcription, and use the AhR pathway to produce toxic effects on the NL-20 human bronchial cell line (Zaragoza-Ojeda, Eguia-Aguilar, Perezpena-Diazconti, & Arenas-Huertero, 2016).

It is well known that mammalian oocytes originate from primordial germ cells, undergo complex processes of mitosis and meiotic maturation, and then arrest at the MII stage. Only after fertilization or parthenogenetic activation, the oocytes initiate the early embryonic development. During the first meiotic process, with the germinal vesicle breakdown, the chromosome is directly exposed to the cytoplasm of the oocytes, opening a very sensitive window of time when chemical substances, such as toxins or drugs, can cause the loss of nuclear and cytoplasmic maturation, affecting the development potential. Exposure to chemicals can significantly induce excessive reactive oxygen species (ROS) production (Y. L. Miao et al., 2012), causing DNA damage (Ma et al., 2013), resulting in disruption of cytoskeletal integrality and early apoptosis, which can lead to poor embryo quality and delay or even prevent embryo development (Hu, Li, Ali, Xu, & Fang, 2017).

Melatonin is a natural hormone released from the pineal gland into the circulation. It has the activity of regulating circadian rhythm and periodic rhythm and participates in seasonal reproductive activities (Reiter et al., 2016). In addition, accumulating studies have shown that melatonin also plays a role in antioxidants (Galano, Tan, & Reiter, 2011), antiapoptosis (El-Missiry, Othman, Al-Abdan, & El-Sayed, 2014), anti-inflammatory (Mauriz, Collado, Veneroso, Reiter, & Gonzalez-Gallego, 2013), and regulation of cytoskeletal organization and other biological functions (Benitez-King, 2006). For example, melatonin removes peroxy radicals, nitric oxide, and singlet oxygen to prevent cell membrane degradation and reduce lipid peroxidation (Hardeland, 2005). It is worth noting that melatonin has been found in human follicular fluid and may be produced by the granulosa cells to protect oocytes from oxidative stress during ovulation (Itoh, Ishizuka, Kuribayashi, Amemiya, & Sumi, 1999). In conclusion, melatonin can alleviate the damage that toxic compounds bring to oocytes.

So far, there have been no reports of the effects of B[ghi]P on the female reproductive system. In this study, we investigated the toxic effects of B[ghi]P on the quality of cultured mouse oocytes and the rescue functions of melatonin. The results showed that B[ghi]P significantly impaired the quality of oocytes by assessing the rate of first polar body exclusion, parthenogenetic activation ability, and the number of sperm binding to the zona pellucida. It is caused by disrupting cytoskeletal integrality, chromosome euploidy, and mitochondrial functions after B[ghi]P exposure. Whereas melatonin can reduce all these damages, which indicates that melatonin has certain protective effects on preventing the reduced oocyte quality caused by B[ghi]P.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

All the experimental procedures in this study were in line with the guiding principles of the Ethics Committee of Qingdao Agricultural University. Institute of cancer research (ICR) mice were raised in a temperature-controlled room for 12 hr in the light/dark cycle and fed with regular diet. During oocytes collection, mice were treated humanely to alleviate the suffering.

2.2 | Oocyte collection and culture

For the collection of oocytes, 4–6 weeks old ICR mice were killed by cervical dislocation to collect ovaries in M2 medium supplemented with 2.5 μM milrinone. Germinal vesicle intact oocytes were picked by a pipetted tube under a stereoscope. Groups of 25–30 oocytes were placed in 30 μl droplets of the M16 culture medium under mineral oil in a cell-culture dish at 37°C in 5% humidified CO_2 atmosphere for maturation in vitro.

2.3 | B[ghi]P and melatonin treatment

B[ghi]P (191-24-2; J&K Chemical Ltd., Beijing, China) was dissolved in dimethylsulfoxide (DMSO, with the ultrasonic shake for 20 min) as stock solution and diluted in the M16 medium as working solution. Melatonin (73-31-4; J&K Chemical Ltd.) was dissolved in ethyl alcohol as stock solution and diluted with the M16 medium as working solution. The highest ultimate concentration of DMSO and ethyl alcohol is less than or equal to 0.1%. To test the toxic effects of B[ghi]P exposure on the meiotic maturation of oocyte, we studied the extrusion rate of the first polar body by five groups of B[ghi]P concentrations: 25, 50, 100, 200, and 400 μM. To prove the protective effect of melatonin, oocytes were randomly assigned to three groups: a control group, a B[ghi]P-exposed group, and a "B[ghi]P+melatonin" group. The concentrations of melatonin in the last group were 25, 50, 100, 200, and 400 μM, respectively. According to the results, 100 μM B[ghi]P and 100 μM melatonin were used for subsequent experiments.

2.4 | Immunofluorescence and confocal microscopy

At room temperature (RT), oocytes were fixed with 4% paraformaldehyde (PFA) for 30 min. After washing, oocytes were permeabilized in D-PBS supplemented with 0.5% Triton X-100 for 20 min and blocked in Dulbecco's phosphate buffered saline (D-PBS) containing 1% bovine serum albumin for 1 hr. Then, oocytes were incubated with different primary antibodies at 4°C overnight. Primary antibodies include Phalloidin (1:1,000; 23122; AAT Bioquest, CA),

anti-α-tubulin antibody (1:500; SC-5286; Santa Cruz Biotechnology, TX), and anticentromere CREST antibody (1:200; 15-234-0001; Antibodies Incorporated, CA); oocytes were incubated with an appropriate secondary antibody at RT for 1 hr. The DNA was counterstained with 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI, C1022; Beyotime Institute of Biotechnology, Shanghai, China) for 20 min. After washing three times, oocytes were installed on a glass slide with a 5 μl DABCO drop. Representative images were captured with a laser-scanning confocal microscope (Leica TCS SP5 II, Mannheim, Germany).

2.5 | Parthenogenetic activation

After maturation culture, approximately 30 oocytes with the first polar body were collected and treated in preequilibrated Ca^{2+}-free Chatot, Ziomek, Bavister (CZB) medium that contained 10 mM $SrCl_2$ (A500908; Sangon Biotech, Shanghai, China) for 4–6 hr at 37°C. The presence of pronucleus was regarded as successful parthenogenetic embryos.

2.6 | Sperm binding assay

Cauda epididymal sperms were isolated from wild-type ICR mouse and then placed under mineral oil in human tubal fluid medium equilibrated previously at 37°C with 5% CO_2 to a density of 1×10^6 cells/ml. After capacitated by an extra 1 hr, 25 μl of the capacitated sperm solution was added to the fertilized droplets containing a certain number of oocytes to give a final sperm concentration of 2.5×10^5 cells/ml, followed by incubation for 1.5 hr (Y. Miao et al., 2018). The sperm binding to oocytes was fixed in 4% PFA for 30 min, and then stained with DAPI. Bound sperms were quantified from z projections acquired by a laser-scanning confocal microscope.

2.7 | Chromosome spread

The oocytes were exposed to acid Tyrode's solution (pH = 2.5) about several seconds. After removing the zona pellucida, the oocytes were transferred into M2 medium drops for 3–5 times washes as soon as possible. Then, approximately 25–30 oocytes were transferred onto a glass slide and fixed in a solution of 1% PFA in distilled water (pH = 9.2) containing 0.15% Triton X-100 and 3 mM dithiothreitol. After air drying, chromosomes were counterstained with propidium iodide (PI, E607306; Sangon Biotech) and observed with a laser-scanning confocal microscope.

2.8 | Mitochondria distribution

To evaluate the distribution of mitochondria in oocytes, MitoTracker Deep Red (M22426; Invitrogen, CA) was used to stain mitochondria. Oocytes were incubated in prewarmed staining solution for 30 min at 37°C. After washes, oocytes were counterstained with DAPI to visualize the nucleus. A laser-scanning confocal microscope was used to observe and capture images.

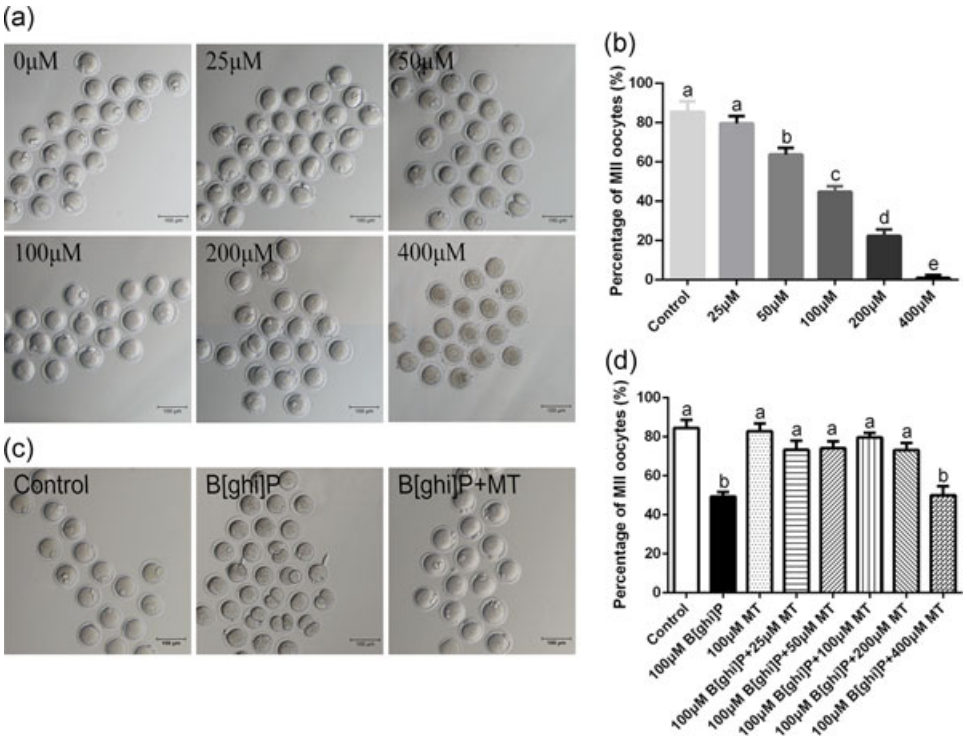

FIGURE 1 Effects of melatonin (MT) on the meiotic maturation in the benzo[ghi]perylene (B[ghi]P)-exposed oocytes. (a) Oocytes morphologies after 12-hr culture with B[ghi]P treatment. (b) The rates of first polar body extrusion with B[ghi]P treatment. (c) Oocyte morphologies in the control, B[ghi]P, and B[ghi]P+MT groups. (d) The rates of first polar body extrusion in the control, B[ghi]P, and B[ghi]P+MT groups. Scale bar = 100 μm. Data were presented as mean ± standard error of the mean. $^{a-e}$ Means not sharing a common superscript are different ($p < 0.05$)

2.9 | ROS generation detection

ROS Assay Kit (S0033; Beyotime Institute of Biotechnology) was performed to detect ROS content generation in oocytes. Briefly, after incubation with dichlorofluorescein diacetate probe for 20 min at 37°C in darkness, the oocytes were mounted on slides after washes and observed under a laser-scanning confocal microscope with the same scanning parameters for all measurements.

2.10 | Annexin-V staining

The apoptosis assay was conducted by using annexin V-FITC, fluoresceine isothiocyanate, Apoptosis Kit (C1063; Beyotime Institute of Biotechnology). Oocytes were stained with 5 μl annexin V-FITC diluted in 195 μl binding buffer for 15 min in darkness at RT. After washes, oocytes were installed on glass slides to monitor fluorescence signals and capture images by using a laser-scanning confocal microscope with the same scanning parameters.

2.11 | Fluorescence intensity analysis

For fluorescence intensity analysis, all the pictures were captured with the same scanning settings. Image J software (NIH, Bethesda, MD) was applied to analyze the relative average fluorescence intensity per unit area within the region of interest.

2.12 | Statistical analysis

Each experiment was replicated at 3 times, and at least 25 oocytes were observed for each group. The data were taken as mean ± standard error of the mean and analyzed by one-way analysis of variance with SPSS software (IBM Co., NY). Differences were considered statistically significant at $p < 0.05$.

3 | RESULTS

3.1 | Melatonin improves the meiotic maturation in B[ghi]P-exposed oocytes

First, we examined the toxic effects of B[ghi]P exposure on the meiotic progression of mouse oocytes in vitro. We cultured mouse oocytes with increasing B[ghi]P concentrations (25, 50, 100, 200, and 400 μM) to detect the rate of first polar body extrusion (PBE). As shown in Figure 1a, most oocytes excluded the first polar body and reached the metaphase II (MII) stage after 12 hr culture in the control group. However, B[ghi]P exposure significantly reduced the PBE rate, which suggests that B[ghi]P exposure inhibits the meiotic maturation of mouse oocytes in a dose-dependent manner (Figure 1b; $n = 573$). According to the results, we selected 100 μM B[ghi]P for subsequent experiments because this concentration not only caused meiotic arrest but also allowed a proper amount of oocytes to develop into the MII

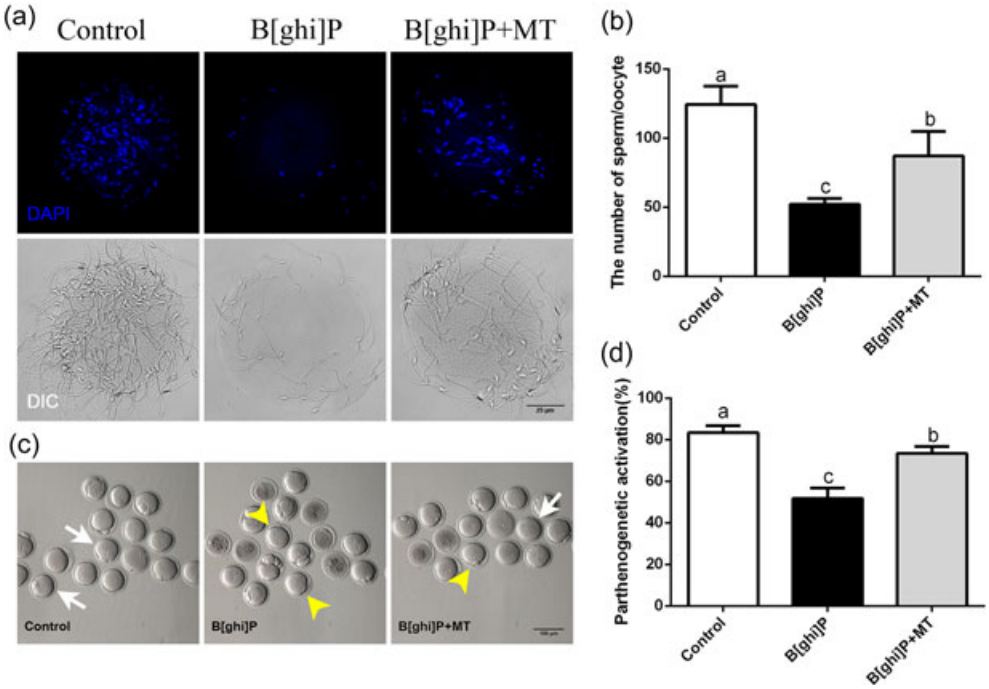

FIGURE 2 Effects of melatonin on the ability of sperm binding to the zona pellucida and parthenogenetic activation in the B[ghi]P-exposed oocytes. (a) Representative images of sperm binding in the control, B[ghi]P, and B[ghi]P+MT groups. Blue, sperm chromosomes. Scale bar = 25 μm. (b) The number of sperm binding to the zona pellucida in the control, B[ghi]P, and B[ghi]P+MT groups. (c) Representative images of parthenogenetic activation in the control, B[ghi]P, and B[ghi]P+MT groups. The white arrow shows an oocyte with a pronucleus and the yellow arrowhead shows an oocyte without a pronucleus. Scale bar = 100 μm. (d) The rate of parthenogenetic activation is recorded in the control, B[ghi]P, and B[ghi]P+MT groups. Data were presented as mean ± standard error of the mean. $^{a-c}$ Means not sharing a common superscript are different ($p < 0.05$). B[ghi]P: benzo[ghi]perylene; MII: metaphase II; MT: melatonin [Color figure can be viewed at wileyonlinelibrary.com]

maturation stage for other investigations. To investigate whether melatonin can alleviate the meiotic arrest caused by B[ghi]P exposure, melatonin was added into the 100 μM B[ghi]P-treated medium to yield a final concentration of 25, 50, 100, 200, and 400 μM, respectively. After 12-hr culture, we found that 100 μM melatonin significantly increased the rate of PBE in B[ghi]P-exposed group compared with the exclusive treatment with B[ghi]P (Figure 1c,d; $n = 730$). There was no difference between the control and melatonin-rescue group (100 μM melatonin+100 μM B[ghi]P). Higher concentration of melatonin did not obviously promote the meiotic maturation in the B[ghi]P-exposed group. According to the results, we chose 100 μM melatonin treatment for the further exploration.

3.2 | Melatonin elevates the ability of sperm binding to the zona pellucida and parthenogenetic activation in B[ghi]P-exposed oocytes

The study of sperm binding ability is of great significance for assessing the fertilization potential of oocytes. Thus, we tested the rate of sperm binding to the zona pellucida by DAPI staining to count the number of sperm. As shown in Figure 2a,b ($n = 270$), the number of sperm binding to the zona pellucida in the B[ghi]P-exposed group reduced significantly compared with that in the control group, but increased partially in the melatonin-rescued group. The results implied that melatonin can improve the fertilization potential of B[ghi]P-exposed oocytes. The study of parthenogenetic activation ability is of great significance for the production and application of embryos in vitro. Then, we investigated the effects of melatonin on the parthenogenetic activation ability in B[ghi]P-exposed oocytes. The results showed that the rate of parthenogenetic activation in the B[ghi]P-exposed group decreased dramatically compared with that in the control group, however, increased partially in the melatonin-rescued group (Figure 2c,d; $n = 279$). Taken together, all these results implied that melatonin rescues the reduced ability of sperm binding to the zona pellucida and parthenogenetic activation in the B[ghi]P-exposed group.

3.3 | Melatonin restores the actin dynamics in B[ghi]P-exposed oocytes

The actin filaments, one of the cytoskeletons, play a key role in the cortical polarization and asymmetric spindle positioning during oocytes maturation. We investigated the effect of B[ghi]P on the progression of oocyte meiosis, specifically the actin dynamics. As shown in Figure 3a, actin is evenly distributed on the plasma membrane with a stronger fluorescent signal in the control group. In contrast, the B[ghi]P-exposed oocyte shows the intermittent distribution of actin filaments with weaker fluorescent signals, and the B[ghi]P+melatonin treatment group could be rescued to a level indistinguishable from the controls (Figure 3a). Correspondingly, quantitative analysis of fluorescence intensity also confirmed the results (Figure 3b; $n = 272$). All the results suggested that melatonin prevents the actin dynamics from damage induced by B[ghi]P exposure.

3.4 | Melatonin protects the spindle defects and chromosome misalignment in B[ghi]P-exposed oocytes

Meiotic arrest is largely caused by the defective spindle assembly, so we detected the formation of spindle apparatus in metaphase I-stage oocytes. After being cultured for 8 hr, oocytes were immunostained with anti-α-tubulin antibody to label the spindle and counterstained with DAPI to visualize the chromosome. As shown in Figure 4a, most of the control oocytes exhibited a typical barrel-shape spindle apparatus with well-aligned chromosomes on the equatorial plate. In contrast, the rate of disorganized spindle and misaligned chromosomes significantly increased in the B[ghi]P-exposed group (Figure 4a, middle). As expected, melatonin treatment in B[ghi]P-exposed group reduced partially the abnormal rates (Figure 4b,c; $n = 270$). All these data indicated that melatonin restores the spindle defects and chromosome misalignment in B[ghi]P-exposed oocytes.

3.5 | Melatonin maintains euploidy by restoring defective kinetochore–microtubule attachment in B[ghi]P-exposed oocytes

Aneuploidy is often associated with the abnormal spindle formation and chromosome alignment, which is usually caused by the defective kinetochore–microtubule attachment. Thus, we next tested the stability of kinetochore–microtubule attachments in the B[ghi]P-exposed group. As shown in Figure 5a, the kinetochores were completely attached by microtubules in the control group. Nevertheless, the oocytes exhibited defective kinetochore–microtubule attachments in the B[ghi]P-exposed group, whereas the melatonin-rescued group reduced the rate of aberrant kinetochore–microtubule attachments caused by B[ghi]P to an indistinguishable level compared with the control group (Figure 5a,b; $n = 270$). Moreover, we analyzed the MII oocytes karyotype by the chromosome spread assay. Less or more than 20 univalents is regarded as aneuploidy. As shown in Figure 5c,d ($n = 261$), the aneuploidy rate significantly increased in the B[ghi]P-exposed group compared with the control group, whereas melatonin significantly reduced the aneuploidy rate induced by B[ghi]P exposure. All these combined results indicated that meiotic defects induced by B[ghi]P treatment eventually lead to an increase incidence of aneuploidy in oocytes, whereas melatonin can significantly reduce this damage.

3.6 | Melatonin increases the rate of homogeneous mitochondria distribution in B[ghi]P- exposed oocytes

Mitochondrion is a vital organelle during oocyte meiosis maturation. Next, we detected the mitochondria distribution in MII oocytes to investigate whether B[ghi]P exposure would cause mitochondria dysfunction. As shown in Figure 6a, typical images represent

homogenous distribution, perinuclear distribution, and cluster distribution, respectively. The rate of homogeneous distribution in the B[ghi]P-exposed group decreased significantly compared with that in the control group, whereas melatonin could partially restore the rate of homogeneous distribution in the melatonin-rescued group (Figure 6b; $n = 270$). These results suggested that melatonin repairs the disrupted mitochondrial distribution to some extent caused by B[ghi]P exposure.

3.7 | Melatonin reduces ROS level and limits early apoptosis in B[ghi]P-exposed oocytes

Oxidative stress is often induced by mitochondrial dysfunction. Then, we detected the extent of ROS generation and melatonin rescue with B[ghi]P exposure. As shown in Figure 7a, the signals of oxidative stress in the B[ghi]P-exposed group increased obviously compared with those in the control group, while decreased partially in the melatonin-rescue group. The fluorescence intensity analysis was also consistent with the above observations (Figure 7b; $n = 272$). The results indicated that melatonin is able to reduce the level of ROS in the B[ghi]P-exposed oocytes. Cumulative data suggest that high levels of ROS could induce apoptosis, and thus, we next studied the early apoptosis in oocytes. Notably, only oocytes with fluorescent signals simultaneously in zona pellucida and cytoplasmic membranes are considered to have undergone early apoptosis (Duan et al., 2015). Our results indicated that the number of early apoptotic oocytes in the B[ghi]P-exposed group was significantly higher than that in the control group, and in the melatonin-rescued group declined to a level indistinguishable from that in the control group (Figure 7c,d; $n = 252$). Taken together, all the results illustrated that melatonin could reduce the level of oxidative stress and the early apoptotic rate in B[ghi]P-exposed oocytes.

4 | DISCUSSION

B[ghi]P in haze has been reported in some research studies to produce cytotoxic effects in NL-20 human bronchial cells and human pulmonary alveolar epithelial cells (Ke, Liu, Yao, Zhang, & Sui, 2018; Zaragoza-Ojeda et al., 2016). However, there are still no relevant reports about its toxic effects on mammalian oocytes. Studies have shown that melatonin could improve metabolic risk factors, regulate related apoptotic proteins, and reduce ROS damage (Amin, El-Missiry, & Othman, 2015; Y. Miao et al., 2018). Therefore, we investigated the toxic effects of B[ghi]P (100 μM) exposure on meiotic maturation of mouse oocytes and the protective roles of melatonin (100 μM) in this study.

We hypothesized that melatonin could improve B[ghi]P-induced oocyte deterioration. To verify our assumption, we first examined the meiotic maturation of mouse oocytes, which is a significantly important indicator of high-quality oocytes. Then, we tested the ability of sperm binding to the zona pellucida and parthenogenetic activation of oocytes, both of which are also vital indicators for high-standard oocytes (Y. Miao et al., 2018; M. Zhang et al., 2017). Fertilization can initiate embryonic development, which is a complex multistep event, whereas sperm binding to zona pellucida is the key first-step in fertilization (Aydin, Sultana, Li, Thavalingam, & Lee, 2016). Furthermore, as embryonic stem cells formed by parthenogenetic activation have the same pluripotency and proliferation as formed by fertilization, and they are capable of performing

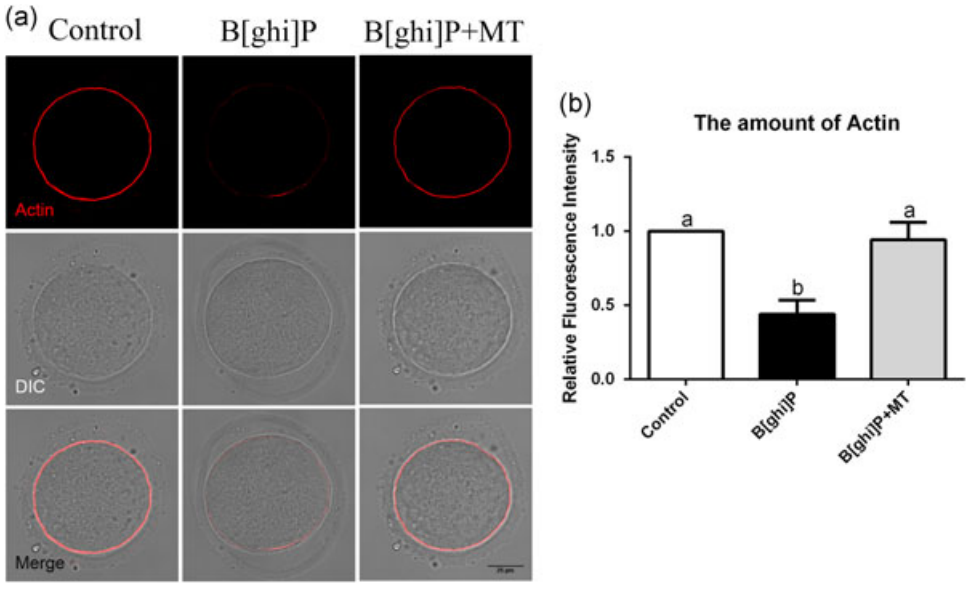

FIGURE 3 Effects of melatonin on the actin dynamics in the B[ghi]P-exposed oocytes. (a) Representative images of actin filaments (red) in the control, B[ghi]P, and B[ghi]P+MT groups. Scale bar = 25 μm. (b) The relative fluorescence intensity of actin in the control, B[ghi]P, and B[ghi]P+MT groups. Data were presented as mean ± standard error of the mean. [a,b] Means not sharing a common superscript are different ($p < 0.05$). B[ghi]P: benzo[ghi]perylene; DIC, differential interference contrast; MII: metaphase II; MT: melatonin [Color figure can be viewed at wileyonlinelibrary.com]

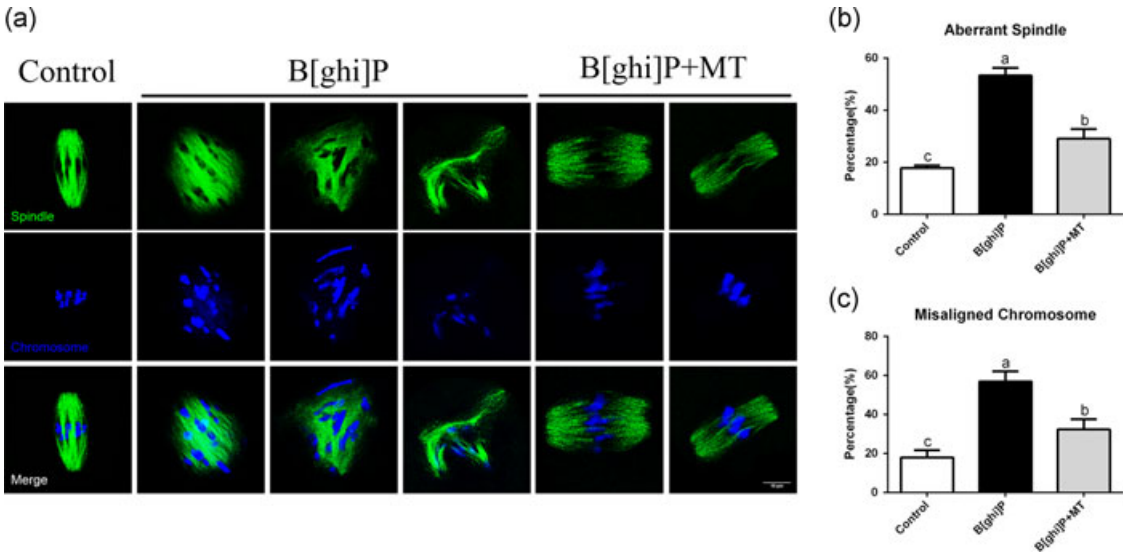

FIGURE 4 Effects of melatonin on the spindle assembly and chromosome alignment in the B[ghi]P-exposed oocytes. (a) Representative images of spindle morphologies and chromosome alignment in the control, B[ghi]P, and B[ghi]P+MT groups. Spindle (green) and DNA (blue). Scale bar = 10 μm. (b) The rates of aberrant spindles in the control, B[ghi]P, and B[ghi]P+MT groups. (c) The rates of misaligned chromosomes in the control, B[ghi]P, and B[ghi]P+MT groups. Data were presented as mean ± standard error of the mean. $^{a-c}$ Means not sharing a common superscript are different ($p < 0.05$). B[ghi]P: benzo[ghi]perylene; MII: metaphase II; MT: melatonin [Color figure can be viewed at wileyonlinelibrary.com]

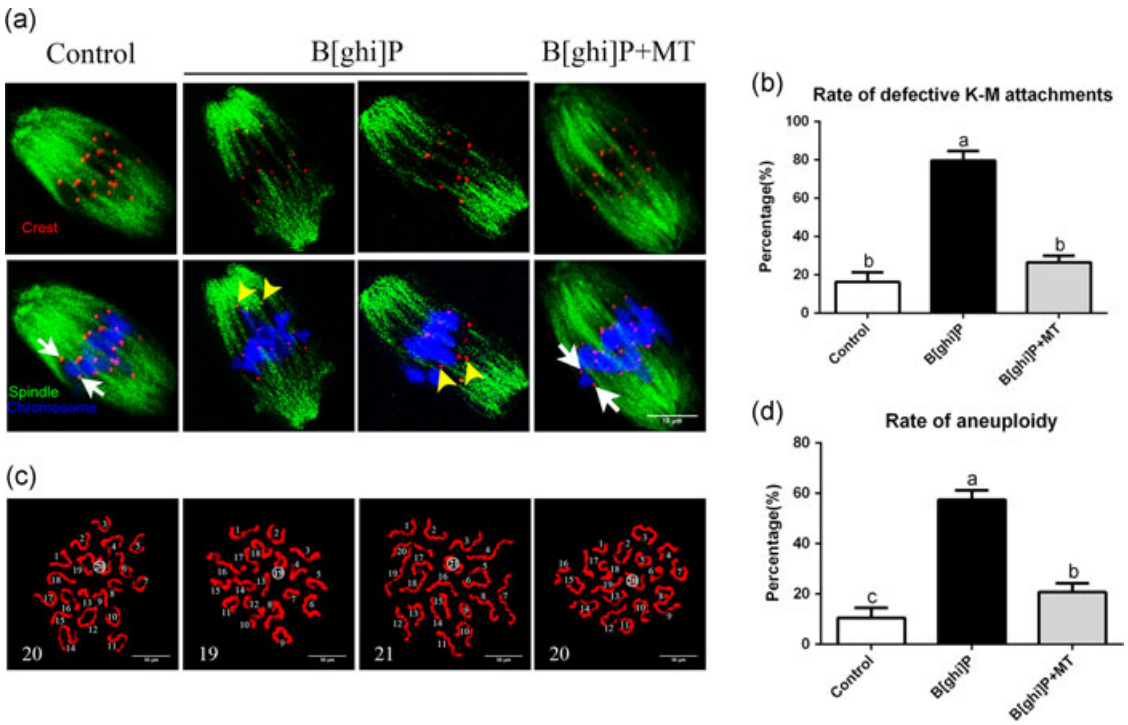

FIGURE 5 Effects of melatonin on the kinetochore–microtubule (K–M) attachment and karyotype in the B[ghi]P-exposed oocytes. (a) Representative images of K–M attachment in the control, B[ghi]P, and B[ghi]P+MT groups. Kinetochore (red), microtubule (green), and DNA (blue). The white arrow indicates normal attachments; the yellow arrowhead indicates abnormal polar attachments. Scale bar = 10 μm. (b) The rates of defective K–M attachments in the control, B[ghi]P, and B[ghi]P+MT groups. (c) Representative images of euploidy (20 univalents) and aneuploidy (less or more than 20 univalents) in MII oocytes. Scale bar = 10 μm. (d) The rates of aneuploid oocytes in the control, B[ghi]P, and B[ghi]P+MT groups. Data were presented as mean ± standard error of the mean. $^{a-c}$ Means not sharing a common superscript are different ($p < 0.05$). B[ghi]P: benzo[ghi]perylene; MII: metaphase II; MT: melatonin [Color figure can be viewed at wileyonlinelibrary.com]

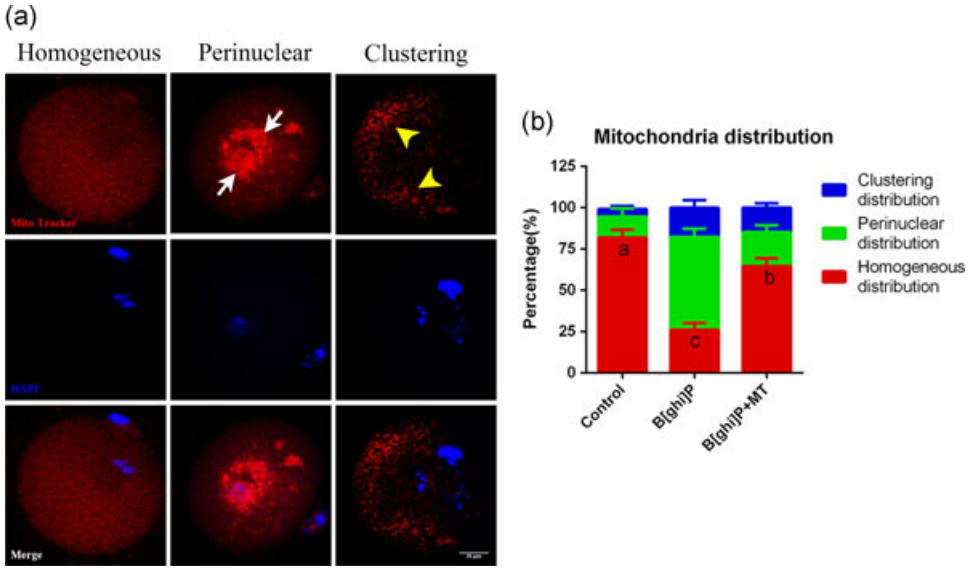

FIGURE 6 Effects of melatonin on mitochondrial distribution in the B[ghi]P-exposed oocytes. (a) Representative images of homogenous, perinuclear, and clustering mitochondrial distribution in MII oocytes: mitochondria (red) and DNA (blue). The white arrow shows perinuclear mitochondrial distribution, and the yellow arrowhead shows clustering mitochondrial distribution. Scale bar = 25 μm. (b) The rates of homogeneous, perinuclear, and clustering distribution pattern. Data were presented as mean ± standard error of the mean. $^{a-c}$ Means not sharing a common superscript are different ($p < 0.05$). B[ghi]P: benzo[ghi]perylene; MII: metaphase II; MT: melatonin [Color figure can be viewed at wileyonlinelibrary.com]

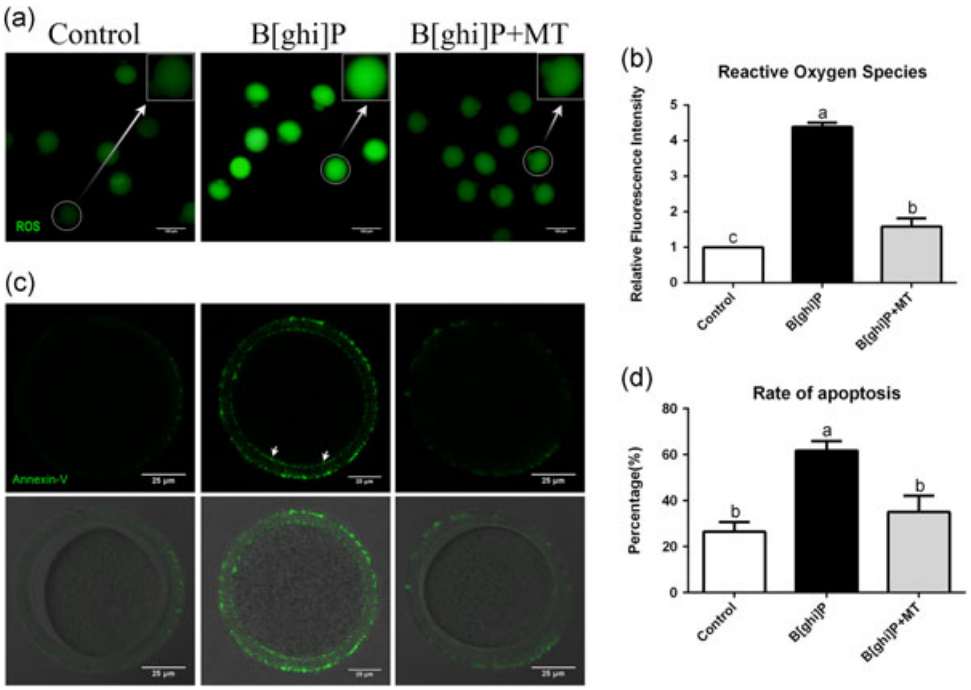

FIGURE 7 Effects of melatonin on oxidative stress and early apoptosis in the B[ghi]P-exposed oocytes. (a) Representative images of reactive oxygen species (ROS) levels (green) in the control, B[ghi]P, and B[ghi]P+MT groups. Scale bar = 100 μm. (b) Relative fluorescence intensity of ROS in the control, B[ghi]P, and B[ghi]P+MT groups. (c) Representative images of early apoptotic oocytes in the control, B[ghi]P, and B[ghi]P +MT groups. Oocytes with fluorescence signals (green) at both membrane (white arrow) and zona pellucida are regarded as early-stage apoptosis. Scale bar = 25 μm. (d) The rate of early apoptosis oocytes in the control, B[ghi]P, and B[ghi]P+MT groups. Data were presented as mean ± standard error of the mean. $^{a-c}$ Means not sharing a common superscript are different ($p < 0.05$). B[ghi]P: benzo[ghi]perylene; MT: melatonin [Color figure can be viewed at wileyonlinelibrary.com]

differentiation and development (Han & Gao, 2013). Our data illustrated that B[ghi]P exposure significantly reduces the rate of first PBE (Figure 1b), the number of sperm binding to the zona pellucida (Figure 2b), and the rate of parthenogenetic activation (Figure 2d), indicating that B[ghi]P exposure disturbs the normal meiotic progression and declines the fertilization potential and developmental ability of oocytes. As expected, with melatonin treatment, the rate of first PBE was returned completely to normal levels (Figure 1d), the amount of sperm binding to the zona pellucida and the ability to parthenogenetic activation were also recovered partially (Figure 2b,d). All these combined results suggested that B[ghi]P exposure impairs the oocyte quality and melatonin is able to restore these damage.

Next, we investigated possible mechanisms that B[ghi]p exposure causes meiotic arrest in oocytes. Actin filament is an important cytoskeletal protein that has been shown to be critical for meiotic progression and first PBE (Field & Lenart, 2011). The spindle is another indispensable component of the cytoskeleton that plays a key role in ensuring faithful chromosome segregation (Sanfins, Lee, Plancha, Overstrom, & Albertini, 2003). Previous studies have shown that abnormal spindle assembly and chromosomal misalignment impair the female reproductive capacity of different species (Herrick, Brad, & Krisher, 2006). Our data indicated that B[ghi]P exposure destroys actin filament dynamics and spindle assembly (Figures 3 and 4), suggesting that B[ghi]P exposure perturbs cytoskeletal integrality. Whereas melatonin restored the actin dynamics in B[ghi]P-exposed oocytes to an indistinguishable level compared with the controls (Figure 3b). Also, melatonin partially restored the spindle defects and chromosome misalignment in B[ghi]P-exposed oocytes (Figure 4b,c). Therefore, melatonin is able to protect the cytoskeletal integrality in B[ghi]P-exposed oocytes.

Abnormal bipolar spindle assembly and chromosome alignment are always accompanied by errors in kinetochore–microtubule attachment, which usually leads to the oocyte aneuploidy (Shomper, Lappa, & FitzHarris, 2014). So, we investigated the toxic effects of B[ghi]P exposure on the kinetochore–microtubule attachment stability and euploidy. Our results showed that the stability of the kinetochore–microtubule attachment was impaired and the amount of aneuploidy was increased, which were recovered with melatonin supplementation. Therefore, melatonin can maintain euploidy by restoring defective the kinetochore–microtubule attachment in B[ghi]P-exposed oocytes.

Mitochondria are important organelles that produce ATP in oocytes, and previous studies have shown that they play a crucial role during the procedure of meiotic maturation and fertilization (Lai et al., 2015; Sun et al., 2001). During meiotic maturation of oocytes, mitochondria are uniformly transferred from the perinuclear region to the cytoplasm, and their failure to migrate usually leads to failure of cytoplasmic maturation (Sun et al., 2001). Thus, the mitochondrial distribution was tested in B[ghi]P-exposed oocytes. Our data confirm that disturbed mitochondrial distribution may be a vital reason for meiotic arrest and failure of parthenogenetic activation in B[ghi]P-exposed oocytes (Figure 6). Moreover, studies have shown that high levels of oxidative stress disrupt oocyte maturation and embryonic development. Meanwhile, accumulation of ROS leads to mitochondrial dysfunction and induction of oocyte apoptosis (Liu, Trimarchi, & Keefe, 2000; Tiwari et al., 2015; H. Zhang et al., 2009). Our data validate that B[ghi]P exposure impairs mitochondria functions, which induce high levels of ROS and oocyte apoptosis (Figure 7). Of course, all these mitochondria-related damages are restored by melatonin (Figures 6b and 7b,d). So, B[ghi]P exposure leads to mitochondrial dysfunctions, all of which can be restored after melatonin is administered.

Collectively, our research demonstrates that B[ghi]P exposure significantly impairs the mouse oocyte quality by assessing the meiotic maturation, fertilization potential, and parthenogenetic activation ability. These are caused by disrupting cytoskeletal integrality and chromosome euploidy, as well as perturbing mitochondrial functions. Whereas melatonin is able to reduce these damages, which indicates that melatonin has a certain protective effect on preventing the reduced oocyte quality caused by B[ghi]P exposure.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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