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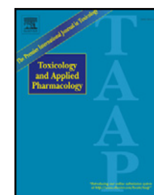

Methyl parathion inhibits the nuclear maturation, decreases the cytoplasmic quality in oocytes and alters the developmental potential of embryos of Swiss albino mice

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A B S T R A C T

Methyl parathion (MP) is one of the most commonly used and extremely toxic organophosphorous group of pesticide. A large number of studies in the literature suggest that it has adverse effects on the male reproductive system. However, there is limited information about its toxicity to the female reproductive system. In the present study we report the toxic effects of methyl parathion on the female reproductive system using Swiss albino mice as the experimental model. The female mice were administered orally with 5, 10 and 20 mg/kg of MP. One week later, the mice were superovulated with pregnant mare serum gonadotrophin (PMSG) and human chorionic gonadotrophin (hCG) to study the quality of the oocytes, spindle organization, developmental potential of early embryos and the DNA integrity in blastocysts. MP exposure resulted in a non-significant decrease in the number of primordial follicles and increased DNA damage in granulosa cells. Though MP did not have any effect on the ovulation it had a significant inhibitory effect on the nuclear maturity of oocytes which was associated with spindle deformity. In addition, the oocytes had higher cytoplasmic abnormalities with depleted glutathione level. Even though it did not have any effect on the fertilization and blastocyst rate at lower doses, at 20 mg/kg MP it resulted in a significant decrease in blastocyst hatching, decrease in cell number and high DNA damage. While low body weight gain was observed in F1 generation from 5 mg/kg group, at higher dose, the body weight in F1 generation was marginally higher than control. Post-natal death in F1 generation was observed only in mice treated with 20 mg/kg MP. In conclusion, we report that MP has adverse effects on the oocyte quality, developmental potential of the embryo and reproductive outcome.

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Introduction

Organophosphorous group of pesticides are commonly used in the agricultural system due to their rapid action on the pests or insects. Increased exposure of these pesticides to public is mainly thought to be due to their illegal indoor exposure (Ruckart et al., 2004). Methyl parathion (*O*,*O*-dimethyl-*O*-4-*p*-nitrophenyl phosphorothioate, MP), commonly known as "cotton poison" (Ruckart et al., 2004), is one of the widely used organophosphorous pesticide classified by the U.S. Environmental Protection Agency as Class I restricted use pesticide (Jaga and Dharmani, 2006). It has a wide application as insecticide and herbicide in the agricultural field (Garcia et al., 2003). Despite its rapid degradation in soil, it can be transported to the surrounding areas through natural agents such as rain, fog and wind thus increasing the risk of exposure to human (Sakellarides et al., 2002).

Human beings are exposed to methyl parathion through the respiratory tract, skin and gastrointestinal tract (Rehner et al., 2000; Zhu et al., 2001). Studies from various parts of the world have shown that vegetables and fruits collected from the market are contaminated with organophosphorous pesticides like methyl parathion exceeding the maximum residue limits (MRLs) (Bai et al., 2006; Bhanti and Taneja, 2007; Chowdhury et al., 2014; Curl et al., 2003; Darko and Akoto, 2008; Dogheim et al., 1996; Mukherjee and Gopal, 1996; Sapbamrer and Hongsibsong, 2014). In addition, studies demonstrating the presence of methyl parathion in milk samples (Srivastava et al., 2011), cheese (Mallatou et al., 1997) and infant formulas (Melgar et al., 2010) are available in the literature. A study conducted on residents of Mississippi, USA, who were exposed to MP due to spraying of the pesticide on the residences by unlicensed pesticide applicators found that the level of *p*-nitrophenol (PNP), a metabolite of MP was ranging from 25 to 1100 μg/g in 50.3% of the residents (Imtiaz and Haugh, 2002).

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In the body methyl parathion is oxidized to methyl paraxon which is an extremely potent metabolite (Hollingworth et al., 1973). It irreversibly inhibits the function of acetyl cholinesterase (AchE) by phosphorylating the enzyme at the substrate binding site (Tang et al., 2003). Apart from its neurotoxic effect, substantial data available in the literature suggest that methyl parathion exposure has adverse effects on the male reproductive system. Testicular atrophy (Narayana et al., 2006a), altered structure and function of the epididymis and accessory sex glands (Narayana et al., 2006b), decrease in sperm count (Narayana et al., 2005; Piña-Guzmán et al., 2009), increase in sperm head abnormalities (Mathew et al., 1992), high incidence of sperm DNA damage (Sánchez-Peña et al., 2004), aneuploidy in spermatozoa (Padungtod et al., 2000; Recio et al., 2001) and decreased fertility (Rupa et al., 1991) have been reported in MP exposed subjects. Prolonged estrous cycle (Sortur and Kaliwal, 1999), decreased ovarian weight (Asmathbanu and Kaliwal, 1997; Dhondup and Kaliwal, 1997) and embryo toxicity (Tanimura et al., 1967) have been observed in MP exposed female rats. In addition, exposure of methyl parathion during the gestation period has shown to cause fibrosis and hemorrhage in the endometrium (Güney et al., 2007) and fetal mortality (Crowder et al., 1980). The available reports on the female reproductive function hint the possible influence of MP exposure on the female gametes. However, we did not come across any study elucidating the effects of MP on the functional competence of the oocytes, its developmental potential and transgenerational changes associated with maternal MP exposure. Our study for the first time reports the effect of methyl parathion on the quality and function of female gametes, and its consequences on early embryonic development and reproductive outcome using Swiss albino mice as the animal model.

Material and methods

Animal model. Inbred female Swiss albino mice of 6–8 weeks old, weighing 25 ± 2 g, maintained in the Central Animal Research Facility, Manipal University, were used for study. The animals were maintained under standard conditions of temperature (25 ± 2 °C), humidity (45–55%) and light (12:12 h of light and dark). The mice were fed with food and water ad libitum. A prior approval was obtained from the Institutional Animal Ethical Committee to carry out the experiment.

Methyl parathion. Karacid-50, the commercial grade of methyl parathion (*O*, *O*-dimethyl-*O*-4-nitrophenyl phosphorothionate) (Jayakrishna Pesticides Pvt. Limited, Salem, India) was freshly diluted in Milli-Q water and administered orally to female mice at different concentrations (5, 10 and 20 mg/kg body weight, single dose). Control animals were fed with Milli-Q water. The doses selected for this study are based on the previous studies (DU et al., 2012; Güney et al., 2007; Tanimura et al., 1967) and the LD_{50} value, which was reported to be 19.5 mg/kg, orally (Haley et al., 1975). In the present study we have taken three doses — 1/4 of LD_{50}, 1/2 of LD_{50} and LD_{50} value to understand the consequences of acute exposure of MP on the female gonads.

Superovulation. To understand the early response of the ovary to acute MP exposure, we assessed the ovulatory function, oocyte quality and its developmental potential after a gap of 10 days. Since earlier studies have shown that MP prolongs the estrous cycle (Sortur and Kaliwal, 1999) and induces degenerative changes in the follicle in the ovary after 10 days of chronic MP administration (Asmathbanu and Kaliwal, 1997) we have selected this interval. For this, one week after MP administration, the mice were superovulated by intraperitoneally (i.p) injecting 5 IU pregnant mare serum gonadotropin (PMSG, Cat. No. G4877, Sigma) followed by 10 IU of human chorionic gonadotropin (hCG, Ovutrig, India) 48 h later. At 12–13 h after the hCG injection (10 days after MP injection), mice were humanely euthanized by cervical dislocation and the ovaries and oviducts were dissected out in an M2 medium.

Ovarian tissue histology. The ovaries were dissected out from mice and were kept in Bouin's fixative for 24 h and dehydrated using graded alcohol in the order of 50%, 70%, 95% and absolute ethanol. The tissues were embedded in paraffin and 5 μm thin sections were taken using a rotatory microtome. The sections were fixed on albumin coated slides and air dried. Prior to staining, the sections were deparaffinized in xylene, rehydrated and stained with hematoxylin and eosin. The slides were then mounted in a DPX mounting medium and observed under a light microscope to assess whether MP induces any histological changes in the ovary such as follicular atresia, edema and vascular damage.

Terminal deoxy nucleotidyl nick end labeling (TUNEL) assay in ovarian tissue section. The paraffin embedded ovarian sections were processed for TUNEL assay as described by Martinez-Madrid et al. (2007) with minor modifications. Briefly, the slides were pre-warmed at 60 °C for 30 min, deparaffinized in xylene and rehydrated using gradients of ethanol. Sections were digested with 20 μg/mL proteinase k (Cat. No. P2308, Sigma-Aldrich, Germany) for 10 min at room temperature followed by permeabilization with 0.1% Triton X-100 in PBS with 0.1% sodium citrate for 2 min at 0 °C. The sections were later incubated with a TUNEL reaction mixture (Roche, USA, 1215792910, TMR Red) at 37 °C in the dark for 60 min, counterstained with 4′6′ diamidino-2-phenylindole (DAPI) and observed under a fluorescent microscope (Axio Imager A1, Zeiss, Germany).

Follicle count. The ovaries were enzymatically digested as described by Roy and Greenwald (1985) with minor modifications. Briefly, the ovaries were transferred to a pre-incubated dissociation medium [containing 5 mg/mL collagenase (Cat. No. 17104019, Invitrogen), 1 mg/mL trypsin (Cat. No. RM713, Himedia) and 0.1 mg/mL hyaluronidase (Cat. No. H4272, Sigma) in a Dulbecco's modified Eagle's medium (DMEM, Cat. No. D5648, Sigma)] and after 1 h the follicles were dispersed by gentle mechanical agitation. Follicles were later transferred to a fresh DMEM medium and observed under a stereomicroscope fitted with a micromanipulator (Olympus IX 70, 200× magnification) for scoring. The number of primordial (oocyte surrounded by a single layer of flattened epithelial cells), primary (oocyte with thin zona and multiple layers of follicular cells), antral (oocytes with zona covered with multiple layers of granulosa cells and antral cavity or free oocytes at the germinal vesicle (GV) stage), and atretic follicles (follicle with degenerated oocyte) was counted separately.

Oocyte collection. The oviducts were carefully teased with a blunt needle in an M2 medium to release clutch of cumulus oocyte complexes (COC) by observing under the stereomicroscope maintained at 37 °C. The cumulus cells were stripped off from the oocytes by incubating the COC in hyaluronidase (1 mg/mL) for 30 s followed by gentle pipetting. Denuded oocytes were washed in an M16 medium and used for further analysis.

Assessment of oocyte maturity and quality. The oocytes were observed under the stereomicroscope fitted with a micromanipulator (Olympus IX 70, 200× magnification) for quality assessment. Based on the degree of cytoplasmic fragmentation, degeneration, granulation and presence of vacuoles the quality of the oocytes was assessed. The nuclear maturity was assessed by staining the oocytes with DAPI and observing under the fluorescent microscope and were classified as germinal vesicle (GV) stage oocyte (oocyte with a nucleus and without polar body), metaphase I oocytes (oocytes with germinal vesicle breakdown (GVBD) and without polar body in the perivitelline space) and MII oocytes (oocytes with single polar body extruded in the perivitelline space).

Mitochondrial distribution pattern (Johnson et al., 1980). Denuded oocytes were kept in a 50 μL droplet of rhodamine 123 (20 μg/mL, R8004, Sigma) and incubated in an incubator (37 °C and 5% CO_2) for 20 min. After incubation oocytes were washed 4–5 times in an M16

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