



Effects of Quinazolinones on the Number and the Size of Balb/C Mice Embryonic Oocytes

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Summary

Heterocyclic compounds such as quinazolinones have variety of biological and pharmacological properties (anticancer, antiinflammatory, antimicrobial, antimalaria, etc.). In this study, effects of a new quinazolinone, 4(3H) quinazolinone-2-ethyl-2-phenyl ethyl (QEPE), were investigated on the ovaries of Balb/C mice embryos. Pregnant Balb/C mice were divided into three groups of control, sham and experimental. Control mice remained intact, sham and experimental groups received 0.05% methyl cellulose and 100 mg/kg/body weight of QEPE, intraperitoneally (IP), on days 16.5th and 18.5th of gestation. Morphological observations showed abnormal embryos, underdeveloped embryos and severe hemorrhage in the neck region of QEPE treated embryos. Histological and pathological studies demonstrated that QEPE reduced the number and size of the oocytes of the embryonic ovaries. In conclusion the present results demonstrate QEPE is teratogenic and toxicogenic chemical, affecting internal organs such as ovaries at histological level.

Key Words: Embryonic ovary, Oocyte size, Oocyte number, Quinazolinones

Introduction

Teratogens are medications, chemicals, and infectious diseases or environmental agents that may interfere with normal development of embryos or fetuses which result in loss of pregnancies, pregnancy complications or birth defects. There are relatively common varieties of teratogens and the word teratogen is used to denote the result of a hazard assessment on a particular agent (for purpose of this guidance, a drug). The use of this term indicates that drugs have the capacities to produce abnormal development in an embryo or fetus under certain exposure conditions [1,2].

Quinazolinones are extensively used in the treatment of prevalent diseases [3-6]; They belong to hypnotic [7,8] and potent anticonvulsant drugs [9-11] and act strongly to inhibit human immunodeficiency virus [12-14]; Anticancer and antimicrobial activities of these drugs have been well documented [15-17]. MCI-176, a quinazolinone derivative, acts as calcium antagonist [18] and can inhibit histamine dependent HCL secretion [19]. They are non-peptide antagonist cholecystokinin receptors (CCR-B, CCR-A) and are used for the treatment of several anxiety disorders [20,21].

Reports demonstrated that the exposures of embryonic ovaries to the toxicants reduces oocytes' pools determining the ovarian lifespans.

In continuation of our previous studies [22-26], the present study has been undertaken with an aim to investigate the effects of a new derivative of quinazolinones, QEPE, on oocytes' number and size of Balb/C mice embryonic ovaries.

Materials & Methods

Chemicals and reagents: All the materials including Methyl Cellulose, Ethanol, Formaldehyde, Paraffin, Hematoxylin and Eosin were purchased from Merck.

Animals: Balb/C mice (3-4 months old) [27] originally obtained from Pars Company (Tehran, Iran), and randomly bred in Department's animal

house were housed at 24±1°C, 50±0.5% RH and 12:12 h L:D controlled conditions. They were provided with lab chow and tap water. Virgin females (about 30g) which mated males overnight were considered to be on day 0.5 of pregnancy. The pregnancy was confirmed by presence of vaginal plugs.

Pregnant mice were divided into three groups. Randomly chosen mice of control groups (n=9) remained intact; They were killed by cervical dislocation on 16.5 or 18.5 days post coitum (dpc) . Mice of sham groups (n=9) received 10 mL/kg/body weight of 0.05% methyl cellulose (the solvent) intraperitoneally (IP), on 13.5 or 16.5 dpc and were killed by cervical dislocation on 16.5 or 18.5 dpc. Experimental groups (n=9) were injected with the most effective dose (100 mg/kg/body weight) of QEPE [22] synthesized at the Department of Chemistry, University of Shahid-Beheshti, Tehran, Iran [28], on 13.5 or 16.5 dpc (critical days of mouse fetal ovary development [29]) and were killed on 16.5 or 18.5 dpc by cervical dislocation.

Histological studies: Ovaries were extracted from randomly chosen 16.5 and 18.5 days old embryos of the mice of control, sham and experimental groups and fixed in formaldehyde (4%). Ovary sections (5µm thick) from formaldehyde -fixed samples were stained with hematoxylin and eosin H&E. The total number of healthy oocytes in every section were counted. By considering the mean values from three sections, the mean number of oocytes per section was determined for each ovary. Oocytes' size was measured in three sections of each embryonic ovary by using Motic soft wares.

Statistical analysis: The experiments were independently replicated at least three times. Graphs depict the mean±SEM of combined data from the replicate experiments. The data were analyzed with statistical packages for social sciences (SPSS, version 16). Specifying differences between experimental, control and/or sham groups were determined with Repeated Measure Test. Level of significance difference was P<0.05.

Results

Morphological observations of randomly chosen 16.5 and 18.5 days old embryos from control, sham and experimental groups showed abnormal embryos (Fig.1, A), underdeveloped embryos (Fig.1, B) and severe hemorrhage in the neck region (Fig.1, C) of QEPE treated embryos.

Light microscope observations demonstrated normal ovaries in 16.5 and 18.5 days old embryos of mice of control (Fig.2,A,D) and sham (Fig.2,B,E) groups with healthy oocytes in cysts but a decrease in the numbers and size of healthy oocytes were observed in the ovaries of the embryos of the mice treated with QEPE (Fig.2,C,F). Cells possessing lightly stained round nuclei with normal cytoplasmic volumes and easily discernible spherical plasma membranes were considered healthy oocytes, whereas those with nuclear condensations (basophilia), cytoplasmic shrinkages, and convoluted plasma membranes were considered apoptotic [30]. Repeated Measure test confirmed that the number and size of healthy oocytes in the ovaries of 16.5 and 18.5 dpc embryos of the mice of control and sham groups did not reveal any significant difference, but the numbers and size of healthy oocytes were significantly reduced in the ovaries of 16.5 and 18.5 dpc embryos of the mice of experimental groups ($P < 0.05$) (Fig.3). In addition, it was shown that treatment of mice with QEPE on day 16.5th was more effective on embryonic oocytes' size.

Discussion

Environmental teratogens, defined as any agent or substance which is capable of interfering with the development of a fetus, causing birth defects or deaths of the fetuses, include vitamins and minerals deficiencies or imbalances, numerous therapeutic drugs, chemicals such as herbicides and pesticides, noxious plants and viral and bacterial infections. The impact a teratogen will have on the fetus depends on several factors. The type of agent involved may determine the area of body or the

developmental process which will be affected. If several substances are involved, their relationships may also have a significant effect on the type of resulting abnormality [31].

Observation of morphological and skeletal abnormalities, and histological surveys by our groups demonstrated that quinazolinones pass through placenta, affect Balb/C mice embryos' and newborns' brain, liver, intestine, kidney, stomach, heart and spleen morphological and histological structures, although some may have normal appearances. In this study, ovaries of embryos of control and sham groups, showed no malformations, proved that quinazolinones were the reason for abnormalities created in treated mice embryos and their organs. Underlying mechanisms of this effect are not understood yet. Since quinazolinones are lipophilic components they can pass through cell membrane and interact with cytosolic and nuclear receptors.

It is well known that exposure to toxicant is associated with decreased female fertility, causing damage to ovarian function and disturbing follicle development [32]. Previous studies were shown that exposure to cigarette toxicants, impaired oocyte growth and decreased oocyte diameter in different stages of follicular development after birth [32-34]. In spite of the evidence of the harmful effects of toxicants on ovarian function and folliculogenesis after birth, few studies so far have investigated the consequences of the exposures of embryonic ovaries to the toxicants. The present study showed that QEPE significantly reduced the number and size of the healthy oocytes in the ovaries of 16.5 and 18.5 dpc embryos of the mice of experimental groups. Additionally, it was shown that treatment of mice with QEPE on day 16.5th was more effective on embryonic oocytes' size. It has been shown that exposure of fetal ovaries to the toxicants such as polycyclic aromatic hydrocarbons, heavy metals, and ionizing radiation reduces the number of oocytes via apoptosis induction [35-37]. Apoptosis is a form of controlled and programmed cell death in physiological and pathological conditions, during development of fetal ovaries.

Occurrence of germ cell apoptosis is likely aimed at removing abnormal oocytes [38]. Recent studies have introduced some derivatives of quinazolinones with anticancer activities inducing apoptosis in various cancer cell lines by changing expressions of some pro and anti apoptotic genes [39]. In conclusion the present results demonstrate a new derivatives of quinazolinones, QEPE, is teratogenic and toxicogenic chemical, affecting embryonic oocytes size and number.

Acknowledgements

Authors are grateful to unconditional help of Miss Mojgan Sarhadi, M.Sc. in Developmental Biology, for Statistical analysis.

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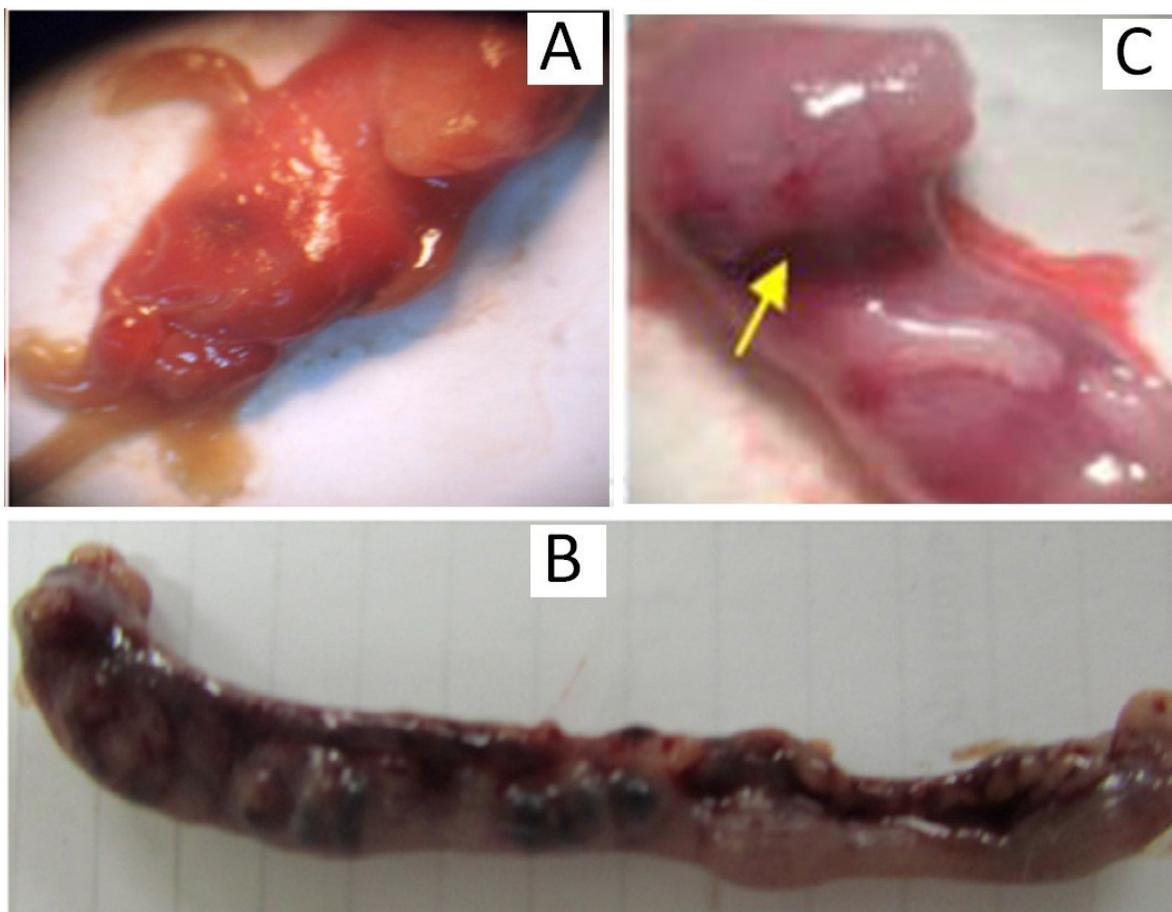


Fig.1: Morphological observations showed abnormal embryos (A), 16.5 dpc underdeveloped embryos in uterus (B) and severe hemorrhage (C) in the neck region of QEPE treated embryos.

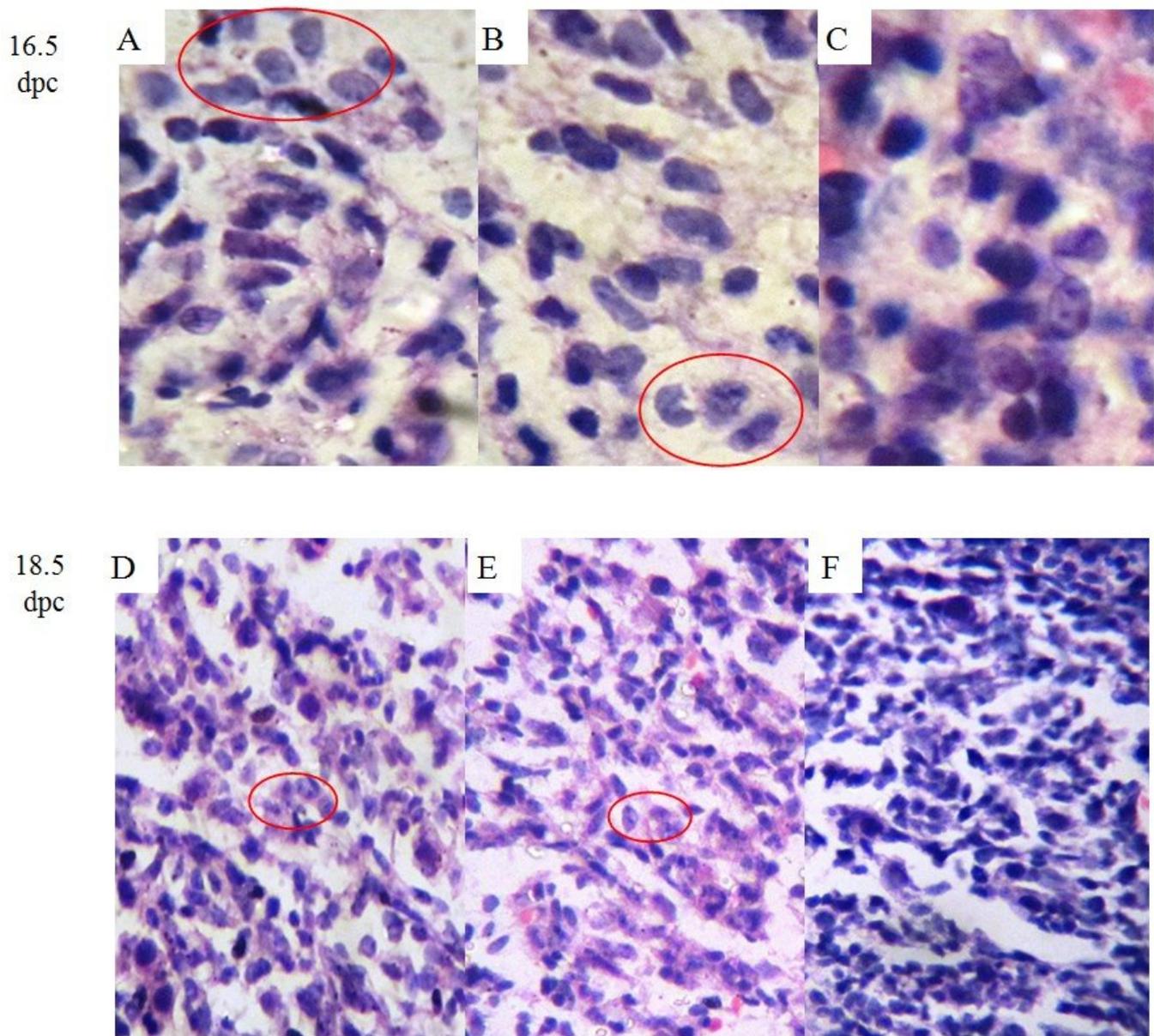


Fig.2: Light microscope observations demonstrated normal ovaries in 16.5 and 18.5 days old embryos of mice of control (A,D) and sham (B,E) groups with healthy oocytes in cysts but a decrease in the numbers and size of healthy oocytes were observed in the ovaries of the embryos of the mice treated with QEPE (C,F). Original magnification: panels A,B,C *1000, D,E,F*400.

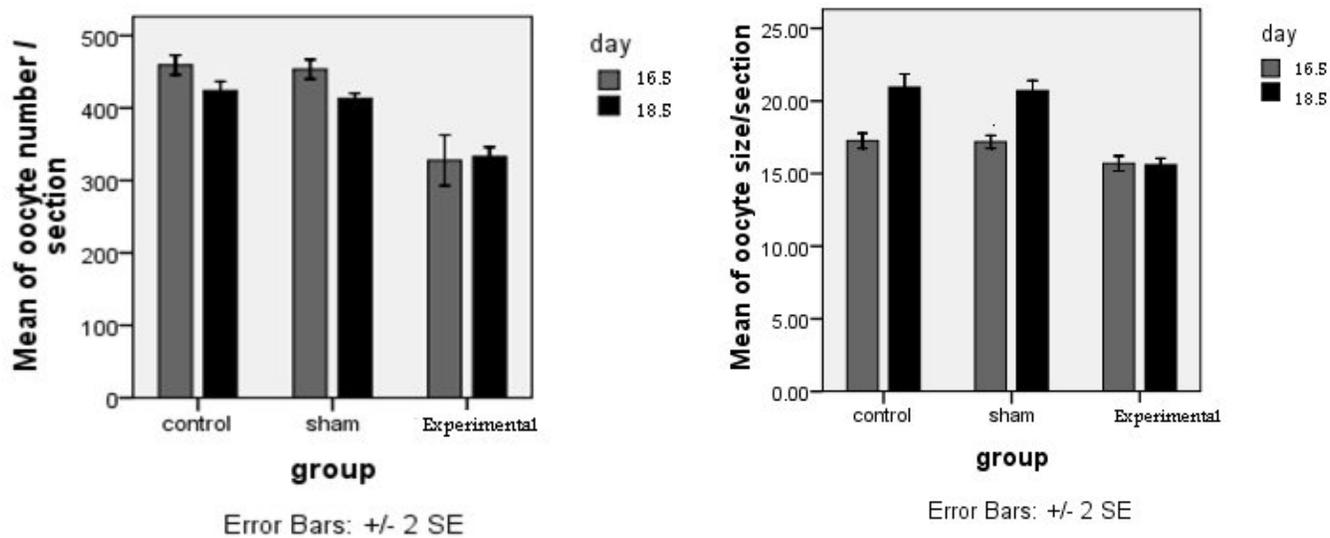


Fig.3: Repeated Measure test confirmed that the number and size of healthy oocytes in the ovaries of 16.5 and 18.5 dpc embryos of the mice of control and sham groups did not reveal any significant difference, but the numbers and size of healthy oocytes were significantly reduced in the ovaries of 16.5 and 18.5 dpc embryos of the mice of experimental groups ($P < 0.05$)