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Research Article

Teratogenic Effects of Two New Derivatives of *Quinazolinones* on Balb/C Mice Embryos and Newborns: A Literature Review

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Abstract: Teratogens are medicines or chemical factors which can affect various stages of organogenesis, change normal cell growth, induce abnormalities and create several defects. Therapeutic use of multiple drugs causes lots of concerns about injuries to the embryos/newborns. The aim of this study was to focus on the impact of two derivatives of *quinazolinones* including 4(3*H*)-*quinazolinone*-2-*propyl*-2-*phenylethyl*) (*QPPE*)and 4(*3H*)-*quinazolinone*-2-*ethyl*-2-*phenyl ethyl* (*QEPE*), as heterocyclic compounds with biological and pharmaceutical properties at different levels including morphological, skeletal, histological, cellular, biochemical and genetic aspects of embryos and newborns of *Balb/C mice* in crucial days of differentiations and organogenesis. These investigations have been done at the Faculty of Sciences (Developmental Biology, Animal Sciences), University of *Shahid-Beheshti*(*SBU*) in this field for many years. This literature review expresses and interprets the devastating effects of these compounds, such as abnormal and underdeveloped embryos, abnormal placenta, necrosis in many organs, splenomegaly, apoptosis in testes and ovary, deformed nucleoli, Damaged mitochondria, change in the level of alkaline phosphatase and many other injuries.

Keywords: Anomalies, embryo, mice, newborn, quinazolinone, teratogen

INTRODUCTION

Quinazolinones are water insoluble heterocyclic compounds with different pharmaceutical properties including analgesic, sedative, anti-tumor, anti-convulsive and anti-microbial benefits and also are effective drugs against cancer and *HIV* (Baek*et al*., 2004; Boumendjel*et al*., 2005; Buyuktimkin*et al*., 1992; Corbett *et al*., 2000; Connolly *et al*., 2005; Wolfe *et al*., 1990; Jatav*et al*., 2006; Pines *et al*., 2000).

Considering the role of *quinazolinones* as cholecystokinin receptor antagonists (*CCR-B, CCR-A*), these compounds are useful for anxiety treatment (Varnavas*et al*., 1996; Yu *et al*., 1992).

Historically, Scientists believed that the physical and chemical factors do not affect human embryos, because they used to consider placenta as an impermeable barrier against these factors and human fetuses (in uterus) were thought to be protected against their effects; but, Thalidomide tragedy put an end to this illusion (Greek *et al*., 2011).

Most developmental disorders occur in early pregnancy, whencellsare differentiatingand specializing to form different structures. Based on specific physiological reactions during pregnancy, therapeutic consumption of various drugs has caused lots of concerns regarding damages to the embryos/newborns (Sachdeva*et al*., 2009). Teratogens are drugs or chemical agents which can affect different stages of embryogenesis, induce abnormalities and impair growth and normal functions by creating various defects (Xing *et al*., 2015).

Chemical properties, doses, directions and mechanisms and mechanisms of influences on fetuses are all among the determining factors for identifying such substances as teratogens (Baily *et al*., 2005;Meschke*et al*., 2003; Polifka and Friedman, 2002).

Teratogens stop or alter the normal cell growth in one area, therefore change normal pattern of embryonic growth. Although the structural defects are clearly visible after delivery, many dysfunctions may not be found out even years after birth (Shams Lahijani and Aounegh, 2007). So far, many studies have been conducted to identify possible impact mechanisms of these materials in order to inform pregnant women about the risks of using such drugs (Sachdeva*et al*., 2009).

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Table 1: Days of *quinazolinones* injections and organs' extractions from *Balb/C mice*enmbryos.

Organ	Injection	Extract
Skeletal system	6th day of pregnancy	Embryo: 17th day of pregnancy
Kidney	Embryo: 10th day of pregnancy Newborn 8th day of pregnancy	Embryo: 17th day of pregnancy Newborn: 5 days after birth
Liver	Embryo: 10th day of pregnancy Newborn 8th day of pregnancy	Embryo: 17th day of pregnancy Newborn: 5 days after birth
Intestine	8th day of pregnancy	Newborn: 5 days after birth
Stomach	8th day of pregnancy	Newborn: 5 days after birth
Brain	8th day of pregnancy	Newborn: 4 days after birth
Spleen	Embryo: 13th day of pregnancy Newborn: 8th day of pregnancy	Embryo: 17th day of pregnancy Newborn: 4 and 5days after birth
Ovary	13.5th and 16.5th days of pregnancy	16.5th and 18.5th days of pregnancy
Testes	(13th day of pregnancy, the critical day for development of testes) and (16th day of pregnancy, when gonocytes enter dormant stage)	15th and 18th days of pregnancy

Due to the similarity between the genomes of some mammals such as mice, results can be generalized to humans too. Therefore, in these researches, *Balb/C mice* were used as a model. According to the *quinazolinones*' properties (such as tranquilizers, anti-microbial and effects against diabetes and prostate tumors, etc.) and because of little or no existing information about the effects of *quinazolinones* on embryos especially on critical days of embryogenesis (Shams Lahijani and Aounegh, 2007), investigation about the impact of two new *quinazolinones* combinations (*QEPE* and *QPPE*) on Pregnant mice started in 2006 (Dabiri*et al*., 2004; Shams Lahijani*et al*., 2006). Therefore, after administrating identified harmful doses (75 mg/kg and 100 mg/kg) (Shams Lahijani*et al*., 2006), destructive effects of them have been studied at different histopathological levels in mice organs (Amiri*et al*., 2012; Shams Lahijani*et al*., 2006; Shams Lahijani and Aounegh, 2007; Shams Lahijani and Estakhr, 2009; Shams Lahijani*et al*., 2009a, 2009b;Maryam*et al*., 2010, Shams Lahijani*et al*., 2010; Shams *et al*., 2011; Shams Lahijani*et al*., 2012a, 2012b).

MATERIALS AND METHODS

For this literature review, we carried out a literature search in Google Scholar and Pub Med, in addition to all researches done in (SBU) by previous researchers (Amiri*et al*., 2012; Shams Lahijani*et al*., 2006; Shams Lahijani and Aounegh, 2007; Shams Lahijaniand Estakhr, 2009; Shams Lahijani*et al*., 2009a, 2009b;Maryam*et al*., 2010, Shams Lahijani*et al*., 2010; Shams *et al*., 2011; Shams Lahijani*et al*., 2012a, 2012b) and electronic database collected for principal articles about this subject. Sources of selected articles were also studied.

It should be noted that all images presented in this study have been taken from the original papers in this field.

RESULTS AND DISCUSSION

In studies performed about *quinazolinone* derivatives (*QEPE and QPPE*), pregnant *Balb/C mice* had been divided into four groups as follow:

- Control groupswhich had received only distilled water (10 mg/kg/body weight, Intraperitoneally (IP)
- Sham groups which had received 10 mL/kg of methyl cellulose (0.05% solvent *quinazolinone)* (IP)

And treatment groups including:

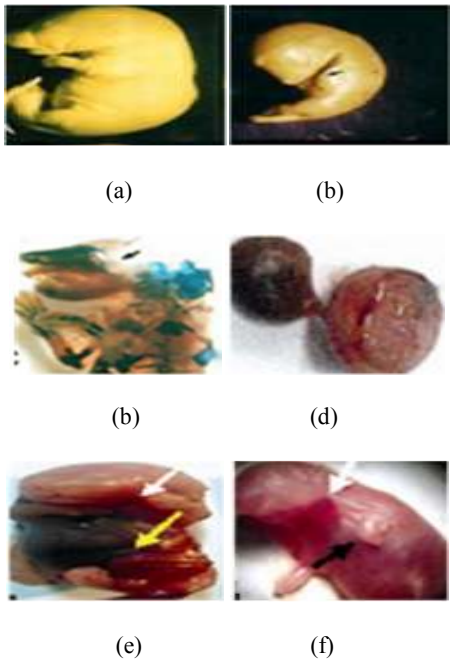
- *QEPE* groups that had received 75 mg and 100 mg/kg/body weight (IP).
- *QPPE* groups that had been treated with 75 mg and 100 mg/kg/body weight (IP).

Based on the purpose of each research, drug doses and effective days might be different, all of which have been mentioned in original articles (Table 1).

There were no malformations reported in sham groups (Amiri*et al*., 2012; Shams Lahijani*et al*., 2006; Shams Lahijani and Aounegh, 2007; Shams Lahijani and Estakhr, 2009; Shams Lahijani*et al*., 2009a, 2009b;Maryam*et al*., 2010;Shams Lahijani*et al*., 2010; Shams *et al*., 2011; Shams Lahijani*et al*., 2012a, 2012b). As a result, *QPPE* and *QEPE*, enter the systemic circulation after injection; cross the placenta (Perretti and Zilletti, 1969), then reach the embryo, cause morphological, skeletal, histological, biochemical and cellular abnormalities and inflammations comes after. Thus, considering the ability of these compounds to cross the placenta, the toxic effects of *quinazolinones* or their metabolites on the fetus is justified.

Researches in this field have demonstrated that *quinazolinones* and their metabolites are lipophilic compounds, such as *methaqualone* (a member of *quinazolinones* family), which passes through the cell membrane and probably interacts with aromatic hydrocarbon receptors (*AHR*) (*androgen*, *estrogen*, *thyroid hormone receptors*), so that it is transferred into nucleus as a complex with the receptor and activates different genes (Zaher *et al*., 1998; Peters and Wiley, 1995; Nebert*et al*., 1972).

After injecting 75 and 100 mg of *QPPE* and *QEPE* respectively on days 6-11, 13 and 16 of pregnancy (Table 1), morphological and multiple skeletal abnormalities (*exencephaly*, *exophthalmia*,



(a) (b)

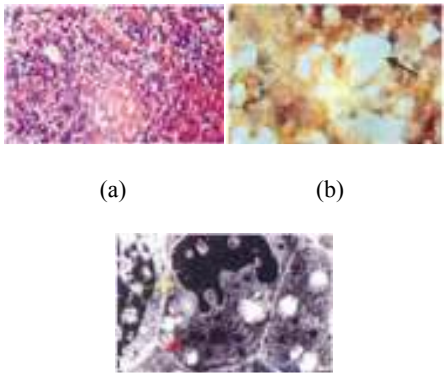
(b) (d)

(e) (f)

Fig. 1: Normal embryo (control group, A), underdeveloped embryo (B, black arrow), malformed parietal (B, black arrow), enlarged disfigured placentas (D), *gastroshesis* (E, yellow arrow), *meromelia* (F, black arrow) and very large swollen embryo with hemorrhages in the neck (F, white arrow) in *Balb/C mice* treated with *QEPE* and *QPPE* (Shams Lahijani andAounegh, 2007; Shams *et al*., 2011)

scaphocephaly and C-shaped embryo, *synductily*, *meromelia*) have been reported. Studies imply that injection after day 6 of pregnancy leads into abnormal and underdeveloped embryos, abnormal placenta (regarding diameter and weight), significantly reduced weights and lengths of fetuses and severe bleeding in the region of neck of the fetus (Fig. 1a to f) (Amiri*et al*., 2012; Shams Lahijani*et al*., 2006; Shams Lahijani and Aounegh, 2007; Shams Lahijani and Estakhr, 2009; Shams Lahijani*et al*., 2009a, 2009b;Maryam*et al*., 2010;Shams Lahijani*et al*., 2010; Shams *et al*.,2011; Shams Lahijani*et al*., 2012a, 2012b).

Since *quinazolinones* are known to inhibit normal function of some proteins such as tubulin and reverse transcriptase enzyme (by binding to specific sites), these compounds can act as antagonists to certain proteins and impair developmental processes (Corbett *et al*., 2000; Raffa *et al*., 2004). Regarding the role of thyroid hormones in fetus growth and development, negative impact of *QPPE* and *QEPE* on activity of this hormone may slowdown growth and embryonic development. On the other hand, due to the direct dependency of fetus on placenta, partial placenta in fetuses causes defects in fetal circulation and creates embryos with low weight (Shams Lahijani*et al*., 2009a).



(a) (b)

(c)

Fig. 2: Light microscope and TEM electromicrograph evaluations on hepatocytes of 17-days old embryos of *Balb/C mice* showed hemorrhages in mice livers treated with *QEPE* (A, 100X), disintegrated reticular fibers in hepatocytes treated with *QPPE* (B, black arrow, 400X), irregular and abnormal heterochromatins (C, yellow arrow), disintegrated abnormal round without cristae, enlarged swollen mitochondria (C, blue arrow, lipid droplets (C, red arrow, 1200X). H&E staining (A), reticulin staining (B) and TEM (C) (Shams *et al*., 2011)

Following observation of morphological abnormalities, further researches have been done to assess the damage inflicted by the two new derivatives of *quinazolinones* at the cellular and tissue level. Results are affirmative of the devastating effects of harmful doses of *QEPE*and *QEEP* on fetuses and infants' internal organs, which had even healthy appearance.

Histopathological and TEM electron microscopy examinations showed that internal organs of the fetuses and newborns were damaged in treatment groups, while the control and sham groups were all normal.

Liver: The effects of these two drugs on liver included reticular fibers disintegrations around hepatocytes, accumulation of fat droplets, steatosis, increase in the diameter of liver and the number of band cells, congestion, increased diameter of sinusoids and the diameter of central vein, bleeding, necrosis, myelin figures, disrupted cytoplasm, large and swollen mitochondria without clear cristas, autophagy, heterochromatin DNA, abnormal nuclei in hepatocytes of treated groups (Fig. 2a to c) (Shams Lahijani*et al*., 2009b; Shams *et al*., 2011).

A number of chemicals are as free radicals or produce free radicals during metabolism; since *quinazolinones* do not have chemically active groups, it seems that getting metabolized in liver and kidneys by cytochrome P450 creates harmful active metabolites (free radicals, or reactive oxygen molecules) which can be toxic for cells, via damaging cell membranes

orintracellular organelles such as mitochondria and endoplasmic reticulum (the main position of this enzyme), causing changes in their structures and protein, lipid oxidation and eventually necrosis (Arici*et al*., 2003; Morgan *et al*., 1984; Tzen*et al*., 2001).

Steatosis or accumulation of fat in hepatocytes occurs as the result of interruption in metabolism and movement of fats inside the cells, increased fat arrival from environmental sources, reduced transfer of fat toward out of the liver as lipoprotein particles (*VLDL*), decreased fatty acid oxidation and finally storage of triglycerides in the cells (Recknagel*et al*., 1973; Vankoningsloo*et al*., 2005). It is likely that *quinazolinones* cause oxidation and damage mitochondrial DNA by producing *ROS*, which is a consequence of metabolic disorders and increased concentrations of fatty acids in the hepatocytes.

Swollen mitochondria could be a symptom of injured mitochondria and organelles accumulation around the nuclei has also been observed; on the other hand, by reduction in the amount of ATP (energy resources), activation of proteases, inflammation and ruptured cell membranes would occur (necrosis) (Muriel, 2009;Nuño-Lámbarri*et al*., 2016). Images of treated groups taken by electron microscopy are evidences of the toxicity of these drugs and destruction of organelles within cells.

Moreover increase in the number of band cells, not only happens at the time of infection, but also due to inflammation, tissue damage, necrosis, metabolic disorders, bleeding, hemolysis and the effects of drugs (Kodali*et al*., 2006).

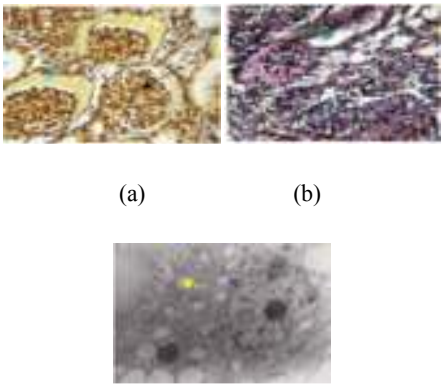
Myelin figures which are rounded and dense with concentric layers arise by taking drugs, effects of hormones or metabolic and infectious diseases as structures surrounding damaged organelles such as destroyed mitochondria and their remnants or autophagy vacuoles (Castejón, 2008).

Autophagy is a mechanism by which the cells get rid of damaged excess organelles with creating two-layered structures around them. Because of the destruction of organelles in treated groups, cells begin to self-eat (autophagy) damaged organelles (Maiuri*et al*., 2007).

Hepatic macrophages secrete cytokines such as *TNF-α,interleukin-6* (*IL-6*) and *ROS* intermediates which are toxic for hepatocytes and injure liver and cause inflammation (Wu and Cederbaum, 2003).

Liver is an active center of hematopoiesis in the fetal period, hence because of increased number of band cells and hyperemia in the sinusoids, it can be said that the elevation in hematopoietic activity of liver has caused an increase in the amount of alkaline phosphatase in the treatment groups (Gaskill *et al*., 2005).

Kidney: Increase in the diameter of glomeruli, proximal tubule lumen Bowman's capsule,



(a) (b)

(c)

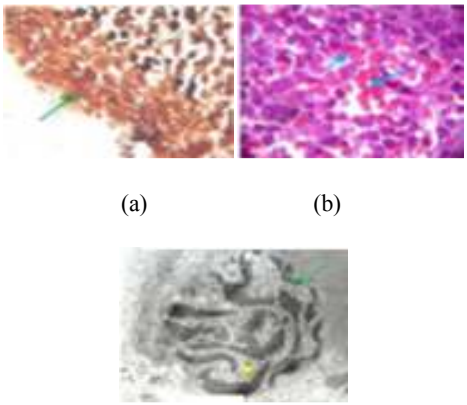
Fig. 3: Light microscopic and TEM studies on kidneys in 17-day old *Balb/C mice* embryos. Accumulation of proteins in renal spaces (A, blue arrows, 400X), damaged convoluted distal tubules (B, green arrows, 400X), increase in glomeruli diameters (B, black arrows, 400X), large vesicles on the surface of epithelial cells (C, yellow arrow) and deformed nucleus (C, purple arrow) were observed. Reticulin staining (A), jones staining (B), TEM (C) (Shams Lahijani*et al*., 2009b).

accumulation of proteins in convoluted proximal tubules, damaged convoluted proximal tubules cells, fragmented epithelial cells, necrosis, damaged mitochondria and autophagy were observed (Fig. 3a to c) (Shams Lahijani*et al*., 2009a, 2009b).

Depending on the types of damages, reactions were different in various parts of the kidney (such as reduction or increase in the volume of glomeruli in different parts of the kidney tissue). These compounds affect capillaries and result in reduced glomerular filtration in addition to small glomeruli (Moritz *et al*., 2003; Shams Lahijani*et al*., 2009a). On the other hand, due to the accumulation of proteins in the kidneys, it can be noted that *quinazolinones* may have effect on Podocin function, which consequently destroys podocytes, creating large vacuoles and causing leakage of proteins in the kidneys.

Heart: Necrosis and increased connective tissue were seen in the heart. These two compounds of *quinazolinones* caused myocyte necrosis and replacement of heart cells by connective tissues (Cohen-Gould *et al*., 1987).

Intestine: Reduced length of villi in proximal, middle and distal parts of small intestine and reduced deep crypts in the proximal part of the small intestine have been observed (Maryam*et al*., 2010). By damaging and decreasing the cell divisions in all parts of small intestine, quinazolinones caused reduction in the length of villi and nutrients absorption (Van Griekenet *al*., 2003).



(a) (b)

(c)

Fig. 4: Light microscopic and TEM electromicrograph studies on the spleens of 17-days old *Balb/C mice* embryos. Increase in the thickness of spleens' capsules treated with *QEPE* (A, green arrow, 400X), subcutaneous hemorrhages and polymorphonuclear cells treated with *QPPE* (B, blue arrow, 400X) (C, green arrow, 12000x) and fractured cells with damaged nuclei (C, green arrow, cells, yellow arrow, nucleus, 12000x) were observed (Maryam*et al*., 2010)

Stomach: Accumulation of the free radicals formed by *QEPE* and *QPPE* caused damages to the mucous tissues (necrosis, atrophy and reduced thickness of mucosal layer and *hyperaemia*), inflammatory response, followed by increasing apoptosis in the lining of the stomach surface (Bayiroğlu*et al*., 2009; Shams Lahijani*et al*., 2010).

Brain: Increase in the diameter of microglia, abnormal myelin sheet and increased number of astrocytes in the cortex and medulla have been reported (Shams Lahijani*et al*., 2010). Astrocytes play role in the exchange of substances and nutrients between blood and nerve cells. Also, they can respond to hormones and environmental stimuli because of having receptors. They absorb excess neurotransmitters and are involved in neuronal survival through neurotrophic factors. When the central nervous system is damaged, astrocytes proliferate and create scar tissue. So, astrocyte hyperplasia occurs in response to these drugs (*QPPE* and *QEPE*), in order to eliminate excitotoxins (Dihné*et al*., 2001; Hailer *et al*., 2001).

Spleen: An increase in the size of spleen compared to the control groups, thickening of the capsule of spleen, bleeding beneath skin and elevated number of cells (macrophages, monocytes, neutrophils, eosinophils and megakaryocytes) have been reported (Fig. 4a to c) (Shams Lahijaniand Estakhr, 2009;Maryam*et al*., 2010;Shams Lahijani*et al*., 2010).

Compounds with toxic properties cause accumulation of iron in the spleen, poisoning through iron-mediated free radicals which leads into hyperplasia, extramedullary hematopoiesis and increase in fibrous tissues (capsule) (Fujitani*et al*., 2004).

Based on mitochondrial damage and free radical formations by *QPPE* and *QEPE*, these effects are justified. Mitogenic factors increased the amount of alkaline phosphatase in spleen cells under the influence of these two drugs and elevated the number of blood cells in spleen. Consequently, an increase occurred in the level of alkaline phosphatase enzyme, which can be considered as a kind of defense against these combinations (Koyama *et al*., 2002).

Liver and heart diseases accompanied by portal hypertension, are major factors for increase in capsule thickness, matrix contents and blood cells in spleen; considering the heart failure and liver injury in the treated groups, results are acceptable (Borojevic, 1987; Wanless and Bernier, 1983).

Ovary: According to the previous studies about women infertilities after exposure to toxic substances, reduced fertility rate, ovarian dysfunction, reduction in diameters of oocytes at different stages of follicular growth have happened (Neal *et al*., 2007; Paixão*et al*., 2012). Naturally, most cell death in mice primordial germ cells/oogonia and oocytes occurred on days 12-13 postcoitum (*dpc*) and also at *zygotene/pachytene* stages from 16 dpc (Bakken and McClanahan, 1978). Also, defects in meiotic recombination and loss of growth factors have been reported to increase and accelerate the death ofoocytes (Matikainen*et al*., 2002; Morita*et al*., 2001).

Based on reports, fetal ovaries of control and sham groups were normal, contained healthy oocytes with round normal clear nuclei, uniform cytoplasm and recognizable spherical plasma membranes. Moreover, very few apoptotic oocytes have been observed (Amiri*et al*., 2012; Shams Lahijani*et al*., 2012a).

In the *QEPE* treated groups, reduction in the number and size of healthy oocytes, basophilic (dark) and dense oocytes nuclei and the volume of the cytoplasm, wrinkled plasma membranes, increase in the number of apoptotic oocytes compared to the number of apoptotic oocytes in control and sham groups in the ovaries were observed (Amiri*et al*., 2012; Shams Lahijani*et al*.,2012a).

Previous research implies that *Bax* gene is a target for *AHR* in fetal oocytes and *Caspase 2* gene plays the major role in female germ cells death (Matikainen*et al*., 2002; Morita*et al*., 2001). Actually, *Caspase 2* null oocytes flunk to undergo apoptosis. Furthermore, inclusion or deletion of *exon 9* results in formation of two splicing isoforms with antagonistic functions in cell death, one of which is *Caspase-2L* (transcript mRNA, promoting apoptosis) and the other is *Caspase-2S*(transcript mRNA, inhibits apoptosis) (Kumar *et al*., 1997; Wang *et al*., 1994).

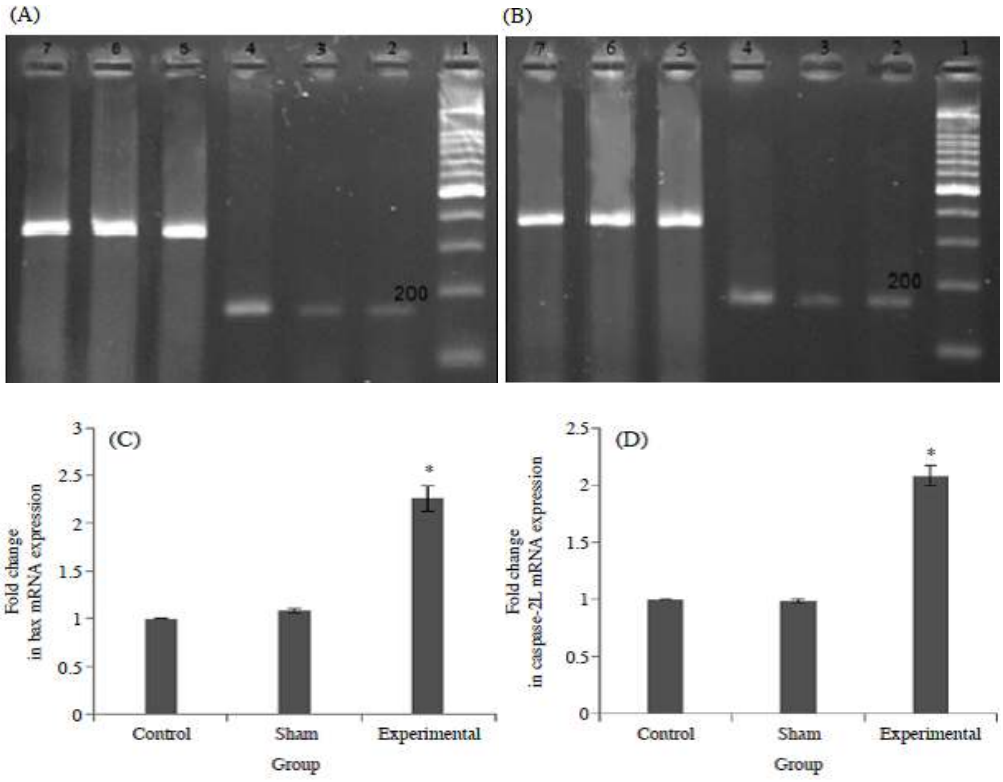


Fig. 5: mRNA levels of *Bax* (A) and *Caspase-2L* (B) in the embryonic ovaries of 18.5 days old *Balb/C mice* measured by RT-PCR. No changes were observed in the *β-actin* level as housekeeping gene in the ovaries of 18.5 days old embryos of treated groups, control and sham (wells: 5-7), Increase in the expression of *Bax*and *Caspase-2L* mRNAs in the embryonic ovaries treated with *QEPE* (well: 4), compared with control and sham groups (wells: 2, 3) were observed, DNA ladder (well: 1) (Shams *et al*., 2011)

According to the results obtained in this research, some *quinazolinones* which have anti-cancer activity induce apoptosis in cell lines accompanied by changes in the expression of some genes (pro-and anti-apoptotic genes). Thus, it can be concluded that by increasing the expressions of *Bax* gene and *Caspase-2L*, *QEEP* caused apoptosis and reduced the number of oocytes (Fig. 5) (Shams Lahijani*et al*., 2012a).

Testes: During the process of development in male *Balb/C mice* testicular germ cells, apoptosis is the mechanism for removing abnormal spermatogenic cells and maintaining a proper ratio between Sertoli and mature germ cells (Print and Lovel, 2000).

In addition, toxins can enhance apoptosis and testicular atrophy, hence apoptosis is the current way for cell death in normal and damaged testes. To our knowledge, apoptosis occurs in two ways of mitochondrial (internal) and external pathways, through ligands such as *TRIR*,*FasL*, *TNF* in one cell and affecting their receptors (*DR5*,*Fas*, *TNF*R) located on other cell (Lin*et al*., 2010; Locksley*et al*., 2001).

Binding of ligand to receptor activates *Caspase* cascade via *Caspase* 8 or 10and initiates apoptosis process. Additionally, proteins such as inhibitors of apoptosis proteins (*IAP*)and cellular *FLIC* inhibitoryprotein (*c-Flip*) act as inhibitors of apoptotic pathway (Ashkenazi and Dixit, 1998; Perlman*et al*., 1999).

Researchers have demonstrated that *Fas/FasL* genes are involved in the regulation of apoptosis in germ cells in various conditions. Fas expression has been detected in both Sertoli and germ cells and *FasL* expression in Sertoli and Leydig cells (Giampietri*et al*., 2006).

Investigations have shown that *QEPE* results in reduced diameter and irregular shapes of seminiferous tubules in the treated groups, compared with sham and control groups. Moreover, the effect of this compound leads into reduction in the number of Leydig cells and gonocytes and increases apoptosis, especially in day 15 compared to the group treated in day 18. Increased apoptosis in day 15 coincides with the manifestation of proliferation in gonocytes, while in day 18, gonocytes proliferation decreases because of entering a period of stagnation. Actually, apoptosis increase happens after the high expression of *Fas/FasL* genes and decreased expression of *C-Flip* in fetal testes of treated groups (Shams Lahijani*et al*., 2012b).

CONCLUSION

These new derivatives of *quinazolinones* create several morphological, histopathological and

cellulardisorders and abnormalities (as mentioned in the result section) in embryos and newborn *Balb/C mice*, imposing their teratogenic effects on internal organs. Therefore, using these compounds during pregnancy period causes adverse and irreparable effects. This research and similar studies might help pharmacists and physicians about the risks and benefits of various drugs during pregnancy, so that the patients will be protected against the harmful effects of detrimental substances.

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