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Original article

Effects of Quinazolinones on the Development of BALB/c Mice Embryonic Kidneys

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Abstract

Objective(s)
Quinazolinones are heterocyclic components (able to form cyclized compounds) which have several medical effects such as anti-malarial, spasmolytic, anti-microbial, sedative, etc. They are also known for their fungicidal properties, inhibition of tyrosine-kinase and DNA repair enzyme poly (ADP-ribose) polymerase (PARP) and are also effective in treatment of cancer, diabetes, and parkinsonism complications.

Materials and Methods
In this study, for the first time different aspects of developmental effects of two new Quinazolinone components (QPPE and QEPE), on kidneys of BALB/c mice embryos were investigated. Pregnant BALB/c mice were divided into four groups of control (n=30), sham (n=30), experimental 1 (n=30) and experimental 2 (n=30). Control mice remained intact, sham and two experimental groups received 0.05% methyl cellulose and 100 mg/kg/body weight (most effective dose) of QPPE and QEPE, intraperitoneally (IP), on day 10th of gestation. Kidneys were removed by c-sections, stained with H&E, PAS, trichrome, reticholin and jones staining. Some embryonic kidneys were prepared for measurements of level of alkaline phosphatase and TEM studies.

Results
Light and TEM microscopes, and level of enzyme surveys demonstrated that QPPE and QEPE are toxic components, creating protrusions at the surface of convoluted proximal tubules, protein casts, renal necrotic cells, pseudothyroidezation, mitochondria degeneration, hyperemia, glomeruli hypertrophy, widening of renal spaces, vacuolization, as well as decrease in the number of brush border villi and level of alkaline phosphatase.

Conclusion
By being teratogens and toxins, these two new derivatives affected development of embryonic kidneys at histological, biochemical and intracellular levels; QEPE had more effects and convoluted proximal tubules were more sensitive than convoluted distal tubules.

Keywords: BALB/c Mice Embryos, Kidneys, Quinazolinones, Toxicogenesis

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Introduction

Different teratogens and toxins have different developmental effects on different species with different severity (1, 2). By passing through placenta (3, 4), treatments with teratogens such as alcohol, quinazolinones, etc create early death, malformations and irregularities in different parts of developing embryos (5-11).

Quinazolinones are heterocyclic components with various characteristics; such as: anti-inflammation, anti-malaria, anti-spasm, anti-microbial, anti-hypertensive, anti-allergic, sedative, anti-tuberculosis, anti-hyperlipidemic, anxiolytic, analgesic, anti-convulsant, as well as hypnotic activities. They are also known for their fungicidal properties, inhibition of tyrosine-kinase (involved in tubulin and 8-hydroxy-2-methyl quinazolinone polymerization), DNA repair enzyme poly (ADP-ribose) polymerase (PARP), and hhs signalling pathways. They are also effective in treatment of osteoarthritis, cancer, diabetes and parkinsonism complications (12-15).

Based on few other reports about specific properties of quinazolinones, and our previous (for the first time) observations of abnormalities at morphological, skeletal and histological levels, in this study we evaluated the effects of intraperitoneal (IP) injections of two new derivatives of quinazolinones: QPPE and QEPE (16), on the level of alkaline phosphatase (which is active during embryonic development) (17-22), histology (using different staining methods), and intracellular structures (TEM) of one of the most sensitive organs of BALB/c mice embryos: kidneys (which different aspects of its abnormalities have been studied in adult organisms) (17-37).

Materials and Methods

3-4 months old BALB/c mice (38) were originally obtained from Pars Company (Tehran, Iran). Random breading was implemented in our local facility. They were housed in room temperature (24±1 ˚C, 50±0.5% humidity) and light controlled room (12 hr light-dark), provided with lab chow and tap water. After overnight mating of virgin females (about 30 g) with males, those with vaginal plugs were considered to be on day 0 of pregnancy.

Pregnant BALB/c mice of experimental groups 1 (n=30) and 2 (n=30), received 100 mg/kg/body weight of QPPE and QEPE, synthesized at Department of Chemistry, University of Shahid-Beheshti, Tehran, Iran (28), intraperitoneally (IP), on day 10 of pregnancy (6-8). Sham (n=30) and control groups (n=30) were injected with 10 ml/kg/body weight of 0.05% of methyl cellulose (the solvent) (6-8), and distilled water (10 ml/kg/body weight), respectively. Effects of these two new components (QPPE and QEPE) on the level of alkaline phosphatase (using spectrophotometer), histology (with H&E, and reticholin, jones, PAS and trichrome staining methods to prove if there were any precipitation or increase in connective tissues and making reticulin stroma distiguishable), and intracellular structures of kidneys of randomly chosen normal and abnormal 17-day old embryos of pregnant BALB/c mice of different groups were studied by light and transmission electron microscopes (Center of Electron Microscopy, Medical School, Shahid-Beheshti University) (23).

Data were analyzed with statistical packages for social sciences (SPSS, version 15). Histograms were drawn with excel software. Level of significant difference was considered meaningful at $P<0.05$.

Results

Morphological surveys of randomly chosen 17-day old embryos showed abnormal and underdeveloped embryos (Figure 1A, B), normal (Figure 1C) and abnormal placentas (Figure 1D), and severe hemorrhages in the neck regions of QEPE treated mice embryos (Figure 1E). An embryo with one kidney on the right side was also observed (Figure 1F).

Analysis of parametric data showed significant differences between diameters (Histogram1) and weights of placentas (Histogram 2), significant decrease in weights (Histogram 3)and lenghts of embryos (Histogram 4), significant differences between diameters of Bowman's capsule (widening of renal spaces, Histogram 5) and their glomeruli

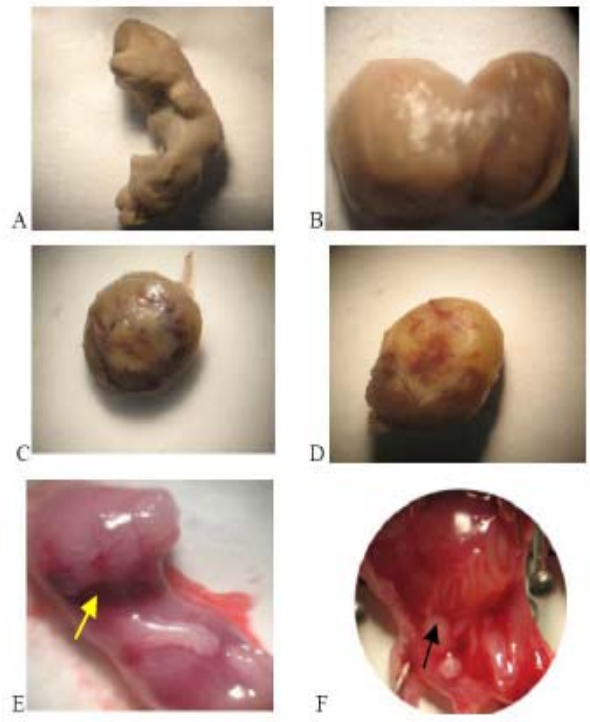
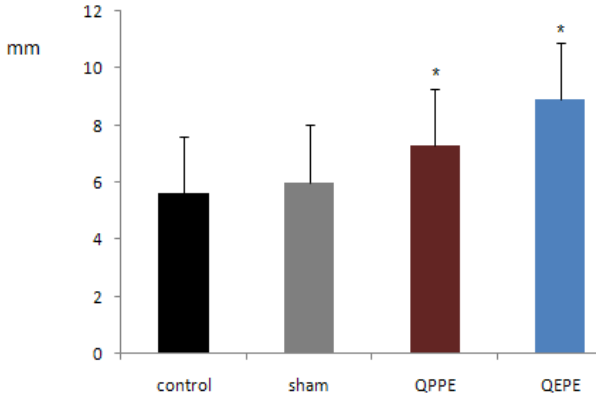
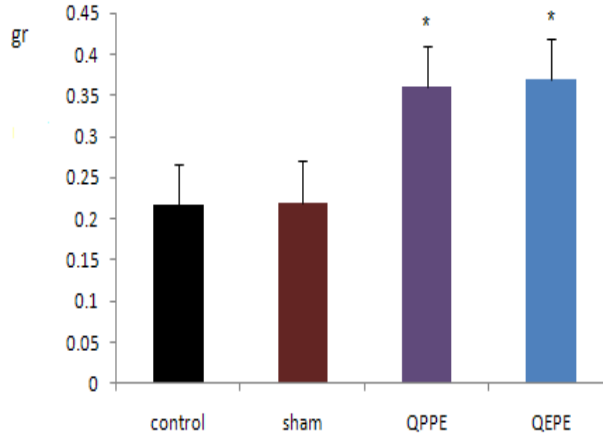


Figure 1. Demonstrating abnormal and underdeveloped embryos (A, B), normal placenta (C), abnormal placenta (D), severe hemorrhage in the neck region of an embryo (E, yellow arrow), and single kidney on the right side of an embryo (F, black arrow) of BALB/c mice treated with QEPE (400×).

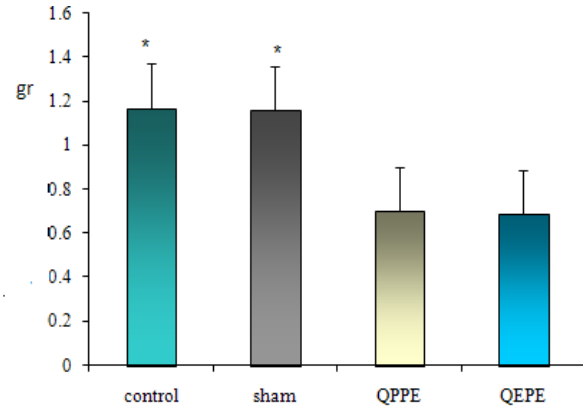
(Histogram 6), in groups treated with QPPE and QEPE (P<0.05), comparing with control and sham groups, while there were no significant differences amongst diameters of kidneys and the glomeruli of QPPE and QEPE treated groups.



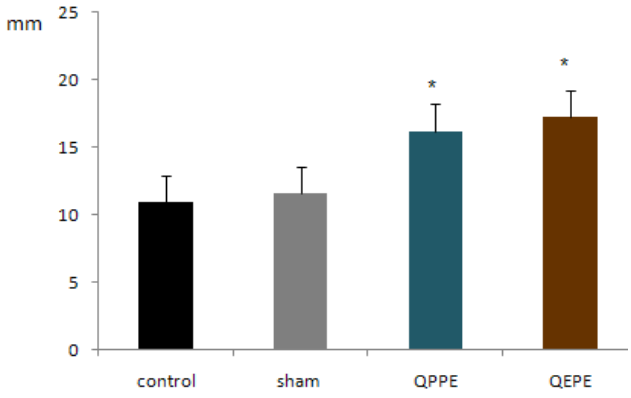
Histogram 1. Comparison between diameters of placentas of 17-day old embryos of BALB/c mice of control, sham, QEPE and QPPE treated groups, with significant differences between diameters of treated groups, comparing with sham and control groups. QEPE's effect was more significant ($^{*}P$<0.05).



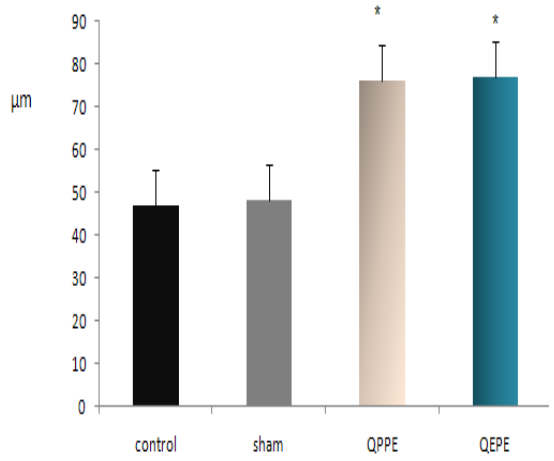
Histogram 2. Comparison of average weights of placentas of 17 day old embryos of BALB/c mice of control, sham, QEPE and QPPE treated groups, with significant differences between treated, sham and control groups. QEPE's effect was more significant ($^{*}P$<0.05).



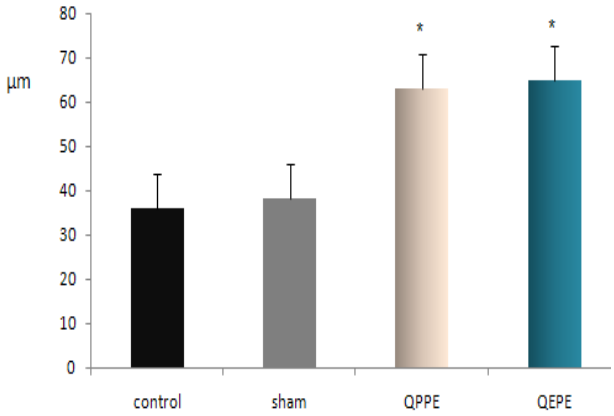
Histogram 3. Comparison of average weights of 17 day old embryos of BALB/c mice of control, sham, QEPE and QPPE treated groups, with significant decrease in weights of treated, sham and control groups. QEPE's effect was more significant ($^{*}P$<0.05).



Histogram 4. Comparison of average lenghts of 17 day old embryos of BALB/c mice of control, sham, QEPE and QPPE treated groups, with significant decrease between weights of treated, sham and control groups. QEPE's effect was more significant ($^{*}P$<0.05).



Histogram 5. Comparison of average diameters of Bowman's capsule (renal spaces) of 17 day old embryos of BALB/c mice of control, sham, QEPE and QPPE treated groups, with significant differences between diameters of renal spaces of treated, sham and control groups. QEPE's effect was more significant ($^{*}P<0.05$).

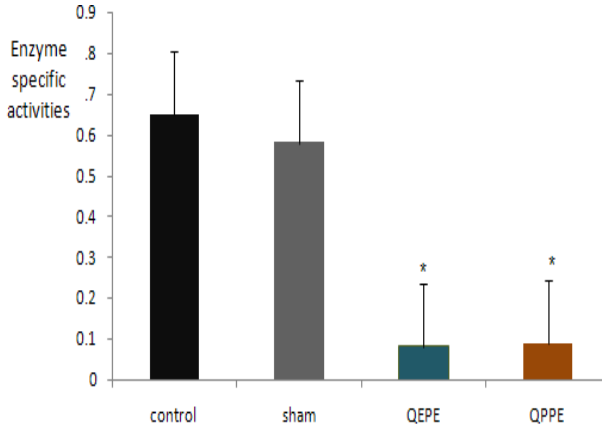


Histogram 6. Comparison of average diameters of glomeruli of kidneys of 17 day old embryos of BALB/c mice of control, sham, QEPE and QPPE treated groups, with significant differences between diameters of glomeruli of treated, sham and control groups. QEPE's effect was more significant ($^{*}P<0.05$).

Hematoxylin and eosin staining showed no changes in kidneys of 17-day old BALB/c embryos of control and sham groups (Figure 2A, B), but demonstrated accumulation of protein casts in convoluted proximal tubules (Figure 2, blue arrows), widening of renal spaces (Figure 2C, D), increase in diameters of glomeruli (Figure 2, black arrows), vacuolization of parietal layer of Bowman's capsule (Figure 2, grey arrows), damages in convoluted proximal tubules cells (Figure 2C, F green arrows) of kidneys of 17-day old BALB/c embryos of treated mice.

PAS (Figure 3 A, B), trichrome (Figure 3C, E), reticulin (Figure 3F) and jones (Figure 3G, H) stainings confirmed the observations by hematoxylin and eosin staining (Figure 2C, F).

There were reductions in the level of alkaline phosphatse in QEPE and QPPE groups compared to sham and control groups ($P<0.05$), while there was no significant difference amongst experimental groups (Histogram 7).



Histogram 7. Comparisons of average level of activities of alkaline phosphatae of kidneys of 17-day old embryos of BALB/c mice of control,sham and treated groups. Reductions in the levels of alkaline phosphatse in QEPE and QPPE treated groups, comparing with sham and control groups occurred. QEPE effect was significantly different in four groups ($^{*}P<0.05$).

TEM investigations of tubules of 17-day old embryos of BALB/c mice showed mass of prutrusions from convoluted proximal tubules, fragmented cells, debris, decrease in the number and size of microvilli, and abnormal large vesicles in degenerated cells (Figure 4A-M).

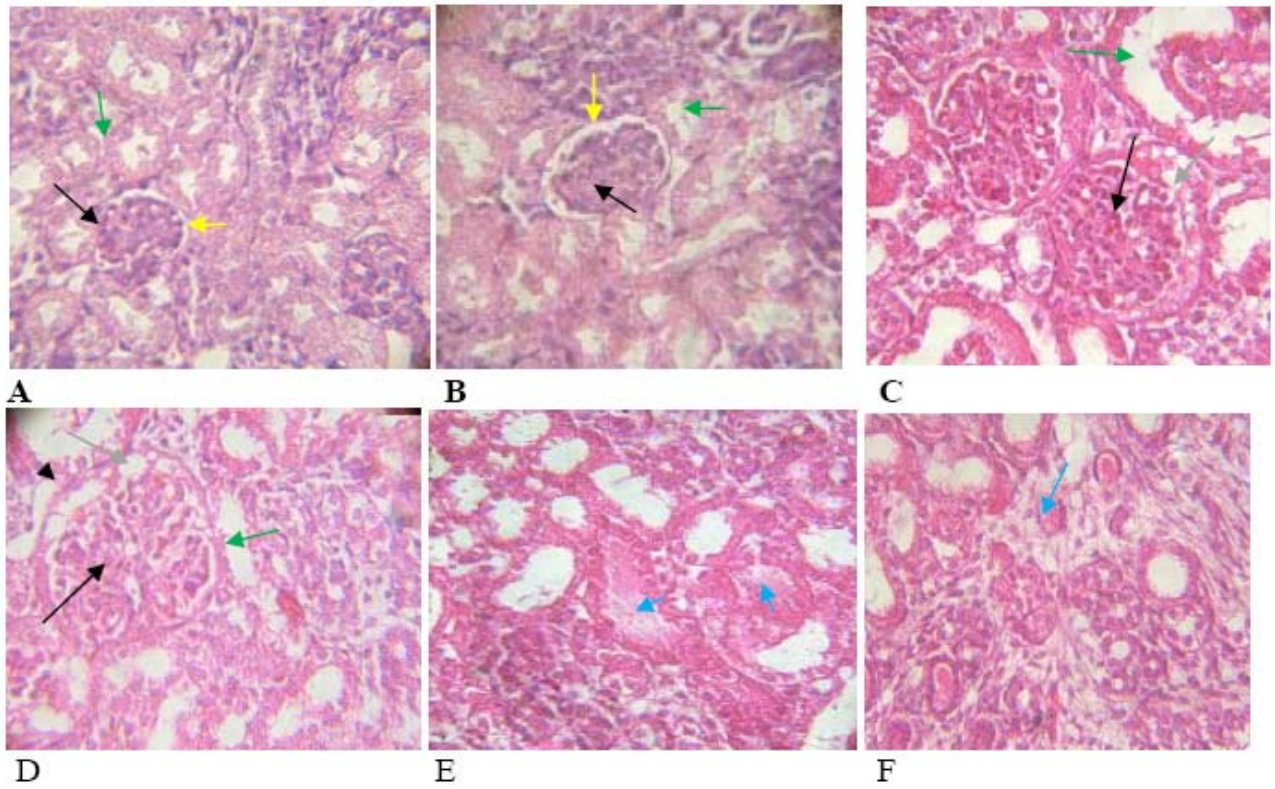


Figure 2. No changes was found in the kidneys of 17-day old embryos of BALB/c mice of control and sham groups (A, B), but an increase in diameters of glomeruli (black arrows, C), vacuolization of parietal layer of Bowman's capsule (grey arrows, C, D), damages of convoluted proximal tubules cells (green arrows, C, D), accumulation of protein in convoluted proximal tubules (blue arrows, E) and Henle loops (blue arrow, F) of kidneys of 17-day old embryos of BALB/c mice treated with QEPE and QPPE was noticed (H&E staining, 400×). Yellow arrows are Bowman's capsules.

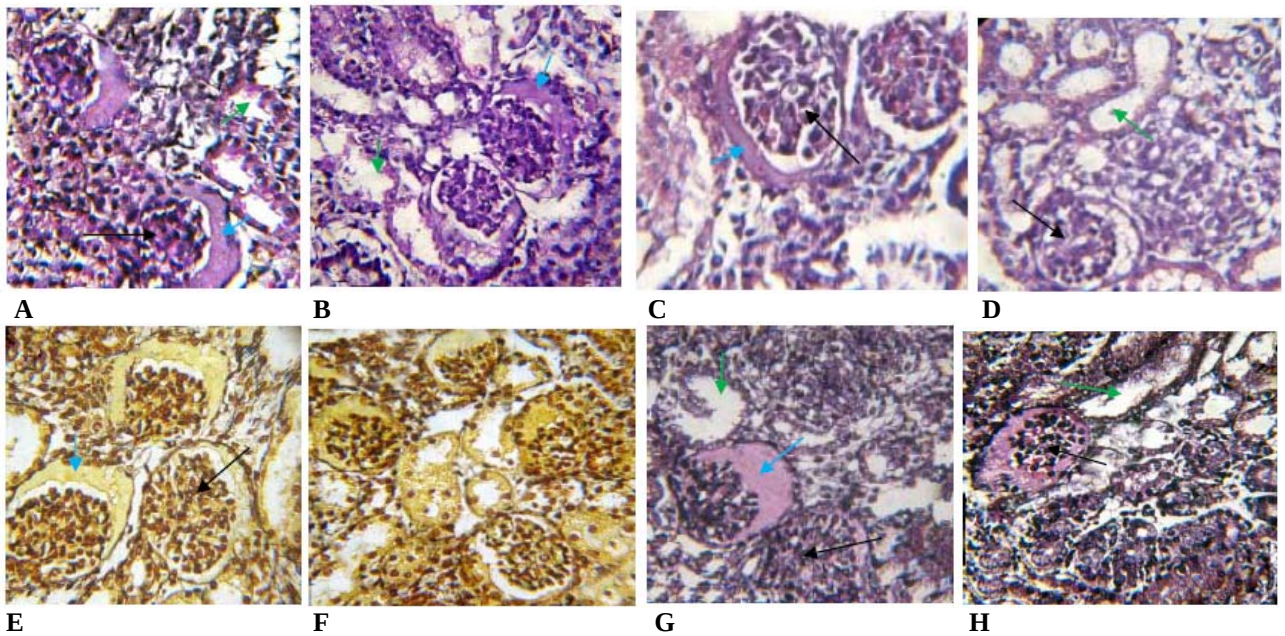


Figure 3. Kidneys of 17-day old embryos of BALB/c mice treated with QEPE and QPPE, with increase in glomeruli diameters (black arrows), vacuolized parietal layer (grey arrows) and protein accumulation in renal spaces (blue arrows), damaged convoluted distal tubules (green arrows), after staining with PAS (A, B), trichrome (C, D), reticulin (E, F), and jones (G, H), confirming the results demonstrated by H&E staining (Figure 2 C, F) (400×). Green arrows are proximal tubules.

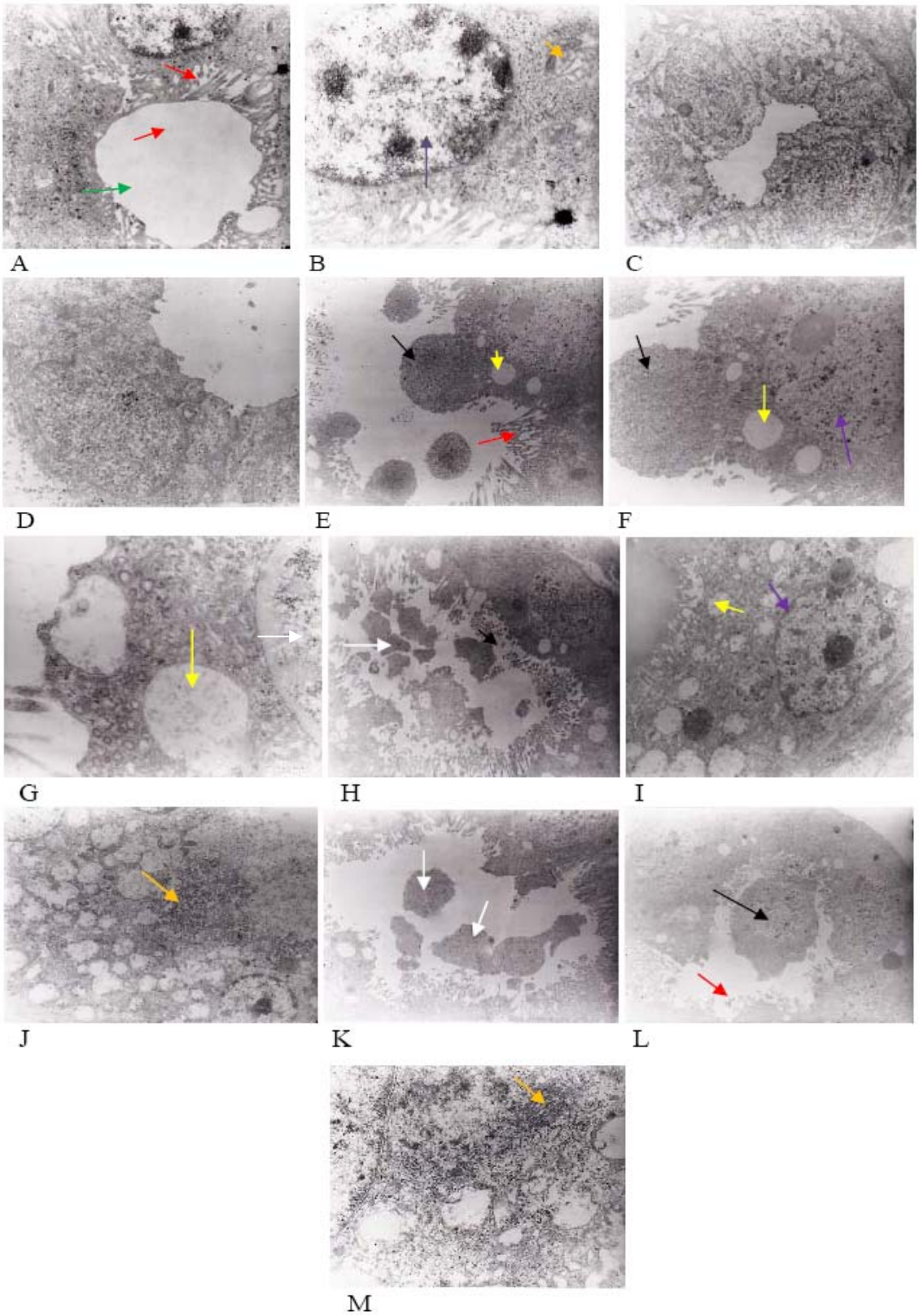


Figure 4. Electromicrograph of convoluted proximal tubules (green arrow) of normal kidneys of 17-day old embryos of BALB/c mice of control groups with normal brush border microvilli (A, red arrow, 7000×), nucleus (purple arrow), mitochondria (B, orange arrow, 12000×), and convoluted distal tubules (C, 3000×, D, 7000×). Convoluted proximal tubules of kidneys of 17-day old embryos of BALB/c mice treated with QEPE had prutrusions (black arrow) on the surface and large vesicles (E, yellow arrow, 4400×, F, 7000×, G, 2000×), fragmented epithelial cells (white arrow) and damaged mitochondria (H, red arrow, I, 4000×, J, 12000×). Convoluted proximal tubules of kidneys of 17-day old embryos of BALB/c mice treated with QPPE had protrusions (K, black arrow, 3000×) inside the tubules (L, 3000×) and degenerated mitochondria (M, orange arrow, 12000×).

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.

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' brains, livers, intestines, kidneys, stomachs, hearts and spleens morphological and histological structures, although some may have normal appearances.

Kidneys of embryos of control and sham groups, showed no malformations, proved that quinazolinones were the reason for abnormailities created in treated mice embryos and their organs. Underlying mechanisms of this effect are not understood yet.

Quinazolinones are lipophilic components which can pass through cell membrane and interact with cytosolic and nuclear receptors. On the other hand, thyroid hormones are involved in embryonic development and quinazolinones have interactions with their receptors, decreasing their activities which can slow down the embryonic growth (underdeveloped embryos).

By using these kinds of drugs, amounts of embryonic proteins would decrease, producing smaller and lighter embryos, which confirm our results. Meanwhile, direct dependence of embryos to placentas on one side, having malformed placentas on the other side, would disturbe blood circulations, creating embryos with lighter weights, since glucuronosyltransferase, sulfotransferase and CYP_{450} metabolise varieties of drugs and chemicals.

It is shown that although kidneys, as a whole, are the most sensitive organs to toxins, glomeruli, convoluted proximal and distal tubules, interstitial tissues and blood vessels are the most reactive parts. They are active in detoxications and excretions, and are the best samples for investigating histological, biochemical and intracellular changes affected by these components.

Quite a few factors, such as increase in glucocorticoid, decrease of vitamins, malnutrition, etc would produce abnormal kidneys after treatment of mother with quinazolinones. By affecting sulphydril groups, protein structure disrupts with resulting effects on catalytic, regulatory and binding sites of macromolecules. Inhibition of protein activities such as proteins of anti-protease system of kidney cells creates necrosis.

Changes in size of glomeruli of damaged kidneys (hypertrophic changes, increase in glomeruli volume, mesengial tissue, capillaries' diameters of filtration part) have also occurred. By considering the kind of damages, reactions will be different (decrease or increase of glomeruli volumes). In some cases, compensation of damages and hypertrophic changes are quite common processes.

Drugs in blood stream, affect capillaries, and decrease glomeruli filtrations. Reaction is the formation of atrophied or smaller glomeruli. On the other hand, contractions of contractive filaments, affect function of mesengial cells, and after a period of time hypertrophic glomeruli by proliferation and stimulation of angiotansin II will ensue.

Proteinuria also damages glomeruli. While podocin is necessity for filtration, absence of podocin will create large vacuoles in podocytes. So, by affecting podocin, quinazolinones facilitate infiltration of proteins into renal spaces, and damaged podocytes will be the result. Nephrin expression also increases renal spaces.

Cell death (necrosis and apoptosis) in epithelial cells of convoluted proximal and distal tubules demonstrated that convoluted proximal tubules (immediately after glomeruli) are more sensitive to teratogens. Swellings and changes in nuclei are the beginning of necrosis, which happened more often in

kidneys of embryos of QEPE treated BALB/c mice. In contrast, apoptosis happens in sickness and normal conditions, while necrosis appears when the cells are severely damaged.

Necrosis, an inactive process, is the consequence of ATP declines with disruption of ionic channels and inbalances. As a result, cytoskeleton ruptures, membrane swellings, prutrusions and disrutions occur.

Quinazolinones lack active chemical group. By metabolization (in kidney) and production of active metabolites and free oxygen radicals cell membrane and organelles such as mitochondria and peroxisom will be damaged and necrosis will appear in renal tubule cells.

On the other hand, filtration by glomeruli, and transformation processes and biosynthetic reactions in tubules epithelial cells require energy. Mitochondria provide 90% of the energy and it is the first organelle to produce ATP in kidney cells.

Toxins damaging mitochondria, inhibit chain of electron complex, oxidative phophorylation, transcription duplication and DNA translation. Disruption of Ca^{+2} heomostasis, creates necrosis with mitochondria playing an important role. Any change in this organelle increases Ca^{+2} ion and necrosis will happen.

Mitochondria were damaged as illustrated in TEMs, and it is possible that quinazolinones were the reason why level of ATP declined, and processes dependent on energy in kidney were disrupted. Meanwhile, convoluted proximal tubules absorb toxins through anionic transporters, inhibit protein synthesis, disrupt energy production by mitochondria, and chang the cells (nephropathy), which is why the cell death happened in convoluted proximal tubules.

Regarding the results of TEMs and disrupted mitochondia with destroyed cristae, there is a high possibilty that these two new components have nephropathy and nephrotoxic effects. As it happened in mice treated with ochratoxin A(84), brush border villi of convoluted proximal tubules' epithelium were damaged severely. It seems that damaged mitochondria are the major causes.

There are large amounts of alkaline phosphatase in kidney's cortex, which change in different experiments, visible not only in convoluted proximal tubule cells but also in their nuclei. Level of this enzyme decreases in necrotic cells, and its activity reduces in atrophied and regenerating cells subsequently. Results confirmed that because of its reduction in kidney tissues and increase in blood stream, level of alkaline phophatase activity reduces in kidneys of BALB/c mice embryos treated with QEPE and QEPE, since it infiltrates into extracellular fluids and circulates into blood stream. On the other hand, reduction of folic acid decreases level of this enzyme too.

Conclusion

Two new derivatives of quinazolinones are teratogenic and toxinogenic chemicals, affecting internal organs such as kidneys at histological, biochemical and intracellular levels. Effects are more severe by QEPE than QPPE, which has one ethylen ($-CH_3$) group less (16), and lighter molecular weight.

Acknowledgment

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References

1. Derelanko MJ, Hollinger MA. Handbook of toxicology. Boca Raton: CRC Press; 2002.
2. Gardella JR, Hill JA. Environmental toxins associated with recurrent pregnancy loss. Semin Repro Med 2000; 18:407-424.
3. Fowden AL, Ward JW, Wooding FPB, Forhead AJ, Constancia M. Programming placental nutrient transport capacity. J Physiol 2006; 572:5-15.
4. Coan PM, Angiolini E, Sandovici I, Burton GJ, Constancia M, Fowden AL. Adaptations in placental nutrient transfer capacity to meet fetal growth demands depend on placental size in mice. J Physiol 2008; 15:4567-4576.
5. Goodlett CR, Horn KH, Zhou FC. Alcohol teratogenesis: mechanisms of damage and strategies for intervention. Exp Biol Med 2005; 230:394-406.

6. Shams Lahijani M, Ahmadzadeh F, Dabiri M. Teratogenic effects of a new derivative of quinazolinone on the development of BALB/c mice embryos,on days 9,10 and 11 of gestation. Iran J Sci Technol 2006; $30A_1$:1- 8.
7. Shams Lahijani M, Aounegh R. Teratogenic effects of quinazolinone on BALB/c mice fetuses. J Med Sci Res 2007; 1:25-30.
8. Shams Lahijani M, Rajabi H, Etemad S, Fadavi Eslam M. Qualitative and quantitative analysis of the effects of quinazolinones on internal organs of newborn BALB/c mice. Iran J Basic Med Sci 2009; 12:112-120.
9. Shams Lahijani M, Hamidi S. Birth Defects caused by quinazolinones in BALB/c mice stomachs. ISPD, Vancouver, Canada, 2008; 1- 4.
10. Shams Lahijani M, Moayer F, Shah Hossein Pour Shoushtary E. Pathological effects of quinazolinones on the brains of newborn BALB/c mice. ESVP, Dubrovnick, Croatia, 2008; 17-21.
11. Estakhr J, Shams Lahijani M. Birth defects in spleen of BALB/c newborns mice treated with quinazolinones. ICBES, Hurghada, Egypt, 2008; 13-16.
12. Abdel-Alim M, El-Shorbag Abdel-Nasser A, El-Gendy Mahmoud A, El-Shareif Hosny AH. Quinazolinone derivatives of biological interest: V. Novel 4(3H)-Quinazolinones with sedative-hypnotic, anticonvulsant and anti-inflammatory activities. Coll Cze Che Comm2008; 58:1963-1968.
13. Jiang S, Zeng Q, Gettayacamin M, Tungtaeng A, Wannaying S, Lim A, *et al*. Anti-malaria activities and therapeutic properties of febrifugine analogs. Antimicrob Agents Chemother 2005; 49:1169-1176.
14. Refaie FM, Esmat AY, Abdel Gawad SM, Ibrahim AM, Mohamed MA. The anti-hyperlipidemic activities of 4(3H) quinazolinone and two halogenated derivatives in rats. Lipids Health Dis 2005; 4:22-30.
15. Yadav MR, Shirude ST, Parmar A, Balaraman R, Giridhar R. Synthesis and anti-inflammatory activity of 2,3-diaryl-4(3H)-quinazolinones. Chem Heter Com 2006; 42:1038-1045.
16. Dabiri M, Salehi P, Khajavi MS, Mohammadi A. Microwave-assisted one- pot three component synthesis of some new 4(3H)- quinazolinone derivatives. Hetero 2004; 36:1417-1421.
17. Browne MJ, Pitts MW, Pitts RF. Alkaline phosphatase activity in kidneys of glomerular and aglomerular marine teleosts. Biol Bull 1950; 99152-156.
18. Dehghani H, Narisawa S, Millan JL, Hahnel AC. Effects of disruption of the embryonic alkaline phosphatase gene on preimplantation development of the mouse. Dev Dyn 2000; 217:440–448.
19. Sun XM, Li D, Bai ZL, Jin WR. Electrochemical detection of alkaline phosphtase in BALB/c mice fetal liver stromal cells with capillary electrophoresis. Chin Chem Lett 2004; 15:212–213.
20. Gyrd-Hansen N. Alkaline phosphatase histochemistry and early renal cortical damage**.** Histochem J 1974; 6:199-209.
21. Schmidt U, Schlumpf V, Jösch W, Dubach UC. Acute renal failure in the rat after folate intoxication: diagnostic value of lactate dehydrogenase and alkaline phosphatase measurements in serum and urine. Clin Nephrol 1974; 2:106-112.
22. Udobre A, Edoho EJ, Eseyin O, Etim EI. Effect of artemisinin with folic acid on the activities of aspartate amino transferase,alkanine amino transferase and alkaline phosphatase in rat. As Biochem 2009; 4:55-59.
23. Reimer L, Kohl H. Transmission electron microscopy. 5th ed. Springer, 2008.
24. Solhaug MJ, Bolger PM, Jose PA. The developing kidney and environmental toxins. Pediatrics 2004; 113:1084-1091.
25. Bonventre JV, Weinberg JM. Recent advances in the pathophysiology of ischemic acute renal failure. J Am Soc Nephrol 2003; 14:2199-2210.
26. Camp V, Martin P. The role of macrophages in clearing programmed cell death in developing kidney. Anat Embryol 1996; 194:341-348.
27. Markowitza GS, Perazella MA. Drug-induced renal failure:a focus on tubulointerstitial disease. Clin Chim Acta 2005; 351:31–47.
28. Roselli S, Heidet L, Sich M, Henger A, Kretzler M, Gubler MC, *et al*. Early glomerular filtration defect and severe renal disease in podocin-deficient mice. Mol Cell Biol 2004; 24:550-560.
29. Nony PA, Schnellmann RG. Mechanisms of renal cell repair and regeneration after acute renal failure. J Pharmacol Exp Ther 2003; 304:905-912.
30. Woolf AS, Hillman KA. Unilateral renal agenesis and the congenital solitary functioning kidney: developmental, genetic and clinical perspectives. BJU Int 2007; 99:17–21.
31. Schrier RW. Diseases of the kidney and urinary tract. 8th ed. Vol II, Lippincott Williams & Wilkins, 2006.
32. Doublier S, Ruotsalainen V, Salvidio G, Lupia E, Biancone L, Conaldi P, *et al*. Nephrin redistribution on podocytes is a potential pathomechanism for proteinuria in patients with primary acquired nephritic syndrome. Am J Pathol 2001; 152:1723-1731.
33. Brady HR, Kone BC, Stromski ME, Zeidel ML, Giebisch G, Gullans SR. Mitochondrial injury: an early event in cisplatin toxicity to renal proximal tubules**.** Am J Physiol Renal Physiol 1990; 258:1181-1187.
34. Lee SC, Beery JT, Chu FS. Immunohistochemical fate of ochratoxin A in mice. Toxicol Appl Pharmacol 1984; 72:218-227.

35. Brzóska MM, Kamiński M, Dziki M, Moniuszko-Jakoniuk J. Changes in the structure and function of the kidney of rats chronically exposed to cadmium. Arch Toxicol 2004; 78:226-231.
36. Homma-Takeda S, Takenaka Y, Kumagai Y, Shimojo N. Selective induction of apoptosis of renal proximal tubular cells caused by inorganic mercury *in vivo*. Envin Toxicol Pharmacol 1999; 7:179-187.
37. Lee H, Shoda R, Krall JA, Foster JD, Selhub J, Rosenberry TL. Folate binding protein from kidney brush border membranes contains components characteristic of a glycoinositol phospholipid anchor. Biochemie 1992; 31:3236–3243.
38. Moser M, Leo O, Hiernaux J, Urbain J. Idiotypic manipulation in mice: BALB/c mice can express the crossreactive idiotype of A/J mice. J Leukoc Biol 2005; 12:32-38.