

## Qualitative and Quantitative Analysis of the Effects of Quinazolinones on Internal Organs of Newborn Balb/C Mice

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### Abstract

#### Objective(s)

Quinazolinones are heterocyclic compounds, with biological and pharmacological activities, such as inhibiting some proteins, enzymes and reducing blood lipids.

#### Materials and Methods

Following previous results of our group, effects of two new derivatives of quinazolinones 9(3)-quinazolinone-2-propyl-2-phenylethyl (QPPE) and 9(3)-quinazolinone-2-ethyl-2-phenylethyl (QEPE) on livers, intestines and kidneys of newborn Balb/C mice were investigated. Pregnant mice were divided into four groups of control, sham, experimental 1, treated with QPPE, and experimental 2, treated with QEPE. Experimental groups received 100 mg/kg body weight (most effective dose) of QPPE and QEPE, sham groups received methyl cellulose 0.05% (the solvent) and control groups received distilled water, intraperitoneally (IP), on day 8 of gestation. Five days after birth, livers, intestines and kidneys were removed, fixed in formalin 10%, stained with hematoxyline and eosin for histological and pathological studies.

#### Results

Results showed appearance of fatty changes in livers, an increase in diameters of hepatocytes and central veins of livers, and reduction in the lengths of villi of proximal, middle and distal segments of newborn Balb/C mice intestines. Furthermore, there was a diminished diameter of the lumen of the proximal tubules, and average diameter of the lumen of distal tubules which led to an increase in the number of glomeruli cells of newborn Balb/C mice kidneys.

#### Conclusion

Regarding inflammation in different parts of the kidneys, livers and intestines, our investigations suggest that quinazolinones may have some toxic effects on embryos.

**Keywords:** Abnormalities, Intestine, Kidney, Liver, Mice fetuses, Quinazolinones

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## Introduction

Quinazolinones have various biological and pharmacological properties such as inhibiting some proteins and enzymes (such as PgP (P- glycoprotein), MRP (Multidrug resistance associated protein), PARP (poly ADP-ribose polymerase) and reducing blood lipids (1, 2).

A few structural and functional disorders, created in newborn animals with birth defects, are associated with drugs and chemicals consumptions during embryonic development, affecting its development through changes in molecular mechanisms. The placenta does have the capability to screen out many injurious substances on the basis of size, but countless teratogens such as disease-causing organisms and chemicals are small enough to readily cross the barrier and enter the developing fetus. In general, molecules small enough to pass through the gastrointestinal tract will also be able to cross the placental barrier. Most developmental abnormalities occur during early pregnancy when cells are differentiating to form specialized body structures. During this time of extreme vulnerability, teratogens damage the cells by arresting cell growth or by changing their normal growth patterns for a particular area. Growth and differentiation is a continuous process with the periods of susceptibility of different organs or tissues difficult to pin point. As the pregnancy progresses, the susceptibility of the fetus to various agents changes, with the developing systems becoming functionally rather than structurally damaged. While structural defects are generally obvious soon after parturition, many functional impairments may not be evident until several days, weeks or even years after birth (3, 4).

Previous investigations in our laboratory resulted in creating malformed morphology and skeleton in embryos of Balb/C mice treated with QPPE and QEPE, two new derivatives of quinazolinones synthesized for the first time (3-10).

Livers, derived from endomesoderm, intestines, from endoderm, and kidneys (pronephros, mesonephros and metanephros), from mesoderm (nephrogenic cords), develop on day 10<sup>th</sup> and 11<sup>th</sup> of pregnancy, in mice.

No studies have so far been carried out, demonstrating teratogenic effects of quinazolinones on mice livers, intestines and kidneys. According to our previous studies and observations of morphological and skeletal abnormalities in embryos of Balb/C mice treated with quinazolinones (3, 4), high rates of absorptions of another derivative of quinazolinone by these organs (12), and the role they have in absorption and metabolism of chemicals and drugs, our research was based on investigating the qualitative and quantitative effects of these new components (10) on livers, small intestines and kidneys of newborn Balb/C mice.

## Materials and Methods

Balb/C mice were originally obtained from Razi Institute (Tehran, Iran); Random breeding was carried out in lighted controlled rooms (12 hr light-dark), provided with lab chow (pellets) and tap water. Virgin females (about 30 g), after mating males overnight and with vaginal plugs, were considered to be on day 0 of pregnancy (3, 4).

Two new derivatives of quinazolinones (10): 4 (3H)-quinazolinone-2-propyl-2-phenylethyl (QPPE) and 4 (3H)-quinazolinone-2-ethyl-2-phenylethyl (QEPE), were used for IP injections. Experimental groups 1 and 2 (n=20) received 100 mg/kg body weight of QPPE and QEPE, sham (n=20) and control groups (n=20) received 0.05% methyl cellulose (10 mg/kg body weight)-a solvent- and distilled water (10 ml/kg), respectively, on day 8th of pregnancy (3, 13). Morphological, qualitative and quantitative effects of two new components (QPPE and QEPE) on the internal organs of newborn Balb/C mice were studied. The mice were anesthetized, their internal organs were fixed in formalin 10%, stained with hematoxyline and eosin and their morphological and pathological structures were examined (Table 1).

Table 1. Number of newborn Balb/C mice of four groups.

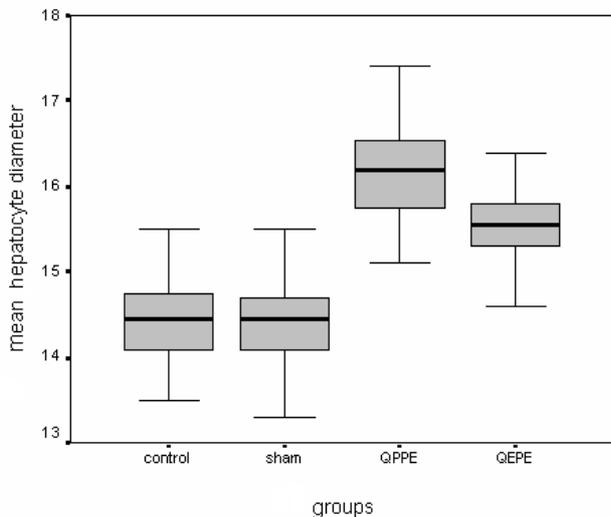
Organs	Control	Sham	QPPE	QEPE
livers	30	34	32	34
kidneys	30	30	30	30
Intestines	30	30	30	30

Lengths of villi and depths of crypts of small intestine (13), diameters of malpighian glomeruli, lumens of proximal and distal tubules, gloemerular hypercellularity of kidneys, and diameters of the hepatocytes, central veins, and bile duct as well as fatty changes in newborn Balb/C mice livers were measured and analyzed with statistical packages for social sciences (SPSS, version 9). One-way ANOVA (CRD) was used for quantitative studies, followed by post hoc LSD multiple comparison test. Chi-square test was used for comparison of categorical variables between two or more groups. Level of significant difference was set at  $P<0.05$ .

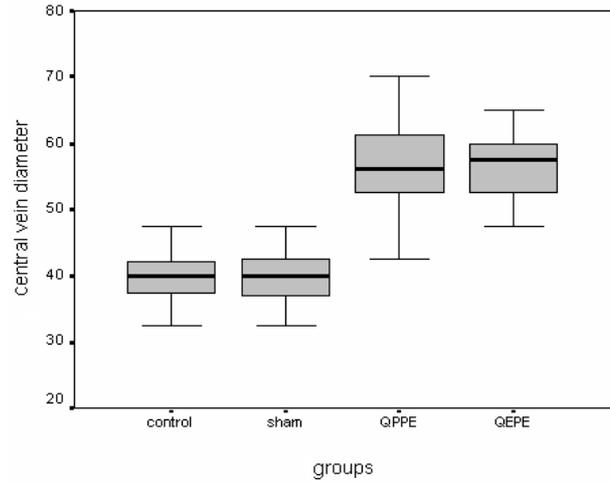
**Results**

No significant difference was observed between control and sham (treated with the solvent) groups. There was no significant difference between the morphology of livers in four groups.

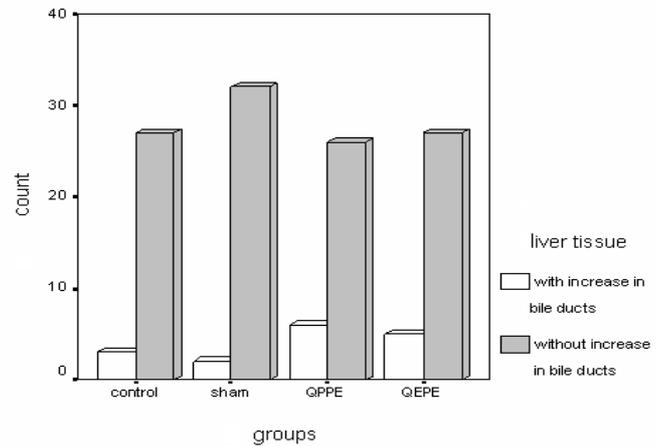
One way ANOVA and LSD tests demonstrated significant differences between average diameters of hepatocytes and central veins of treated and control groups ( $P<0.05$ ); in other words, quinazolinones QPPE and QEPE increased these diameters, with larger effect in the group treated with QPPE (Histograms 1 and 2). Chi square test did not show any significant difference between groups considering the increase in diameters of bile ducts (Histogram 3).



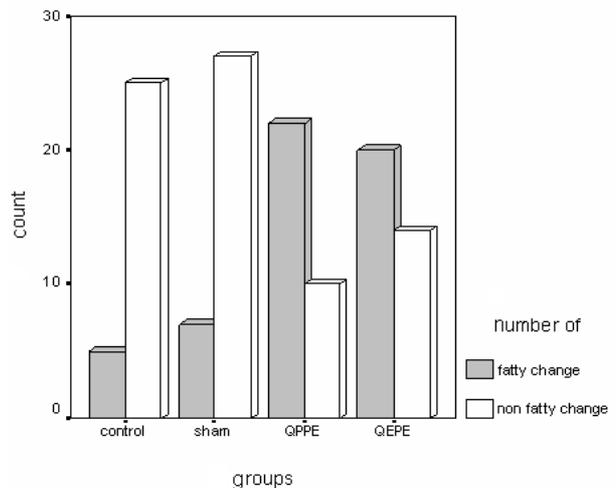
Histogram 1. Comparison of diameters of hepatocyte of newborn Balb/C mice, using CRD and LSD tests.



Histogram 2. Comparison of diameters of central veins of newborn Balb/C mice livers, using CRD and LSD tests.



Histogram 3. Balb/C mice livers with and/or without increase in bile ducts diameters. Using chi-square test, there was no significant difference between groups.



Histogram 4. Newborn Balb/C mice livers with and/or without fatty changes. Using chi-square test, there was a significant difference between groups.

## Quinazolinones and Internal Organ of Newborn Mice

Comparison between the number of newborn Balb/C mice livers with and/or without fatty changes, exhibited a significant difference in treated groups ( $P < 0.05$ ). As illustrated in histogram 4, quinazolinones led to the accumulation of fat in hepatocyte vacuoles (Figures 1 and 2).

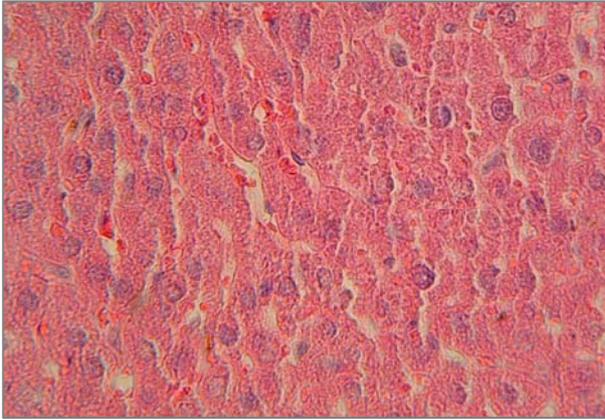


Figure 1. Liver of newborn Balb/C mouse of control group (H&E, 400 $\times$ ).

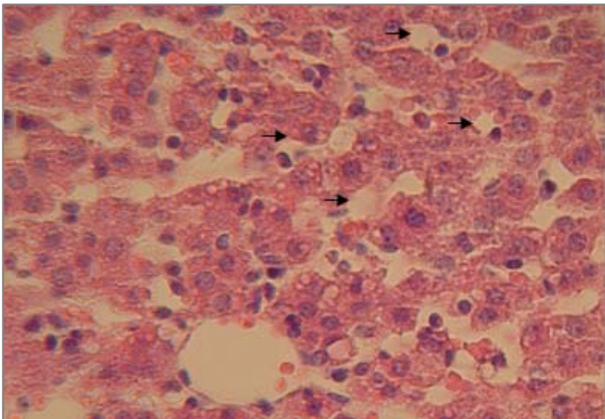
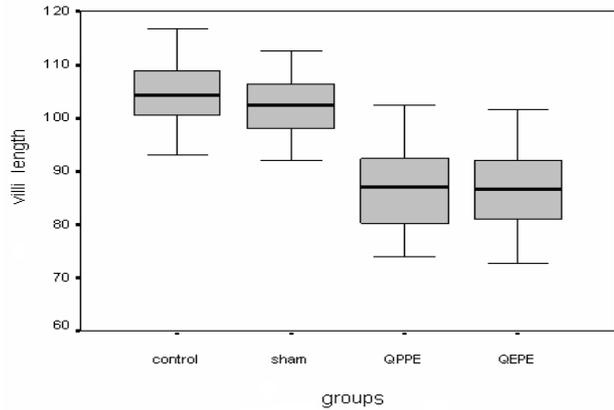
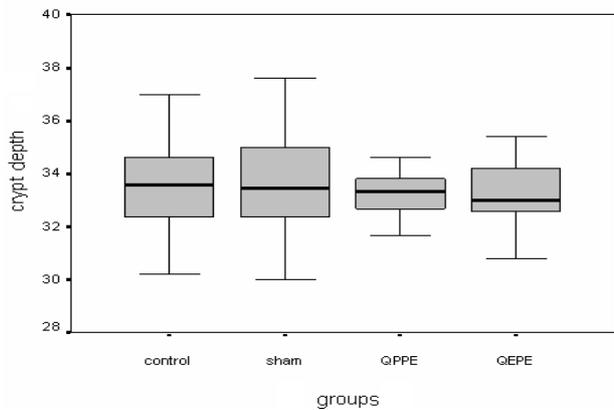


Figure 2. Liver of newborn of Balb/C mouse treated with QPPE, showing vacuoles with fat (black arrows, H&E, 400 $\times$ ).

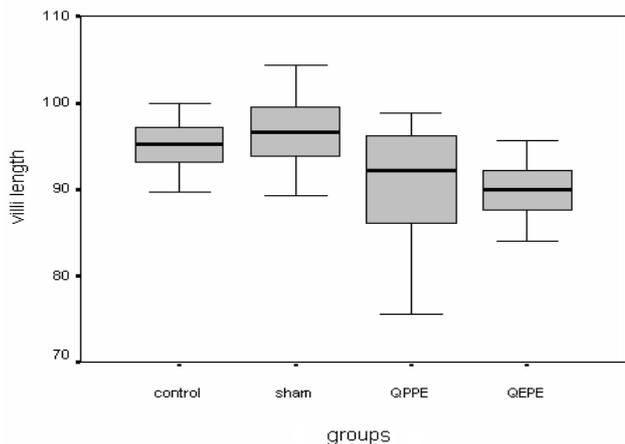
QPPE and QEPE caused a reduction in the lengths of the villi of proximal portion of the experimental newborn Balb/C mice intestines ( $P < 0.05$ ), compared with control groups. Intensity of the effects was very close in two experimental groups (Histogram 5). Comparison of depths of crypts of proximal portions of the intestines amongst control, sham and experimental groups (treated with QPPE and QEPE) did not show any significant difference (Histogram 6). Slight reduction was observed in the lengths of the villi of the middle segment of the experimental intestines compared to control groups, although it was very close in two experimental groups (Histogram 7).



Histogram 5. Comparison of average lengths of villi of proximal part of newborn Balb/C mice intestines ( $\mu\text{m}$ ). Using CRD and LSD tests, there was a significant difference between groups.



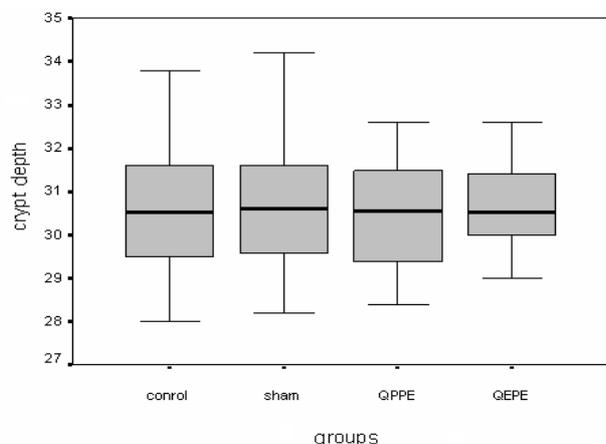
Histogram 6. Comparison of average depths of crypts of proximal parts of newborn Balb/C mice intestines ( $\mu\text{m}$ ). Using CRD test, no significant difference was noted between groups.



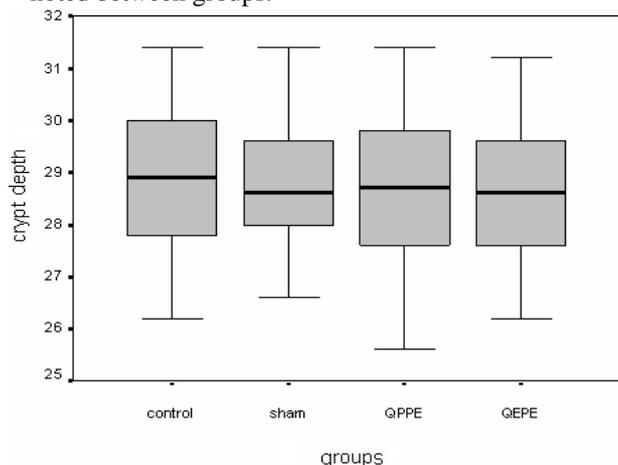
Histogram 7. Comparison of average lengths of villi of middle parts of newborn Balb/C mice intestines ( $\mu\text{m}$ ). Using CRD and LSD tests, there was a significant difference between groups.

Comparison of the depths of crypts in the middle and distal portions of the intestines of control, sham and experimental groups did not show any significant difference (Histograms 8

and 9). No reduction was observed in the lengths of the villi of distal portions of the experimental intestines of newborn mice, compared with control groups.



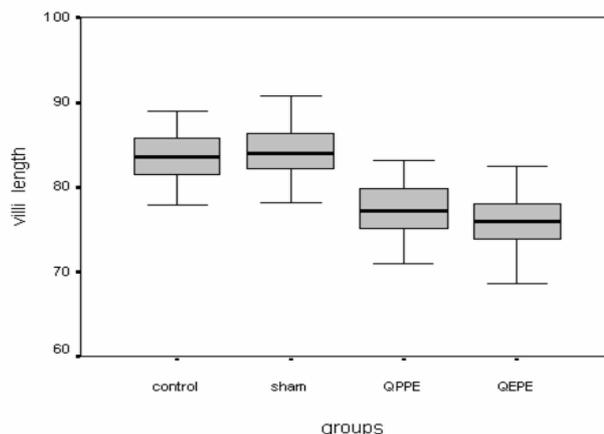
Histogram 8. Comparison of average depths of crypts of middle portions of newborn Balb/C mice intestines ( $\mu\text{m}$ ). Using CRD test, no significant difference was noted between groups.



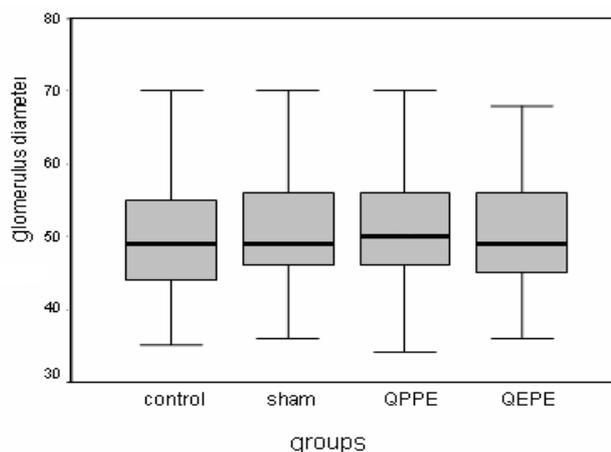
Histogram 9. Comparison of average depths of the crypts of distal portions of newborn Balb/C mice intestines ( $\mu\text{m}$ ). Using CRD test, no significant difference was noted between groups.

The intensity of the effects was nearly the same between experimental groups (Histogram 10).

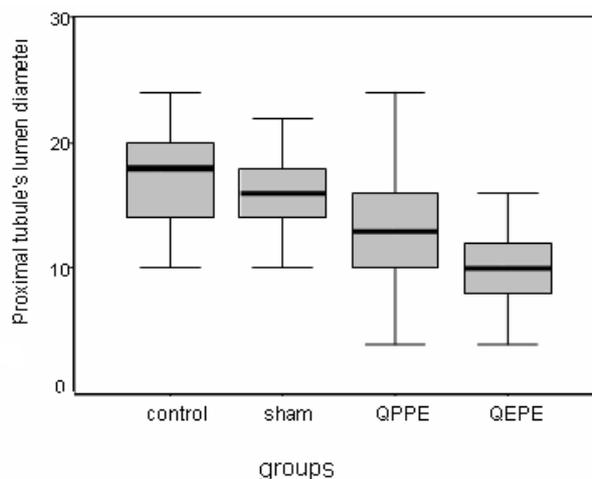
Comparison between average diameters of glomeruli of experimental kidneys showed no significant difference. However, there was a significant difference between average diameter of lumen of proximal tubules of experimental, sham and control groups. Two experimental groups had been significantly affected differently regarding these structures (Histograms 11 and 12).



Histogram 10. Comparison of average lengths of villi of distal segments of newborn Balb/C mice intestines ( $\mu\text{m}$ ). Using CRD test, no significant difference was noted between groups.



Histogram 11. Diameters of glomeruli of newborn Balb/C mice kidneys. Using CRD test, no significant difference was noted between groups.

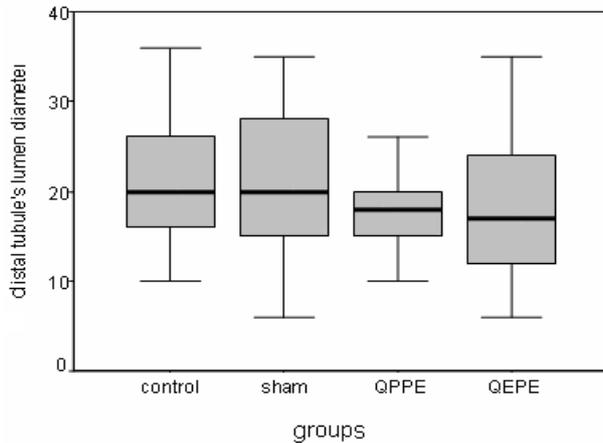


Histogram 12. Diameters of the lumen of proximal tubules of newborn Balb/C mice kidneys. Using CRD and LSD tests, there was a significant difference between groups.

On the other hand, QPPE and QEPE did diminish the diameters of lumen of distal

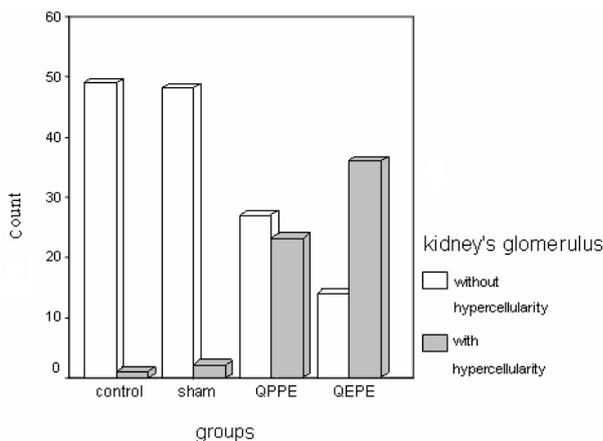
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tubules, but this effect was larger in the group treated with QEPE ( $P<0.05$ ). Although the level of reduction was higher but it was not statistically significant in experimental groups compared to the control and sham groups (Histogram 13).



Histogram 13. Diameters of lumen of distal tubules of newborn Balb/C mice kidneys. Using CRD test, no significant difference was noted between groups.

No difference was observed between the number of glomeruli with and/or without hypercellularity in control and sham groups, and there was no significant difference in the treated groups either. In general, treatments with QPPE and QEPE led to an increase in the number of glomeruli cells (hypercellular) in kidneys ( $P<0.05$ ) (Histogram 14, Figures 3 and 4).



Histogram 14. Glomeruli with and/or without hypercellularity in newborn Balb/C kidneys. Using chi-square test, there was a significant difference between groups.

Unilateral renal agenesis (URA) in a treated newborn Balb/C mice is shown in Figure 5.

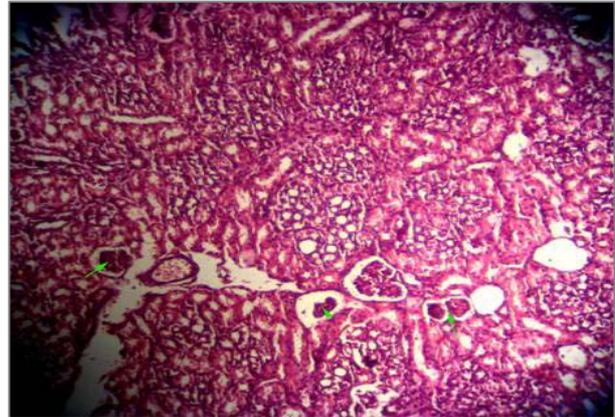


Figure 3. Kidney of a newborn Balb/C mice treated with QPPE. Green arrows show hypercellular glomeruli (H&E, 100 $\times$ ).



Figure 4. Kidneys of a newborn Balb/C mice treated with QEPE. Green arrows show hypercellular glomeruli (H&E, 100 $\times$ ).

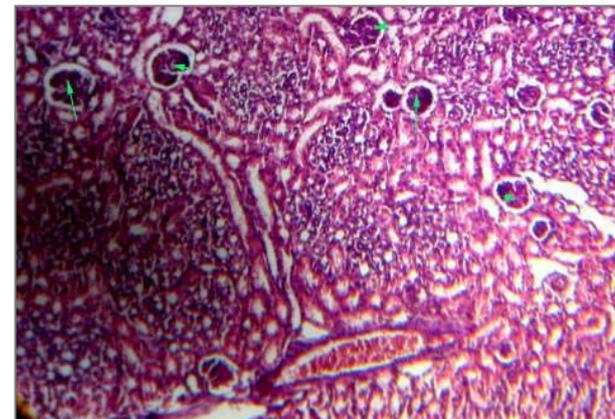


Figure 5. Unilateral renal agenesis (URA) in a newborn Balb/C mice treated with quinazolinones. Yellow arrow shows the larger right unilateral kidney in experimental group (10 $\times$ ).

## Discussion

According to our previous investigations, methyl cellulose (injected to the sham groups) had no teratogenic effects on newborn Balb/C mice (4), which was confirmed in our

research, by not causing malformations in sham and control groups; so the observation of high percentages of disorders in newborn Balb/C mice of experimental groups is due to the injections of QPPE and QEPE.

Our earlier studies demonstrated abnormalities caused by injections of 100 mg/kg of quinazolinones on day 8th of pregnancy. In this investigation, the pathological effects of QPPE and QEPE on the internal organs were carried out with most effective dose and period (3 and 4).

Since quinazolinones have no active groups, they produce active groups after being affected by cytochrome P<sub>450</sub> and metabolized in livers and/or kidneys. Thus, injuring cells and their organelles' membranes, causing necrosis in hepatocyte, renal tubule and glomerulus (14-17).

One way of cell adaptation is hypertrophy-synthesis of structural particles as the result of cell enlargement- since they (especially QEPE) need products of quinazolinones metabolisms. Abnormal accumulations of fat in vacuoles (fatty changes, steatosis), which is the build up of fat in liver, is due to the blockage in one of the lipids metabolic pathways in hepatocytes.

In general, an increase in fat influx from outside sources and/or decrease of lipid outflux from the cell, due to the deficiency or lack of apoproteinal portions (lipid-binding proteins) of LDL or phospholipids, can cause steatosis. Apparently, quinazolinones biotransformation produces active metabolites, which brings about lipid peroxidation and subsequent decrease in hepatocytes VLDL secretion and accumulations of fat vacuoles in liver cells. On the other hand, it might be due to the damages to protein synthesis systems, so that the apoproteinal portion of secretory lipoproteins could not be formed (18).

Some of intracellular accumulations, fatty changes for instance, are sources of cell adaptations. As is the case for stored lipids in fasting or decreased food absorption, which are the principal energy sources. In this case, large amount of lipids enter the liver, thus, fat vacuoles are visible in the liver of hungry person.

Quinazolinones shorten the lengths of the villi in all parts of newborn Balb/C mice intestines, which would reduce the level of absorptions of

nutrients. Consequently, body uses more fat as a fuel, and eventually fat droplets appear in liver. It is possible that quinazolinones react with tubulins, inhibit the formation of spindles, block cell divisions, and cause reduction in the lengths of intestinal villi (19).

Our results indicate that teratogenic effects of QPPE and QEPE on tissues of proximal portions of intestines are more severe than those of middle and distal portions, which may be due to the fact that the proximal part of the intestine (duodenum) is fed by celiac and mesenteric veins (embryonic phase), while other sections are fed only by branches of the upper mesenteric veins. So, there is a good possibility that the most proximal part of intestine (duodenum) is exposed to higher concentrations of these components due to being fed by two blood vessels (20).

Studies showed that unilateral renal agenesis (URA), a congenital abnormality, is the result of disturbances in normal development of kidneys. Mutation of genes and growth factors, teratogenic drugs (specially angiotensin-renin inhibitors), high consumption of vitamin A derivatives, and diabetic parents are the major factors which most often result in missing left kidney and enlargement of right kidney (without any changes in its function). So, the case of left kidney agenesis and enlargement of the right kidney in a newborn Balb/C mouse of a mother treated with QPPE and QEPE in our results could be explained by this fact (21).

Results of these research showed that QPPE and QEPE enter mother blood circulations, pass through placenta into blood system and embryo's kidney, cause kidney inflammations in the form of abnormalities in glomerular, tubular and interstitial tissues (laboratory observations).

As embryonic kidneys are affected by mother and other factors like lack of vitamin A and increase in glycocorticoids, these also could create malformations in embryonic kidneys. Treatments of mother with these drugs can explain the side effects (22).

The results also indicated that most of the affected glomeruli had hypercellularity without any change in their diameters. In addition, amacite cells could be seen in all parts of the glomeruli. Glomerular cells (epithelial, mesangial, and endothelial) usually

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act as proinflammatory molecules during immune responses and because of having more access to blood, mesangial cells are more vulnerable than others.

According to previous studies related to possible mechanisms of glomerulonephritis, after stimulating immune system, numerous leukocytes infiltrated through glomeruli, injured glomerular mesangium, and caused necrosis. Following damages to glomerulus, mesangial hypercellularity begins with mesangial cell proliferation and extracellular matrix accumulations without altering glomerulus size. Subsequently, proliferative glomerular lesions progresses to the chronic renal failure. Although the number of proliferating cells and infiltrating leukocytes gradually decrease, glomerular inflammation results in sclerosis and fibrosis of mesangial cells (18, 19).

Proximal tubules are more sensitive to toxins than other parts of the nephron, because of their juxta-glomerular location. Outcome of this study indicated a reduction in diameter of lumen of proximal tubules of treated newborn Balb/C mice. So, it can be suggested that observed decrease in diameter of lumen is the consequence of swelling of proximal tubule cells, accompanied by alterations in cells' nuclei and initiation of necrosis.

No effect was observed in the cells of distal and other tubular parts. This can be due to two possible reasons: 1) excretion of quinazolinones into urine, through proximal cells, would not

expose these parts, and 2) insignificant damageable effects of 100mg/kg/body weight of quinazolinones.

An increase in the number of mitochondria occurred in kidney tubular cells. Mitochondria act as an important organelle in producing superoxides, reactive metabolites and especially as the metaboliser of fat. Inhibition and nonfunctional mitochondria are the consequences of reactive products of quinazolinone's metabolism in cells, resulting in kidney nephritis and fatty changes in hepatocytes (23-28).

### Conclusion

In brief, pathological study of teratogenic effects of QPPE and QEPE showed that QEPE had more effects than QPPE. Having smaller molecules, and being more penetrable through phospholipid layer than QPPE are the major factors, causing more teratogenic effects by QEPE (3). Regarding inflammation of different parts of newborn Balb/C mice kidneys, livers and intestines, we suggest that quinazolinones may have some toxic effects (nephrotoxic and hepatotoxic) on pregnant mice. More research, at cellular and biochemical levels are in progress.

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