Preliminary Evaluations of the Effects of Quinazolinones on Internal Organs of Newborn Balb/C Mice

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Summary

Different properties of water insoluble heterocyclic compounds such as quinazolinones, with various biological and pharmacological properties (anti-bacterial, anti-inflammation, sedation, antidepression) are also used for the treatments of cancer and HIV. Our previous results proved that two new derivatives of quinazolinones are toxogenic and teratogenic chemicals for embryos.Pregnant Balb/C mice(n=25)were divided into 4 groups of control,receiving distilled water, sham,treated with 0.05% methyl cellulose (the solvent)and two experimental groups 1 and 2, receiving one of the most effective dose of 100mg/kg/body weight of 4(3H)quinazolinone-2-propyl-2-phenyl ethyl(QPPE)and 4(3H)quinazlonones-2-ethyl-2-phenyl ethyl (QEPE),by IP injections, on one of the most effective day 8 of gestation.After anesthetising mothers, brains,stomachs,hearts and spleens of 4-day old newborn Balb/C mice were removed,fixed and stained with H & E for light microscopic and quatitative studies.Pathological observations demonstratded an increase in the number of astrocytes of cerebral cortex and medulla of newborn mice of treated groups.Comfirming the results,one-way ANOVA,LSD and chi square tests(*$P<0.05$)showed no significant differences between the morphology of newborn mice of control, sham and treated groups'astrocytes,but there were significant differences in the number of astrocytes of newborn mice of groups treated with QPPE and QEPE. Results showed symptoms of gastritis (hyperaemia and decrease in thickness of mucus layer) in newborn Balb/C mice of treated groups.Quinazolinones also created necrotic cells and an increase in connective tissues of hearts of newborn mice of treated groups,but there were no significant differences amongst newborn mice of QPPE and QEPE treated groups.Statistical data indicated significant differences between thickness of capsules and number of macrophage cells of spleens of experimental groups, in comparison with sham and control groups.In conclusion: 1)Astrocyte hyperplasia decrease toxic effects of QPPE and QEPE,by passing through blood-brain barrier;2)Oxygen-derived free radicals play pathological roles in gastritis and radical scavengers such as alfa tocopherol.

Carotenoids glutathione redox system also plays a significant role in protecting membranes from oxidative damages;3)By having no active chemical groups and production of active groups and free radicals,after being affected by cytochrome P_{450} and metabolized in heart,release of intracellular components(such as lysosomal enzymes and lipid peroxidation),cells,organelles,membranes injuries and myocyte necrosis happen;4)Appearance of connective tissues, between myocytes,is due to the stimulation of multiplication of fibroblasts because of necrosis and their annihilation;So,necrotic cardiac cells will be replaced by connective tissues and 5)Damages observed in newborn mice livers, intestines, kidneys, hearts, stomachs and brains,in previous and this report,could have been the reason why the number of macrophage cells have been increased in spleens.Some studies reported damages to the organs such as livers and hearts can cause an increase in the thickness of spleen capsule,consequently, its weight and length.

Keywords: Internal organs, abnormalities, Quinazolinones, Toxicity, Newborn Balb/C mice.

Introduction

During past few decades, it has become increasingly evident that human and animal embryos are subjected to variety of environmental influences that could have deleterious effects on their development.

Since thalidomide tragedy,attention has been focused on drugs or chemicals as potential teratogens, to which pregnant women might be exposed to(1,2).Developmental toxicity is the consequence of toxicity in mother;So, as the result of effects on pregnant mother,embryos will be affected secondarily.Earlier investigations indicated that consumption of critical doses of some drugs,during pregnancy,is harmful to fetuses (3-8).Considering the valuable data presented, reported abnormalities are very important for identifying and preventing them from happening .

More than 40 alkaloid composed of 4(3H) – quinazolinones moiety were isolated from nautral sources (9,10). Quinazolinones,frequently encountering heterocycles in medical chemistry(11),with two conjoined aromatic rings incorporating two nitrogen atoms and one of the carbons oxidized with a keto oxygen,have wide applications,and are extensively used in treatments of prevalent diseases(12-14).They belong to hyponic chemicals,are immunopathological and anti-tumor agents, inhibitores of tubulin polymerization, DNA repair enzyme Poly(ADP-ribose) polymerase (PARP), mixed lineage kinases, mammalian aspartate transcarbamylase, HIV reverse transcriptase , some proteins and enzymes such as PgP(P-glycoprotein),MRP(multidrug resistance associated protein),PARP(poly ADP-ribose polymerase), polymerization of tubuline, decrease midbrain dopamine unit activity,causing stress, anti-inflammatory activity,active in synthesis and reduction of prostaglandin E2 production, antagonists of CXCR3, and acting strongly to inhibit human immunodeficiency viruses and other activities mentioned in previous papers (15- 30).They pass through placental barriers,which make them chemicals of interest for research and there is a high possibility that they will create toxic and teratogenic effects on embryos and newborns as a whole.

The mechanisms of the effects of quinazolinones on embryonic cells are not clear yet, but there are few reports showing its toxic characteristics. Following our earlier demonstrations of their toxic effects at morphological and skeletal levels of Balb/C mice fetuses and embryos(31,32),the pathological effects of QPPE and QEPE,have been investigated on the morphological and histological structures of newborn Balb/C mice internal organs,such as brain,stomach,heart and spleen,on GD_8(most effective day)(33-39) .

Materials and Methods

Balb/C mice ,obtained from Razi Institute (Tehran, Iran),were housed in lighted controlled room(24±1^{o} C ,65 ± 0.5% humidity, 12h light-dark),provided with lab chow(pellets)and tap water. Males mated virgin females, randomly ,in our local facility(animal room)overnight ;Vaginal plugs presented day 0 of pregnancy.

IP injections were performed on day 8th of pregnancy(40,41).Pregnant mice were divided into 4 random groups of control , receiving 10 ml/kg/body weight of distilled water,sham, treated with 10 mg/kg/body weight of 0.05% methyl cellulose(the solvent)(420,and experimental groups 1 and 2,receiving 100 mg/kg/body weight of QPPE and QEPE(43). Brains,hearts,spleens and stomachs of 4-days old Balb/C mice were removed, fixed in %10 formaldehyde, stained with H and E,studied with light microscope. Parametric data were analyzed by statistical packages for social sciences (SPSS,version 9.0).One-way analysis of variance(ANOVA)was also used, followed by post hoc LSD,multiple comparison and chi square tests.

Level of significance difference was $P<0.05$.

Results

Detailed observations showed increase in the diameters of cerebral microglia of 22 newborn Balb/C mice brains of mothers treated with QPPE and 23 newborn Balb/C mice brains of mothers treated with QEPE ;There were no increase in the volume of brains of 3 newborn mice brains of mothers treated with QPPE ,and 2 newborn Balb/C mice brains of mothers treated with QEPE.

Abnormal myelin sheaths were observed in 18 newborn Balb/C mice of mothers treated with QPPE and 20 newborn Balb/C mice of mothers treated with QEPE.7 newborn Balb/C mice brains of mothers treated with QPPE and 5 newborn Balb/C mice brains of mothers treated with QEPE,had normal myelin sheaths(Figs. 1,2).

Chi-square tests showed significant differences ($P<0.05$)between the number of astrocytes of newborn Balb/C mice cerebral cortex and medulla of 4 different groups,which were more severe in group treated with QEPE (Fig. 3-6).

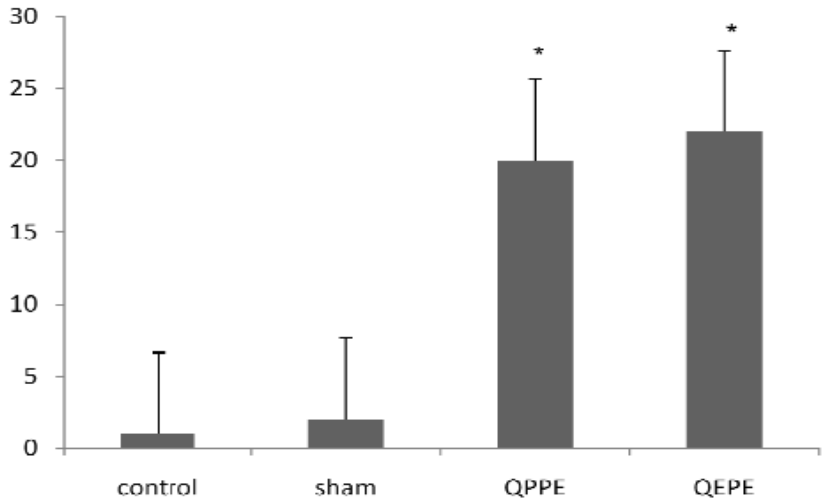

Figure 1. Comparison of the average increase in diameters of cerebral microglia of four different groups of newborn Balb/C mice brains. QEPE had more effects (P<0.05).

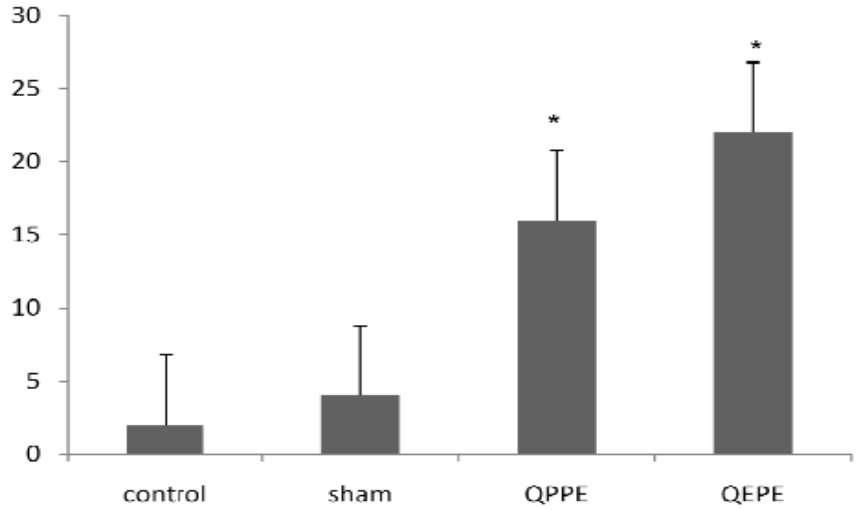

Figure 2. Comparison of the mean number of abnormal myelin sheaths of four different goups of newborn Balb/C mice brains. QEPE had more effects (P<0.05).

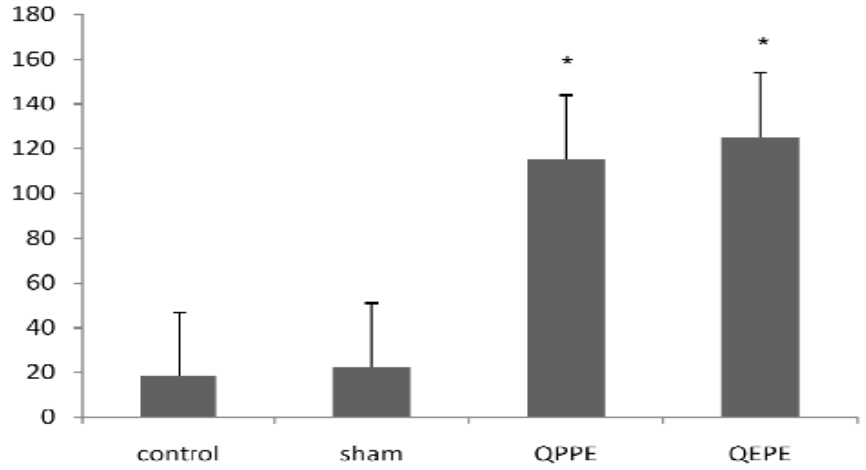

Figure 3. Increase in the mean number of astrocytes of newborn Balb/C mice cerebral cortex of four different groups of of newborn Balb/C mice brains.QEPE had more effects (P<0.05).

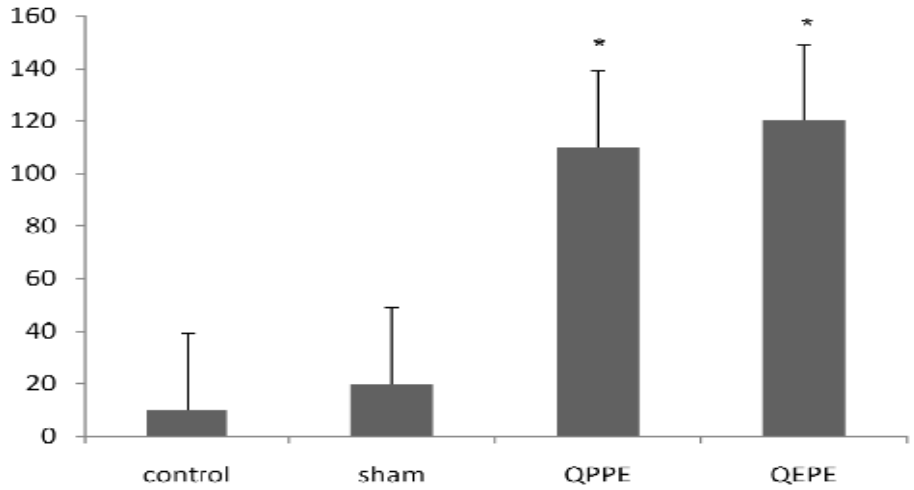

Figure 4. Increase in the mean number of astrocytes of newborn Balb/C mice cerebral medulla of four different groups of newborn Balb/C mice brains.QEPE had more effects(P<0.05).

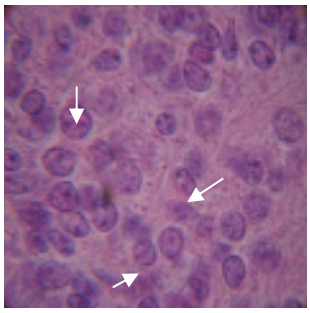

Figure 5. Increase in the mean number of astrocytes of newborn Balb/C mice cerebral cortex treated with QPPE (white arrows) .QPPE had less effects.

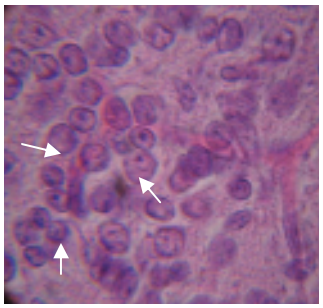

Figure 6. Increase in the mean number of astrocytes of newborn Balb/C mice cerebral medulla treated with QEPE(white arrows) .QEPE had more effects.

No morphological abnormalities were observed in the stomachs of newborn Balb/C mice of experimental groups 1 and 2, in comparison with sham and control groups. There were no significant differences between control and sham groups.

Injection of 100mg/kg/body weights of QPPE and QEPE,resulted in the formation of normal(without hyperaemia)and abnormal(with hyperaemia)stomachs in newborn Balb/C mice(Table1); As ANOVA, LSD and chi square tests revealed, significant decrease occurred in the thickness of mucosal layer of stomachs of newborn Balb/C mice of experimental groups 1 and 2(Figs.7-9),comparing with stomachs of newborn Balb/C mice of sham and control groups.There were no significant differences between mucosal layers of stomachs of newborn Balb/C mice of QPPE and QEPE treated groups.

LSD

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Sham	1.6400	1.1645	.162	-.6715	3.9515
	QPPE	9.6000*	1.1645	.000	7.2885	11.9115
	QEPE	11.4400*	1.1645	.000	9.1285	13.7515
Sham	Control	-1.6400	1.1645	.162	-3.9515	.6715
	QPPE	7.9600*	1.1645	.000	5.6485	10.2715
	QEPE	9.8000*	1.1645	.000	7.4885	12.1115
QPPE	Control	-9.6000*	1.1645	.000	-11.9115	-7.2885
	Sham	-7.9600*	1.1645	.000	-10.2715	-5.6485
	QEPE	1.8400	1.1645	.117	-.4715	4.1515
QEPE	Control	-11.4400*	1.1645	.000	-13.7515	-9.1285
	Sham	-9.8000*	1.1645	.000	-12.1115	-7.4885
	QPPE	-1.8400	1.1645	.117	-4.1515	.4715

*. The mean difference is significant at the .05 level.

Table 1. Comparison of normal and abnormal stomachs of four different groups of newborn Balb/C mice,using chi square test(P<0.05).

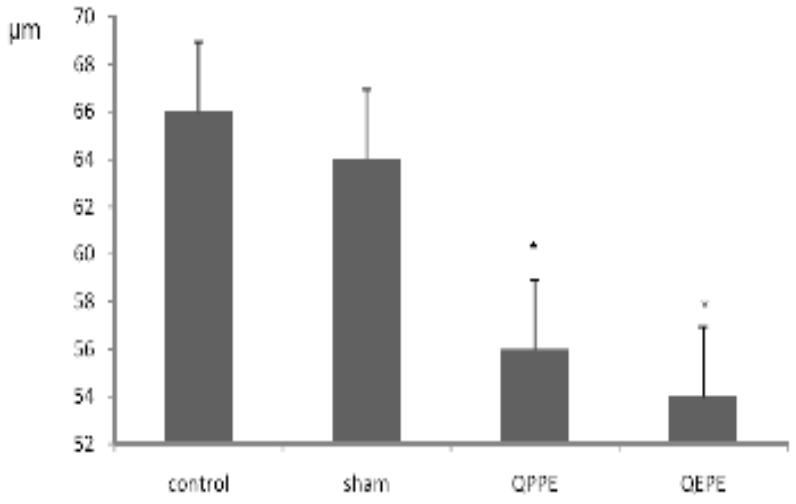

Figure 7. Comparison of the thickness(□m)of mucus layer of stomatchs of four different groups of newborn Balb/C mice. QEPE was more effective(P<0.05).

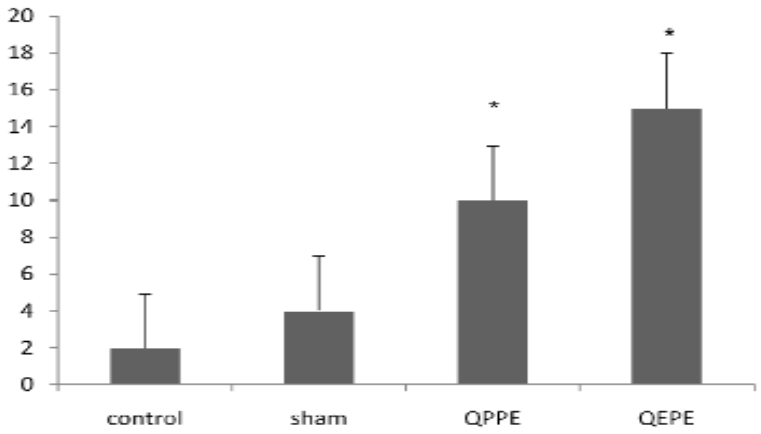

Figure 8. Comparison of the mean number of newborn Balb/C mice with abnormal stomachs in four different groups($P<0.05$).

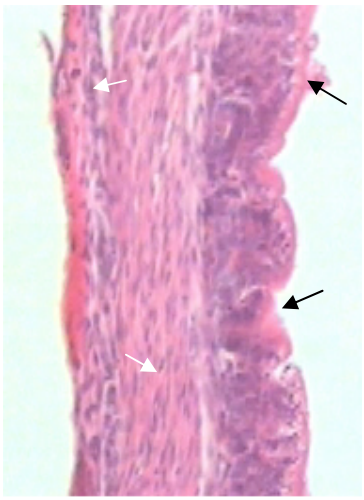

Figure 9. Hyperaemia(white arrows) and decrease in the thickness of mucus layers(black arrows) of stomachs of groups treated with QPPE(40X).

No significant differences were observed between morphological and histological structures of hearts of newborn Balb/C mice of control and sham groups.Injection of 100mg/kg/body weights of QPPE and QEPE created abnormal hearts(Fig.10) , hearts with necrotic cells(Figs. 11-13)and connective tissues between myocytes of newborn Balb/C mice (Figs.14,15),comparing with newborn Balb/C mice hearts of sham and control groups;Abnormalities were more severe in

group treated with QEPE;There were no significant differences between QPPE and QEPE effects.

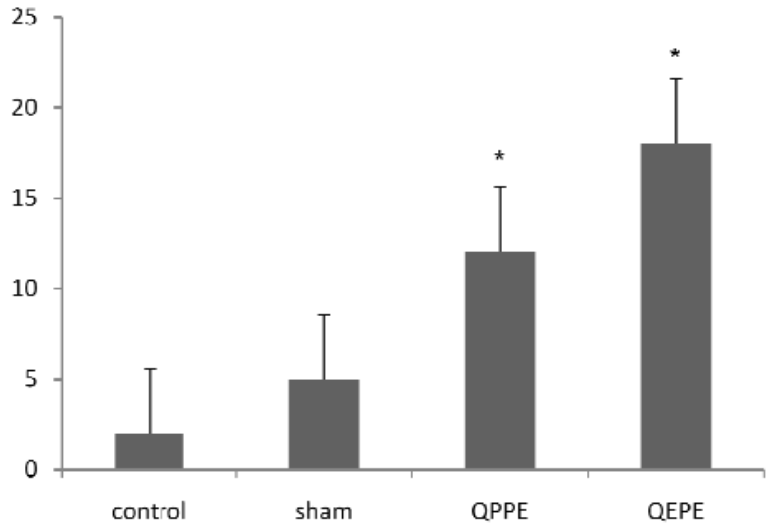

Figure 10. Comparison of the mean number of newborn Balb/C mice of four different groups ,with normal and abnormal hearts.QEPE had more effects($P<0.05$).

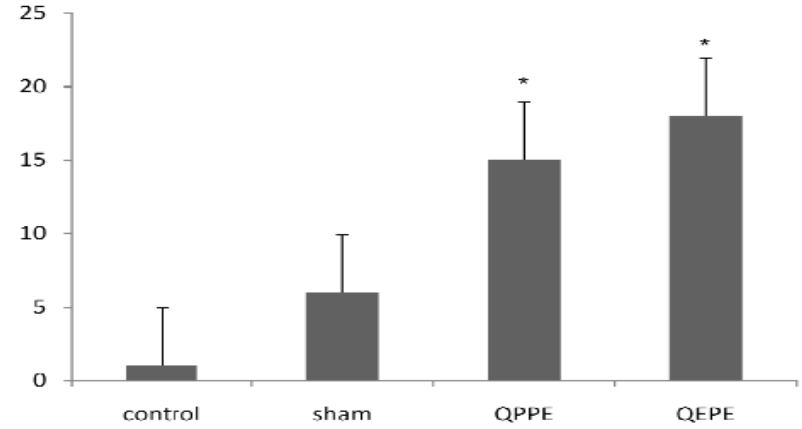

Figure 11. Comparison of the mean number of newborn Balb/C mice of four different groups with normal(without necrotic cells) and abnormal(with necrotic cells) hearts($P<0.05$).

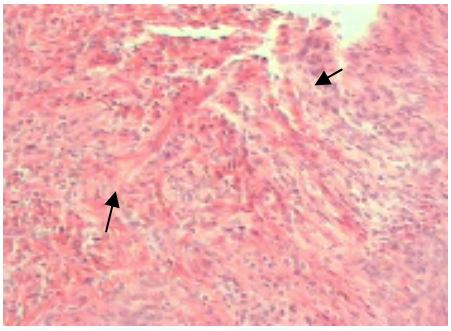

Figure 12. Necrosis (arrows) in hearts of newborn Balb/C mice of mother treated with QPPE.

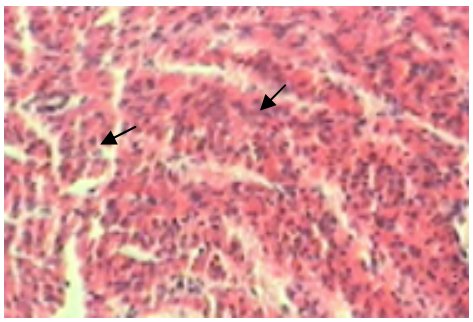

Figure 13: Necrosis(arrows) in hearts of newborn Balb/C mice of mother treated with QEPE.

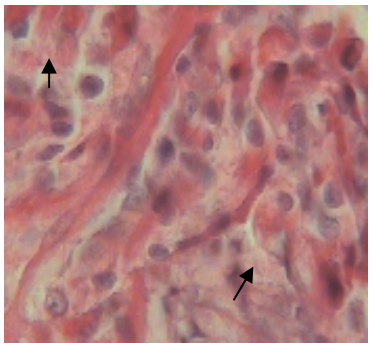

Figure 14. Connective tissues(arrows) in hearts of newborn Balb/C mice of mother treated with QPPE.

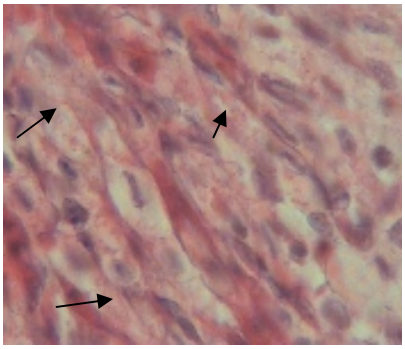

Figure 15. Connective tissues (arrows)in hearts of newborn Balb/C mice of mother treated with QEPE.

Statistical analysis in this research demonstrated no significant differences between weights and lengths of spleens of newborn Balb/C mice of control and sham groups,but differences were significant between weights and lengths of spleens of newborn Balb/C mice of experimental groups 1 and 2;There were also significant differences between the number of macrophage cells and thickness of spleens'capsules of newborn Balb/C mice of experimental groups 1 and 2(Table 2, Figs. 16-19).QPPE had more effects.

Table 2. Comparison of the spleens' weights(gr) of newborn Balb/C mice of four different groups using LSD test.

Multiple Comparisons

Dependent Variable: weight

LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Sham	.0000733	.0005754	.899	-.001079	.001226
	QPPE	-.0118933*	.0005754	.000	-.013046	-.010741
	QEPE	-.0037133*	.0005754	.000	-.004866	-.002561
Sham	Control	-.0000733	.0005754	.899	-.001226	.001079
	QPPE	-.0119667*	.0005754	.000	-.013119	-.010814
	QEPE	.0037867*	.0005754	.000	.004939	.002634
QPPE	Control	.0118933*	.0005754	.000	.010741	.013046
	Sham	.0119667*	.0005754	.000	.010814	.013119
	QEPE	.0081800*	.0005754	.000	.007027	.009333
QEPE	Control	.0037133*	.0005754	.000	.002561	.004866
	Sham	.0037867*	.0005754	.000	.002634	.004939
	QPPE	-.0081800*	.0005754	.000	-.009333	-.007027

*. The mean difference is significant at the .05 level.

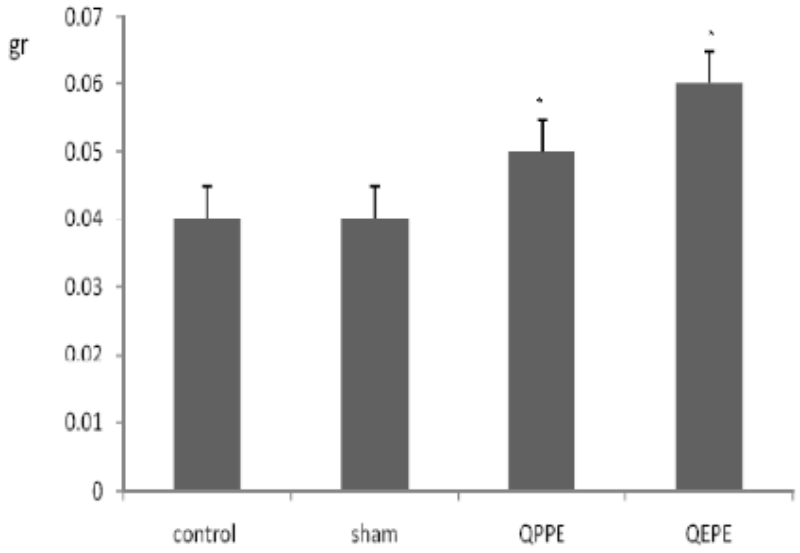

Figure 16. Comparison of the weights(gr) of spleens of newborn Balb/C mice .

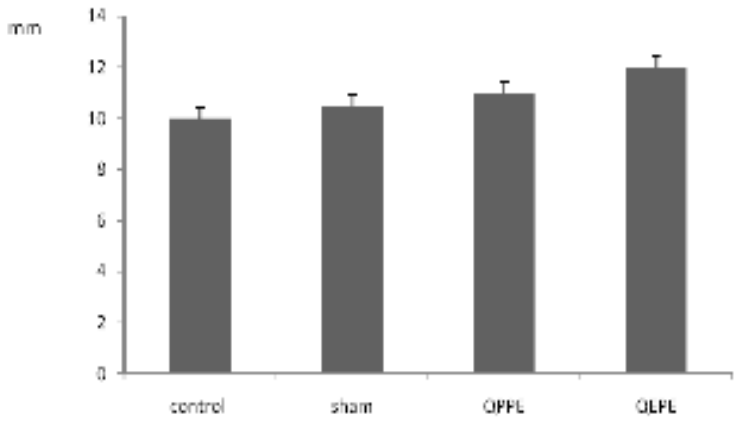

Figure 17. Comparison of the lengths of spleens of newborn Balb/C mice.

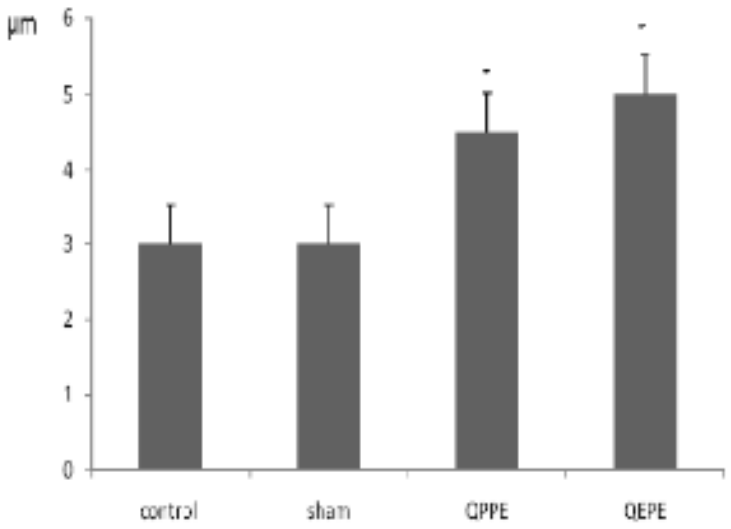

Figure 18. Comparison of the thickness of capsules of spleens of newborn Balb/C mice

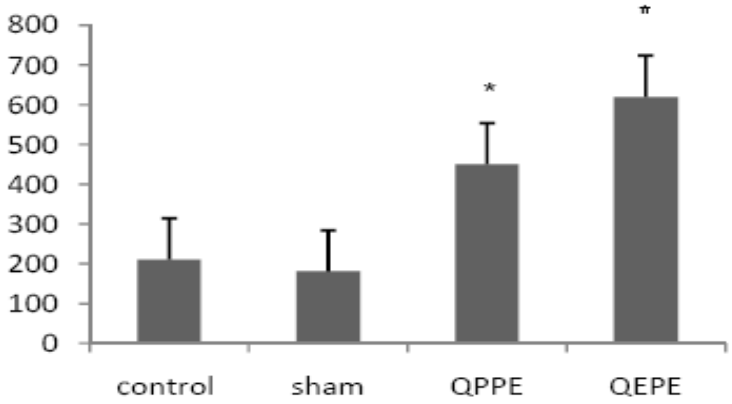

Figure 19. Comparison of the number of macrophage cells of spleens of newborn Balb/C mice.

Discussion

In current study, there was no significant difference between internal organs of control and sham groups , indicating methyl cellulose 0/05% (the solvent) had no teratogenic effects on newborn mice.

Results of this investigation proved that quinazolinones pass through placenta barrier,affecting brain and other organs.In response to QPPE and QEPE treatments, astrocytic hyperplasia was observed in the brains of newborn Balb/C mice,so that their processes,surrounding synapses with

efficient uptake systems, removed excitatoxins;There are growing evidences suggesting that astrocytes play critical role in the regulation of excitatoxicity and inflammatory processes during evolution of Alzheimer's disease (AD)(44).

Neurones are not injured because of astrocytes' involvement in homeostasis of CNS,regulating ionic and water balances,anti-oxidant concentrations,uptake and metabolism of neurotransmitters,and sequestration of potential neurotoxins(ammonia,heavy metals,and excitatory amino acid neurotransmitters such as glutamate and aspirate). Since astrocytes are responsible for major part of glutamate uptake and…… ,this turns glutamate from an excitatory neurotransmitter to a powerful neurotoxin.On the other hand, astrocytic factors down-regulate the expression of major histocompatibility complex-class-II and intercellular adhesion molecule-1 on human monocytes **,** which modulate inflammatory events in CNS and function as antigen-presenting cells (APC) upon expression of class-II major histocompatibility complex (MHC) antigens,in response to QPPE and QEPE.They also secrete growth factors and extracellular matrix molecules which play roles not only in development but also in repairing CNS(45, 46) .

In response to cytokines produced by T lymphocytes and macrophages, activated astrocytes form networks in the areas of inflammation,BBB, prevascular and subarachnoid regions recognize QPPE and QEPE as foreign antigens(47, 48).

Increase in the number of astrocytes (astrocytosis),in response to IL-1 (released from microglia)leads to the secretion of FGF, IGF, IL-6 and TGF (endocrine and paracrine)from astrocytes,maintening extracellular matrix and homeostasis of functioning neurons.Previous investigations indicated that IL-6,released from astrocytes, corresponded with gliosis, inducing NGF from astrocytes(49,50).On the other hand,QPPE and QEPE enter the brain as antigen,causing release of chemotaxics from macrophages. Thereafter,macrophages produce lysozymal enzymes,chemical mediatores and free radicals which would not bring about severe swellings,however,as in our experiments, brain could repair itself(51).

As results demonstrated, quinazolinones pass through placenta barrier by simple diffusion, along a chemical gradient entering gastric tissues, causing inflammation (gastritis) , because of atrophy in mucus layer, likely as the result of creating necrosis in stomach cells.QPPE and QEPE are without active chemical groups but generate free radicals and active metabolites after metabolization leading to lipid peroxidation ,destroying cell membranes after releasing intracellular components such as lysosomal enzymes, causing further tissue damages.Oxygen-derived free radicals play pathological roles in gastritis and radical scavengers such as alfa tocopherol, carotenoids glutathione redox system play a significant role in protecting membranes from oxidative damages. Depletion of gastric mucus GSH may result in accumulation of free radicals, initiating membrane damages by lipid peroxidation,ultimately leading to necrosis in specially parietal cells ,because of their numerous surface receptors and pumps specializing for gastric acid production(52).Apoptosis is uncommon in normal mucosal layer ,but chronic inflammation is associated with increased apoptosis, happening mainly and only in mucus surface (53) .

Appearance of connective tissues between myocytes is due to the stimulation of the multiplication of fibroblasts because of necrosis and their annihilation,so,necrotice heart cells will be replaced by connective tissue (54).

Increase in thickness of spleen capsule is often seen in patients with hepatic cirrhosis or portal hypertension from other causes in livers and hearts [55]. It could also be the result of increase in matrix contents ,blood and macrophage cells. So, capsule thickening created by damages in livers and hearts, increase the number of macrophage cells.

In conclusions,because of the its lower molecular weight (having less CH_2),QEPE could pass through phospholipid layer of cells quite easily,therefore its effect was more severe than QPPE.

Acknowledgements

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