Quercetin Preventive Effects on Theophylline-Induced Anomalies in Rat Embryo

Neda Sistani Karampour 1; Ardeshir Arzi 1,2,*; Hossein Najafzadeh Varzi 3; Babak Mohammadian 4; Mohsen Rezaei 1

1Department of Pharmacology and Toxicology, School of Pharmacy, Jundishapur University of Medical Sciences, Ahvaz, IR Iran
2Department of Pharmacology and Toxicology, Physiology Research Center, School of Pharmacy, Jundishapur University of Medical Sciences, Ahvaz, IR Iran
3Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamram University, Ahvaz, IR Iran
4Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamram University, Ahvaz, IR Iran

*Corresponding author: Ardeshir Arzi, Department of Pharmacology and Toxicology, Physiology Research Center, School of Pharmacy, Jundishapur University of Medical Sciences, Ahvaz, IR Iran. Tel:+986113738378, E-mail:Ardeshir.Arzi@gmail.com

Received: January 26, 2014; Revised: February 16, 2014; Accepted: February 23, 2014

Background: Theophylline has been shown to cause heart anomaly in animal and human fetus.

Objectives: The present study aimed to investigate the protective effect of quercetin on theophylline-induced heart disorders in rat embryo.

Materials and Methods: Theophylline-induced teratogenicity in rats was used as the animal model. Pregnant rats were administered theophylline (259 mg/kg, po) or theophylline plus quercetin (259 mg/kg, po and 100 mg/kg, ip, respectively) on 9th and 10th days of pregnancy. On day 19, cardiac changes were assessed, measuring malondialdehyde (MDA) and glutathione peroxidase (GPx) activity levels in blood samples and also the fetus heart weight. Histopathological examination was also performed on all specimens.

Results: Theophylline-treated rats showed MDA level elevation and GPx activity reduction. Quercetin treatment improved heart conditions and resulted in a significant reduction in MDA levels and elevation in GPx activity. Moreover, co-administration of quercetin and theophylline increased the heart weight significantly. Furthermore, histopathological study showed no changes in the treated groups.

Conclusions: This study demonstrated that quercetin have beneficial effects on theophylline-induced anomalies in rat embryo.

Keywords: Theophylline; Quercetin; Malondialdehyde; Glutathione peroxidase

1. Background

Congenital anomalies are among the most important causes of infant mortality. Genetic disorders and environmental factors, like maternal illness, receiving harmful chemicals and physical injuries during pregnancy, are of the most important causes of congenital anomalies. Certain medications are of the most prominent anomaly factors, during pregnancy (1). There are reports of anomalies in fetuses of mothers who had used theophylline during pregnancy, the most important of which are cardiovascular disorders (1, 2). Theophylline is a phosphodiesterase enzyme inhibitor, widely used for asthma (3).

The importance of theophylline in the treatment of asthma has decreased, due to more powerful effects of beta-receptor agonists and inhalation forms of anti-inflammatory compounds, in acute and chronic asthma, respectively. However, theophylline may be chosen as the third line treatment, in the absence of a good response to beta-receptor agonists or corticosteroids. It also of use in the treatment of patients with poor economic status and in countries with limited healthcare measures, due to its very low cost (3).

The most common complications of theophylline observed in asthmatic patients are restlessness, dizziness, sinus tachycardia, nausea, emesis and convulsion. It also easily crosses the placenta and harms the fetus (4, 5). Theophylline has dose-dependent teratogenic effects on the cardiovascular system development. Anomalies observed in infants born from mothers with asthma, using oral theophylline include double outlet right ventricle (DORV), heart murmurs, aortic displacement, ventricular septal defect (VSD), pulmonary valve stenosis and hypoplasia of the left ventricle and the mitral valve (1, 2).

Theophylline-induced complications in laboratory animals (rabbits, rats and mice) include cleft palate, abnormalities in fingers, small organs, micrognathia, bandy legs, DORV, aortic displacement and VSD (6, 7).

Fetal anomalies may be attributed to oxidative stresses, increasing the level of free radicals. Weakness of antioxidant defense and reduced antioxidant enzyme activity, like superoxide dismutase and glutathione peroxidase, are some of the causing factors (8). Nowadays, antioxidant activity and the amount of body oxidized biological products can be determined, in order to evaluate oxidative stress. The amount of serum malondialdehyde is a
The test groups received theophylline (259 mg/kg, po), quercetin (100 mg/kg, ip) and theophylline plus quercetin (259 mg/kg, po and 100 mg/kg, ip), respectively on the 9th and 10th days of gestation. The control group received normal saline (0.5 mL/100g, po) on the 9th and 10th days of gestation.

On day 19, pregnant females from all groups (control and study) were anesthetized with ether and blood samples were collected from their hearts for biochemical assay. The offsprings were then recovered, number of absorbed fetuses was measured and their hearts were removed. All blood samples were centrifuged at 2500 rpm, for 15 minutes, at temperature of 4°C. Serum samples were collected to assay the malondialdehyde and glutathione peroxidase levels.

3.4. Malondialdehyde Assay
Serum malondialdehyde (MDA) was determined according to the method of Buege and Aust, using bioassay laboratory ELISA kit (China). Standard and samples are aspirated into wells and the target protein is bound to specific immobilized monoclonal capture antibodies. The biotinylated secondary specific antibody is used for detection. To avidin-biotin-peroxidase complex, TMB substrate solution is added. The solution color changes after adding acidic TMB stop solution. Each step needs sufficient amount of washing.

3.5. Glutathione Peroxidase Assay
Serum glutathione peroxidase activity was assessed, according to the method of Paglia and Valentine, using Bioassay laboratory ELISA kit (China). Standard and samples are aspirated into wells, the target protein is bound to specific immobilized monoclonal capture antibodies. The biotinylated secondary specific antibody is used for detection. To avidin-biotin-peroxidase complex, TMB substrate solution is added. The solution color changes after adding acidic TMB stop solution. Each step needs sufficient amount of washing.

3.6. Histologic Techniques
The hearts were fixed in 10% buffered formalin, embedded in paraffin, sectioned stained with hematoxylin and eosin and E stained for microscopic studies.

3.7. Statistical Analysis
The data are expressed as mean ± SE. Statistical differences between means were determined by one-way analysis of variance (ANOVA), followed by LSD test and a threshold of significance of P < 0.05.

4. Results
No significant difference was observed in heart weights and the percentage of absorbed fetuses, between the groups receiving quercetin alone, quercetin plus theophylline and the normal saline group (Table 1 and Figure 1).
<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Embryos</th>
<th>Percentages of Live Embryos, %</th>
<th>Percentage of Absorbed Embryos, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>68</td>
<td>98.53</td>
<td>1.47</td>
</tr>
<tr>
<td>Theophylline</td>
<td>37</td>
<td>58.20</td>
<td>41.8</td>
</tr>
<tr>
<td>Quercetin</td>
<td>53</td>
<td>94.33</td>
<td>5.66</td>
</tr>
<tr>
<td>Theophylline + Quercetin</td>
<td>61</td>
<td>85.25</td>
<td>14.75</td>
</tr>
</tbody>
</table>

Table 1. Number and Percentage of Live and Absorbed Embryos in Control and Test Groups

No apparent morphological or specific pathological changes were observed during the heart macroscopic and microscopic examinations of the rat fetuses, in the normal saline (control) group, the theophylline receiving group, the quercetin receiving group and the group receiving both quercetin and theophylline. Pericardium, bilobate and mitral valves and tricuspid and pulmonary valves were found to be normal. Cardiac muscle cells were also normal; their large purple nucleus was located in the center of their pink colored cytoplasm (Figure 2).

Figure 2. The Quercetin and Theophylline-Induced Histopathological Changes in Rat Heart

5. Discussion
Promotion of free radicals and oxidative stress by medications is one of the main mechanisms of developing anomalies. The present research investigated the effect of quercetin (as an antioxidant factor) on the prevention of theophylline-induced heart anomalies in rat embryos. No significant difference was observed in the percentage of absorbed fetuses and heart weight, between groups receiving quercetin alone and quercetin plus theophylline and the control group. Moreover, quercetin negated the...
teratogenic effect of theophylline (increased percentage of absorbed fetuses and fetal heart weight).

In Lindstrom et al. study, following the theophylline administration with the doses of 24, 218 and 259 mg/kg, during the 6th to 15th days of rats' pregnancy, maternal womb weight decrease was observed in a dose dependent manner. Moreover, the number of fetuses decreased by 0.4% and their weight decreased by 0.3 and 0.4% in males and females, respectively. However, no macroscopic malformations were observed in these fetuses. This result is inconsistent with that of the present research.

Cardiac anomalies including DORV, heart murmur, aortic displacement, VSD, pulmonary valve stenosis and hypoplasia of the left ventricle were observed in a female infant, born to a 19-year-old mother who had consumed 800 mg/kg of oral theophylline, daily, since the age of 7 due to asthma. Moreover, cardiac failure, hypoplasia of the ascending aorta, aortic and left ventricle arch, aortic valve atresia and heart hypoplasia syndrome were observed in an infant born to a 32-year-old mother with asthma, who had used theophylline, orally, 300 mg/kg, twice a day.

Cardiac conditions, observed in the above studies are inconsistent with the results of the present research, which can be due to using animal models. The hypothesis that the consumption of antioxidants can reduce fetal complications of drugs is confirmed in other studies. This result is rather consistent with that of the present study.

Administration of vitamins A, C and E as antioxidants in the 9th and 10th days of rats' pregnancy can prevent nitrophen-induced conditions like the heart tissue hypoplasia, accompanied with diaphragmatic hernia. In this study, the administration of vitamins A, C and E prevented decreased fetal weight and cell count and increased apoptosis.

The administration of vitamin E was observed to have preventive effects on nitrophen-induced pulmonary hypoplasia in rat fetuses. In this study, the serum malondialdehyde level was significantly higher in the group receiving theophylline, compared to other groups. Since malondialdehyde is the final composition of lipid peroxidation and indicates the sensitivity of lipids to oxidation, its increase is indicative of increased oxidation level in theophylline receiving rats. Decreased malondialdehyde level in the theophylline and quercetin receiving group is indicative of quercetin ability, in increasing the antioxidant and decreasing the lipid oxidation levels.

Another study indicated that the administration of quercetin (15 mg/kg) for four weeks, decreases the serum malondialdehyde level in streptozotocin-induced diabetic rats. Another study indicated that the administration of quercetin in rats with monosodium urate-induced gout, decreases serum malondialdehyde level and increases serum antioxidant enzymes. Annapurna et al. stated that the administration of quercetin (50 mg/kg), ten minutes before stroke, decreases the malondialdehyde level in rats. Atef et al. observed that daily oral administration of 20 mg/kg of quercetin for seven consecutive days, significantly decreases the malondialdehyde level in diabetic rats.

In this study, the comparison of average glutathione peroxidase level indicated that the amount of this enzyme, in the theophylline receiving group is lower than the other groups. It is also significantly higher in the group receiving quercetine. Decreased glutathione peroxidase level in the theophylline receiving group is induced by increased oxidation process. The glutathione peroxidase level in the quercetin receiving group and lack of a significant difference, regarding this level, between the group receiving quercetin and the one receiving both theophylline and quercetin is related to quercetin antioxidant properties. The result of the present study is consistent with that of the other studies.

Quercetin increases the glutathione peroxidase activity gene expression in the liver of old rats. Moreover, the administration of quercetin to little white mice with the daily doses of 75, 50 and 25 mg/kg, for 15 consecutive days, increases the glutathione peroxidase level.

A study indicated that intraperitoneal administration of 50 mg/kg of quercetin 10 minutes before ischemia, increases the glutathione peroxidase level, in rats. Moreover, oral administration of 10 mg/kg of quercetin to rats with hypertension increases the glutathione peroxidase activity.

The antioxidant effect of quercetin on rats was investigated in another study, using hepatocyte cultivation. To induce oxidative stress, H$_2$O$_2$ was used. They concluded that the quercetin antioxidant activity is due to the glutathione peroxidase activation. Quercetin is a natural antioxidant, preventing free radicals formation and applies its antioxidant effect by inhibiting lipid peroxidation, blocking xanthine oxidase, chelating iron and eliminating free radicals.

The role of quercetin in decreasing the teratogenic effects of theophylline in rat embryos was demonstrated in this study for the first time. Regarding the role of quercetin in the elimination of free radicals and oxidative stress, it seems that as an antioxidant, quercetin can decrease some of the teratogenic effects of theophylline and also has an effect in the prevention of fetal complications. Further studies on different animal models and ultimately adapting the existing laws and regulations to human models are essential to ensure the beneficial effects of quercetin.

Acknowledgements

We would like to thank Mr. Mohammad Nokhbeh and Ms. Fakhri Kiani for laboratory work.
Authors’ Contributions
Supervision: Ardeshir Arzi; study concept and design: Ardeshir Arzi and Hossein Najafzadeh Varzi; drafting of the manuscript: Neda Sistani Karampour; statistical analysis: Kambiz Ahmadi Engali; administrative, technical, and material support: Hossein Najafzadeh and Babak Mohamadian and Mohsen Rezaie.

Funding and Support
This research was supported by a grant (PRC30) from the Physiology Research Center of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran.

References