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Troponin T and Caspase-3 Changes Induced by Theophylline and Quercetin in Heart Tissue of Rat Embryo

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Abstract: Theophylline induces heart anomaly in human and animal fetus. Intracellular oxidative stress might be one of the mechanisms of action of the teratogens. The aim of this study was to investigate the protective effect o quercetin on theophylline induced-heart disorder in rat embryo. For this study, pregnant female rats were randomly divided into a controlled and 3 experimental groups. Experimental groups received theophylline (259 mg kg^{-1}, orally), quercetin (100 mg kg^{-1}, ip), theophylline and quercetin (259 mg kg^{-1}, orally and 100 mg kg^{-1}, ip), respectively on 9th and 10th days of pregnancy. The control group received an equal volume of normal saline on 9th and 10th days (ip). The fetuses were recovered on day 19, then their weight and length of fetus were measured and their hearts were removed to assay the level of troponin T and caspase-3 in heart tissues. The result indicated that the mean weight and length were significantly decreased by theophylline in comparison with control group. Amount of troponin T was not different in any groups. Theophylline increased caspase-3 activity in comparison with control group.

Key words: Theophylline, quercetin, troponin T, caspase-3, heart tissue, rat embryo

INTRODUCTION

The methylated xanthines including caffeine, theophylline and theobromine are known to occur in such beverages as coffee, tea, cocoa and cola drinks. Because of this and some clinical usage of each of these drugs, there is a great deal of human exposure. Indeed, it is reasonable to assume that almost everyone is exposed at least to some extent. Of the three pointed materials, theophylline is more used for its therapeutic effects (Tucci and Skalko, 1998; Brunton *et al*., 2011). Theophylline plays an important role in the management of asthma both, as a prophylactic drug and in the treatment of prolonged attacks and status asthmatics. It is one of the drugs of choice in the treatment of asthma during pregnancy which occurs with an incidence of 0.4-1.3% and it has gained increasing popularity as a treatment of idiopathic apnea in the premature infants (Lindstrom *et al*., 1990; Kaysar and Andcupit, 1978; Lucey, 1975). Theophylline has been shown to cross the human placenta and it is secreted in breast milk (Lindstrom *et al*., 1990; Labovits, 1982). It has been suggested that therapeutic doses in mothers may be toxic to embryo and infants (Labovitz and Spector, 1982).

Several laboratories have investigated the teratogenic potential of theophylline in rodents. In mice, a single dose of theophylline (100 mg kg^{-1}, ip) produced cleft palate, digital abnormalities, micromelia, micrognathia, clubfoot, subcutaneous hematoma, open eyelids and embryo lethality (Morrissey *et al*., 1988).

In rats, theophylline (150 mg/kg/day, orally) produced digital abnormalities whiles embryo lethality occurred at a subcutaneous dose of 200 mg/kg/day (Morrissey *et al*., 1988). In rabbits, administration of theophylline (60 mg/kg/day, ip) caused cleft palate and embryo lethality (Shibata *et al*., 2000).

Administreation of theophylline induced aortic aneurysms and ventricular septal defects in embryonic chick (Shikawa *et al*., 1978; Matsuoka *et al*., 1992; Gilbert *et al*., 1977).

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Previous documents were reported that theophylline is a potent cardiovascular teratogen in human fetus. The cardiovascular anomalies were aortic anomalies, double-outlet right ventricle and transposition of the great arteries, total anomalous pulmonary venous connection and hyperplasia of the left ventricle (Matsuoka *et al*., 1985; Park *et al*., 1990; Watanabe *et al*., 2008).

Some studies indicate that the mechanism of acting the teratogen could be intracellular oxidative stress and subsequent apoptotic cell death. Apoptosis is a process that is regulated by prosurvival signaling cascades. Various proteins (caspases) involved in apoptosis. Experimental evidence demonstrated that activated caspase-3 was considered to be a hallmark of apoptosis (Jeong *et al*., 2009; Zhong *et al*., 2010).

Base on the earlier mentioned data, this evidence leads us to hypothesize that possibly administration of antioxidant agents could act positively on some of the malformations.

One of the most important groups of antioxidants are flavonoids that may help prevent creation of anomalies (Asl *et al*., 2006; Khaki *et al*., 2010). Quercetin is a flavonoid that forms the backbone for many other flavonoids including the citrus flavonoids, rutin, hesperidin, naringin and tangeritin (Asl *et al*., 2006).

Quercetin has demonstrated a significant anti-inflammatory activity, it inhibits release of histamine and other allergic mediators. Quercetin also shows remarkable anti-tumor properties (Asl *et al*., 2006; Khaki *et al*., 2010). Measurements of serum levels of cardiac Troponin-T (cTnT), a component of the troponin complex of muscle cells have begun to be used for the diagnosis of myocardial damage in various conditions including acute myocardial infarction, acute myocarditis, unstable angina and isoproterenol-induced myocardial necrosis (Herman *et al*., 1999; Koh *et al*., 2004). Accordingly, measurement of troponin T can be a suitable hallmark of heart anomalies.

The aim of this study, was to investigate the protective effects of quercetin on theophylline induced-heart disorder in rat embryo.

MATERIALS AND METHODS

Theophylline preparation: Theophylline was obtained from Daroopakhsh Company (Iran). It was dissolved in normal saline. This solution was prepared immediately preceding its use (Lindstrom *et al*., 1990).

Quercetin preparation: Quercetin powder was obtained from Sigma Chemical Company (St. Louis, MO, USA). It was dissolved in 0.9% normal saline, mixed vigorously and stored in a dark bottle at 4°C. The quercetin solution was freshly prepared each day (Khaki *et al*., 2010).

Animals: The Wistar rats were obtained from animal house of Jondishapour Medical Sciences University of Ahvaz and were kept under specific conditions on a constant 12 h light/dark cycle and at a controlled temperature of 23±2°C. Standard pellet food and tap water were available *ad labium*. Mature female Wistar rats were mated overnight at a 3:1 ratio of female:male. Then pregnant female Wistar rats were randomly divided into 4 equal groups (n = 10), a control group and 3 experimental groups: Experimental groups recived theophylline (259 mg kg^{-1}, orally), quercetin (100 mg kg^{-1}, ip), theophylline and quercetin (259 mg kg^{-1}, orally and 100 mg kg^{-1}, ip), respectively on the 9th and 10th days of gestation. The control group received an equal volume of normal saline (ip) on the 9th and 10th days of gestation (Vieira *et al*., 2011; Gonzalez-Reyes *et al*., 2005; Overmans and Beaudoin, 1971).

On day 19, pregnant females from all groups (control and experimental) were anesthesia with ether then offsprings were recovered and weight and length of them were measured and their hearts were removed. The heart samples were homogenized with PBS (pH:7.4) and centrifuged at speed of 3000 rpm at 4°C for 20 min. Supernatants were transferred to clean tubes and stored at -70°C. Then, the level of troponin T and caspase-3 were measured in heart tissues.

Troponins T assay: The level of cardiac Troponin T (cTnT) in tissue was mesasured by using a rat cTnT LISA kit (Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions (Herman *et al*., 1999).

Caspase-3 assay: Tissue level of caspase-3 was mesasured by using Bioassay laboratory ELISA kit (China).

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 $p<0.05$.

RESULTS AND DISCUSSION

The means of weight and length of fetuses in the group that received theophylline was significantly lesser than the other group ($p<0.05$). But, the difference was not significant among the other groups ($p>0.05$) (Fig. 1 and 2). The difference mean trponin T of heart fetus was not significant in all the groups (Fig. 3).

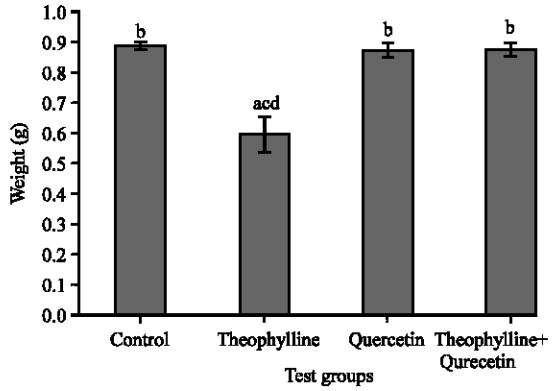

Fig. 1: Weight (mean±SE) of fetuses in normal saline and test groups: Normal saline (control) (1 mL/100 g, ip); Theophylline (259 mg kg^{-1} po); Quercetin (100 mg kg^{-1}, ip); Theophylline+Quercetin (259 mg kg^{-1}, po; 100 mg kg^{-1}, ip). Different letters show significant difference between groups ($p<0.05$, n = 30)

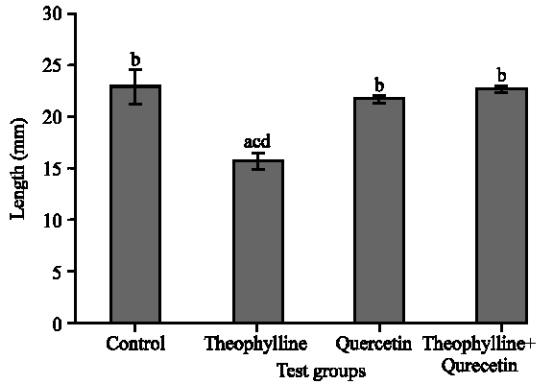

Fig. 2: Length (mean±SE) of fetuses in normal saline and test groups: Normal saline (control) (1 mL/100 g, ip); Theophylline (259 mg kg^{-1}, po); Quercetine (100 mg kg^{-1}, ip); Theophylline+Quercetine (259 mg kg^{-1}, po ;100 mg kg^{-1}, ip). Different letters show significant difference between groups ($p<0.05$, n = 30)

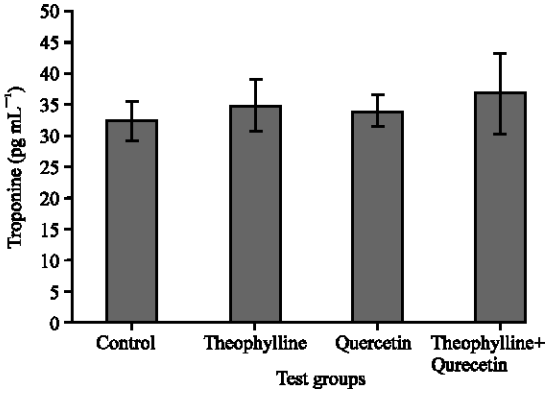

Fig. 3: Troponin T (mean±SE) of fetuses heart in normal saline and test groups: Normal saline (control) (1 mL/100 g, ip); Theophylline (259 mg kg^{-1}, po); Quercetine (100 mg kg^{-1}, ip); Theophylline+ Quercetine (259 mg kg^{-1}, po; 100 mg kg^{-1}, ip). There is no significant difference between group, n = 30)

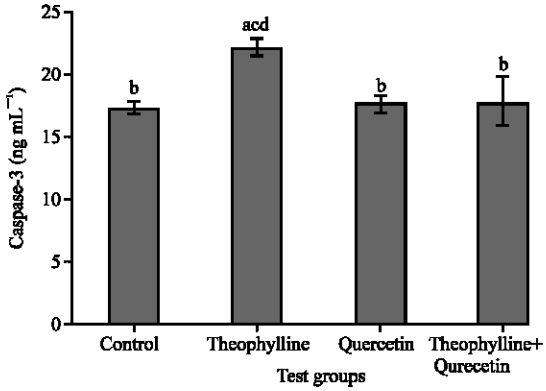

Fig. 4: Caspase-3 (mean±SE) of fetuses heart in normal saline and test groups: Normal saline (control) (1 mL/100 g, ip); Theophylline (259 mg kg^{-1}, po); Quercetine (100 mg kg^{-1}, ip); Theophylline+ Quercetin (259 mg kg^{-1}, po; 100 mg kg^{-1}, ip). Different letters show significant difference between groups ($p<0.05$, n = 30)

The means caspase-3 of heart fetuses in the group that received theophylline was significantly ($p<0.05$) more than other groups. But, the difference was not significant among the other groups (Fig. 4).

In the present study, administration of theophylline (259 mg kg^{-1}, orally) on 9th and 10th days of gestation significantly reduced weight and length of fetuses in comparison with control group (normal saline) but there was not seen macroscopic anomalies.

Another study showed that administration of theophylline (124, 218 and 259 mg/kg/day) on 6 through 15 of gestation in rat induced a dose-related decreasing trend in gravid uterine weight. The number of live fetuses decreased and the average male and female fetal weight decreased at the range of 0.30 and 0.40% (Lindstrom *et al*., 1990). As well as administration of theophylline (100, 150 and 200 mg kg^{-1}, ip) on days 10 through 13 of gestation in mice caused moderately embryo lethal, cleft palate, ectrodactyly, syndactyly and micromelia as the predominant malformations (Tucci and Skalko, 1998).

Caffeine (a biotransformation product of theophylline in the human fetus) with dose of 150 mg kg^{-1} (ip) indused

ventricular septa1 defect in embryo rats (Matsuoka *et al*., 1987). Also, the teratogenic and fetal toxicity of theophylline (60 mg/kg/day, IV) on 6 through 18 of gestation in rabbit was reported. Fetuses exhibited teratogenic toxicity, such as celeft palate and skeletal variation of 13th rib. Fetal toxicity was observed including abortion, increased number of late deaths and decreased body weight appearing on day 29 of gestation (Shibata *et al*., 2000).

Adminestration of tedral (theophylline, Ephedrine and Phenobarbital) in chick embryos showed that theophylline, a major component of tedral, produce cardiovascular anomalies in embryonic chick heart (Matsuoka *et al*., 1987).

In human, it was reported that three infants with severe unusal congenital cardiovascular anomalies born to asthmatic mothers who had taken theophylline throughout pregnancy (Park *et al*., 1990).

There was no significant difference among groups that received quercetine alone, theophylline along with quercetin and normal saline (control), thus it showed that quercetine could prevent from effect of theophylline on weight and length of fetus.

The level of cardiac Troponin T (cTnT) in tissue cTnT had no significant deference in all the groups in the study (Sato *et al*., 2001). While, another study showed a positive correlation between serum levels of cardiac troponin T and the severity of the chronic cardiomyopathy induced by doxorubicin (adriamycin) in rat (Sato *et al*., 2001; Herman *et al*., 2001). Experimental evidence demonstrated a positive correlation between serum levels of cardiac troponin T and cardiomyopathy induced by isopernolol (Bleuel *et al*., 1995).

Discrepancies between this study and others can be related to our teratogen (theophylline) and its dosage. The result of this study showed that administration of theophylline increased significantly tissue level of heart caspase-3 in comparison with other groups.

Previous research has shown that teratogens, such as hyperthermia, 4-hydroperoxycyclophosphamide and staurosporine induce cell death in mouse embryos by activating the mitochondrial apoptotic pathway. Key to the activation of this pathway is the activation of the caspase cascade involving the cleavage-induced activation of an initiator ptocaspase, caspase-9 and the downstream effecter procaspase, caspase-3 (Little *et al*., 2003). In mouse embryonic limbs cultured with 4-hydroperoxycyclophosphamide (4-OOHCPA), a teratogenic metabolite of CP, the activation of caspase-3 has been observed (Huang and Hales, 2002).

Also, the activation of caspase-3 was observed in mouse embryonic limbs cultured with another teratogen, vitamin A (Ali-Khan and Hales, 2003). One of the previous studies showed that quercetin inhibited caspase-3 activation in retinal pigment epithelial cells in age-related macular degeneration (Kook *et al*., 2008).

CONCLUSION

Based on the results of this study, quercetin prevent from decrease of weight and length of rat embryos which is induced by theophylline, also quercetin as an antioxidant prevent from increase of tissue level of caspase-3 induced by theophylline. It is mentionable, no change was seen in tissue level of troponin T among all groups.

Regarding the role of quercetin in omitting free radicals and oxidative stress, it seems that most of teratogenic effects of theophylline can be decreased by using quercetin (antioxidant).

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REFERENCES

Ali-Khan, S.E., B.E. Hales, 2003. aspase-3 mediates retinoid-induced apoptosis in the organogenesis-stage mouse limb. Birth Defects Res. A: Clin. Mol. Teratol., 67: 848-860.

Asl, B.H., A. Delazar and M. Assady, 2006. Effects of quercetin and ACTH on morphine-induced tolerance and dependence in mice. Iran. J. Pharm. Sci., 2: 137-142.

Bleuel, H., U. Deschl, T. Bertsch, G. Bolz and W. Rebel, 1995. Diagnostic efficiency of troponin T measurements in rats with experimental myocardial cell damage. Exp. Toxicol. Pathol., 47: 121-127.

Brunton, L.L., B. Chabner and B.C. Knollmann, 2011. Goodman and Gilmans the Pharmacological Basis of Therapeutics. 12th Edn., MacMillan, New York, USA., ISBN-13: 9780071624428, pp: 367-375.

Gilbert, E.F., H.J. Jr. Bruyere, S. Ishikawa, M.O. Cheung and R.J. Hodach, 1977. The effects of methylxanthines on catecholamine-stimulated and normal chick embryos. Teratology, 16: 47-52.

Gonzalez-Reyes, S., L. Martinez and J.A. Tovar, 2005. Effects of prenatal vitamins A, E and C on the hypoplastic hearts of fetal rats with diaphragmatic hernia. J. Pediatric Surg., 40: 1269-1274.

Herman, E.H., J. Zhang, S.E. Lipshultz, N. Rifai and D. Chadwick *et al*., 1999. Correlation between serum levels of cardiac troponin-T and the severity of the chronic cardiomyopathy induced by doxorubicin. J. Clin. Oncol., 17: 2237-2243.

Herman, E.H., J. Zhang, N. Rifai, S.E. Lipshultz and B.B. Hasinoff *et al*., 2001. The use of serum levels of cardiac troponin T to compare the protective activity of dexrazoxane against doxorubic in- and mitoxantrone-inducedcardiotoxicit y. Cancer Chemother. Pharmacol., 48: 297-304.

Huang, C.F. and B.F. Hales, 2002. Role of caspases in murine limb bud cell death induced by 4-hydroperoxycyclophosphamide, an activated analog of cyclophosphamide. Teratology, 66: 288-299.

Jeong, J.J., Y.M. Ha, Y.C. Jin, E.J. Lee and J.S. Kim *et al*., 2009. Rutin from *Lonicera japonica* inhibits myocardial ischemia/reperfusion-induced apoptosis *in vivo* and protects H9c2 cells against hydrogen peroxide-mediated injury via ERK1/2 and PI3K/Akt signals *in vitro*. Food Chem. Toxicol., 47: 1569-1576.

Kaysar, S.R. and G.C. Andcupit, 1978. Teratogenicity of selected asthmatic medications. Drug Intell. Clin. Pharm., 5: 162-173.

Khaki, A., F.F. Azad, H.R.A. Ashtiani, S.H. Rezazadeh, H. Rastegar and A.M. Imani, 2010. Compartments of quercetin and *Allium cepa* (onion) on blood glucose in diabetic rats. J. Med. Plans, 9: 107-112.

Koh, E., T. Nakamura and H. Takahashi, 2004. Troponin-T and brain natriuretic peptide as predictors for adriamycin-induced cardiomyopathy in rats. Circ. J., 68: 163-167.

Kook, D., A.H. Wolf, A.L. Yu, S.A. Neubauer, S.G. Priglinger, A. Kampik and U.C. Welge-Lussen, 2008. The protective effect of quercetin against oxidative Stress in the human RPE *in vitro*. Invest. Ophthalmol. Vis. Sci., 49: 1712-1720.

Labovitz, E. and S. Spector, 1982. Placental theophylline transfer in pregnant asthmatics. J. Am. Med. Assoc., 247: 786-788.

Lindstrom, P., R.E. Morrissey, J.D. George , C.J. Price, M.C. Marr, C.A. Kimmel and B.A. Schwetz, 1990. The developmental toxicity of orally administered theophylline in rats and mice. Fundam. Applied Toxicol., 14: 167-178.

Little, S.A., W.K. Kim and P.E. Mirkes, 2003. Teratogen-induced activation of caspase-6 and caspase-7 in early postimplantation mouse embryos. Cell Biol. Toxicol., 19: 215-226.

Lucey, J.F., 1975. The xanthine treatment of apnea of prematurity. Pediatrics, 55: 584-586.

Matsuoka, R., E.F. Gilbert, H. Bruyers Jr. and J.M. Optiz, 1985. An aborted human fetus with truncus arteriosus communis-Possible teratogenic effect of Tedral. Heart Vessels, 1: 176-178.

Matsuoka, R., H. Uno, H. Tanaka, C.S. Kerr, K. Nakazawa and B. Nadal-Ginard, 1987. Caffeine induces cardiac and other malformations in the rat. Am. J. Med. Genet Suppl., 3: 433-443.

Matsuoka, R., T. Nishikawa, H.J. Bruyere Jr. and E.F. Gilbert, 1992. Teratogenic effect of tedral (theophylline, ephedrine and phenobarbital) on cardiac development in chick embryos. Congenital Anomalies, 32: 65-75.

Morrissey, R.E., J.J. Collins, J.C. Lamb, A.G. Manus and D.K. Gulati, 1988. Reproductive effects of theophylline in mice and rats. Fundam Applied Toxicol., 10: 525-536.

Overmans, D.O. and A.R. Beaudoin, 1971. Early biochemical changes in the embryonic rat heart after teratogen treatment. Teratology, 4: 183-190.

Park, J.M., V. Schmer and T.L. Myers, 1990. Cardiovascular anomalies associated with prenatal exposure to theophylline. South Med. J., 12: 1487-1488.

Sato, Y., T. Yamada, R. Taniguchi, K. Nagai and T. Makiyama *et al*., 2001. Persistently increased serum concentrations of cardiac troponin T in patients with idiopathic dilated cardiomyopathy are predictive of adverse outcomes. Circulation, 103: 369-374.

Shibata, M., M. Wach, M. Kawaguchi, J. Kojima and K. Onodera, 2000. Teratogenic and fetal toxicity following intravenous theophylline administration in pregnant rabbits is related to maternal drug plasma levels. Methods Find Exp. Clin. Pharmacol., 22: 101-107.

Shikawa, S., E.F. Gilbert, H.J. Bruyere Jr. and M.O. Cheung, 1978. Aortic aneurysm associated with cardiac defects in theophyline stimulated chick embryos. Teratology, 18: 23-30.

Tucci, S.M. and R.G. Skalko, 1998. The trratogenic effect of theophylline in mice. Toxicol. Lett., 1: 337-341.

Vieira, E.K., S. Bona, F.C.D. Naso, M. Porawski, J. Tieppo and N.P. Marroni, 2011. Quercetin treatment ameliorates systemic oxidative stress in cirrhotic rats. ISRN Gastroenterol., Vol. 2011. 10.5402/2011/604071.

Watanabe, S., J. Yamakami, M. Tsuchiya, T. Terajima, J. Kizu and S. Hori, 2008. Anti-inflammatory effect of theophylline in rats and its involvement of the glucocorticoid-glucocorticoid receptor system. J. Pharm. Sci., 106: 566-570.

Zhong, H., H. Xin, L.X. Wu and Y.Z. Zhu, 2010. Salidroside attenuates apoptosis in ischemic cardiomyocytes: A mechanism through a mitochondria-dependent pathway. J. Pharmacol. Sci., 114: 399-408.