Effect of Cigarette Smoke Exposure and Exercise on Abortion, Placental Vascularization, Human Chronic Gonadotropin Hormone and Birthweight

Shadi Barzanjeh[1] Mehdi Eskandari[1]*, Hosein Mostafavi[1], Mohsen Alipour[1] and Saeed Ghanbarzadeh[2]

[1]Department of Physiology Medicinal and Pharmacology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
[2]Zanjan Nanotechnology Research Center, and Department of Pharmaceutics, Faculty of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran.

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The purposes of this investigation were to examine the effects of exercise and smoking throughout pregnancy on placenta growth and vascularization, human chorionic gonadotropic hormone (HCG) level and birthweight. Rats in group 1 were selected as control group and were not in exposure of smoke and not exercised. Rats in group 2 were exercised for 5 days in week for 3 weeks. Rats in group 3 were in the cigarette smoke exposure without exercise. Finally, rats in group 4 were forced to exercise and were in the exposure of cigarette smoke, simultaneously. Six rats from each group were killed after 3 weeks of pregnancy and the number of blood vessels, placenta decidual thickness, and HCG level were analyzed. Birthweight and weight of offspring after 60 days in each group were also evaluated. Results showed that the number of blood vessels and HCG level were decrease in animals which exposed to smoke and exercise ($P<0.05$), however, the placenta decidua thickness did not changed significantly. The offspring birthweight in group 4 was decreased significantly ($P<0.001$) in comparison with control group. Accordingly, the offspring birthweight in group 3 and 4 was decrease significantly compared to group 2 ($P<0.01$). On the other hand, the weight of offspring in day 60 in group 3 was less than group 4. In conclusion, both exercise and smoking have negative effect on blood vessels, HCG level, and offspring birthweight, however, there is synergic effect when rats were in exposure of smoke and exercise, simultaneously.

Keywords: Cigarette Smoke, Exercise, Pregnancy, Placenta, Birthweight.

Cigarette smoking in the around the world is a critical challenge for women and children's health. Cigarette smoke contains about 4800 compounds of which at least 60 are classified as carcinogens. Cigarette smoking causes 30% of all cancer deaths in developed countries. It is estimated that cigarette smoking kills over 1000000 people each year by causing lung cancer and many other neoplasmas. In addition to lung cancer, cigarette smoking is an important cause of esophageal, oral, oropharyngeal, hypopharyngeal, and laryngeal cancers as well as pancreatic cancer, bladder cancer, liver, colon, nose, and myeloid leukemia, and cancer of the renal[1-5]. Some other components have adverse effects on the cardiovascular, respiratory, reproductive, and nervous systems[6-10]. The World Health Organization reports that the smoking rates for women (≤15-year old) range between 7-24% in the U.S., Europe, and Australia. It has been widely reported that smoking during pregnancy or secondhand smoke, as involuntary smoking, have

* To whom all correspondence should be addressed.
Tel: +982433342767; Fax: +98243 33424769;
E-mail: Mehdiesk@zums.ac.ir

negative reproductive effects including reduced fetal growth, low birth weight, premature delivery, spontaneous abortion, placental abruption, perinatal mortality and ectopic pregnancy as well as increased risk of fetal mortality. Furthermore, maternal smoking accounts for an estimated 20–30% of all low birthweight babies, as well as for 14% of all preterm deliveries, and some 10% of all infant death[11-14]. In several animal models and the human, regular maternal exercise reduces birthweight. Recent findings suggested that regular, sustained, moderate to high intensity exercise during pregnancy might change vessel growth, blood flow, surface area and the configuration of the villous tree. Furthermore, the concern was heightened by the other reports indicating to reduction in all villous dimensions in placenta from human pregnancies at high altitude, and morphometric outcome is similar to that seen with regular exercise throughout pregnancy[15-18]. Thus, the current study was undertaken to determine if exercise can attenuate the negative effect of cigarette smoke exposure during pregnancy.

MATERIAL AND METHODS

Animals

All animal experiments were conducted according to the Guide for Care and Use of Laboratory Animals of Zanjan University of Medical Sciences, Zanjan-Iran (National Institutes of Health Publication No 85-23, revised 1985).

Experimental Design

Thirty two adult female Wistar rats (weighing 200-250 g, aged 8 weeks) were divided into four groups (n=8). In group 1 (Control group) animals were not exposed to smoke and not exercised. To investigate the effect of smoke and exercise, animals were exposed to smoke or forced to exercise 5 days in a week for 3 weeks, respectively. Rats in group 4 were both in exposed to smoke (rats were exposed, for 6 hours for 5 days in a week for 3 weeks to tobacco smoke,) and exercised (swimming program was used as an exercise model) [19, 20]. Animals had free access to food and water under standard lighting conditions (12-h light: 12-h darkness). The presence of vaginal plug proved the natural pregnancy and this day was considered as day one of the pregnancy[21].

Blood sampling and hormonal assay

After 3 weeks 6 rats from each group were sacrificed (by cervical dislocation) and their placenta decidual thickness and the number of blood vessels and abortion were evaluated. The number of identified blood vessels was counted by a built-in function in ImagePro software (Media Cybernetics, Bethesda, MD, USA). Human chorionic gonadotropin is a pregnancy hormone secreted by the placental synctiotrophoblast cell layer that has been linked to fetal growth and various placental, uterine and fetal functions[22]. The anticoagulated blood samples using sodium citrate were collected and HCG level was measured with enzyme-linked immunosorbent assay (ELISA) method (Diaplus, USA) . The offspring birthweight and their weight after 60 days of remained rats were also measured[23, 24].

Tissue preparation and morphological staining

To evaluate the morphology of the tissues, the sections were stained by routine hematoxylin and eosin technique and examined under light microscope. Tissues from each group were fixed in formaldehyde, embedded in paraffin wax, sectioned at 6 micrometer and stained using hematoxyline and eosin (H&E) technique. After preparation of the sections, 3 slides were chosen randomly from each sample and at least four fields of view were measured from each slide. The following blood vessels number and the placenta decidual thickness were measured in each field of view[25].

Statistical Analysis

The number of blood vessels in each group was compared using paired t-test. Independent Student's t-test was used to compare the proportion of cells in the same region of the tissue between the treated and control groups. The sufficient number of our samples was approved by statistically normality test. Data were expressed as mean ± SD and P values of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Effect of smoke and exercise on HCG level

Fig. 1 represents the effect of exposure to smoke and exercise on the HCG level in rats in four investigated groups. As shown in both smoke

Table 1. Effect of cigarette smoke and exercise on the placenta decidua thickness and blood vessels number

Group	Control	Smoke	Exercise	Smoke/Exercise
Placenta decidua thickness (µm)	502.28 ± 148.8	489.5 ± 517	517.9 ± 119.7	456.5 ± 112.1
Blood vessels number	17.3 ± 7.1	15.1 ± 3.9	11.1 ± 5.4*	9.3 ± 4.6**

Values are expressed as mean±SEM of each group. The results were analyzed to be statistically significant at *P<0.05, **P<0.01, ***P<0.001 significantly different from control group.

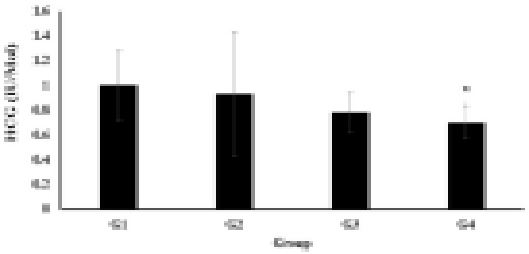
exposed and smoke exposed-exercised groups the level of HCG were decreased, however, the decrease in HCG level was significant in smoke exposed-exercised group (P<0.05). Furthermore, results indicated that the level of HCG did not decrease in exercised rat which were not in exposure of smoke.

Effect of cigarette smoke and exercise on the abortion

As shown in Fig. 2 the exercise and smoke alone did not have significant effect on number of abortion. However, in rats in group which exposed to cigarette smoke and exercised simultaneously, the number of abortion was increased significantly (P<0.001). This finding indicated that the exercise in the smoke exposure had negative effect on the fetal growth.

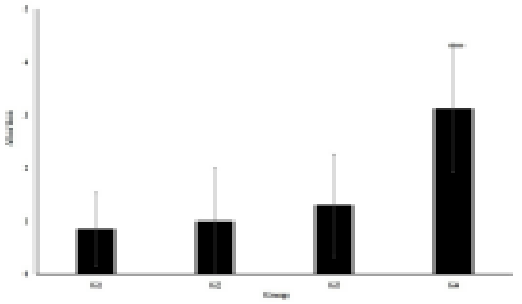
Effect of cigarette smoke and exercise on the birthweight and offspring weight after 60 days

Effects of cigarette smoke on birthweight are mainly as a results of fetal growth limitation.



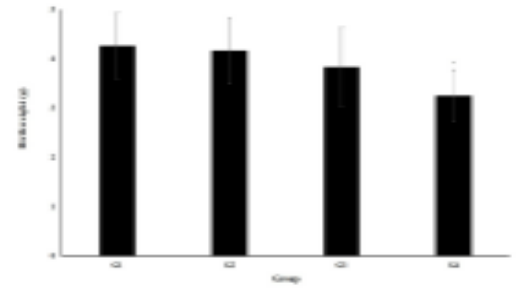
Values are expressed as mean±SEM of each group. The results were analyzed to be statistically significant at *P<0.05, **P<0.01, ***P<0.001 significantly different from control group.

Fig. 1. The effect of smoke and exercise on HCG level



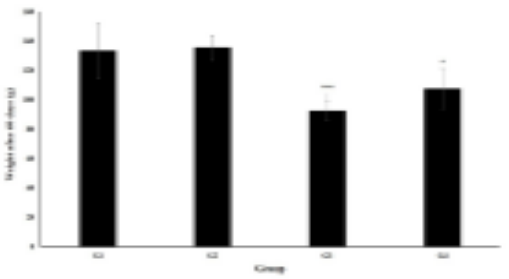
Values are expressed as mean±SEM of each group. The results were analyzed to be statistically significant at *P<0.05, **P<0.01, ***P<0.001 significantly different from control group.

Fig. 2. The number of abortion in different groups



Values are expressed as mean±SEM of each group. The results were analyzed to be statistically significant at *P<0.05, **P<0.01, ***P<0.001 significantly different from control group

Fig. 3. Effect of cigarette smoke and exercise on the birthweight of offspring



Values are expressed as mean±SEM of each group. The results were analyzed to be statistically significant at *P<0.05, **P<0.01, ***P<0.001 significantly different from control group.

Fig. 4. Effect of cigarette smoke and exercise on the offspring weight after 60 days

The mechanisms underlying the effects of cigarette on fetal growth are not well defined and are likely to be multifactorial. Fig. 3 represents the effect of cigarette smoke and exercise on the birthweight of offspring. Results indicated that simultaneous cigarette smoke exposure and exercise, during pregnancy, resulted in decrease in birthweight of offspring. These results were in accordance with earlier results which indicated that, exercised rats which were in exposure of smoke during pregnancy resulted in lower offspring birthweight. Accordingly, exercise or smoke exposure alone showed negative effect on birthweight of offspring, however were not significant.

Fig. 4 also represents the effect of smoke and exercise alone and simultaneously on the offspring weight after 60 days. Like earlier results, exercise alone did not show significant effect on offspring weight after 60 days. However, surprisingly in the case of offspring weight after 60 days, exposure with smoke during pregnancy showed higher negative effect than rats which exercised in the exposure of cigarette smoke.

Effect of cigarette smoke and exercise on the placenta tissue

It is well recognized that smoking during pregnancy resulted in fetal hypoxia and increases the risk of perinatal problems. The placental pathologies related to maternal smoking are essential in many of the adverse developmental effects. Maternal smoking alters the placenta blood flow and balance between proliferation and differentiation of the cytotrophoblast. Furthermore, the changes in gene and protein expression in the cytotrophoblast, regulates cellular responses to oxygen tension, are also observed. These changes were seen in the placentas of smokers in less severe than women exposed to secondhand smoke during pregnancy[26-30]. Previous studies showed that smoking induces a generalized dysfunction of both villous and invasive trophoblasts in early pregnancy. Biochemical markers of placental function show that maternal levels of oestriol, oestradiol, HCG, and human placental lactogen (HPL) hormones were lower in smokers than non-smokers pregnant women[31-33]. Literature review showed that placental development is influenced by a change of environmental factors which alter oxygen tension and placental perfusion such as chronic pulmonary disease, diabetes, smoking, diet and sustained exercise. Furthermore, many reports indicated that maternal smoking has been associated with thickening of the trophoblastic basement membrane, increased collagen in the villous mesenchyme and decreased vascularization of the placenta[34-36]. Placenta decidual thickness was measured in the all of four groups. Results indicated that, placenta decidual thickness in rats which were in the cigarette smoke exposure, exercised, or rats which exercised in the exposure of smoke did not change significantly in comparison with rats in control group. Results also showed that, in all of treated groups the number of blood vessels was decreased, however, only the number of blood vessels in rats exposed in cigarette smoke and exercised was decreased significantly ($P<0.05$).

CONCLUSIONS

Cigarette smoke is a complex mixture of toxic chemicals including nicotine, carbon monoxide, and several renowned carcinogens and mutagens which absorbed through the pulmonary vasculature and transported via the bloodstream. These toxins cause cytotoxicity, genotoxicity, and tumorigenicity throughout the body. Furthermore, in addition to the harmful effects of on cardiovascular and pulmonary physiology, cigarette smoking affects the reproductive system. Smoking have been found to be associated with infertility, ectopic pregnancy, and spontaneous abortion menstrual abnormalities. Results of the present study indicated that, exposure to cigarette smoke and exercise simultaneously showed synergic negative effect on placenta tissue, HCG hormone level, birthweight and weight of offspring weight after 60 days. Therefore, it is suggested that animals or humans which are in the exposure of cigarette smoke avoided from exercise.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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