Genistein preserves the lungs of ovariectomized diabetic rats: addition to apoptotic and inflammatory markers in the lung

Faeze Daghigh [1], Alireza Alihemmati [2], Pouran Karimi [3], Parisa Habibi [4], Naser Ahmadiasl [1]*

[1] Tuberculosis and Lung Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2] Department of Histology & Embryology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[3] Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[4] Department of Physiology, Hamadan University of Medical Sciences, Hamadan, Iran

A R T I C L E I N F O

A B S T R A C T

Objective(s): The role of isoflavones in pulmonary structure and function during menopause is not well studied. Moreover, the important role of estrogen in the physiological function of respiratory system has been revealed. Genistein, as an isoflavone, mimics estrogenic in diabetic and ovariectomized rats. Here, we hypothesized that genistein would reverse changes in the protein expression levels related to estrogen deficiency in the lung of ovariectomized diabetic rats.

Materials and Methods: Wistar female rats were assigned to four experimental groups (n=10 in each group): sham, rats underwent laparotomy without removing the ovaries; OVX, rats that underwent ovariectomy; OVX.D, rats underwent bilateral ovariectomy and were fed a high-fat diet (HFD); OVX.D.G, ovariectomized diabetic rats with genistein administration (1 mg/kg /day). After ovariectomy, rats continued to feed HFD for a 4-week period. After 4 weeks of HFD feeding, a single dose of 30 mg/kg of streptozotocin was administered in the diabetic group. Genistein was administered for eight weeks. At the end of the experiment, lung tissue was removed and Western blotting technique and hematoxylin-eosin staining were used for evaluation of the lung.

Results: Treatment with genistein significantly decreased inflammatory and apoptotic biomarkers in the ovariectomized diabetic rats compared to non-treated animals (P<0.05). Also, genistein exerted a protective effect in the lung architecture.

Conclusion: Genistein partly reversed ovariectomy-induced changes in apoptotic and inflammatory biomarkers in the lung. Our data suggest that genistein treatment as a natural replacement therapy may prevent the estrogen deficiency effects in the lung of diabetic menopausal women.

▶ *Please cite this article as:*

Introduction

The effects of diabetes mellitus on the pulmonary system have been studied over the past few decades. Increasing evidence has suggested that diabetes is strongly associated with reduction in pulmonary function (1). Accordingly, the risk of pulmonary disorders increases in patients with diabetes (2). Recently, a meta-analysis research has established that the ratio of forced expiratory volume through the first sec and forced vital capacity decreased in type 1 and type 2 diabetes (3). Diabetes can lead to a marked augmentation in caspase 3 activity, as a final biomarker of apoptosis, in the lung of diabetic rats (4). Also, inflammatory biomarkers including Tumor necrosis factor-alpha (TNF-α) and C-reactive protein were shown to increase in diabetic subjects (5). Increasing evidence in the past decades suggests that gender exerts an impact on the incidence, prevalence, and severity of multiple pulmonary diseases among other factors. Moreover, the important role of sex hormones in respiratory health has been established.

Estrogen deficiency in ovariectomized (OVX) mice is associated with an increase in the number of apoptotic cells in the lung (6). Likewise, estrogen replacement has been shown to improve lung architecture in OVX mice (7). Moreover, the anti-inflammatory effect of estrogen has been demonstrated in the lung tissue (7).

Hormone replacement therapy (HRT) has been shown to improve insulin resistance in postmenopausal diabetic subjects (8). However, HRT raises serious concerns such as breast cancer and vascular complications (9, 10). Some studies have focused on other compounds that exert estrogenic effects in menopausal women. Phytoestrogens have been reported to exert similar effects as HRT without having unwanted side effects (11). Genistein, as a phyto-estrogen, exerts a protective effect against metabolic disorders related to diabetes mellitus (12). Accordingly, anti-apoptotic and anti-inflammatory effects of genistein have been suggested in previous research (13). Also, our research team previously has reported the protective effect of genistein in the pancreas of OVX rats (14).

Corresponding author: Naser Ahmadiasl. Tuberculosis and Lung Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. Tel: +98-411-3364664; Fax: +98-4133364664; email: n.ahmadiasl@gmail.com

Therefore, in this study, we induced ovariectomy and diabetes in rats and hypothesized that genistein could prevent OVX-induced tissue injury in vital organs such as the lungs by attenuating the proinflammatory cytokine response and apoptotic biomarker generation in animal models of diabetes.

Materials and Methods

Animal care

Forty adult female Wistar rats, aged about 10 weeks with average weight of 180–220 g, were used in the current study. The animals were obtained from the Animal Research Institute, Faculty of Medicine, Tabriz University of Medical Sciences (Tabriz, Iran). This study was approved by the Animal Ethics Committee (code no.: IR.TBZMED.REC.1396.450). All rats were kept at a 22–24 °C temperature range in a 12 hr light/dark cycle and maintained in a standard condition with access to water *ad libitum* and standard pellet diet for a week.

Chemical

Genistein and streptozotocin (Sigma St. Louis, Mo, USA), polyclonal rabbit anti- Bcl-2 (sc-492), anti-IL1β (sc-7884), anti-caspase 3 (sc-7148), anti-ERK (sc-292838), and anti-P-ERK (sc-101760) (Santa Cruz, CA, USA) were used in the experiment.

Experimental design

Adult female Wistar rats were assigned to four experimental groups: sham (n=10), these animals underwent surgery without ovariectomy and received the vehicle (DMSO 100 μl/day); OVX (n=10), rats underwent bilateral ovariectomy; OVX.D (nμ=10), diabetes was induced in OVX rats with HFD; OVX.D.G (n=10), in this group OVX diabetic rats were treated with genistein. Ovariectomy was performed under anesthesia with xylazine chloride (5 mg/kg) and ketamine chloride (50 mg/kg). All animals except those in the sham group underwent ovariectomy.

Ventral hair was shaved approximately 1 cm above the imaginary line of ovaries. The site was then cleaned with povidone-iodine and alcohol 70%. A paralumbar lateral incision was made using a sharp knife and the ovaries were removed. The wound was sutured using catgut and covered with sterile gauze. Each rat was injected with gentamicin (60–80 mg/kg, IM) and cleaned with povidone-iodine for 3 days after surgery to prevent postoperative infection.

Bilateral ovariectomy was performed with two incisions on both sides of the backbone. After incision of skin and muscle walls, the abdominal cavity was exposed and ovaries were removed. At the end of the operation, oviducts were located with minimal disruption of the soft tissues. Two layers of skin and muscles were sutured with sterile suture (15). Ten days after surgery, rats received an HFD consisting of 17% carbohydrate, 58% fat, and 25% protein in the diabetic group for four weeks. After 4 weeks of HFD, a single dose of 35 mg/kg of streptozotocin was dissolved freshly in citrate buffer (0.1 M, pH 4.5) and injected intraperitoneally for inducing diabetes type II (16, 17). Blood sugar level was checked with a glucometer, and rats were confirmed diabetic when blood glucose levels exceeded 200 mg/dl (17). After 4 weeks on HFD, animals received a normal diet for a period of four weeks. Genistein dissolved in DMSO was administered daily for up to eight weeks (1 mg/kg; subcutaneously), concurrent with the onset of diabetes induction (18). After 8 weeks, lung tissue was removed under anesthesia and prepared for molecular and histological analysis.

Plasma glucose measurements

Measurement of fasting blood glucose (FBG) was done at the beginning of the experiment by using a glucometer (Roche) in order to ensure rats were euglycemic. After injection of streptozotocin, the blood glucose concentration was checked and the levels higher than 200 mg/dl were considered as hyperglycemia.

Immunoblotting analysis

Evaluation of ERK1/2 phosphorylation, Bcl-2, IL1β, and caspase 3 was performed by using the Western blotting technique in the lung (19). Briefly, all samples after snap freezing were homogenized in RIPA lysis buffer comprising proteinase inhibitor cocktail (aprotinin, pepstatin, antipain, leupeptin, and chymostatin) on ice and left 20 min at 4 °C. The samples were then centrifuged at 4 degrees centigrade at 12,000 rpm for 10 min, then supernatant was collected and kept at -80 °C. The proteins were separated in SDS-PAGE. After separation, proteins were transferred electrophoretically onto polyvinylidene difluoride (PVDF). Incubation of membranes was done with 5% w/v nonfat dry milk in Tris-Buffered Saline (pH 7.5) for 2 hr in order to block non-specific binding sites. Blots were located at the room temperature for 2 hr with anti- Bcl-2, anti- P-ERK1/2, anti- caspase 3 , anti-ERK1/2, and anti-IL1β (primary antibody) in antibody dilution buffer comprising 1% w/v nonfat dry milk in 0.05% v/v Tris-Buffered Saline with 0.05% v/v Tween 20). Then samples were washed with TBST 3 times, incubation was prepared for one hr with goat Anti-Rabbit (secondary antibody) in an antibody dilution buffer. Chemiluminescence (ECL) detection kit (Pierce, Rockford, IL) was used for detection of blotting substrate. The density of the bonds on immunoblots was quantified using the ImageJ software.

Histological evaluation

At the end of the experiment, lung tissue was fixed in 10% formaldehyde after isolation, sectioned with 5 μm thicknesses, stained with hematoxylin and eosin (H&E), and examined using light microscopy.
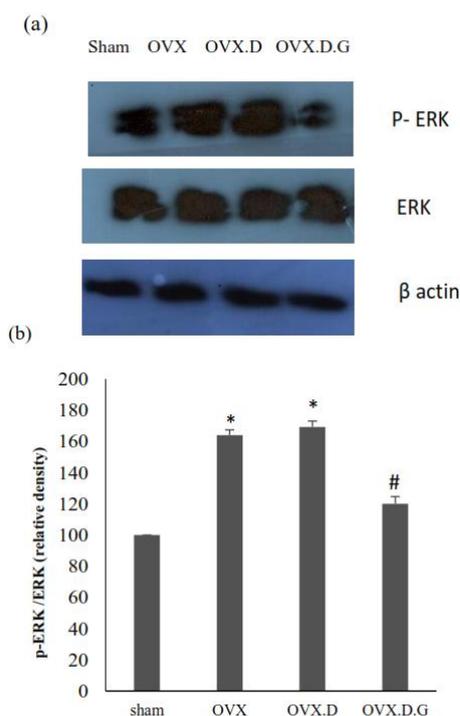
Figure 1. ERK1/2 proteins hyperphosphorylation with genistein treatment in the lungs of experimental groups. ERK immunoblotting among different studied groups, (a) Quantitation of immunoblotting of P-ERK expression levels against ERK, (b) OVX: ovariectomized group, OVX.D: ovariectomized diabetic group, OVX.D.G: ovariectomized diabetic with genistein treatment. Data expressed as mean±SEM. *P<0.05 versus sham group; # P<0.05 versus OVX.D groups

Figure 2. Increase in Bcl-2 and decrease in caspase 3 expression levels with genistein treatment, in the lungs of experimental groups. Immunoblotting of caspase 3 and Bcl-2 among different studied groups (a), Quantitation of caspase 3 and Bcl-2 immunoblotting against β actin expression in the lungs (b). OVX: ovariectomized group, OVX.D: ovariectomized diabetic group, OVX.D.G: ovariectomized diabetic with genistein treatment. Data expressed as mean±SEM. *P<0.05 versus sham group; # P<0.05 versus OVX.D group

Data analysis and statistics

Data are presented as the mean±SEM. One way ANOVA with *post hoc* Tukey's test was used for statistical analysis of all data. *P*-values<0.05 was considered as statistically significant.

Results

Genistein treatment on ERK1/2 level in the lung

As shown in Figure 1, the levels of ERK1/2 increased significantly in the OVX rats compared with the sham group and this increase was higher in the OVX diabetic rats (*P*<0.05). Treatment with genistein markedly decreased ERK levels in the genistein treatment group as compared with OVX and OVX.D rats (*P*<0.05).

Genistein treatment on caspase 3 and Bcl-2 levels in the lungs

When compared with the sham group, the level of activated caspase 3 was markedly elevated in the OVX group and this increase was more in the OVX.D rats (*P*<0.05) (Figure 2). Also, the Bcl-2 protein was significantly reduced in the ovariectomized and ovariectomized diabetic groups as compared with the sham (*P*<0.05). Genistein treatment significantly
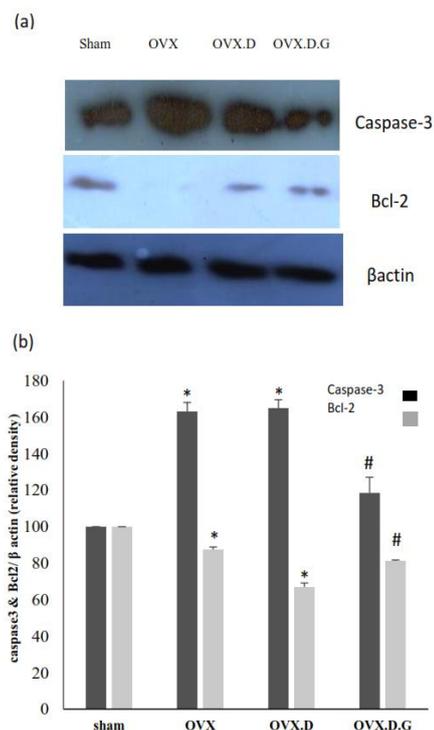
increased Bcl-2 and decreased caspase 3 expression levels as compared with ovariectomized diabetic groups (*P*<0.05).

Effect of genistein on IL1β level in the lungs

As shown in Figure 3, ovariectomy with diabetes noticeably increased the expression level of IL1β as compared with the sham (*P*<0.05). Genistein treatment significantly reduced IL1β expression level when compared with the ovariectomized diabetic group (*P*<0.05).

Genistein treatment on histological changes in the lung

Histological study of the sham group revealed lung tissue without macrophage and leucocytes infiltration and perivascular edema (Figure 4A). In the OVX group, cellular infiltration and perivascular edema were obviously observed (Figure 4B). Diabetes and ovariectomy exerted severe perivascular edema and severe macrophage and leucocyte infiltration (Figure 4c). Treatment with genistein was associated with obviously decreased in cellular infiltration. Perivascular edema was prominently diminished in the genistein treatment group (Figure 4D).
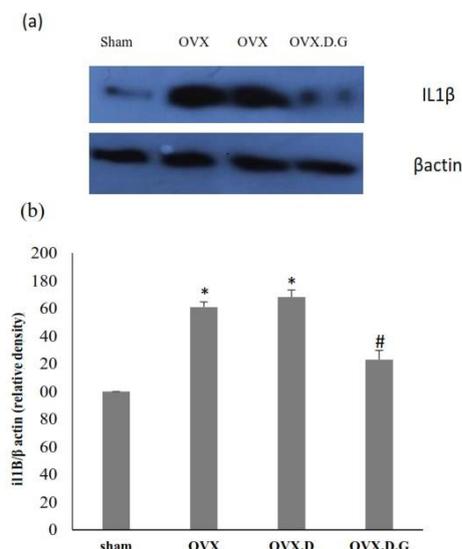
Figure 3. Down-regulation of IL1β protein with genistein administration, in the lungs of experimental groups. IL1β immunoblotting within different groups, (a) Immunoblot quantitation of IL1β against the expression level of β actin in the lungs, (b) OVX: ovariectomized group, OVX.D: ovariectomized diabetic group, OVX.D.G: ovariectomized diabetic with genistein treatment. Data expressed as mean±SEM. *P<0.05 versus sham group; #P<0.05 versus OVX.D groups

Figure 4. Histological micrograph in the lungs of different studied groups. Sham (4A) normal lung tissue without cellular infiltration, OVX (4B) leukocyte and macrophage infiltration in OVX group (blue and red arrow), respectively, and perivascular edema (brown arrow) observed in OVX group, OVX.D (4C) severe cellular infiltration (blue and red arrow) presented in OVX.D group and marked perivascular edema (brown arrows). OVX.D.G (4D) cellular infiltration markedly reduced (blue and red arrow) and decrease of perivascular edema (brown arrow) observed in OVX.D.G group against the OVX.D group

Discussion

Diabetes mellitus alters cellular function in different types of tissues; it causes unsuitable effects called diabetic complications. Inflammation and apoptosis are two important cellular processes that are affected by diabetes. On the other hand, reduction in ovarian function has been shown to induce inflammation and apoptosis in the lungs of OVX mice (6, 20). In this work, we studied the protective role of genistein in the lungs of OVX diabetic rats.

The Ras/Raf/ERK pathway has a key role in lung injury induced by hyperoxia (21). It has also been shown that inhibition of Raf-1 kinase protects retinal endothelial cells against increased apoptosis under hyper glycemic conditions (22). The effect of estrogen on apoptosis in the lung cells is not well known, however, some studies have revealed that estrogen may exert a protective role in the development of pulmonary apoptosis in the OVX mice. Accordingly, estrogen deficiency in old female mice has been associated with increase in ERK activity, which is prevented by estrogen replacement in the lung (6). In this study, the expression levels of ERK were elevated in the ovariectomized diabetic group compared to the sham rats. Estrogen deprivation also induced an increase in caspase 3 activity as a final biomarker of apoptosis in the lungs of OVX mice (6). Moreover, elevated levels of caspase 3 activity have been found in the lungs of diabetic rats (4). It is now well known that ERK activity can induce apoptosis through enhancing group as
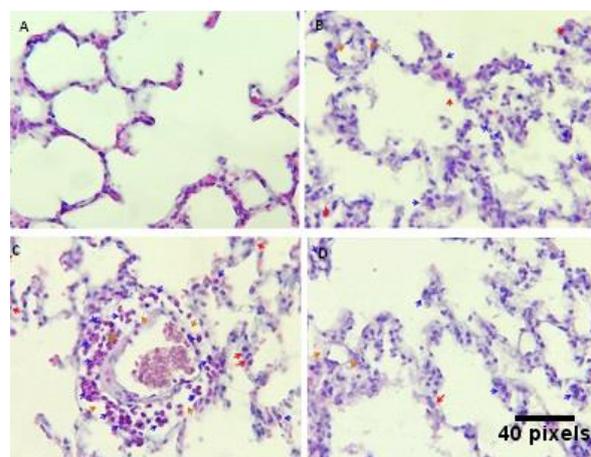
compared with the sham. Estrogen deprivation also induced an increase in Caspase 3 as a final biomarker of apoptosis in the lung of OVX mice (6). Moreover, elevated levels of caspase3 activity have been found in the lung of diabetic rats (4). Zhuang and colleagues showed that ERK activity can induce apoptosis through enhancing in caspase3 activity in the renal epithelial cells (23). Additionally, ERK/MEK activity has been found to be associated with down-regulation of the Bcl-2 protein, as an anti-apoptotic marker, in osteoblasts (24). In our study, caspase 3 was elevated and Bcl-2 declined in OVX diabetic rats as compared with the sham.

On the other hand, investigators have suggested that IL1β, an inflammatory mediator, has a key role in lung inflammation. Accordingly, neutralization of IL1β is associated with decreased silica-induced inflammation by inhibiting other inflammatory mediators in the lung (25). Additionally, increasing evidence reveals that IL1β may be involved in tissues damaging by induction of apoptosis (26). It has become apparent cytokine IL1β has also the ability to trigger the ERK/ MAP kinase signaling in the astrocytes (27). The pathogenesis of diabetes in different tissues is associated with chronic inflammation. Moreover, increased levels of IL1β have been found in the retina of diabetic rats (28). Consistent with previous reports, we observed markedly increased IL1β levels in OVX.D group compared to the sham.

Genistein, a naturally occurring isoflavonic phyto - estrogen, can bind to estrogen receptors; it has been proposed as a natural replacement to estrogen. It has also been shown to have beneficial effects on the prevention of metabolic disorder related to diabetes

(12). Similarly, the anti-inflammatory effect of genistein has been proposed in diabetic mice. Genistein has been shown to reduce inflammatory markers including NFκB in diabetes induced renal damage (29). Also, another study reported that genistein reduces P-ERK production in diabetic nephropathy in mice (30). Consistent with the previous studies, we observed significantly decreased inflammatory and apoptotic markers inclu - ding IL1β and ERK in genistein treated rats compared to the OVX.D group. We hypothesized that OVX diabetic rats would be protected against developing lung inflammation and apoptosis with genistein treatment. Genistein has also attracted much attention for its anti-apoptotic properties in diabetes. It is now well known that high glucose concentration triggers endothelial cell apoptosis, and genistein treatment decreases cellular apoptosis by the Bcl-2 dependent pathway (31).

Results of previous studies by our group revealed that genistein exerts a strong protective effect against inflammatory and apoptotic markers in the pancreas of OVX diabetic rats (14). In our study, genistein significantly decreased caspase 3 and increased Bcl-2 expression levels in the genistein treatment group compared with the OVX.D group. In this study improving the effect of genistein on pulmonary apoptosis and inflammation could result in protection from lung damage in OVX diabetic rats. Although the beneficial effects of genistein on pulmonary function is not well known, our results encourage further works in this area.

Conclusion

Our study revealed increased levels of IL1β, ERK, and caspase 3 , and also decreased Bcl-2 expression level in the OVX.D group compared to the sham. Estrogen deficiency alone or with diabetes increased inflammatory and apoptotic biomarkers in the lungs. However, administration of genistein prevented these apoptotic and inflammatory changes in the lungs of OVX diabetic rats. The results of this work support the beneficial effects of genistein for prevention of lung injury induced by estrogen deficiency in OVX diabetic subjects.

Acknowledgment

Conflict of interest

There are no competing interests in the present work.

References

1. Sandler M. Is the lung a'target organ'in Diabetes mellitus? Arch Intern Med 1990; 150:1385-1388.
2. Goldman MD. Lung dysfunction in diabetes. Diabetes Care 2003; 26:1915-1918.
3. van den Borst B, Gosker HR, Zeegers MP, Schols AM. Pulmonary function in diabetes: a metaanalysis. Chest 2010; 138:393-406.
4. Wu J, Jin Z, Yan L-J. Redox imbalance and mitochondrial abnormalities in the diabetic lung. Redox Biol 2017; 11:51-59.
5. Dennis RJ, Maldonado D, Rojas MX, Aschner P, Rondón M, Charry L, *et al*. Inadequate glucose control in type 2 diabetes is associated with impaired lung function and systemic inflammation: a cross-sectional study. BMC Pulm Med 2010; 10:38.
6. Glassberg MK, Choi R, Manzoli V, Shahzeidi S, Rauschkolb P, Voswinckel R, *et al*. 17β-estradiol replacement reverses age-related lung disease in estrogen-deficient C57BL/6J mice. Endocrinology 2014; 155:441-448.
7. Massaro D, Massaro GD. Estrogen regulates pulmonary alveolar formation, loss, and regeneration in mice. Am J Physiol Lung Cell Mol Physiol 2004; 287:L1154-L9.
8. Bitoska I, Krstevska B, Milenkovic T, Subeska-Stratrova S, Petrovski G, Mishevska SJ, *et al*. Effects of hormone replacement therapy on insulin resistance in postmenopausal diabetic women. Open Access Maced J Med Sci 2016; 4:83-88.
9. Chen C-L, Weiss NS, Newcomb P, Barlow W, White E. Hormone replacement therapy in relation to breast cancer. JAMA 2002; 287:734-741.
10. Mosca L, Collins P, Herrington DM, Mendelsohn ME, Pasternak RC, Robertson RM, *et al*. Hormone replacement therapy and cardiovascular disease. Circulation 2001; 104:499-503.
11. Serock MR, Wells AK, Khalil RA. Modulators of vascular sex hormone receptors and their effects in estrogen - deficiency states associated with menopause. Recent Pat Cardiovasc Drug Discov 2008; 3:165-186.
12. Stephenson T, Setchell K, Kendall C, Jenkins D, Anderson J, Fanti P. Effect of soy protein-rich diet on renal function in young adults with insulin-dependent diabetes mellitus. Clin Nephrol 2005; 64: 1-11.
13. Zhao L, Wang Y, Liu J, Wang K, Guo X, Ji B, *et al*. Protective effects of genistein and puerarin against chronic alcohol-induced liver injury in mice via antioxidant, anti-inflammatory, and anti-apoptotic mechanisms. J Agric Food Chem 2016; 64:7291-7297.
14. Yousefi H, Alihemmati A, Karimi P, Alipour MR, Habibi P, Ahmadiasl N. Effect of genistein on expression of pancreatic SIRT1, inflammatory cytokines and histological changes in ovariectomized diabetic rat. Iran J Basic Med Sci 2017; 20:423-429.
15. Irigoyen M-C, Paulini J, Flores LJ, Flues K, Bertagnolli M, Moreira ED, *et al*. Exercise training improves baroreflex sensitivity associated with oxidative stress reduction in ovariectomized rats. Hypertension 2005; 46:998-1003.
16. Srinivasan K, Ramarao P. Animal models in type 2 diabetes research: an overview. Indian J Med Res 2007; 125:451-472.
17. Su X, Meng X, Sun C, Liu L, Su B. Intramuscular injection of soluble receptor for advanced glycation end products expression

vector prevents the development of streptozotocin-induced diabetes mellitus in rats on high fat diet. J diabetes 2011; 3:309-316.

18. Matori H, Umar S, Nadadur RD, Sharma S, Partow-Navid R, Afkhami M, *et al*. Genistein, a soy phytoestrogen, reverses severe pulmonary hypertension and prevents right heart failure in rats. Hypertension 2012; 60: 425-430.

19. Faramoushi M, Sasan RA, Sarraf VS, Karimi P. Cardiac fibrosis and down regulation of GLUT4 in experimental diabetic cardiomyopathy are ameliorated by chronic exposures to intermittent altitude. J Cardiovasc Thorac Res 2016; 8:26-33.

20. Elfattah LIA. A comparative study between the effects of dietary soya and estrogen replacement therapy on the lung of ovariectomized albino rats: histological and immunohistochemical study. Egyptian J Histol 2012; 35:34-42.

21. Zhang X, Shan P, Sasidhar M, Chupp GL, Flavell RA, Choi AM, *et al*. Reactive oxygen species and extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase mediate hyperoxia-induced cell death in lung epithelium. Am J Respir Cell Mol Biol 2003; 28:305-315.

22. Rayappa SP, Kowluru RA. Role of Raf-1 kinase in diabetes-induced accelerated apoptosis of retinal capillary cells. Int J Biomed Sci 2008; 4:20-28.

23. Zhuang S, Yan Y, Daubert RA, Han J, Schnellmann RG. ERK promotes hydrogen peroxide-induced apoptosis through caspase 3 activation and inhibition of Akt in renal epithelial cells. Am J Physiol Renal Physiol 2007; 292:F440-F447.

24. Tanaka Y, Nakayamada S, Fujimoto H, Okada Y, Umehara H, Kataoka T, *et al*. H-Ras/mitogen-activated protein kinase pathway inhibits integrin-mediated adhesion and induces apoptosis in osteoblasts. J Biol Chem 2002; 277:21446-21452.

25. Guo J, Gu N, Chen J, Shi T, Zhou Y, Rong Y, *et al*. Neutralization of interleukin-1 beta attenuates silica-induced lung inflammation and fibrosis in C57BL/6 mice. Arch Toxicol 2013; 87:1963-1973.

26. Falk R, Hacham M, Nyska A, Foley JF, Domb AJ, Polacheck I. Induction of interleukin-1β, tumour necrosis factor-α and apoptosis in mouse organs by amphotericin B is neutralized by conjugation with arabinogalactan. J Antimicrob Chemother 2005; 55:713-720.

27. Gurjar MV, Deleon J, Sharma RV, Bhalla RC. Role of reactive oxygen species in IL-1β-stimulated sustained ERK activation and MMP-9 induction. Am J Physiol Heart Circ Physiol 2001; 281:H2568-H2574.

28. Carmo A, Cunha-Vaz J, Carvalho A, Lopes M. L-arginine transport in retinas from streptozotocin diabetic rats: correlation with the level of IL-1β and NO synthase activity. Vision Res 1999; 39:3817-3823.

29. Kim MJ, Lim Y. Protective effect of short-term genistein supplementation on the early stage in diabetes-induced renal damage. Mediators Inflamm 2013; 2013: 510212.

30. Elmarakby AA, Ibrahim AS, Faulkner J, Mozaffari MS, Liou GI, Abdelsayed R. Tyrosine kinase inhibitor, genistein, reduces renal inflammation and injury in streptozotocin-induced diabetic mice. Vascul Pharmacol 2011; 55:149-156.

31. Zhong W-W, Liu Y, Li C-L. Mechanisms of genistein protection on pancreas cell damage in high glucose condition. Intern Med 2011; 50:2129-2134.