

Original Article

ESTROGENS IMPROVE THE CARDIOVASCULAR ALTERATIONS IN FRUCTOSE-INDUCED INSULIN RESISTANT OVARIECTOMIZED RATS

DALIA O. SALEH^{b*}, SALLY A. EL-AWDAN^b, SALWA M. NOFEL^b, WAFAA I. EL-ERAKY^b, AIMAN S. EL-KHATIB^a, SANAA A. KENAWY^a

^aDepartment of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt, ^bDepartment of Pharmacology, National Research Centre, Giza, Egypt
Email: doabdelfattah@yahoo.com

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ABSTRACT

Objective: The present study aimed to investigate the possible improving effects of 17- β estradiol (EST) and genistein (GEN) on the cardiovascular changes associated with fructose (21% in drinking water for 8 weeks)-induced insulin resistance.

Methods: Sham-operated and ovariectomized mature female rats were included in this study. Insulin-resistant ovariectomized animals were *sc* treated with EST (100 μ g/kg) or GEN (1 mg/kg) on the daily basis for 21 consecutive days.

Results: Induction of insulin resistance in both sham-operated and ovariectomized rats decreased the vascular responsiveness of isolated aortic rings towards the vasoconstrictor norepinephrine and the vasodilator acetylcholine (Ach) with no changes towards the vasodilator sodium nitroprusside. Fructose-induced insulin resistance was also associated with an elevation in the blood pressure (BP) with decreased serum level of nitric oxide (NO). Treatment of insulin-resistant ovariectomized rats with either EST or GEN improved the vascular responsiveness of isolated aortic rings towards Ach and succeeded to reduce the elevated BP. Moreover, both EST and GEN decreased the insulin resistance/compensatory hyperinsulinaemia. Treatment with EST increased serum NO level.

Conclusion: EST and GEN have the ability to improve the endothelium-dependent relaxation in insulin-resistant ovariectomized rats and modulate the elevated BP.

Keywords: Insulin resistance, 17- β estradiol, Genistein, Vascular responsiveness, Aortic rings.

INTRODUCTION

Insulin resistance and its consequent hyperinsulinemia have been increasing worldwide. It is a metabolic disorder that plays a role in the pathophysiology of many common human diseases including type 2 diabetes mellitus (DM), hypertension, obesity, and coronary heart disease [1]. It most often precedes the onset of type 2 DM by many years, is present in a large segment of the general population, and is multifactorial [2, 3]. There are convincing data to show that there is a genetic component associated with insulin resistance [4].

Endothelial dysfunction and insulin resistance closely coexist and can be detected in the pathogenesis of atherosclerosis [5]. Several studies have confirmed that insulin resistance are commonly accompanied with endothelial dysfunction and that the exposure of vascular endothelium to high circulating levels of lipids and glucose is associated by reduced nitric oxide (NO) availability [6-8]. These observations have given rise to the theory that endothelial dysfunction is both a cause and a consequence of the metabolic disturbances observed in insulin resistance [9].

The incidence of cardiovascular diseases is lower in premenopausal women than men of similar age, protection is lost soon after women reach menopause [10]. Interestingly, exogenous estrogen replacement therapy can decrease the incidence of myocardial infarction and stroke in postmenopausal women, which suggests that estrogen protects against dysfunction of the heart and/or blood vessels [11, 12]. 17- β estradiol treatment also have protective effects in animal models of atherosclerosis and vascular injury [10]. Estrogens are natural vaso protective agents due to the presence of ERs which have been detected in smooth-muscle cells of coronary arteries and endothelial cells [13].

The present study was devoted to investigate further the possible protective effect of estrogens on some cardiovascular, metabolic and biochemical complications accompanying insulin resistance. To achieve this aim both sham-operated and ovariectomized rats were

used. EST and genistein (GEN) (a phytoestrogen that is similar in structure and function to EST) were given subcutaneously on daily basis for three weeks to ovariectomized insulin-resistant animals.

The responsiveness of the isolated aortic rings towards various vasoactive agents was tested. Norepinephrine was chosen as a vasoconstrictor agent while acetylcholine and sodium nitroprusside were employed as endothelium-dependent and endothelium-independent vasodilators, respectively.

Moreover, the blood pressure and heart rate were measured non-invasively. For the assessment of insulin resistance-induced metabolic and biochemical changes, the glucose tolerance, hyperinsulinemia and lipid profile were investigated. Serum glucose levels in oral glucose tolerance test (OGTT) were taken to examine the glucose tolerance, the serum insulin levels were used to examine hyperinsulinemia and insulin resistance. Serum nitrate/nitrite level was also determined and used as a convenient marker for NO formation.

MATERIALS AND METHODS

Drugs and chemicals

17- β estradiol (EST) and genistein (GEN) were purchased from Sigma-Aldrich (St Louis, MO, USA) and L.C Laboratories (MA, USA), respectively. Both drugs were initially dissolved in DMSO and then freshly diluted in distilled water to be *sc* given. The concentration was adjusted so as to each 100 g animal body weight received 0.25 ml, containing the required dose of either drug.

D-Fructose was purchased from Isochem (France). Acetylcholine perchlorate and N-(1-Naphthyl) ethylene-diamine dihydrochloride (NEDD) were purchased from Sigma-Aldrich (St Louis, MO, USA). Norepinephrine hydrochloride, sodium nitroprusside, and sulphanilamide were purchased from Fluka (Italy), Oxford Laboratory (India), and Merck (Germany), respectively. All other chemicals were of the highest commercially available grade.

Animals

Female albino rats, initially weighing 130-150 g, were used in this study. They were obtained from the Animal House Colony of the National Research Center (Dokki, Giza, Egypt) and were housed under conventional laboratory conditions throughout the period of experimentation. The animals were fed a standard rat pellet diet and allowed free access to water. The study was conducted in accordance with ethical procedures and has been approved by the National Research Center-Medical Research Ethics Committee (NRC-MREC) for the use of animal subjects (approval number 09/110; date 07/2009). Rats were anaesthetized with thiopental sodium (20 mg/kg; *ip*) and then ovariectomized according to the method described by Turner *et al.* (2000) [14]. Sham-operated control group of animals was also included.

Induction of insulin resistance

Three weeks after the operation, insulin resistance was induced by adding 21% fructose in drinking water for both ovariectomized and sham-operated rats for 8 weeks as described by Yadav *et al.* (2007) [15]. At the end of the 8th week, insulin resistance was confirmed by the oral glucose tolerance test (*vide infra*). Insulin-resistant rats were those whose serum glucose level reached 140 mg/dl or more after 30 min from the oral glucose loading dose [16].

Experimental design

While still keeping animals on fructose in drinking water, the effects of 3 weeks *sc* treatment with EST and GEN on the cardiovascular alterations induced by insulin-resistance were assessed. Two sets of experiments were carried out. In each set the following 6 groups (8-12 rats each) were used:

Group I: sham-operated rats

Group II: ovariectomized rats

Group III: sham-operated rats with insulin resistance

Group IV: ovariectomized rats with insulin resistance

Group V: ovariectomized rats with insulin resistance treated with EST (100 µg/kg/day) [17, 18]

Group VI: ovariectomized rats with insulin resistance treated with GEN (1 mg/kg/day) [19]

The first set of experiments was used for systolic blood pressure (BP), heart rate and vascular reactivity assessment while the second set of experiments was used for the biochemical determinations. The aforementioned parameters of the two sets of experiments were assessed 24 h after the administration of the last dose of either EST or GEN.

Blood pressure and heart rate measurements

Systolic blood pressure and heart rate of animals were indirectly measured by non-invasive BP monitor (ML 125 NIBP, AD Instruments, Australia) from the tail of conscious rats by the tail-cuff technique for which all animals were pre-trained until BP was steadily recorded with minimal stress and restraint. In the tail-cuff technique, animals were warmed for 30 min at 28 °C in a thermostatically controlled heating cabinet (Ugo Basille, Italy) for better detection of tail artery pulse, the tail was passed through a miniaturized cuff and a tail-cuff sensor that was connected to an amplifier (ML 125 NIBP, AD Instruments, Australia). The amplified pulse was recorded during automatic deflation of the cuff. Systolic BP was defined as the cuff inflation pressure at which waveform become indistinguishable from the baseline noise. The average of at least 5 measurements was taken at each occasion. Heart rate was recorded automatically by a counter triggered by the pulse wave. The Chart for windows (v 7) software was used to record and elaborate data.

Assessment of vascular reactivity

The vascular reactivity towards NE as a vasoconstrictor, Ach as an endothelium-dependent vasodilator, and SNP as an endothelium-independent vasodilator was assessed using the isolated aortic ring

preparation described by Cocks *et al.* (1988) [20]. Briefly, segments of thoracic aortas were rapidly excised from cervical dislocated animals. They were placed in warm Krebs' solution and dissected free of surrounding tissue before being cut into transverse rings of 3-5 mm length. An aortic ring was mounted in 10 ml water jacketed automatic multi-chamber organ bath system (Model no. ML870B6/C, Panlab, Spain) containing Krebs' solution of the following composition (g/l): NaCl 6.9, KCl 0.35, KH₂PO₄ 0.16, MgSO₄·7H₂O 0.3, CaCl₂·2H₂O 0.37, NaHCO₃ 2.1, and glucose 1.05. The organ bath solution was continuously aerated with carbogen (a mixture of 95% O₂ and 5% CO₂) and its temperature was kept at 37°C. The mounted aortic ring was suspended horizontally between 2 hooks passed through its lumen, care being taken not to injure the luminal surface. The bottom hook was attached to a support leg while the upper one was attached to a force-displacement transducer (Model no. MLT0201, Panlab, Spain) connected to an amplifier (Power Lab, AD Instruments Pty. Ltd.) which is connected to a computer. The chart for windows (v 3.4) software was used to record and elaborate data. The preparation was allowed to equilibrate for about 2 h under a resting tension of 2 g during that time any change in the resting tension was readjusted.

NE, Ach, and SNP were freshly prepared by dissolving them in Krebs' solution. Serial dilutions of each vasoactive agent were prepared such that cumulative additions to the bath gave a final bath concentration ranging from 10⁻⁹M to 10⁻⁴M. For testing the relaxant effects of Ach or SNP, pre-contraction with NE was carried out first with a concentration that produce approximately 60-70% of the maximum contractile response. Contractile responses to NE were expressed as percentage of maximal response while relaxant responses to the vasodilators Ach and SNP were expressed as percentage relaxation of the pre-contraction value.

Assessment of biochemical parameters

Blood samples were withdrawn from the retro-orbital venous plexus of 18 h food-deprived rats. Collected blood samples were allowed to stand for 10 min at room temperature then centrifuged at 4°C using cooling centrifuge (Laborezentrifugen, 2 k 15, Sigma, Germany) at 3000 xg for 10 min and sera were separated for the assessment of the following biochemical parameters.

Oral glucose tolerance test

After blood sampling from 18 h food-deprived animals (zero time), each animal received a glucose loading dose of 1 ml/100 g of body weight from a 50% w/v glucose solution by oral gavage. Thereafter, blood samples were again withdrawn at 30, 60, and 120 min (16). Serum glucose level was determined as quinineamine using a test reagent kit (Stanbio, USA) according to the method of Trinder (1969) [21]. The absorbance was measured at 510 nm and the results were expressed as mg/dl. A curve was obtained after determining serum glucose level after zero, 30, 60, 120 min for the 6 groups from which the area under the curve (AUC) was calculated for each group using the trapezoidal rule.

Determination of insulin level

Serum insulin level was adopted by the method described by Judzewitsch *et al.* (1982) [22] using solid phase enzyme-linked immunosorbent assay (ELISA) method using a test reagent kit (Labor Diagnostika Nord, Nordhorn, Germany). The absorbance of the samples and the standard solutions were measured at 450 nm using ELISA reader (ELx800, Ultra Microplate Reader BIO-TEK Instruments Inc., USA) against blank and the results were expressed as µIU/ml.

Determination of homeostatic model assessment-insulin resistance index

Insulin sensitivity was determined by HOMA-IR index which was calculated for all groups using the following equation [23]

$$\text{HOMA-IR index} = \frac{\text{insulin } (\mu\text{IU/ml}) \times \text{fasting glucose (mg/dl)}}{405^*}$$

*Constant used when glucose is in mg/dl

Determination of nitric oxide level

The total amount of NO was indirectly estimated in terms of its main metabolites, nitrate and nitrite by the Griess reaction using NEDD and sulphaniamide as described by Miranda *et al.* (2001) [24] after deproteinizing the serum samples with absolute ethanol. The absorbance was measured at 540 nm and the results were expressed as nmol/ml.

Statistical analysis

Data are expressed as means±SEM. Statistical significance was taken as $p < 0.05$ for all experiments, using one way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test to judge the difference between various groups for the parametric parameters. For the non-parametric parameters Kruskal-Wallis non-parametric ANOVA followed by Dunn's multiple comparisons test was used.

Graph Pad Prism software (version 5.00 for Windows, San Diego, California, USA) was used to carry out all statistical tests.

RESULTS

Effects of EST and GEN on blood pressure and heart rate

Induction of insulin resistance with fructose (21% in drinking water for 11 weeks) was associated with an elevation in the BP and a reduction in the heart rate of both sham-operated and ovariectomized rats. Treatment of insulin-resistant ovariectomized rats with either EST (100 µg/kg/day; *sc*) or GEN (1 mg/kg/day; *sc*) for 21 consecutive days succeeded to normalize the elevated BP values which was associated with elevations in the reduced heart rate reaching about 87% and 75% of the normal value, respectively (table 1).

Table 1: Effects of 17-β estradiol and genistein on systolic blood pressure, heart rate, and serum nitric oxide level of insulin-resistant ovariectomized rats

Groups		BP (mm/Hg)	HR (bpm)	NO (µM)
Normal-fed rats	Sham-operated	93.37±4.59	319.77±18.56	63.20±3.70
	Ovx	106.77±1.60	314.13±18.75	61.95±5.72
Insulin resistant rats	Sham-operated	109.96*±1.43	217.35*±16.10	38.94*±2.36
	Ovx	129.70*±11.87	191.51*±18.96	34.66*±1.10
	Ovx+EST	94.78†±5.40	278.94†±16.88	52.78†±5.08
	Ovx+GEN	88.47†±3.55	239.89†±23.13	33.76±3.40

Three weeks after ovariectomization, rats were rendered insulin-resistant by giving fructose (21%) in drinking water for 8 weeks. EST (100 µg/kg/day) and GEN (1 mg/kg/day) were *s. c.* injected for 3 weeks while still keeping animals on fructose in drinking water. Twenty-four hours after the last dose of either drug, BP and HR were measured non-invasively and blood samples from 18 h food-deprived animals were withdrawn and serum NO level was measured.

Insulin resistance was confirmed by oral glucose tolerance test and insulin-resistant rats were those whose serum glucose level reached 140 mg/dl or more after 30 min from the oral glucose loading dose.

Results are expressed as means±SEM (n=6-10).

Statistical analysis was carried out by ANOVA followed by Tukey's multiple comparisons test.

* Significant difference from corresponding normal rats at $p < 0.05$.

@ Significant difference from insulin-resistant sham-operated control at $p < 0.05$.

† Significant difference from insulin-resistant ovariectomized control at $p < 0.05$.

Effects of EST and GEN on the vascular reactivity of isolated aortic rings

Effect of EST and GEN on NE-induced contractions

Addition of cumulative concentrations of NE, starting from 10^{-9} M to 10^{-4} M, to aortic rings isolated from either sham-operated or ovariectomized rats resulted in concentration-dependent contractions. A maximum tension was developed at 1×10^{-5} M NE and 1×10^{-4} M NE, respectively.

There was a marked decrease in the vascular responsiveness of aortic rings isolated from insulin-resistant sham operated and ovariectomized rats towards various concentrations of NE as compared to that isolated their corresponding controls. Treatment of insulin-resistant ovariectomized rats with either EST (100 µg/kg/day; *sc*) or GEN (1 mg/kg/day; *sc*) for 21 consecutive days decreased the vascular responsiveness of isolated aortic rings towards lower concentrations of NE as compared to the respective insulin-resistant ovariectomized untreated group (fig. 1a).

Effect of EST and GEN on Ach-induced relaxations

Addition of cumulative concentrations of Ach, starting from 10^{-9} M to 10^{-4} M, to aortic rings isolated from either sham-operated or ovariectomized rats and subjected to pre-contraction with 10^{-6} M of NE, elicited a dose-dependent relaxation. A maximum relaxation reached about 87% and 75% of the pre-contracted value, respectively. There was a marked decrease in the vascular

responsiveness of aortic rings isolated from insulin-resistant sham operated and ovariectomized rats towards almost all concentrations of Ach as compared to that isolated from their corresponding controls. Treatment of insulin-resistant ovariectomized rats with EST (100 µg/kg/day; *sc*) for 21 consecutive days succeeded to reverse the attenuated vascular responsiveness of isolated aortic rings towards almost all concentrations of Ach. Similar treatment of insulin-resistant ovariectomized rats with GEN (1 mg/kg/day; *sc*) showed a tendency to increase the vascular responsiveness of isolated aortic rings towards Ach as compared to the respective insulin-resistant ovariectomized untreated group (fig. 1b).

Effect of EST and GEN on SNP-induced relaxations

Addition of cumulative concentrations of SNP, starting from 10^{-9} M to 10^{-4} M, to aortic rings isolated from either sham-operated or ovariectomized rats and subjected to pre-contraction with 10^{-6} M of NE, produced a dose-dependent relaxation. A maximum relaxation reached about 139% and 164% of the pre-contracted value, respectively. There was no significant change in the vascular responsiveness of aortic rings isolated from insulin-resistant sham operated rats towards almost all concentrations of SNP as compared to that isolated from its corresponding control. On the other hand, there was a decrease in the vascular responsiveness of aortic rings isolated from insulin-resistant ovariectomized rats towards lower concentrations of SNP as compared to that isolated from its corresponding control.

The responsiveness was, however, increased at high concentrations of SNP starting from 1×10^{-6} M. Treatment of insulin-resistant ovariectomized rats with either EST (100 µg/kg/day; *sc*) or GEN (1 mg/kg/day; *sc*) for 21 consecutive days tended to normalize the decreased vascular responsiveness of isolated aortic rings towards various concentrations of SNP (fig. 1c).

Three weeks after ovariectomization, rats were rendered insulin-resistant by giving fructose (21%) in drinking water for 8 weeks. EST (100 µg/kg/day) and GEN (1 mg/kg/day) were *s. c.* injected for 3 weeks while still keeping on fructose in drinking water. Twenty-four hours after the last dose of either drug, aortas were isolated and suspended as rings (length, 3-5 mm). Relaxation was induced by applying cumulative concentrations of Ach (b) and SNP (b) on aortic rings precontracted with 10^{-6} M of NE.

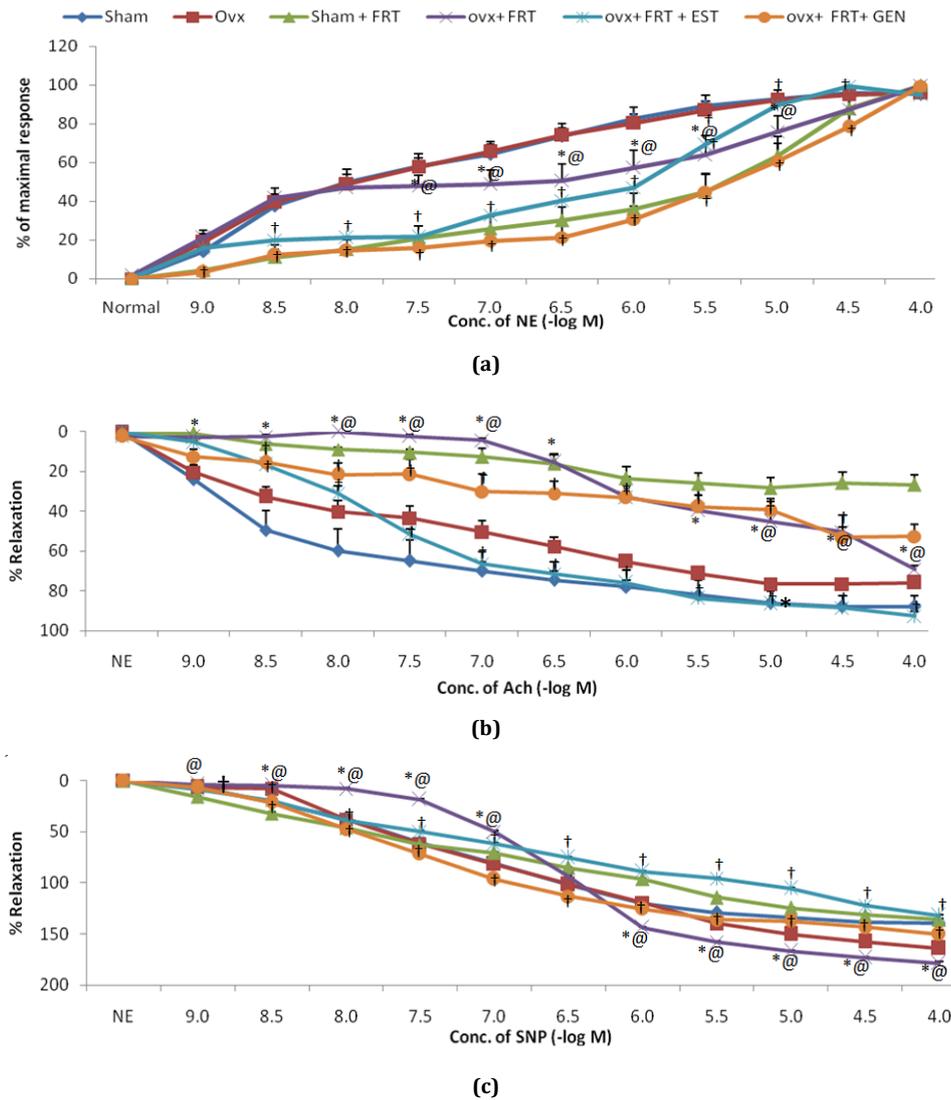


Fig. 1: Effects of 17- β estradiol and genistein on NE-induced contraction (a), Ach (b) and SNP (c)-induced relaxations of aortic rings isolated from insulin-resistant ovariectomized rats

Insulin resistance was confirmed by oral glucose tolerance test and insulin-resistant rats were those whose serum glucose level reached 140 mg/dl or more after 30 min from the oral glucose loading dose.

Results are expressed as means \pm SEM (n=6-10).

Statistical analysis was carried out by ANOVA followed by Turkey's multiple comparisons test.

* Significant difference from corresponding normal rats at $p < 0.05$.

@ Significant difference from insulin-resistant sham-operated control at $p < 0.05$.

† Significant difference from insulin-resistant ovariectomized control at $p < 0.05$.

Effects of EST and GEN on the measured biochemical parameters

Adding 21 % fructose to drinking water of sham-operated and ovariectomized rats for 11 weeks produced an elevation in serum glucose level in OGTT as compared to its corresponding controls. This elevation appears to be prominent throughout the whole experiment and showed an elevation in the AUC of the OGTT in both sham-operated and ovariectomized rats (fig. 2). Additionally, a profound elevation in serum insulin level leading to an elevation in the HOMA-IR index was also demonstrated (fig. 3). Insulin-resistant

animal model was also associated with a decrease in serum NO level as compared to its corresponding control (table 1).

Treatment of insulin-resistant ovariectomized rats with either EST (100 μ g/kg/day; sc) or GEN (1 mg/kg/day; sc) for 21 consecutive days showed a decrease in serum insulin level as compared to the respective insulin-resistant ovariectomized untreated group. Similar treatment with either EST (100 μ g/kg/day; sc) or GEN (1 mg/kg/day; sc) succeeded to decrease the HOMA-IR index (fig. 3), with no change in serum glucose level in OGTT as compared to the respective insulin-resistant ovariectomized untreated group (fig. 2).

Treatment of insulin-resistant ovariectomized rats with EST (100 μ g/kg/day; sc) for 21 consecutive days caused an elevation in serum NO level. On the other hand, similar treatment of insulin-resistant ovariectomized rats with GEN (1 mg/kg/day; sc) showed no change in serum NO level (table 1).

Insulin resistance was confirmed by oral glucose tolerance test and insulin-resistant rats were those whose serum glucose level reached 140 mg/dl or more after 30 min from the oral glucose loading dose.

Results are expressed as means \pm SEM (n=6-10).

Statistical analysis was carried out by ANOVA followed by Tukey's multiple comparisons test.

* Significant difference from corresponding normal rats at $p < 0.05$.

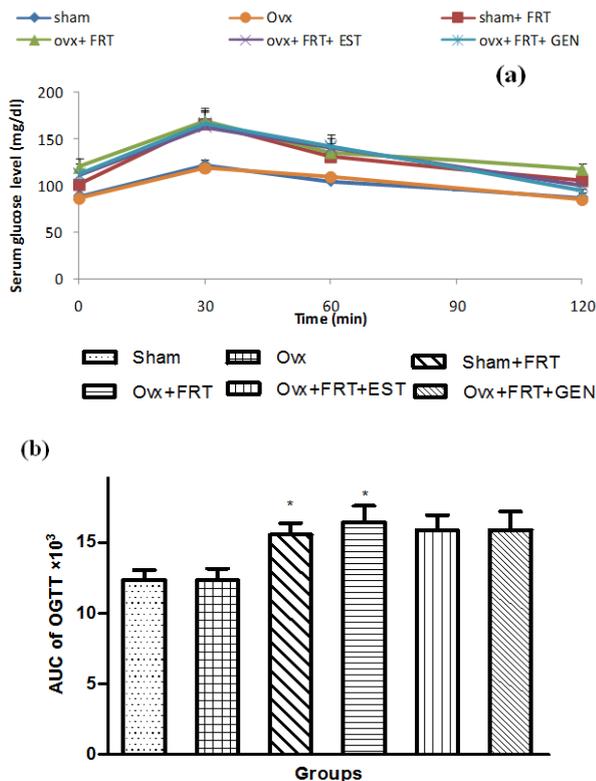


Fig. 2: Effects of 17- β estradiol and genistein on oral glucose tolerance test (a) and its area under the curve (b) in insulin-resistance ovariectomized rats

Three weeks after ovariectomization, rats were rendered insulin-resistant by giving fructose (21%) in drinking water for 8 weeks. EST (100 $\mu\text{g}/\text{kg}/\text{day}$) and GEN (1 mg/kg/day) were *s. c.* injected for 3 weeks while still keeping animals on fructose in drinking water. Twenty-four hours after the last dose of either drug, 18 h food-deprived animals were given a glucose loading dose (1 ml/100 g of body weight from a 50 % w/v glucose solution) and serum glucose levels were determined at 0, 30, 60, and 120 min post loading.

DISCUSSION

The present study provided further evidence that FRT-fed animal model, a common method used for induction of insulin resistance, was accompanied by some cardiovascular alterations. These cardiovascular changes were evidenced as hypertension and alterations in the reactivity of isolated aortic rings towards a vasoconstrictor, as well as endothelium-dependent and independent vasodilators

It has been documented that FRT induces insulin resistance/compensatory hyperinsulinemia, and hypertension [25-29] where insulin resistance precedes hypertension [27, 30]. Several mechanisms have been proposed to mediate the link between insulin resistance/compensatory hyperinsulinemia and hypertension, including the continuous activation of the sympathetic nervous system [31], increased production and/or activity of vasoconstrictors, such as angiotensin II [32], endothelin-1 [33], and thromboxane A₂ (28), as well as impaired endothelium-dependent relaxation [34]. Oxidative stress and abnormal NO activity have been proposed to be a link for the development of hypertension [35]. In addition, oxidative stress has been linked to a decrease in NO availability. Each of these proposed mechanisms can contribute to an increase in the vascular tone, which can lead to the development of endothelial dysfunction and eventually the development of hypertension [30]. Impaired endothelium-dependent vasodilation is considered to be the hallmark of endothelial dysfunction, which is

mediated by NO. A defect in NO production and/or activity has been proposed as a main mechanism of endothelial dysfunction and a contributor to atherosclerosis [36].

In this study, it has been found that there was a marked decrease in the vascular responsiveness of aortic rings isolated from FRT-induced insulin resistant sham operated and ovariectomized rats towards Ach as compared to normal sham-operated rats. This is consistent with previous observations that revealed that endothelium-dependent relaxation response to Ach is impaired in FRT-induced insulin resistant rats [31, 37, 38] and in insulin resistant mice [39].

Treatment of FRT-induced insulin resistant ovariectomized rats with either EST or GEN for three weeks succeeded to reverse the attenuated vascular responsiveness of isolated aortic rings towards Ach and to ameliorate the FRT-induced hypertension. These results were consistent with other studies which showed that EST has an important role in the regulation of vascular function. EST enhances Ach-induced endothelium-dependent relaxation in the guinea pig coronaries [40] and in rat aorta without changing the vascular responses to Ach in endothelium-denuded aortic rings isolated from prepubertal female rats [41].

It has been proposed that the improved endothelium-dependent vasodilation towards Ach after EST treatment is due to an upregulation of NOS III protein expression with a subsequent increase in NO availability [40, 42].

Moreover, it has been documented that the role of vascular ERs is very important in modulating the vascular tone. Both ER α and ER β subtypes have been demonstrated to mediate the vasodilator effects of EST in CVS.

These effects are mediated by genomic as well as nongenomic mechanisms some of which include increases in local NO [43]. Nongenomic activation of eNOS by estrogens via ER α has been reported in endothelial cells of different vascular bed such as pulmonary artery and bovine aorta. This response is rapid, mediated via genomic effects and also occurs in low concentrations, hence influences the vascular tone at physiological concentration [44]. Moreover, the antioxidant property of EST also may attribute to the restored endothelial function [45].

The phytoestrogen, GEN, potentiated endothelium-dependent vasodilation and enhances coronary vascular reactivity in atherosclerotic female monkeys [46] and is able to act as estrogen agonist on ER of the vascular wall [47]. It has been observed that GEN *in vitro* may induce an endothelium-dependent vasodilation in mesenteric bed on NE-precontracted vessels through COX pathway in a dose-dependent manner [48]. GEN can stimulate PGL₂ synthesis in cultured human umbilical vein endothelial cells via ER-dependent mechanism which has the ability to enhance the COX-2 activity [49]. Chronic oral GEN administration improved endothelial function in male spontaneously hypertensive rats by reducing the vascular O₂^{•-} production [50]. It has also been suggested that GEN induced relaxant effect is probably due to inhibition of voltage-dependent Ca²⁺ channels [51].

Previous studies concerned with the contractile response of α 1-adrenoceptor agonists showed, however, inconsistent results, while in some increased reactivity was demonstrated [52, 53], in others decreased [54] or unchanged responsiveness (38) was shown. This discrepancy could be due to difference in the amount and duration of fructose consumption [30].

In the present study, treatment of FRT-induced insulin resistant ovariectomized rats with either EST or GEN for three weeks decreased the vascular responsiveness towards NE as compared to the respective insulin resistant ovariectomized untreated group. This result was consistent with other studies which showed a significant decrease in contractile response of rat tail artery towards NE, after intravenous injection of EST [55]. Pretreatment with EST also decreased contractile responses towards NE in the tail artery [56]. The inhibitory effect of EST on the pressor responses to NE may be related to its modulation of VSMC intracellular free Ca²⁺ through its actions on membrane Ca²⁺ channels [55].

Also, studies had shown that GEN could decrease the contractile responses towards different vasoconstrictors *viz.* KCl, 5-HT and CaCl₂ in isolated porcine coronary arteries [19]. This may be attributed to its inhibitory effect to tyrosine kinase which plays an important role in mediating the responses associated with an intracellular Ca²⁺ release [57].

In the present study, the effect on endothelium-independent relaxation induced by SNP on FRT-treated rats was also assessed. SNP exerts its direct vaso relaxant effect on the vascular smooth muscle cells via spontaneous release of NO, without the involvement of vascular endothelium [58]. There was no change in the vascular responsiveness of aortic rings isolated from hyperglycemic rats towards various concentrations of SNP. This result confirms previous reports indicating that insulin resistance does not significantly influence endothelium-independent vasodilation by SNP [38, 59, 60]. It releases NO independent of NOS and acts directly on guanylate cyclase in the smooth muscle [61]. Treatment of insulin resistant ovariectomized rats with EST or GEN for three weeks showed no significance change in the vascular responsiveness towards SNP as compared to either normal rats or insulin resistant rats. This result is consistent with previous studies that showed that the relaxation response of thoracic aorta rings to SNP after EST treatment had no significant difference among the groups (62). GEN has a direct effect on the aortic smooth muscle [19].

CONCLUSION

Finally, one can conclude from all the previously mentioned findings that estrogens namely, EST (the natural occurring estrogen) and GEN (a phytoestrogen) have a beneficial and protective effect against the vascular dysfunction associated with insulin resistance.

CONFLICT OF INTERESTS

Declared None

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