Chronic estradiol-17β exposure increases superoxide production in the rostral ventrolateral medulla (RVLM) and causes hypertension: reversal by resveratrol

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Abstract

Women are exposed to estrogen in several forms such as oral contraceptive pills and hormone replacement therapy. Although estrogen was believed to be cardioprotective, lately, its beneficial effects are being questioned. Recent studies indicate that oxidative stress in the rostral ventrolateral medulla (RVLM) may play a role in the development of hypertension. Therefore, we hypothesized that chronic exposure to low levels of estradiol-17β (E2) leads to hypertension in adult cycling female Sprague Dawley (SD) rats potentially through generation of superoxide in the RVLM. To test this hypothesis, young adult (3-4 months old) female SD rats were either sham-implanted or implanted (s.c.) with slow release E2 pellets (20 ng/day) for 90 days. A group of control and E2-treated animals were fed lab chow or chow containing resveratrol (0.84 g/ Kg of chow), an antioxidant. Rats were implanted with telemeters to continuously monitor blood pressure (BP) and heart rate (HR). At the end of treatment, the RVLM was isolated for measurements of superoxide. E2 treatment significantly increased mean arterial pressure (mm Hg) and HR (beats/min) compared to sham rats (119.6±0.8 vs. 105.1±0.7 and 371.7±1.5 vs. 354.4±1.3, p<0.0001 respectively). Diastolic and systolic BP were significantly increased in E2-treated rats compared to control animals. Superoxide levels in the RVLM (nmol/min*mg) increased significantly in the E2-treated group (0.833±0.11) compared to control (0.532±0.04, p<0.05). Treatment with resveratrol reversed the E2-induced increases in BP and superoxide levels in the RVLM. In conclusion, these findings support the hypothesis that chronic exposure to low levels of E2 induces hypertension and increases superoxide levels in the RVLM and that this effect can be reversed by resveratrol treatment.
Keywords: Estrogen, blood pressure, superoxide, oxidative stress, resveratrol
Introduction

Cardiovascular disease remains one of the leading causes of morbidity and mortality in women. Postmenopausal increases in blood pressure (BP) in women make hypertension more prevalent in women compared to men of the same age (6, 42). Since premenopausal women have lower blood pressure compared to age-matched men, estrogens were thought to play a protective role against hypertension. Estrogens are reported to improve lipid profile (52), decrease vascular resistance (28) and modulate the activity of brain nuclei involved in cardiovascular regulation (18, 47). However, recent reports from the Women’s Health Initiative study, National Institute of Health (NIH) have provided evidence that Hormone Replacement Therapy (HRT) using estrogen alone or a combination of estrogen and progestin does not confer cardiovascular protection, and may actually increase the risk for coronary heart disease among postmenopausal women (30). These clinical studies have brought to light the importance of clinical and basic research in understanding the role of estrogen in BP regulation (57). Oral estrogen administration, either given alone or in combination with progestins, was found to promote systolic blood pressure in postmenopausal women (34, 46). Although the magnitude of the increase in BP was only between 1 and 2 mm Hg, similar increases in systolic BP and pulse pressure are known to be associated with a higher rate of progression of coronary atherosclerosis (35) and development of cardiovascular events (34) in large clinical trials.

In addition to women who are on HRT, younger women who take oral contraceptives are also at increased risk for potentially developing cardiovascular disorders. Oral contraceptives have been used worldwide for over 30 years. Most
women taking oral contraceptives have been found to have small elevations in BP of ~2 mm of Hg (56). In addition, approximately 5% of patients who take a preparation containing more than 50 μg of estradiol-17β (E2) have significant elevations in BP greater than 140/90 mm Hg (56). Estrogen rather than progesterone, appears to be the primary cause for increases in BP observed with oral contraceptives because women taking progestin-only contraceptives do not have significant elevations in BP (21). Therefore it is important to elucidate the mechanisms by which chronic exposure to E2 increases BP.

The central nervous system (CNS) plays an important role in the development and maintenance of hypertension (4, 9, 25, 26). The cortex, limbic system, hypothalamus, brainstem and the autonomic nervous system are all known to be involved in the maintenance of BP. In the brainstem, the rostral ventral lateral medulla (RVLM) is a critical area involved in the regulation of sympathetic activity and BP. The RVLM integrates sympathetic outflow and provides excitatory input to preganglionic sympathetic cells in the spinal cord (51). The RVLM maintains basal vasomotor tone, and an increase in RVLM activity is associated with hypertension and heart failure (26).

Recently, oxidative stress in the RVLM has been suggested to increase sympathetic nervous system activity and BP in several animal models of hypertension (4, 9, 25). Therefore, we hypothesized that E2 induced increase in blood pressure could be mediated by oxidative stress-related changes in RVLM.

To test this idea, young, adult cycling female Sprague-Dawley rats were exposed to low levels of E2 on a chronic basis. We attempted to reduce oxidative stress in these animals by treating them with resveratrol, an antioxidant.
Materials and Methods

Experimental Animals and Treatment

All the protocols followed in this experiment were approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University. Adult (3-4 months old) female Sprague-Dawley (SD) rats (Harlan Sprague-Dawley, Indianapolis, IN) were housed in temperature (23 ± 2°C) and light controlled (14:10h light/dark cycle) rooms with ad libitum food and water. Estrous cycles were monitored as described previously (22) for 2 weeks and animals that were cycling regularly were used in the experiments. In experiment 1, animals were divided into 2 groups (n=4/group); sham-implanted (control); or implanted with E₂ (20 ng/day, 90-day slow-release pellet; Innovative Research America, Sarasota, FL). After 60 days, sham and E₂-treated rats were implanted with radiotelemetry transmitters to monitor BP as described below. After 90 days of exposure to E₂, all the animals were sacrificed at noon on the day of estrous. Most of the E₂ treated animals were in persistent estrous after 90 days treatment. The control animals were sacrificed on the day of estrous after 90 days of sham implantation for comparison to the treatment group. The brains and brainstem were removed, frozen on dry ice and stored at -70°C. The trunk blood was collected and serum was separated and stored at -70°C until processed.

In experiment 2, animals were divided into 4 groups (n=6/group): sham-implanted (control); implanted with E₂ slow-release pellets (E-90; 20 ng/day for 90 days; (22)); control fed with chow containing 0.84g resveratrol/kg of chow (Res) (3); and E₂ implanted rats fed with chow containing resveratrol (Res+E-90). Resveratrol treatment was started 7 weeks after pellet implantation. Animals were implanted with telemeters
on the 9th week as described below. Food intake, water intake and body weight were monitored weekly throughout the experimental period. The animals were sacrificed at the end of 90 days while in the state of estrous.

**BP Measurement**

In the ninth week of the treatment period, radio-telemetry transmitters (Model TA11-PA-C40, Data Sciences International) were implanted as described previously (24). Briefly, the tip of the transmitter catheter was placed in the abdominal aorta through the femoral artery under general anesthesia. The body of the transmitter was placed in a subcutaneous pocket in the abdomen. Ten days later, BP data from the transmitter were collected continuously for 11 days in experiment 1 and 14 days in experiment 2 (24 h/day; 10 second averages collected every 10 min). Data was stored and analyzed using the Dataquest A.R.T. software (Data Sciences International, St. Paul, Minnesota).

**Brain Microdissection**

Palkovits's microdissection procedure (32) was used to isolate the RVLM. Briefly, 300-µm serial sections of brainstem were obtained using a cryostat (Slee Mainz, London, UK). The sections were transferred to microscope slides and placed on a cold stage maintained at –10°C. The RVLM of the brainstem was microdissected from sections obtained 12.00 mm to 12.48 mm posterior to the bregma using a 500µm diameter punch, using the rat brain stereotaxic atlas as a reference(41). The punches were stored immediately at -70°C until processed for superoxide measurement.

**Superoxide Measurement**
Superoxide (O$_2^−$) levels from the RVLM region were measured using a lucigenin O$_2^−$ chemiluminescence assay adapted from Rey et al. (43). RVLM punches were placed in HEPES buffer [119 mmol NaCl, 20 mmol HEPES, 4.6 mmol KCl, 1.0 mmol MgSO$_4$, 0.15 mmol Na$_2$HPO$_4$, 0.4 mmol KH$_2$PO$_4$, 5 mmol NaHCO$_3$, 1.2 mmol CaCl$_2$, 5.5 mmol glucose (pH 7.4)]. Diethyldithiocarbamate (DDC; a superoxide dismutase inhibitor; 10 mmol) was added and incubated at 37ºC for 30 min. Lucigenin (5µmol/L) was added and the samples were incubated for 10min at 37 ºC. Chemiluminescence measurements were obtained using a model TD 20/20 Luminometer (Turner Designs, Sunnyvale, CA, USA) for 10 readings (30 seconds/reading). Tiron (10 mmol/L; a O$_2^−$ scavenger; Sigma, St. Louis, MO) was added and incubated for 15 min at 37ºC and 10 additional readings were taken. The relative amount of O$_2^−$ was determined by taking the average of 2$^{nd}$-9$^{th}$ readings prior to the addition of tiron, and subtracting the average of the 7$^{th}$-10$^{th}$ readings after the addition of tiron. O$_2^−$ generated was reported as change in chemiluminescence/min/mg tissue weight.

Serum Estradiol

Estradiol levels in serum separated from trunk blood were measured by double antibody radioimmunoassay by Dr. A.F. Parlow, National Hormone and Pituitary Program, NIDDK. Samples were assayed in duplicate.

Statistics

Daily BP measurements and weekly food intake, water intake and body weight were analyzed by repeated measures ANOVA followed by post hoc Fischer’s LSD test. Average BP parameters, serum estradiol and O$_2^−$ data were analyzed by ANOVA, followed by post hoc Fischer’s LSD test. Results were considered significant when
p<0.05. Average food intake, water intake, body weight, heart: body weight ratio were analyzed by ANOVA followed by post hoc Fisher’s LSD test. Results were considered significant when p<0.05.

Results

Chronic exposure to estradiol-17β (E2) causes hypertension

The daily average profiles and the average mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) during the 10-11th week of treatment in control and E2-treated rats are shown in Fig. 1 (A-H). As seen in Fig. 1A, the MAP in control animals remained steady over the entire period of observation. In contrast, E2 treatment increased MAP significantly (p<0.01; Fig. 1A). The average MAP (mean±SE, mm Hg) measured during the 10-11th week of observation in control rats was 105.1±0.7. In contrast, E2 exposure increased MAP significantly to 119.6±0.8 (p<0.0001; Fig. 1E). The HR profile of E2-treated rats had a tendency to increase when compared to control rats during the entire period of observation (Fig. 1B). The average HR (mean±SE, beats/min; Fig. 1F) during the 10-11th week of treatment in E2-treated rats (371.7±1.5) was significantly elevated compared to control rats (354.5±1.4; p<0.0001). Similarly, the SBP and DBP profiles in E2-treated were significantly elevated in E2-treated rats compared to control rats (p<0.0001; Figs. 1 C, D). E2 exposure also increased the average SBP and DBP (mean±SE, mm Hg; 140.0±0.7 and 101.1±0.8 respectively) significantly compared to control rats (125.4±0.7 and 88.0±0.7 respectively; p<0.0001; Figs. 1 G, H).

Chronic exposure to E2 increases O2– levels in RVLM
Chronic E₂ exposure significantly elevated O₂⁻ levels (mean±SE, nmol/min*mg) in the RVLM of E₂-treated rats (0.833±0.1) when compared to control rats (0.532±0.04; p<0.01) (Fig. 2).

**Chronic E₂ exposure increases food intake, water intake and body weight**

The average weekly food intake (mean±SE, g/week), water intake (mean±SE, ml/week) and body weight (mean±SE, g) of control, E₂, resveratrol, resveratrol+E₂ animals are shown in Fig. 3 A-G. There was no difference in food intake between control and E₂-treated groups at the beginning of the experiment. However, food intake in the E₂ treated group appeared to increase on the 2nd week (141.7±4.7), the 5th week (132.1±5.3) and the 7th week (127.6±3.8) compared to control rats (123.8±2.8, 117.7±2.3 and 117.6±2.5 respectively; p<0.05). The average food intake was significantly higher in E₂-treated rats (134.6±1.7) compared to control rats (122±2.3) and those treated with resveratrol alone (123.1±1.3; p<0.05; Fig. 3E). Feeding chow containing resveratrol did not affect food intake in the control and E₂-treated groups.

The average weekly water intake in control and experimental animals are given in Fig. 3B. There was a tendency for increased water intake in E₂-treated rats compared to control rats in the first week and this trend continued throughout the period of observation, though the change was not statistically significant. However, the average water intake (Fig. 3F) was significantly higher in the E₂-treated group (316.5±5.6) compared to control rats (288.2±5.5), sham-implanted resveratrol treated group (279.6±2.9) and the resveratrol and E₂ treated group (257.5±5.8; p<0.005). The E₂-treated group fed chow containing resveratrol consumed less water (257.5±5.8) compared to the control group (288.2±5.5; p<0.05).
Changes in weekly body weight among the different groups are shown in Fig. 3C. There was no difference in body weight between the treatment groups at the beginning of the experiment. Although there was a tendency for body weight to increase with time, the average weekly body weight in the E2 group was not statistically different from that of control rats. Feeding control and E2 rats with chow containing resveratrol for 2 weeks did not produce any change in body weight. The average body weight over the entire period of observation (Fig. 3G) was significantly higher in the E2 group (297.4±2.7) when compared to control rats (287.3±1.6; p<0.005). Feeding resveratrol did not alter the average body weight in control and E2-treated rats. Body weight in E2-treated rats fed chow containing resveratrol (299.6±0.4) was significantly higher compared to control (p<0.05).

There were no significant changes in heart weight or the ratio of heart weight to body weight in any of the treated groups compared to the control group (Fig. 3D).

**Serum E2**

Serum E2 levels (mean±SE, pg/ml) at the end of 90 days of E2 exposure and resveratrol treatment are shown in Fig.4. Exposure to E2 resulted in significant increases in serum E2 of animals that are treated with E2 alone (68.2±4.07) or treated with E2 and resveratrol (59.4±1.5) when compared to control rats (46.7±2.9) or rats treated with resveratrol alone (47.4±4.4, p<0.0005). There was no difference in E2 levels between the E2 treated groups (with or without resveratrol).

**Resveratrol reverses chronic E2-induced hypertension**

The daily average profiles and the average MAP, SBP, DBP and HR during the entire period of observation in control, E2, resveratrol and resveratrol+ E2-treated rats
are shown in Fig. 5 (A-H). Similar to what was observed in Fig. 1, exposure to E_2 for 90 days significantly elevated MAP, HR, SBP and DBP compared to control rats. Feeding chow containing resveratrol to sham-implanted control rats did not alter MAP, HR, SBP and DBP. In contrast, feeding chow containing resveratrol to E-90 rats completely reversed E_2-induced increase in MAP, HR, SBP and DBP in E_2 implanted rats (p<0.01).

**Resveratrol attenuates oxidative stress in RVLM**

Changes in superoxide levels (mean±SE, nmol/min*mg) in the RVLM of control, E_2, resveratrol and resveratrol+E_2 groups are shown in Fig. 6. As seen in Fig. 2, superoxide levels increased significantly in E-90 rats (0.608±0.052) compared to control rats (0.454±0.007; p<0.005). When sham-implanted control rats were fed chow containing resveratrol alone, it did not alter superoxide levels (0.431±0.007) compared to control rats (0.454±0.007). In contrast, feeding E_2-treated rats with chow containing resveratrol completely reversed E_2-induced increase in superoxide levels in the RVLM (0.431±0.007 vs. 0.608±0.052; p<0.0005).

**Discussion**

The results from the present study provide evidence that chronic exposure to low levels of E_2 for 3 months increases superoxide levels in RVLM and results in hypertension. Treatment with an antioxidant, resveratrol, reversed E_2-induced increases in superoxide levels in the RVLM and reversed the increase in BP indicating that chronic exposure to low levels of E_2 is capable of causing hypertension possibly by increasing superoxide generation in the RVLM.

Estrogen was originally believed to prevent hypertension based on the observation that postmenopausal women have a higher incidence of hypertension.
compared to age-matched men (17, 31, 40) and, therefore, HRT containing estrogenic preparations were believed to reduce BP in these women (48). Several possible mechanisms by which estrogens could decrease BP have been suggested. There is evidence that estrogen increases acetylcholine-induced, nitric oxide-mediated relaxation of aorta in male SHR rats (23) through up-regulation of endothelial nitric oxide synthase (eNOS) (20). Also, estrogen is believed to act on some brainstem autonomic centers to decrease sympathetic nervous activity (54, 55), thereby causing reduced vasomotor tone and lowered BP.

Contrary to the belief that estrogens can lower BP, there is a large body of evidence indicating that repeated exposure to low levels of estrogens, as in the case of oral contraceptives, can cause an increase in BP. Numerous human clinical studies have associated chronic use of contraceptive pills with increase in BP (12, 29, 56). Similar results have been seen in animal studies, where exposure to a combination of 1µg of ethinyl estradiol and 10µg of norgestrel or ethinylestradiol alone for 10 weeks resulted in concomitant increases in systolic and mean arterial BP (7, 36, 38). In another study, female SD rats injected with 0.2µg of ethinyl estradiol exhibited significant increases in systolic BP of 17mm of Hg in 6 weeks and 32mm of Hg in 12 weeks (7). Different mechanisms have been proposed for oral contraceptive-induced hypertension. These include impaired renal handling of water resulting in volume-dependent hypertension (37) and hyperactivity of the renin-angiotensin system (7, 38). Increased water intake as observed in the present study could be another contributing factor to volume expansion. However, it is not clear if this is associated with the increased food intake and body weight observed in E₂-treated animals. Interestingly,
there were no measureable increases in heart weight, or the ratio of heart weight to body weight, in E₂ treated rats. This is perhaps due to the rather mild increase in BP caused by E₂, and to the known protective effect of E₂ against pressure-induced cardiac hypertrophy (50).

The increase in MAP, HR, SBP and DBP with chronic E₂ exposure that was observed in the present study is supported by other reports (7, 36, 38). The main difference between these and the present study is the dose of E₂ used and duration of exposure. While the doses used in other studies ranged from 0.2 to 10 µg of various estrogenic preparations, we were able to observe increases in cardiovascular parameters with 10-fold less concentration of E₂ (0.02 µg or 20 ng). In contrast to our present study, Brosnihan et.al, have shown that 3 weeks of estrogen treatment decreased MAP and significantly decreased ANG-II-induced pressor response in female ovariectomized rats which was accompanied by significant reduction in plasma ANG-II levels (5). However, the dose of E₂ used in their study was 1.5 mg/day which yielded plasma concentrations of 190±20 pg/ml. With a similar dosage of E₂, Gimenez et.al, demonstrated enhanced hypotensive responses to administration of an ACE inhibitor in E₂-treated 18 months old female SHR rats (16). When using 1.5 mg/day, the serum E₂ concentrations were approximately 2.5 fold higher than those that have been found in the present study, where serum E₂ concentrations were 68.2±4.07 pg/ml in animals that were treated with E₂ alone. Previous studies also used a much shorter E₂ exposure time frame than those used in the present study, thus complicating the comparisons. In the present study, we monitored changes in BP only 9 weeks after E₂ implantation. Further
studies are needed to determine exactly when the changes in BP become apparent in these animals.

The mechanism by which exposure to low doses of E₂ induces hypertension is not clear. Several brain sites such as the paraventricular nucleus of the hypothalamus, the subfornical organ, nucleus tractus solitarius and the RVLM are known to be involved in the regulation of blood pressure (8). We chose to focus specifically on the RVLM because it is known to be involved in the maintenance of basal vasomotor tone via regulation of sympathetic activity (45). Moreover, brainstem neurons in the RVLM contain estrogen receptor mRNA (1, 27, 49) indicating that this is a potential site of E₂ action. Also, stimulation of the RVLM is known to activate sympathetic preganglionic neurons in the intermediolateral cell column of the spinal cord and increase sympathetic activity resulting in hypertension (14). E₂-induced increase in O₂⁻ levels in the RVLM observed in the present study may act through similar mechanisms. Other studies have shown an association between O₂⁻ in the RVLM and hypertension in multiple animal models (9, 19, 25, 39). Moreover, reversal of hypertension by increasing the expression of superoxide dismutase using gene transfer and adenoviral vectors or decreasing O₂⁻ in the RVLM substantiates the role of oxidative stress in the pathogenesis of hypertension (10, 11, 13, 15). Since our results clearly indicated that E₂ treatment increases O₂⁻ production in the RVLM, we wanted to explore the possibility of reversing this effect using the antioxidant, resveratrol.

Resveratrol is a red wine polyphenol which is known to possess antioxidant and O₂⁻ scavenging properties (53) and exerts strong antioxidant effects in the brain (33). In the current study, treatment with resveratrol reduced MAP, HR, SBP and DBP induced
by E$_2$ treatment with complete quenching of O$_2^-$ in the RVLM. Resveratrol has been shown to reduce SBP in obese Zucker rats by increasing eNOS expression in the aorta (44). Resveratrol treatment also prevented hypertension caused by high fat diet in female rats(2). In the present study, treatment of E$_2$-treated rats with resveratrol reversed E$_2$-induced increase in O$_2^-$ in the RVLM and also decreased BP raising the possibility that E$_2$’s effects on BP could be mediated through increased production of O$_2^-$. In conclusion, our results support the hypothesis that chronic exposure to low levels of E$_2$ causes hypertension in young SD female rats. This hypertensive effect of E$_2$ may be related to increased O$_2^-$ production in the RVLM. Although, this study examines the effect of E$_2$ on O$_2^-$ generation in the RVLM, the involvement of other brain sites and peripheral tissues such as the vasculature and kidney should also be considered. Collectively, this study provides valuable insights into the mechanisms by which chronic low dose exposure to E$_2$ causes hypertension and draws attention to potential cardiovascular risks faced by women on long term oral contraceptives.

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**Disclosure statement:** The authors have nothing to disclose.
References


Figure Legends

Fig. 1. Effect of chronic E2 exposure on BP parameters.
A-D: Line graphs depicting MAP (mmHg), HR (beats/min), SBP and DBP (mmHg) respectively: closed circles represent E2 pellet implanted (20 ng/day, 90-day slow-release pellets) and open circles represent control rats. E-F: Bar graphs showing the average values of the BP parameters shown in A-D, between E2 implanted and control rats. * denotes significant difference (p<0.05) from control rats.

Fig. 2. Effect of chronic E2 exposure on Superoxide in the RVLM.
A: Schematic coronal brain stem section showing the location of micropunches of RVLM (within circles) taken from Paxinos and Watson rat brain atlas, 6th edition. The section coordinate (-12.24mm bregma) represents the distance caudal to the bregma. (Rob- raphe obscurus nucleus; rs- rubrospinal tract; sp5- spinal trigeminal tract; IRt-intermediate reticular nucleus; Sol- nucleus of the solitary tract) B: Bar graph denoting O2\textsuperscript{-} levels measured in RVLM brainstem punches of E2-treated (20 ng/day, 90-day slow-release pellets) and sham-implanted female SD rats (n=4 per group) using a luminometer. * indicates significant difference (p<0.05) from control rats.

Fig. 3. Effect of chronic E2 exposure and resveratrol on food intake, water intake, body weight and heart weight.
A-C: Line Graphs showing food intake (g), water intake (ml), and body weight (g) between different groups of female SD rats (n=6 per group): open circles shows control, closed circle shows E2 pellet implanted (E-90), open inverted triangle shows resveratrol (Res) treatment alone, and closed triangle shows resveratrol treatment on E2 implanted rats (Res+E-90). * indicates significant difference from control (p<0.05). D. Bar graphs
showing the average values of heart weight (g) and heart: body weight ratio in the
different treatment groups (n=6 per group). E-G: Bar graphs showing the average
values of food intake, water intake and body weight shown in A-C respectively. Fig E *
denotes significant difference (p<0.05) from control and resveratrol treated groups. Fig
F: * denotes significant difference (p<0.005) from the rest of the groups. a denotes
significant difference from control (p<0.05). Fig G: * denotes significant difference
(p<0.05) from control.

Fig. 4. Serum E2 levels
Bar graphs showing serum E2 levels in all the groups (n=5-7 per group). * denotes
significant difference (p<0.05) from control and the group treated with resveratrol alone.

Fig. 5. Effect of resveratrol on chronic E2-induced hypertension
A-D: Line graphs depicting MAP (mmHg), HR (beats/min), SBP and DBP (mmHg)
respectively: closed circles represent E2 pellet implanted (20 ng/day, 90-day slow-
release pellets) and open circles represent control female SD rats, filled triangles
represent rats fed with chow containing 0.84g/Kg resveratrol only and open inverted
triangles represent E2 pellet implanted (20ng/day, 90-day slow-release pellets) rats fed
with chow containing 0.84g/Kg resveratrol. # denotes significant difference from control,
* denotes significant difference from all the other groups. E-H: Bar graphs showing the
average values of the BP parameters shown in A-D., * denotes significant difference
(p<0.05) from all the other groups; ‘a’ represents significant difference from control and
Res+E-90; ‘b’ represents significant difference from control.

Fig.6. Effect of resveratrol on chronic E2-induced oxidative stress in RVLM
Bar graph showing superoxide levels measured in RVLM punches from control, E₂ treated (20 ng/day, 90-day slow-release pellets), rats fed with chow containing 0.84g/kg resveratrol only and rats implanted with E₂ pellet (20ng/day, 90-day slow-release pellets) fed with chow containing 0.84g/kg resveratrol (n=4-6 per group). * denotes significant difference (p<0.05) from all the other groups.
Fig 2

A

Bregma -12.24 mm

B

Superoxide (nmol/min/mg)

Control  E-90

0.00  0.50  1.00

*
Fig 5

A

MAP (mm Hg)

Control → E-90 → Res → Res+E-90

B

HR (beats per min)

C

SBP (mm Hg)

D

DBP (mm Hg)

E

MAP (mm Hg)

Control → E-90 → Res → Res+E-90

F

HR (beats/min)

G

SBP (mm Hg)

H

DBP (mm Hg)

Control → E-90 → Res → Res+E-90