RESEARCH ARTICLE

Effects of Estrogen in Treating Myocardial Damages Caused by Ischemia in Adult Female Rats

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ABSTRACT

This study aims to show the role show the role of estrogen in rats with ischemic heart disease. 60 adult female rats were used in the study and divided as follows into five equal groups (each group consists of 12 rats); control female rats group, second group ischemia rats without ovariectomy. Third group rats with only left ovary were removed, fourth group rats both ovaries were removed, fifth group rats with both ovaries were removed and treated (intramuscular injection) with (10 mg/kg) estrogen. The results show a significant increase ($p < 0.05$) in several inflammatory cells and levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), malonedialdehyied (MDA) with significant ($p < 0.05$) reduce in glutathione (GSH) levels in third and fourth group compare with healthy female rats. While, after using estrogen in treatment, the outcomes exhibited non-statistic significant ($p < 0.05$) differences in a number of inflammatory cells, liver enzymes, MDA, and GSH compared with healthy female rats. It was concluded that estrogen has been an important role in the treatment of myocardial damages caused by ischemia.

Keywords: Estrogen, Inflammatory cells, Ischemia, Liver enzymes.

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INTRODUCTION

The disease of myocardial infarction (MI) is defining as an event of cardiac attack. MI happens when the heart muscle is injured by hypoxia (deprived of adequate oxygen supply), which occurs when a coronary artery is plugged.[1,2] Disease of myocardial ischemia, defined as lack of blood flow to cardiac muscles, is the most common reason of signs and symptoms of coronary cardiac disorders- also mentioned like a disease of the coronary artery.[3] The reason of heart ischemia is to narrow the lumen of coronary arteries. When coronary arteries are narrowed or reduced the lumen, it leads to less blood supply and oxygen to cardiac muscles. Cardiac ischemia causes disease of coronary vessels, angina pectoris condition, myocardial infarction, and heart failure.[4,5] Estrogen is defined as a female steroid hormone-producing from the ovary organ responsible for controlling the menstrual cycle in women. Therefore estrogen is essential for women reproduction.[6,7] There is almost twice the ischemic stroke risk in female with natural menopause before age 42 years contrast to those who developed menopause after age 42 years[8] or between 50 to 54 years.[9] The menstrual cycle possesses significant effects and protective impacts on system of heart vascular. The female rats and those receiving an Oophorectomy (called surgery for ovary removal) with the hormone replacement therapy (HRT) female rats have less myogenic tone and less response of pronounced myogenic than male or female rats with oophorectomy and without hormone replacement therapy because estrogen-induced increase nitric oxide (NO) produce and activity.[10] The concept of hormone replacement therapy was based on the useful effects observed in different animal experiments.[10-12] So, the study aims to detect the role of estrogen in myocardial ischemia.

MATERIALS AND METHODS

Animal Model

Rat procedures were achieved in agreement with the proper utilization and laboratory animal care. 24 female rats (*Rattus rattus*) weighing 150 to 200 g with age 4 to 6 months obtains from animal house in Tikrit University. Rats were kept under optimum conditions as optimum temperature (24±3°C), the cycle of 12 hours light-dark, and had access to rat pellets.

Surgical ligation of Left Anterior Descending Artery (LAD)

Female rats were anesthetized with (Intraperitoneal) injection of ketamine (100 mg/kg) and Xylazine (10 mg/kg). After trachea intubation by using a 20 G cannula and tube called

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endotracheal tube was binding tightly to the ventilation machine. The ventilation percent was fixed from 120 to 135 breaths/min with the volume of tidal 20 mL/kg body weight, with 100% oxygen supply. The Left Anterior Descending artery coronary artery was passing ligated 1 to 2 mm under the left auricle tip utilizing a sharpened needle and 8-0 ligature of polypropylene. Ligature tightening may lead to then occlude artery for a 30-minutes ischemic time. The chest cavity was closed by bringing together the fourth and fifth ribs with one silk suture (2-0). Heart reperfusion was done by releasing the applying of tension to the ligature for 120 minutes.[13] Rats were euthanized after the reperfusion through injection anesthesia with high dose and the cavity of the chest was re-opened, then right ventricle was punctured by utilizing a syringe needle and collect about 3 mL of blood was for later estimate some parameters.

Ovariectomy

Female rats usually do not undergo menopausal conditions until 18–24 months of their age.[14] So, OVX is utilized to induce menopause conditions in female rats.[15] Fasted female rats (6–12 hours) were undergone to anesthetize by using ketamine (Bioveta, Inc/Czech) and xylazine (XYLAMAX® Xylazine Injection 100 mg/mL, Bimeda/Canada). Ventral incisions of skin performed OVX in female. The area of skin is then shaved, washed and disinfected. Ovaries were removed from its location and oviducts change with minimum disruption to surrounding soft tissues (abdominal organs) and the incisions in abdominal region were closed with clips.[16] To avoid contamination and any possible complications, ovariectomized rats administration of midazolam (1 mg/kg subcutaneously every 6 hours) gentamicin (intramuscularly 5 mg/kg for 5 days).[17]

Experimental Design

60 female rats were used in this experiment and divided as follow (12 female in each group)

- *Control group:* rats were received a standard pellet diet only for seven days and then killed compared with other groups.
- *Second group:* ischemia rats without ovariectomy
- Monolateral ovariectomy (OVX) group: only left ovary was removed
- *Bilateral ovariectomy (OVX) group:* both ovaries were removed
- *Bilateral ovariectomy (OVX) group:* both ovaries were removed and treated (intramuscular injection) with (10 mg/kg) estrogen

After each week, four rats from each group were dissected and the samples (blood and cardiac tissues) were taken to observe the changes three weeks.

Measurements

Malondialdehyde (MDA) is produced due to lipid peroxidation process of polyunsaturated fatty acids in body tissues.[18] MDA was measured based on the colorimetric reaction with thiobarbituric acid (TBA) using spectrophotometer.[19] Glutathione (GSH) enzymes can stop and prevent destruction to components of cellular that caused by reactive oxygen species (ROS) like free radicals, etc.[20] the levels of GSH evaluated by mixed buffer (2.3 mL) with sample (0.2 mL) and after that added 0.5 mL of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB). The spectrophotometer was utilized to estimate GSH in rat serum.[21] Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were measured by technique according to the instructions of the manufacturer company kit (Randox).

Histological Study

Heart biopsies were taken with 5 mm punch. Heart biopsies were removed, fixed in 10% formalin (China), routinely processed and embedded by using paraffin wax (China) sections that were stained by using hematoxylin and eosin stains (Anamol/ANA-HE/India) and diagnosed under light microscope (Optica/ Italy).

Statistical Analysis

The Data of current work were analyzed utilizing a special statistical program called Minitab program. The statistical difference in the current study between the means of the studied groups was analyzed by utilizing one-way analysis of variance (ANOVA).[22]

RESULTS

Number of Inflammatory Cells

In this study evaluated the cellular infiltration 21 days after coronary I/R by Hematoxylin-Eosin staining. The presence of inflammatory cells in C and D group compared show high significant increase ($p<0.05$) compared with control group and group B as shown in Figure 1. Estrogen administration in E group resulted in decreased cellular infiltrate as shown in Figure 1.

Here, Ctr: control group, ISCH: rats with ischemia, OVX1: rats with monolateral ovariectomy, OVX2: rats with monolateral ovariectomy, OVX3: rats with bilateral ovariectomy and treated with estrogen.

MDA and GSH

Evaluated of MDA and decreased of GSH levels were observed in C & D group and showed high significant changes ($p<0.05$ compared with a control group and group B. Estrogen administration in E group resulted in decreased MDA and increased of GSH levels as shown in Figures 2 and 3.

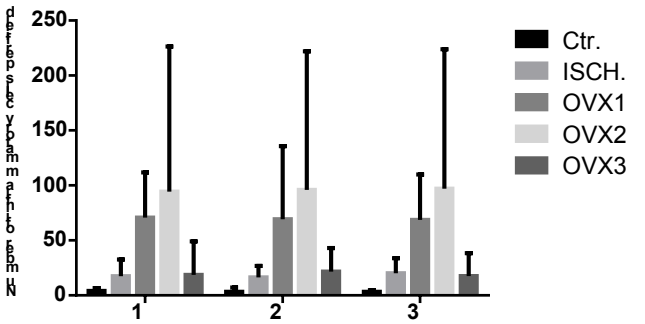

Figure 1: Number of inflammatory cells in all groups

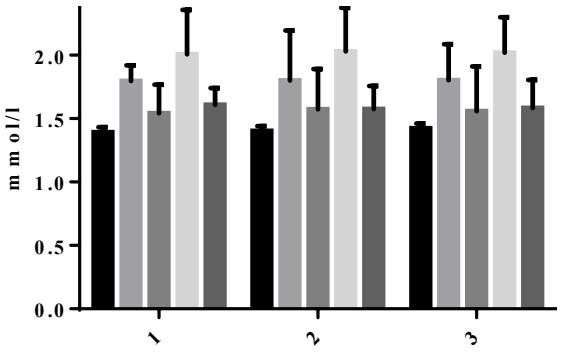

Figure 2: Levels of MDA in all groups

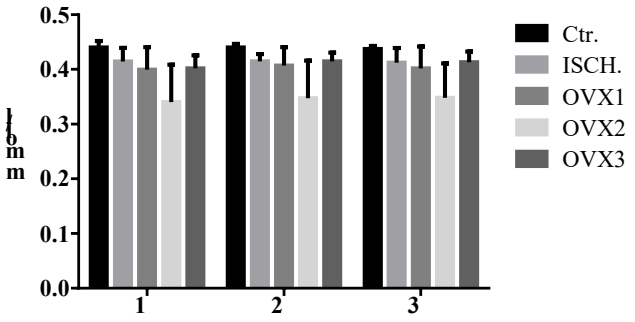

Figure 3: Levels of GSH in all groups

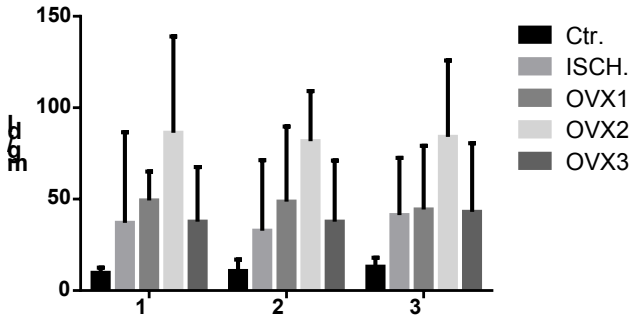

Figure 4: Levels of AST in all groups

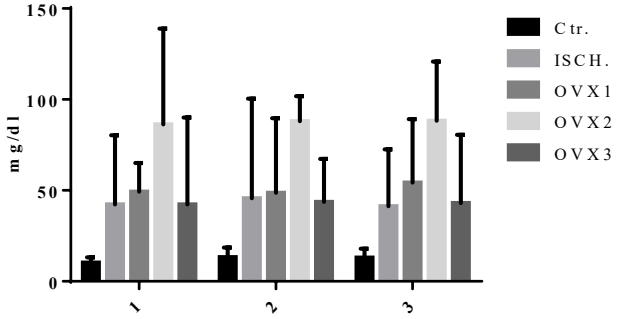

Figure 5: Levels of ALT in all groups

Liver Enzymes

Evaluated of AST and ALT levels were observed in C and D group and showed high significant increase ($p < 0.05$) compared with control group and group B. Estrogen administration in E group resulted in decreasing AST and ALT levels as shown in Figures 4 and 5.

DISCUSSION

The present results demonstrate that ischemic in female rats leads to myocardial injury and increased oxidative stress, AST, ALT, and number of inflammatory cells. Mohammed referred that ischemia/reperfusion leads to myocardial injure and increases oxidative stress by elevating the free radical. About the role of estrogen, previously, it has been reported that 100 µg as single dose of estrogens.[23] decreased the ischemia-induced arrhythmias in dogs. Another study suggested that a bolus dose of 17β-estradiol (10 µg kg^{-1}) causes trend of more experiment animals surviving ischaemia and reperfusion disorders. Other study has evaluated estrogen's effects on reperfusion-induced arrhythmias.[24,25] The antiarrhythmic movement of E2 in myocardial ischemia setting was prominent in females more than in male rats,[26] which could be because of the effect of E2 on the articulation and capacity of particle channels that control cardiovascular cell excitation and repolarization.[27] Estrogen has mitigating and vasoprotective effects when controlled to young female or trial creatures that give off an impression of being changed over to proinflammatory and vasotoxic impacts in more established subjects, especially those without hormone for extensive stretches.[28] Additionally, 17β-Estradiol (E2) offers cardiovascular security in youthful female creatures and postmenopausal ladies. Conversely, randomized preliminaries of menopausal hormones performed in more established ladies have appeared or no cardiovascular advantage.[29]

CONCLUSION

The current study reveals that the treatment by using estrogen hormone protects the heart against ischemia-reperfusion, which induces myocardial injuries.

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