

Original Research Article

Modulatory effects of vitamin B6 on ovariectomy-related cardiovascular dysfunction

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Abstract

Purpose: To investigate the modulatory effect of chronic supplementation with vitamin B6 (pyridoxine) in estrogen-deficiency-induced cardiovascular perturbations in OVX female rabbits.

Methods: A total of 18 adult female rabbits were randomly divided into three groups (n = 6 each). Control group received no treatment; group 1 underwent ovariectomy (OVX) and was left untreated; group 2 was ovariectomized and exposed to pyridoxine (10 mg/kg/day orally over 12 weeks). Serum cardiac biomarkers (cardiac troponin T and lipocalin-2), nitric oxide synthase (NOS), and metabolic indices were measured. Histological examination of myocardial tissue was done to determine structural damage, fibrosis, and inflammation. Also, expression of endothelial nitric oxide synthase gene (NOS3) in cardiac tissue was determined using quantitative real-time PCR.

Results: Treatment with pyridoxine significantly reduced myocardial injury markers and increased NOS activity to similar values as control (p < 0.05). Histopathological analysis following pyridoxine treatment did not damage myocardial structure, with increased fibrosis and inflammatory cell infiltration. At the molecular level, NOS3 was significantly increased following treatment with pyridoxine compared to other groups, indicating better endothelial functionalities (p < 0.05).

Conclusion: The study showed that vitamin B6 significantly reduced myocardial injury improves endothelial function with no damage to myocardial tissues.

Keywords: Ovariectomy, Vitamin B6, Pyridoxine, Estrogen deficiency, Endothelial function

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INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of mortality in women worldwide, with significantly higher incidence after menopause [1]. It is associated with estrogen deficiency, which adversely affects vascular homeostasis, lipid metabolism, glucose tolerance and inflammatory pathways [2]. Estrogen also

has a very significant protective effect. It increases endothelial nitric oxide synthase (eNOS) activity and decreases oxidative stress. Thus, estrogen loss during natural or surgically induced menopause is an important risk factor of CVD [3]. In animals, experimental ovariectomy (OVX) is a validated model to simulate the postmenopausal state and examine estrogen deficiency-associated cardiovascular

dysfunctions [4]. Endothelial dysfunction, myocardial remodelling, insulin resistance, and systemic oxidative stress are associated with ovariectomy and are similar to clinical findings in postmenopausal women [5]. New nutritional and pharmacological treatments have been investigated to alleviate these negative effects. Vitamin B6 (pyridoxine) is a water-soluble vitamin acting as a coenzyme in the metabolic process of amino acids, synthesis of neurotransmitters, and hemoglobin. It is one of the main regulators of homocysteine metabolism through the transsulfuration pathway, in which homocysteine is converted to cysteine. High homocysteine is an already known independent risk factor of CVD, leading to endothelial dysfunction, vascular inflammation, and oxidative stress [6]. Use of pyridoxine to reduce homocysteine levels and enhance endothelial performance is an encouraging cardio-protective therapeutic option. Furthermore, pyridoxine has been shown to exhibit antioxidant and anti-inflammatory effects [7]. Experimental results indicate that pyridoxine may directly affect the expression of eNOS and bioavailability of nitric oxide (NO), thus maintaining vascular integrity [8]. Despite available evidence, there is a gap in previous studies centered on the effect of pyridoxine on the cardiovascular system in estrogen deficiency. Most of the studies centered on clinical outcomes in metabolic syndrome or hypertension, and placed less focus on postmenopausal cardiovascular dysfunction.

Therefore, this study investigated the potential therapeutic effects of pyridoxine on cardiovascular physiology in OVX rabbits by assessing biochemical markers, histopathological alterations, and molecular changes in NOS3 expression.

EXPERIMENTAL

Animal model design

A total of 18 healthy adult female New Zealand white rabbits (*Oryctolagus cuniculus*, strain: NZW) were obtained from the Animal House, College of Veterinary Medicine, Mustansiriyah University, Baghdad, Iraq) aged 6 – 8 months and weighing 2 – 3 kg. The animals were kept under standard laboratory conditions (22 ± 2 °C, 12 h light/dark cycle, 50 – 60 % humidity) with unlimited access to a standard diet (commercial rabbit feed, Al-Nahrain Feed Co., Baghdad, Iraq) and tap water *ad libitum*. Bedding was made up of sterilized wood shavings (local supplier, Baghdad, Iraq), replaced twice weekly. Rabbits were randomly assigned into three groups (n = 6 per group). Group 1 was the control group

(without treatment), group 2 underwent ovariectomy without treatment, and group 3 was ovariectomized and treated with pyridoxine (Vitamin B6, Sigma-Aldrich, St. Louis, MO, USA) orally at 10 mg/kg/day for 12 weeks.

Ethical approval was obtained from the Animal Care and Use Committee of Mustansiriyah University (Approval No. 47). All procedures involving animals were performed in strict accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes (specifically Annex IV concerning euthanasia methods) [9].

Ovariectomy procedure

Bilateral ovariectomy was performed under general anesthesia induced by intramuscular injection of ketamine (35 mg/kg) combined with xylazine (5 mg/kg). An exploratory midline laparotomy was made, and both ovaries were removed. Postoperative analgesia was provided using meloxicam (0.2 mg/kg) (Meloxidyl®, Boehringer Ingelheim, Ingelheim, Germany) and ciprofloxacin (Baytril®, Bayer AG, Leverkusen, Germany; 5 mg/kg) for three consecutive days to minimize pain and prevent infection [10].

Animals were monitored daily, and humane endpoints were predefined to prevent unnecessary distress. At the end of the 12-week experimental period, all animals were euthanized humanely using an overdose of sodium pentobarbital (Euthasol 120 mg/kg, Virbac, Carros, France) via the intravenous route. There was no death recorded.

Evaluation of parameters/indices

Biochemical analysis

At the end of the study, blood samples (5 mL) were collected from all rabbits via cardiac puncture under anesthesia [11]. The serum was separated by centrifugation at 3,000 rpm for 10 min and stored at -80 °C for further analysis (Table 1).

Histopathological analysis

Heart tissues were washed in saline and preserved in 10 % buffered formalin for 24 h. Tissues were processed, embedded in paraffin and sectioned into 5 µm sections. General morphology was assessed by staining tissue sections with hematoxylin and eosin (H & E). One was stained by a pathologist for microscopic analysis.

Table 1: Biochemical markers assessed

Marker	Kit Name/Source	Methodology
Cardiac Troponin T	MyBioSource, USA	ELISA
Lipocalin-2 (Lipcar)	Abcam, UK	ELISA
Nitric Oxide Synthase	Cayman Chemical, USA	Colorimetric assay
Serum Insulin	Roche Diagnostics, Germany	Chemiluminescent immunoassay
Serum Glucose	Spinreact, Spain	Glucose oxidase assay
Fibrinogen	Abcam, UK	ELISA
HOMA-1 IR	Roche Diagnostics, Germany	Calculated from fasting glucose and insulin
HOMA-2 IR	HOMA Calculator Software	Model-based calculation
Urea	Spinreact, Spain	Urease-GLDH method
Creatinine	Roche Diagnostics, Germany	Jaffe method
Uric Acid	Spinreact, Spain	Uricase-Peroxidase method
Aldosterone Hormone	Cayman Chemical, USA	ELISA

Table 2: Primer sequences used for qPCR

Gene	Primer Type	Sequence (5' → 3')	Product Size (bp)
NOS3	Forward	AGGCTGCTGATGGTGAGTGA	150
	Reverse	GCTCCGCTTCCGATGTTGT	150

Each parameter was scored on a scale of 0 to 3, where 0 indicated no detectable abnormality, 1 signified some alterations (such as the presence of few inflammatory cells or mild disorder), 2 showed mild alterations (examples are focal necrosis, moderate fibrosis), and 3 showed severe alterations (most of the structure has been replaced by necrosis or massive fibrosis). Total parameter scores for each sample were obtained by adding up scores of all the parameters, giving an overall measure of the extent of myocardial damage [12].

Gene expression analysis

Expression of NOS3 (endothelial nitric oxide synthase) was analyzed using quantitative real-time PCR (qPCR) to assess the effects of pyridoxine on cardiovascular physiology in ovariectomized rabbits. Cardiac tissues were taken from the experimental groups, and total RNA was isolated using TRIzol reagent. RNA yield and its quality were estimated by a NanoDrop spectrophotometer. Reverse transcription was done using a cDNA synthesis kit following manufacturer's instructions. PCR Primer design for NOS3 was done using Primer-BLAST, and gene-specific primers for NOS3 were designed. The sequences are presented in Table 2.

The qPCR analysis was performed using SYBR Green Master Mix on a StepOnePlus Real-Time PCR system under standard reaction conditions (initial denaturation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak method), normalizing NOS3 expression [13].

Statistical analysis

Data were analyzed using Statistical Packages for Social Sciences (SPSS v26.0, IBM Corp., Armonk, NY, USA). Results were presented in mean $\pm$ standard deviation (SD), and compared using one-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant. The non-significant results were checked using post hoc power analysis to ensure it was not due to lack of statistical power. To make the most effective use of animals and maintain statistical rigor, the design and analysis were conducted in accordance with the design and statistical methods in studies using animal models of development [13].

RESULTS

Biochemical parameters

Group 2 showed significantly higher serum levels of cardiac troponin T, lipocalin-2, glucose, insulin, fibrinogen, HOMA-1 IR, HOMA-2 IR, urea, creatinine, uric acid, aldosterone and lower NOS activity compared to control ($p < 0.001$). Furthermore, treatment with pyridoxine significantly improved these parameters to baseline ($p < 0.001$) (Table 3).

Histopathological findings

Histology of the control group showed normal myocardial tissue architecture (Figure 1 A). Myocardial tissue of group 1 was significantly altered pathologically (Figure 1 B). Histological examination of group 3 revealed significant improvement in myocardial histoarchitecture compared to group 2 (Figure 1 C).

Table 3: Biochemical parameters across groups (mean $\pm$ SD, n = 6)

Parameter	Group 1	Group 2	Group 3
Cardiac Troponin T (ng/mL)	0.18 $\pm$ 0.03	0.38 $\pm$ 0.05*	0.22 $\pm$ 0.04**
Lipocalin-2 (ng/mL)	95 $\pm$ 8	135 $\pm$ 10*	110 $\pm$ 9**
NOS Activity (U/mL)	32.5 $\pm$ 3.1	15.8 $\pm$ 2.5*	28.4 $\pm$ 2.8**
Glucose (mmol/L)	5.2 $\pm$ 0.3	7.8 $\pm$ 0.5*	6.3 $\pm$ 0.4**
Insulin (μ U/mL)	12.3 $\pm$ 1.5	19.5 $\pm$ 2.0*	14.8 $\pm$ 1.8**
Fibrinogen (g/L)	3.1 $\pm$ 0.2	4.2 $\pm$ 0.3*	3.4 $\pm$ 0.2***
HOMA-1 IR	1.2 $\pm$ 0.2	2.8 $\pm$ 0.4*	1.8 $\pm$ 0.3#
HOMA-2 IR	1.1 $\pm$ 0.2	2.5 $\pm$ 0.3*	1.6 $\pm$ 0.2#
Urea (mmol/L)	5.8 $\pm$ 0.4	8.5 $\pm$ 0.6*	6.9 $\pm$ 0.5***
Creatinine (μ mol/L)	72 $\pm$ 6	90 $\pm$ 8 ^{&}	78 $\pm$ 7***
Uric Acid (μ mol/L)	350 $\pm$ 18	420 $\pm$ 20*	370 $\pm$ 15#
Aldosterone (pg/mL)	150 $\pm$ 12	220 $\pm$ 15*	170 $\pm$ 10#

* $P < 0.001$ compared to group 1, ** $p < 0.001$ compared to group 2, # $p < 0.01$ compared to group 2, *** $p < 0.05$ compared to group 2, & $p < 0.05$ compared to group 1. Group 1 (control), group 2 (underwent ovariectomy without treatment), group 3 (ovariectomized and treated with pyridoxine (Vitamin B6))

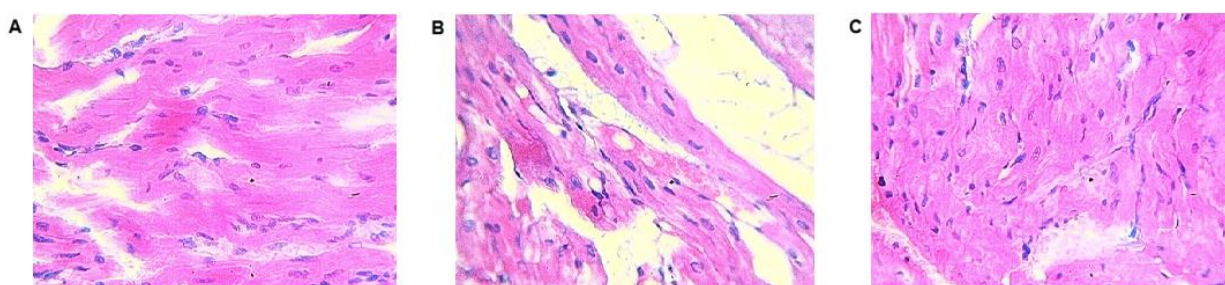


Figure 1: Histoarchitecture of myocardial tissue (H & E stain, 400x). A (Control group): The cardiomyocytes were well organized, with clear striations and intercalated discs, with no pathological modifications (fibrosis, inflammation, and disorganization of cells). B (underwent ovariectomy without treatment): Disarranged myocardial fibers, pervasive interstitial fibrosis and pronounced inflammatory cell infiltration, and presence of cardiomyocyte hypertrophy (structural damage caused by hormonal deficiency). C: (ovariectomized and treated with pyridoxine): The process of fibrosis and inflammatory cell infiltration was significantly diminished and myocardial fibers became more structured. Some of the remaining abnormalities induced in group 2 were also witnessed, but the general tissue pathology was more similar to the control, which proved the benefit of pyridoxine supplementation

Table 5: Relative expression levels of NOS3 in rabbit groups

Group	Mean Relative Expression $\pm$ SD	Fold change	P-value
Control	1.00 $\pm$ 0.15	1.00	Reference
Group 1	0.45 $\pm$ 0.08	0.45	< 0.01
Group 2	1.13 $\pm$ 0.12	2.50	< 0.05

Group 1 (control), group 2 (underwent ovariectomy without treatment), group 3 (ovariectomized and treated with pyridoxine (Vitamin B6))

Gene expression analysis

The NOS3 expression was significantly lower in group 2 compared to control ($p < 0.01$). Treatment with pyridoxine significantly increased the relative expression of NOS3 compared to group 2 ($p < 0.05$) (Table 5).

DISCUSSION

This study showed that ovariectomy in estrogen deficiency raised cardiac troponin T, lipocalin-2 (lipcar), reduced NOS activity, and high cardiac glucose and insulin levels. These findings are in tandem with previous studies confirming that

estrogen deficiency led to higher cardiac troponin T levels, indicative of myocardial damage [14]. Group 2 exhibited high lipocalin-2 compared to control, which is in correlation with other reports, suggesting inflammatory alterations following estrogen deficiency [15]. Also, this study showed that NOS activity was reduced due to estrogen deficiency, which significantly affects the endothelium [16]. These results are in tandem with a previous study revealing that postmenopausal women undergo gradual loss of endothelial-dependent vasodilation, due to a decrease in NOS3 activity and an increase in oxidative stress [17].

These biochemical parameters were improved following pyridoxine administration. Cardiac troponin T levels in the present study were similar to previous studies, which suggested that B-vitamin supplementation reduces oxidative stress and inflammation in cardiovascular diseases [7]. Similar improvements in NO bioavailability following vitamin B6 therapy have been reported in hypertensive and metabolic syndrome models, emphasizing the ability of pyridoxine to directly upregulate NOS3 expression and improve vascular compliance [8]. Group 2 showed a significantly higher level of fibrinogen, which is associated with higher cardiac risk due to low estrogen levels [18], which reduced following pyridoxine administration. Elevated fibrinogen has long been recognized as an independent risk factor for thrombosis and coronary events, especially in postmenopausal women [19]. Therefore, normalization of fibrinogen level following pyridoxine therapy suggested a protective vascular mechanism. Furthermore, group 2 showed insulin resistance, confirming the effect of estrogen deficiency on metabolism. Pyridoxine positively corrected glucose metabolism and insulin resistance by decreasing the inflammatory state and oxidative damage [20]. Similar outcomes have been reported in human trials where B6 supplementation improved glucose homeostasis in overweight patients with metabolic syndrome [6]. As a result, the role of pyridoxine extends beyond cardiovascular protection into metabolic regulation. Pyridoxine has antioxidative effect on renal tissues, which may also be beneficial [21]. Increased serum uric acid concentration in group 1 indicated the presence of oxidative stress. Pyridoxine reduced uric acid levels, with improved antioxidative activity, as shown in previous study [22]. Uric acid is not only a sign of oxidative burden but also a cause of vascular stiffening and endothelial dysfunction [23]. Therefore, dual antioxidative and vasoprotective action is achieved following treatment with pyridoxine by decreasing uric acid. Pyridoxine is also effective in normalizing aldosterone levels, suggesting that it may control hormonal processes disturbed by estrogen deficiency [24]. Since hyperaldosteronism has been linked to myocardial remodeling and hypertension, this result showed a new endocrine control mechanism of pyridoxine [25].

Furthermore, the histoarchitecture of live tissues from group 2 was characterized by fibrosis and inflammatory infiltration. A previous study revealed that the risk of myocardial fibrosis (a symptom of cardiac remodeling) in rats occurred after the ovariectomy procedure [26]. Such

structural change is due to histopathological remodeling of estrogen-deprived myocardium [27]. The histoarchitecture following pyridoxine treatment restored the myocardial alterations induced by ovariectomy. A previous study showed that vitamin B6 reduced inflammatory mediators and increased antioxidants, resulting in the reduction of cardiac fibrosis [28]. The findings from this study are in tandem with earlier studies, which reported that cardiac hypertrophy and collagen deposition were mitigated in experimental heart failure models by pyridoxine supplementation [29]. A decrease in the expression of NOS3 gene was observed in group 1, which was an indication of endothelial dysfunction related to estrogen deficiency [30]. The NOS3 gene expression was restored to levels comparable to those of the control following pyridoxine therapy, suggesting that pyridoxine supplementation may enhance endothelial nitric oxide synthase (eNOS) activity. Similarly, a previous study also reported that vitamin B6 supplementation elevated the activity of NOS3, such that endothelial performance was improved without cardiovascular etiological risks. Also, vitamin B6 was reported to lower homocysteine, which influences endothelial dysfunction and cardiovascular diseases as well [8]. In addition to homocysteine reduction, pyridoxine also regulated epigenetic endothelial genes, suggesting a more widespread genomic activity. These findings suggested that pyridoxine supplementation may enhance endothelial function by restoring NOS3 expression in the estrogen deficiency model, potentially mitigating cardiovascular risks associated with ovariectomy.

CONCLUSION

Pyridoxine (vitamin B6) significantly reduced myocardial injury, improved endothelial function, and reduced myocardial fibrosis and inflammation following ovariectomy.

DECLARATIONS

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Conflicts of interests

The authors declare that they have no conflicts of interest.

Consent to Participate

Not applicable.

Ethical approval

It is stated in the method.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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