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Original Article

Effects of vitamin C on cardiovascular oxidative stress induced by hypothyroidism in neonatal and juvenile rats

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Abstract

Background and aims: Hypothyroidism and cardiovascular diseases are associated with oxidative stress. Vitamin C is widely recognized for its antioxidant properties. This study aimed to investigate the effects of vitamin C during the neonatal and juvenile periods on oxidative stress induced by hypothyroidism in the hearts of rats.

Methods: Sixteen pregnant rats were randomly divided into five groups after delivery: a control group, a group receiving propylthiouracil (PTU), and three treatment groups receiving PTU along with 10, 100, and 500 mg/kg of vitamin C. The experimental treatments continued for the first eight weeks of the offspring's life, following the lactation period, and the offspring were treated in the same manner. At the end of the study, the rats were sacrificed, and serum, aortic, and cardiac tissues were collected for antioxidant evaluation.

Results: In the group receiving PTU, levels of thyroxine and antioxidant enzymes were decreased in serum, heart, and aortic tissues. In contrast, the groups receiving PTU along with various doses of vitamin C demonstrated an attenuation of these parameters.

Conclusion: Based on the findings, vitamin C supplementation improved cardiovascular oxidative stress induced by hypothyroidism in rats.

Keywords: Vitamin C, Propylthiouracil, Hypothyroidism, Cardiovascular, Oxidative stress

Introduction

The production of free radicals is typically balanced by the antioxidants present in a healthy individual (1). An imbalance may occur due to decreased activity of antioxidant systems, leading to elevated levels of free radicals, including reactive oxygen species (ROS) (2). These free radicals can damage biological components, including the elements of the cell plasma membrane (1). Several antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), are well-known for their ability to protect tissues from damage induced by free radicals. Additionally, vitamins such as vitamin C and Vitamin E play a significant role in protecting the body from oxidative stress (1).

Results from both human and animal studies suggest that oxidative stress occurs in both hyperthyroid and hypothyroid conditions (3). Furthermore, an association has been reported between hypothyroidism and the prevalence and mortality of cardiovascular diseases, which may be attributed to an increase in vascular resistance (4-6). A defect in lipid metabolism has also been observed in hypothyroidism, often accompanied by atherosclerosis and, ultimately, cardiovascular dysfunction (7). Additionally, coronary artery disease has been linked to hypothyroidism (8).

Thyroid hormones are known to be key regulators of cellular respiratory capacity (9) and are essential for normal metabolism in mammals (10,11). Low levels of thyroid hormones can lead to defects in mitochondrial function and energy production (9), which may contribute to the cardiovascular complications associated with hypothyroidism. Given these findings, the administration of antioxidants, including vitamins, is suggested to have beneficial effects against oxidative stress induced by

hypothyroidism (12).

Vitamin C, a water-soluble vitamin, is known to act as a powerful antioxidant in extracellular fluids and plasma. To counteract ROS, vitamin C can donate two electrons and oxidize itself to dehydroascorbic acid (13). It has also been reported that vitamin C can be transported into cells, where it collaborates with glutathione as part of the intracellular antioxidant defense system (13). In addition to directly scavenging free radicals, vitamin C exerts its antioxidant effects by enhancing other antioxidant systems (14). Furthermore, vitamin C attenuates the activity of NADPH oxidase, a significant source of ROS (15). It protects cell membranes against lipid peroxidation by preventing the production of free radicals in the aqueous phase (16). Clinical research has demonstrated that by reducing ROS levels, vitamin C can protect the cardiovascular system, improve cardiac and endothelial function, and help prevent coronary artery disease (17). It has also been reported that vitamin C protects vascular endothelium from oxidative stress damage induced by homocysteine (18). Thus, we designed this study to investigate the effects of vitamin C during the neonatal and juvenile periods on oxidative stress induced by hypothyroidism in the aorta and heart of rats.

Materials and Methods

Animals and treatments

Sixteen pregnant Wistar rats (240 ± 10 g) were used in the present study. The rats were maintained under standard conditions in accordance with established guidelines. After delivery, the animals were randomly divided into five groups (n = 12 in each group) as follows:

- Control group: Rats received drinking water.
- PTU group: Rats received water containing 0.005% PTU to induce hypothyroidism (19).
- Treatment Groups: Rats received different doses of vitamin C (10, 100, and 500 mg/kg) in addition to PTU (20).

Mothers received the treatments starting from the first day after delivery and continued through the lactation period. Subsequently, the offspring received the treatments in their drinking water for the first 8 weeks of their lives (20,21). Seven male offspring from each group were randomly selected for the study. After deep anesthesia using ketamine and xylazine, blood samples were collected from the apex of the heart following thoracotomy, after which the rats were sacrificed by decapitation with a guillotine. The heart and aorta were then collected for antioxidant tests.

Chemicals and drugs

Propylthiouracil (PTU) was obtained from Sigma Chemical Company (USA). All chemicals used for the biochemical analyses were sourced from Merck (Germany) and were of analytical grade.

Determination of thyroxin

The total serum thyroxine level was measured using a radioimmunoassay (Daisource, T4-RIA-CT) to confirm the diagnosis of hypothyroidism.

Determination of total thiol concentration

Total thiol levels were measured using DTNB (2,2'-dinitro-5,5'-dithiodibenzoic acid), a compound that interacts with sulfhydryl (SH) groups to form a yellow-colored complex with a peak absorbance at 412 nm (22). The total thiol concentration (mM) was calculated using an equation previously described in the literature (23,24).

Determination of malondialdehyde level

Malondialdehyde (MDA) was measured using thiobarbituric acid (TBA) according to the method described by Mihara and Uchiyama (25). The reaction between MDA and TBA produces a red-colored complex that exhibits a peak absorbance at 535 nm. The concentration of MDA was then calculated using the following formula (26,27):

$$C\ (m) = \text{Absorbance} / (1.65 \times 10^5)$$

Determination of catalase activity

CAT activity was estimated using the method described by Aebi (28). This assay is designed to determine the rate constant (k, with units of s^{-1}) for the decomposition of hydrogen peroxide. The enzyme's rate constant was calculated by measuring the decrease in absorbance at 240 nm/min. The activities were expressed as the rate constant (k) per liter (29).

Determination of superoxide dismutase activity

Madesh and Balasubramanian described a procedure for measuring SOD activity (30). This colorimetric assay involves the generation of superoxide through the auto-oxidation of pyrogallol, along with the inhibition of the superoxide-dependent reduction of the tetrazolium dye MTT (3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide) to its formazan product, which is measured at 570 nm. One unit of SOD activity is defined as the amount of enzyme that causes a 50% inhibition in the MTT reduction rate (31).

Statistical analysis

All data are expressed as means ± SEM. The data were assessed for normality using the Kolmogorov–Smirnov test. Comparisons were made using one-way ANOVA with Tukey's post hoc test (for normally distributed data) or the Kruskal-Wallis test (for non-normally distributed data). Differences were considered statistically significant when $P < 0.05$.

Results

Effect of vitamin C on thyroxin level

Hypothyroidism was confirmed by measuring thyroxine levels. The serum thyroxine concentration was significantly lower in PTU-treated animals compared to

control animals ($P<0.01$; Figure 1). Treatment with doses of vitamin C at 100 mg/kg and 500 mg/kg significantly mitigated the PTU-induced reduction in serum thyroxine levels ($P<0.05$; Figure 1).

Effect of vitamin C on MDA level

MDA levels were significantly increased in the aorta ($P<0.001$; Figure 2A), heart ($P<0.001$; Figure 2B), and serum ($P<0.01$; Figure 2C) of animals treated with PTU compared to the control group. Animals receiving all doses of vitamin C exhibited a decrease in MDA levels in the aorta ($P<0.001$; Figure 2A) and heart ($P<0.05$ – $P<0.01$; Figure 2B) compared to the PTU group. Additionally, the group treated with 500 mg/kg of vitamin C showed a significant reduction in serum MDA levels compared to the PTU group ($P<0.05$; Figure 2C).

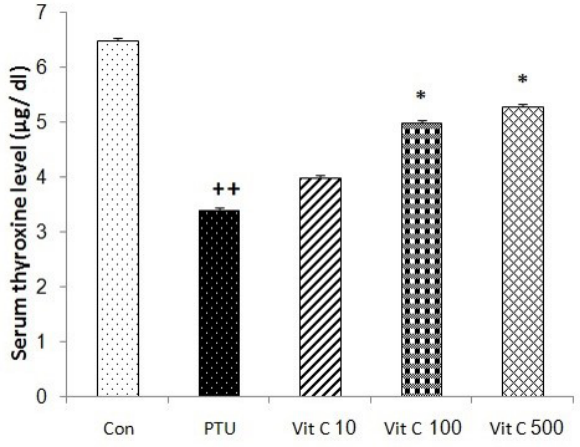

Figure 1. Serum thyroxine levels in the control (Con), propylthiouracil (PTU), and propylthiouracil + vitamin C (10, 100, and 500 mg/kg) groups (Vit C 10, 100, 500). Data are presented as mean ± SEM for 7 animals per group. ++ $P<0.01$ compared to the control group; * $P<0.05$ compared to the PTU group

Effect of vitamin C on total thiol concentration

Total thiol concentration significantly decreased in the aorta ($P<0.01$; Figure 3A), heart ($P<0.01$; Figure 3B), and serum ($P<0.001$; Figure 3C) of animals that received PTU compared to those in the control group. Treatment with all doses of vitamin C significantly improved total thiol concentration in the aorta ($P<0.05$; Figure 3A), heart ($P<0.05$; Figure 3B), and serum ($P<0.05$ to $P<0.01$; Figure 3C) compared to the PTU group.

Effect of vitamin C on CAT activity

CAT activity was significantly decreased in the aorta ($P<0.001$; Figure 4A), heart ($P<0.001$; Figure 4B), and serum ($P<0.001$; Figure 4C) of animals treated with PTU compared to those in the control group. Animals treated with all doses of vitamin C demonstrated a significant improvement in total CAT activity in the aorta ($P<0.01$ – $P<0.001$; Figure 4A) and heart ($P<0.01$ – $P<0.001$; Figure 4B) compared to the PTU group. Additionally, animals treated with 100 and 500 mg/kg of vitamin C exhibited an increase in serum CAT activity compared to the PTU group ($P<0.05$ – $P<0.01$; Figure 4C).

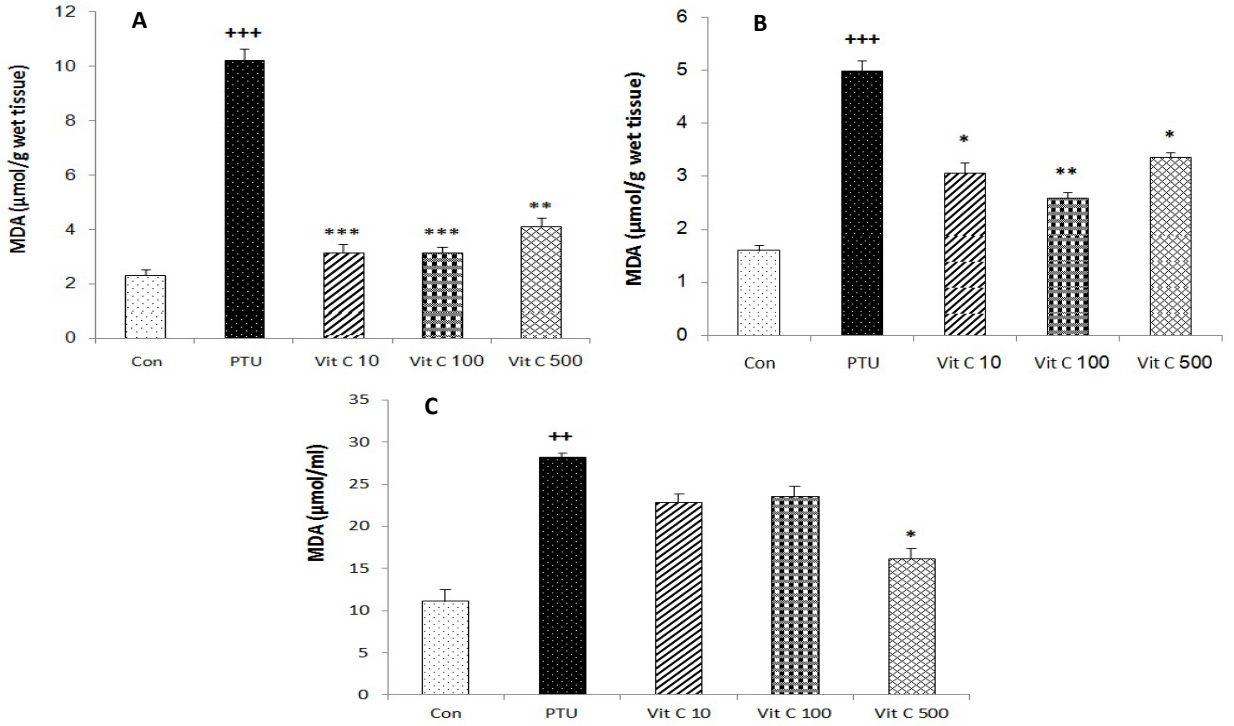

Figure 2. Malondialdehyde (MDA) levels in aortic (A), heart (B) tissues, and serum (C) of control (Con), propylthiouracil (PTU), and propylthiouracil + vitamin C at doses of 10, 100, and 500 mg/kg (Vit C 10, 100, 500) groups. Data are presented as mean ± SEM for 7 animals per group. Statistical significance is indicated as follows: ++ $P<0.01$, +++ $P<0.001$ compared to the control group; * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to the PTU group

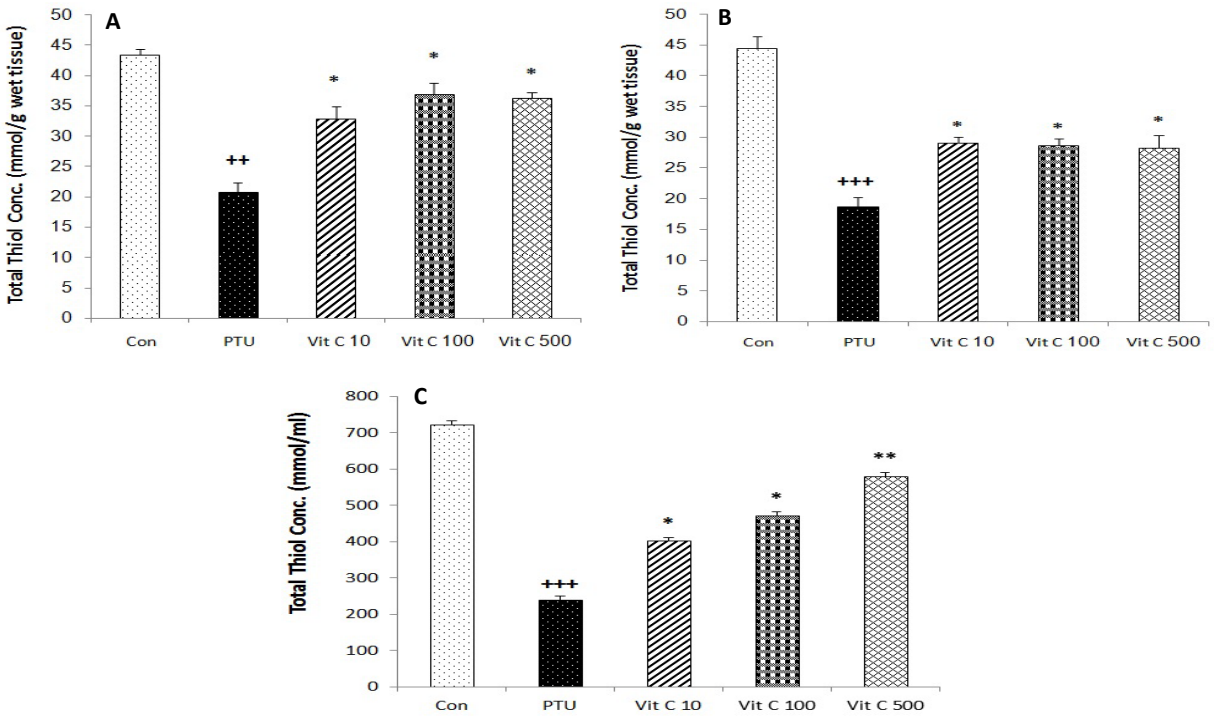

Figure 3. Total thiol concentrations in aortic (A), heart (B) tissues, and serum (C) of control (Con), propylthiouracil (PTU), and propylthiouracil + vitamin C at doses of 10 mg/kg, 100 mg/kg, and 500 mg/kg (Vit C 10, 100, 500) groups. Data are presented as mean ± SEM for 7 animals per group. Statistical significance is indicated as follows: ++ $P<0.01$, +++ $P<0.001$ compared to the control group; * $P<0.05$, ** $P<0.01$ compared to the PTU group

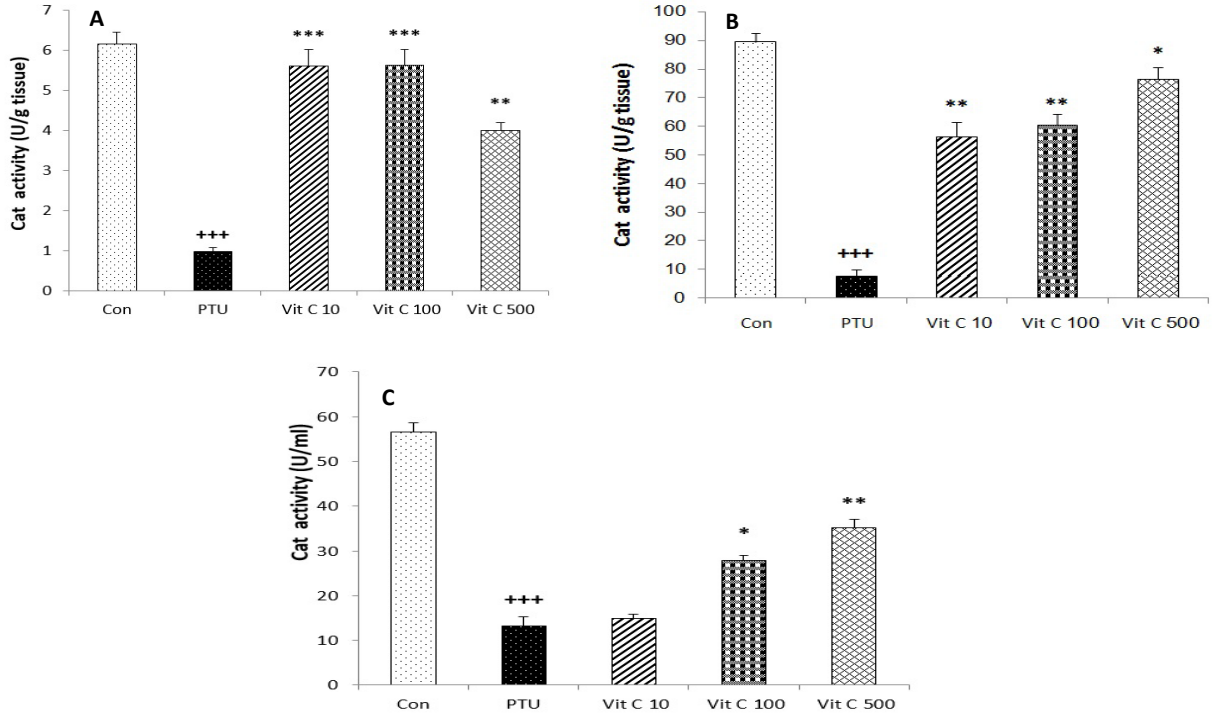

Figure 4. Catalase activity in aortic (A), heart (B) tissues, and serum (C) of control (Con), propylthiouracil (PTU), and propylthiouracil + vitamin C (10, 100, 500 mg/kg) groups (Vit C 10, 100, 500). Data are presented as mean ± SEM for 7 animals per group. Statistical significance is indicated as follows: +++ $P<0.001$ vs. control group; * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs. PTU group

Effect of vitamin C on SOD activity

SOD activity was significantly decreased in the aorta ($P<0.01$; Figure 5A), heart ($P<0.01$; Figure 5B), and serum ($P<0.05$; Figure 5C) of animals that received PTU compared to those in the control group. Animals treated with 100 and 500 mg/kg of vitamin C exhibited a significant increase in SOD activity in the aorta ($P<0.05$; Figure 5A) and heart ($P<0.05$; Figure 5B) compared to the PTU group. Furthermore, animals treated with 500 mg/kg of vitamin C showed a significant increase in

SOD activity in the serum compared to the PTU group ($P < 0.05$; Figure 5C).

Discussion

Recent studies have frequently reported the role of oxidative imbalance in cardiovascular damage induced by hypothyroidism. At the cellular level, hypothyroidism can lead to DNA damage, mitochondrial dysfunction, and the generation of ROS. These effects contribute to myocardial remodeling, cardiac failure, hypercholesterolemia, increased mean arterial pressure, and impaired vascular endothelium (32,33).

The findings of this study clearly indicate that PTU-induced hypothyroidism resulted in a significant increase in MDA levels, along with a notable decrease in total thiol concentration and the activities of SOD and CAT in the serum, aorta, and heart of the animals. These results are consistent with previous research demonstrating oxidative damage in the cardiovascular system due to hypothyroidism. A clinical study by Baskol et al found that hypothyroidism was associated with elevated serum MDA and nitric oxide levels, as well as reduced antioxidant enzymatic activity (34). Similarly, another clinical study showed that hypothyroidism was linked to oxidative stress, potentially mediated by insufficient antioxidant capacity and impaired lipid metabolism (35). Our prior research also indicated that hypothyroidism was associated with lipid peroxidation in the hippocampus during the neonatal and juvenile growth periods in rats (36). Additionally, increased serum MDA levels in hypothyroid patients have been reported in several clinical studies (34,37).

The results of this study demonstrated that chronic administration of vitamin C, an antioxidant compound, significantly reduced MDA levels. All three doses—10, 100, and 500 mg/kg of vitamin C—were effective in both heart and aorta tissues; however, only the 500 mg/kg dose was able to attenuate MDA levels in the serum. MDA is recognized as an indicator of lipid peroxidation and has been shown to be mutagenic and cytotoxic to cells (38,39). Based on these findings, it appears that vitamin C can effectively decrease lipid peroxidation in the cardiovascular system under hypothyroid conditions.

In our study, vitamin C was found to increase total thiol content in the serum, heart, and aorta of hypothyroid rats. The most significant effect on total thiol content in serum was observed at the 500 mg/kg dose of vitamin C. Total thiol groups are highly susceptible to oxidation and are considered one of the most important antioxidants in the body (40). Among all the antioxidants present in the body, thiols represent a major portion of the total antioxidant capacity and play a crucial role in defending against ROS (41).

Our results indicated that vitamin C significantly increased the antioxidant enzymatic activity of CAT and SOD in the serum, heart, and aorta of hypothyroid rats. The most pronounced effect of vitamin C on CAT activity was observed at doses of 10 mg/kg and 100 mg/kg in aorta tissue, and at a dose of 500 mg/kg in heart tissue and serum. Additionally, the greatest increases in SOD activity were noted at the 500 mg/kg dose in aorta tissue and serum, as well as at doses of 100 mg/kg and 500 mg/kg in heart tissue.

Given that studies have demonstrated that increases in

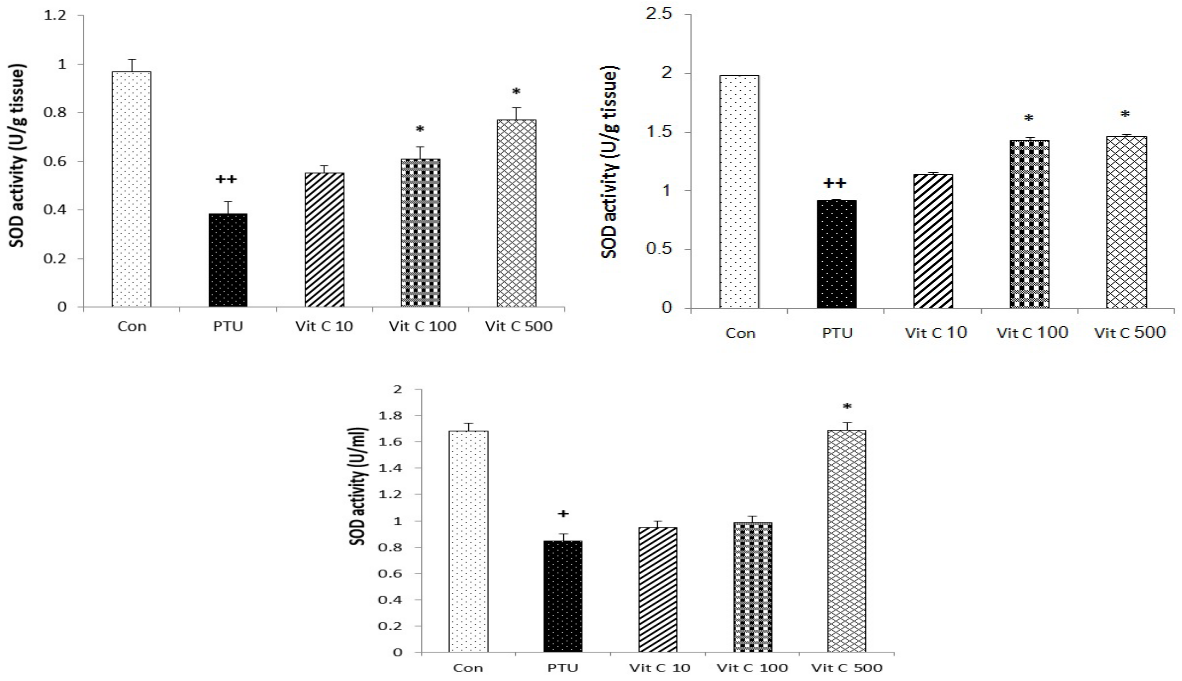

Figure 5. SOD activity in aortic (A), heart (B) tissues, and serum (C) from control (Con), propylthiouracil (PTU), and propylthiouracil + vitamin C at doses of 10 mg/kg, 100 mg/kg, and 500 mg/kg (Vit C 10, 100, 500) groups. Data are presented as mean ± SEM for 7 animals per group. Statistical significance is indicated as follows: + $P < 0.05$, + + $P < 0.01$ compared to the control group; * $P < 0.05$ compared to the PTU group

SOD and CAT activity, as well as total thiol concentration, alongside decreases in MDA concentration, confer protective effects on the cardiovascular system (42,43), it can be concluded that the different doses of vitamin C used in our study, which improved redox status, may also have a protective effect on the cardiovascular system.

Conclusion

In conclusion, the findings of the present experiment highlight the beneficial effects of vitamin C supplementation during the neonatal and juvenile periods in mitigating cardiovascular oxidative damage induced by hypothyroidism in a rat model.

Authors' Contribution

Conceptualization: Farimah Beheshti, Mahmoud Hosseini, Saeed Niazmand.
Data curation: Mahmoud Hosseini, Saeed Niazmand.
Formal analysis: Farimah Beheshti, Maryam Paseban.
Funding acquisition: Mahmoud Hosseini, Saeed Niazmand.
Investigation: Masomeh Mirzaei, Maryam Paseban.
Methodology: Mahdiyeh Hedayati-Moghadam, Maryam Paseban.
Project administration: Mahmoud Hosseini, Saeed Niazmand.
Resources: Mahmoud Hosseini, Maryam Paseban.
Software: Farimah Beheshti, Maryam Paseban.
Supervision: Mahmoud Hosseini, Saeed Niazmand.
Validation: Farimah Beheshti, Maryam Paseban.
Visualization: Reza Mohebbati, Farimah Beheshti, Masomeh Mirzaei.
Writing-original draft: Reza Mohebbati, Maryam Paseban.
Writing-review & editing: Reza Mohebbati.

Competing Interests

The authors declare that there are no conflicts of interest.

Ethical Approval

Animal handling and all related procedures were conducted in accordance with the guidelines approved by the Mashhad University of Medical Sciences Ethical Committee (Code: IR.MUMS.fm.REC.1396.206).

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References

1. Halliwell B, Gutteridge JM. Free Radicals in Biology and Medicine. USA: Oxford University Press; 2015.
2. Grek CL, Tew KD. Redox metabolism and malignancy. Curr Opin Pharmacol. 2010;10(4):362-8. doi: 10.1016/j.coph.2010.05.003.
3. Resch U, Helsel G, Tatzber F, Sinzinger H. Antioxidant status in thyroid dysfunction. Clin Chem Lab Med. 2002;40(11):1132-4. doi: 10.1515/cclm.2002.198.
4. Razvi S, Shakoor A, Vanderpump M, Weaver JU, Pearce SH. The influence of age on the relationship between subclinical hypothyroidism and ischemic heart disease: a metaanalysis. J Clin Endocrinol Metab. 2008;93(8):2998-3007. doi: 10.1210/jc.2008-0167.
5. Klein I, Danzi S. Thyroid disease and the heart. Circulation. 2007;116(15):1725-35. doi: 10.1161/circulationaha.106.678326.
6. Klein I, Danzi S. Thyroid hormone treatment to mend a broken heart. J Clin Endocrinol Metab. 2008;93(4):1172-4. doi: 10.1210/jc.2008-0291.
7. Duggal J, Singh S, Barsano CP, Arora R. Cardiovascular risk with subclinical hyperthyroidism and hypothyroidism: pathophysiology and management. J Cardiometab Syndr. 2007;2(3):198-206. doi: 10.1111/j.1559-4564.2007.06583.x.
8. Auer J, Berent R, Weber T, Lassnig E, Eber B. Thyroid function is associated with presence and severity of coronary atherosclerosis. Clin Cardiol. 2003;26(12):569-73. doi: 10.1002/clc.4960261205.
9. Wrutniak-Cabello C, Casas F, Cabello G. Thyroid hormone action in mitochondria. J Mol Endocrinol. 2001;26(1):67-77. doi: 10.1677/jme.0.0260067.
10. Sarkar PK. In quest of thyroid hormone function in mature mammalian brain. Indian J Exp Biol. 2002;40(8):865-73.
11. Pacheco-Rosado J, Alva-Sánchez C. Thyroid hormone and their possible role as neuron survival factors in the adult rat brain. New Perspectives on Brain Cell Damage, Neurodegeneration and Neuroprotective Strategies, Research Signpost, India. 2007. p. 75-93.
12. Hedayati M, Niazmand S, Hosseini M, Baghcheghi Y, Beheshti F, Niazmand S. Vitamin E improved redox homeostasis in heart and aorta of hypothyroid rats. Endocr Regul. 2017;51(4):205-12. doi: 10.1515/enr-2017-0021.
13. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, et al. Vitamin C as an antioxidant: evaluation of its role in disease prevention. J Am Coll Nutr. 2003;22(1):18-35. doi: 10.1080/07315724.2003.10719272.
14. Robichová S, Slamenová D, Chalupa I, Sebová L. DNA lesions and cytogenetic changes induced by N-nitrosomorpholine in HepG2, V79 and VH10 cells: the protective effects of vitamins A, C and E. Mutat Res. 2004;560(2):91-9. doi: 10.1016/j.mrgentox.2004.01.011.
15. Ulker S, McKeown PP, Bayraktutan U. Vitamins reverse endothelial dysfunction through regulation of eNOS and NAD(P)H oxidase activities. Hypertension. 2003;41(3):534-9. doi: 10.1161/01.hyp.0000057421.28533.37.
16. Antunes LM, Darin JD, Bianchi MD. Protective effects of vitamin C against cisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: a dose-dependent study. Pharmacol Res. 2000;41(4):405-11. doi: 10.1006/phrs.1999.0600.
17. Zhou H, Ma JH, Zhang PH, Luo AT. Vitamin C pretreatment attenuates hypoxia-induced disturbance of sodium currents in guinea pig ventricular myocytes. J Membr Biol. 2006;211(2):81-7. doi: 10.1007/s00232-005-7014-8.
18. On YK, Kim HS, Kim SY, Chae IH, Oh BH, Lee MM, et al. Vitamin C prevents radiation-induced endothelium-dependent vasomotor dysfunction and de-endothelialization by inhibiting oxidative damage in the rat. Clin Exp Pharmacol Physiol. 2001;28(10):816-21. doi: 10.1046/j.1440-1681.2001.03528.x.
19. Hosseini M, Hadjzadeh MA, Derakhshan M, Havakhah S, Behnam Rassouli F, Rakhshandeh H, et al. The beneficial effects of olibanum on memory deficit induced by hypothyroidism in adult rats tested in Morris water maze. Arch Pharm Res. 2010;33(3):463-8. doi: 10.1007/s12272-010-0317-z.
20. Beheshti F, Karimi S, Vafaee F, Shafei MN, Sadeghnia HR, Hadjzadeh MA, et al. The effects of vitamin C on hypothyroidism-associated learning and memory impairment in juvenile rats. Metab Brain Dis. 2017;32(3):703-15. doi: 10.1007/s11011-017-9954-y.
21. Esmaeilizadeh M, Hosseini M, Beheshti F, Alikhani V, Keshavarzi Z, Shoja M, et al. Vitamin C improves liver and renal functions in hypothyroid rats by reducing tissue oxidative injury. Int J Vitam Nutr Res. 2020;90(1-2):84-94. doi: 10.1024/0300-9831/a000495.
22. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-7. doi: 10.1016/0003-9861(59)90090-6.
23. Khodabandehloo F, Hosseini M, Rajaei Z, Soukhtanloo M, Farrokhi E, Rezaeipour M. Brain tissue oxidative damage as a possible mechanism for the deleterious effect of a chronic high dose of estradiol on learning and memory in

ovariectomized rats. Arq Neuropsiquiatr. 2013;71(5):313-9. doi: 10.1590/0004-282x20130027.
24. Mohebbati R, Jalili-Nik M, Paseban M, Shafei MN, Khajavi Rad A. Effects of *Zataria multiflora* extract and carvacrol on doxorubicin-induced oxidative stress in rat brain. Pharm Sci. 2018;24(3):187-92. doi: 10.15171/ps.2018.27.
25. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem. 1978;86(1):271-8. doi: 10.1016/0003-2697(78)90342-1.
26. Hosseini M, Pourganji M, Khodabandehloo F, Soukhtanloo M, Zabihi H. Protective effect of L-arginine against oxidative damage as a possible mechanism of its beneficial properties on spatial learning in ovariectomized rats. Basic Clin Neurosci. 2012;3(5):36-44.
27. Mohebbati R, Paseban M, Beheshti F, Soukhtanloo M, Shafei MN, Rakhshandeh H, et al. The preventive effects of standardized extract of *Zataria multiflora* and carvacrol on acetaminophen-induced hepatotoxicity in rat: - *Zataria multiflora* and carvacrol and hepatotoxicity. J Pharmacopuncture. 2018;21(4):249-57. doi: 10.3831/kpi.2018.21.028.
28. Aebi H. Catalase. In: Bergmeyer HU, ed. Methods of Enzymatic Analysis. New York: Academic Press; 1983. p. 276-86.
29. Paseban M, Mohebbati R, Niazmand S, Sathyapalan T, Sahebkar A. Comparison of the neuroprotective effects of aspirin, atorvastatin, captopril and metformin in diabetes mellitus. Biomolecules. 2019;9(4):118. doi: 10.3390/biom9040118.
30. Madesh M, Balasubramanian KA. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian J Biochem Biophys. 1998;35(3):184-8.
31. Mohebbati R, Paseban M, Soukhtanloo M, Jalili-Nik M, Shafei MN, Jahani Yazdi A, et al. Effects of standardized *Zataria multiflora* extract and its major ingredient, carvacrol, on adriamycin-induced hepatotoxicity in rat. Biomed J. 2018;41(6):340-7. doi: 10.1016/j.bj.2018.10.008.
32. Mishra P, Samanta L. Oxidative stress and heart failure in altered thyroid states. ScientificWorldJournal. 2012;2012:741861. doi: 10.1100/2012/741861.
33. Moulakakis KG, Poulakou MV, Dosios T, Dontas I, Sokolis DP, Vlachos IS, et al. Hypothyroidism and the aorta. evidence of increased oxidative DNA damage to the aorta of hypothyroid rats. In Vivo. 2008;22(5):603-8.
34. Baskol G, Atmaca H, Tanriverdi F, Baskol M, Kocer D, Bayram F. Oxidative stress and enzymatic antioxidant status in patients with hypothyroidism before and after treatment. Exp Clin Endocrinol Diabetes. 2007;115(8):522-6. doi: 10.1055/s-2007-981457.
35. Torun AN, Kulaksizoglu S, Kulaksizoglu M, Pamuk BO, Isbilen E, Tutuncu NB. Serum total antioxidant status and lipid peroxidation marker malondialdehyde levels in overt and subclinical hypothyroidism. Clin Endocrinol (Oxf). 2009;70(3):469-74. doi: 10.1111/j.1365-2265.2008.03348.x.
36. Beheshti F, Hosseini M, Shafei MN, Soukhtanloo M, Ghasemi S, Vafaee F, et al. The effects of *Nigella sativa* extract on hypothyroidism-associated learning and memory impairment during neonatal and juvenile growth in rats. Nutr Neurosci. 2017;20(1):49-59. doi: 10.1179/1476830514y.0000000144.
37. Santi A, Duarte MM, de Menezes CC, Loro VL. Association of lipids with oxidative stress biomarkers in subclinical hypothyroidism. Int J Endocrinol. 2012;2012:856359. doi: 10.1155/2012/856359.
38. Mateos R, Lecumberri E, Ramos S, Goya L, Bravo L. Determination of malondialdehyde (MDA) by high-performance liquid chromatography in serum and liver as a biomarker for oxidative stress. Application to a rat model for hypercholesterolemia and evaluation of the effect of diets rich in phenolic antioxidants from fruits. J Chromatogr B Analyt Technol Biomed Life Sci. 2005;827(1):76-82. doi: 10.1016/j.jchromb.2005.06.035.
39. Bouderbala S, Lamri-Senhadji M, Prost J, Lacaille-Dubois MA, Bouchenak M. Changes in antioxidant defense status in hypercholesterolemic rats treated with *Ajuga iva*. Phytomedicine. 2008;15(6-7):453-61. doi: 10.1016/j.phymed.2007.10.001.
40. Patel BP, Rawal UM, Dave TK, Rawal RM, Shukla SN, Shah PM, et al. Lipid peroxidation, total antioxidant status, and total thiol levels predict overall survival in patients with oral squamous cell carcinoma. Integr Cancer Ther. 2007;6(4):365-72. doi: 10.1177/1534735407309760.
41. Prakash M, Shetty MS, Tilak P, Anwar N. Total thiols: biomedical importance and their alteration in various disorders. Online J Health Allied Sci. 2009;8(2):2.
42. Bonomini F, Tengattini S, Fabiano A, Bianchi R, Rezzani R. Atherosclerosis and oxidative stress. Histol Histopathol. 2008;23(3):381-90. doi: 10.14670/hh-23.381.
43. Fukai T, Ushio-Fukai M. Superoxide dismutases: role in redox signaling, vascular function, and diseases. Antioxid Redox Signal. 2011;15(6):1583-606. doi: 10.1089/ars.2011.3999.

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