DOI 10.26724/2079-8334-2026-1-95-203-207
UDC 577.1:661.29:577.112.3:599.323.4

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EFFECT OF HYDROGEN SULFIDE AND CYSTEINE ON AORTIC TONE IN RATS WITH DIFFERENT THYROID STATUS

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Impaired thyroid function is recognized as a major contributor to endothelial dysfunction and cardiovascular disease. This study examined the vascular effects of sulfur-containing compounds in rats with experimentally induced hypothyroidism and hyperthyroidism. Thoracic aortic segments were analyzed to assess contractile responses to cysteine and hydrogen sulfide. The results demonstrated that hypothyroidism significantly reduced vascular relaxation, as shown by a rightward shift of dose-response curves and increased effective concentrations of the test substances. In contrast, hyperthyroidism did not produce notable changes in vascular tone. These findings indicate that thyroid hormone deficiency leads to pronounced endothelial dysfunction manifested as diminished sensitivity of the vascular wall to vasodilators. The study expands understanding of molecular mechanisms regulating vascular tone under endocrine disorders and suggests new therapeutic targets for cardiovascular conditions associated with thyroid dysfunction.

Key words: hyperthyroidism, hypothyroidism, hydrogen sulfide, cysteine, vascular tone, aorta.

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ВПЛИВ ГІДРОГЕН СУЛЬФІДУ ТА ЦИСТЕЇНУ НА ТОНУС АОРТИ ЩУРІВ З РІЗНИМ СТАТУСОМ ЩИТОПОДІБНОЇ ЗАЛОЗИ

Порушення функціонального стану щитоподібної залози є важливим чинником ризику розвитку ендотеліальної дисфункції та серцево-судинних захворювань. У дослідженні оцінено вплив сірковмісних сполук, зокрема цистеїну та гідрогенсульфіду, на скоротливу активність грудної аорти щурів із експериментально індукованим гіпо- та гіпертиреозом. Результати показали, що дефіцит тиреоїдних гормонів значно знижує здатність судинної стінки до релаксації у відповідь на зазначені сполуки, що підтверджувалося зміщенням кривих «доза-ефект» та підвищенням середньоефективних концентрацій. Натомість гіпертиреоїдний стан не спричинив суттєвих змін у тонусі аорти. Виявлені закономірності свідчать про формування вираженої ендотеліальної дисфункції при гіпотиреозі та розширюють уявлення про молекулярні механізми регуляції судинного тонусу в умовах ендокринних порушень. Отримані дані відкривають перспективи для пошуку нових терапевтичних мішеней у корекції серцево-судинних патологій, пов'язаних із дисфункцією щитоподібної залози.

Ключові слова: гіпертиреоз, гіпотиреоз, гідроген сульфід, цистеїн, судинний тонус, аорта.

Funding. *The work is a fragment of the research project "The role of the H_2S signaling system in modulating the biological activity and toxicity of drugs and biologically active substances", state registration No. 0124U002903.*

Cardiovascular diseases remain one of the main causes of mortality worldwide, affecting over 10 % of the adult population [2]. Disorders of thyroid status are important risk factors for cardiovascular diseases. The prevalence of hypothyroidism in the general population is high and shows a rising trend; in Ukraine, the incidence rate was 22.1 per 100,000 population in 2011, and in 2012, it was diagnosed in 90,884 individuals [4]. The highest incidence of hypothyroidism is observed in older individuals (> 60 years of age), and congenital hypothyroidism affects one in 3000–4000 newborns. It is known that thyroid hormones play a key role in regulating cardiac function and cardiovascular hemodynamics, as thyroid hormone receptors are present in both the myocardium and blood vessels, and changes in their concentrations adversely affect the cardiovascular system [1]. Without appropriate therapeutic intervention, hyperthyroidism or hypothyroidism, both clinical and subclinical, may contribute to the progression of cardiovascular disease. Even moderate changes in thyroid hormone levels increase cardiovascular mortality by 20–80 % [10]. It has been shown that reduced thyroid hormone levels have a serious impact on the cardiovascular system, mediated through a number of mechanisms, including dyslipidemia, hypertension, systolic and diastolic myocardial dysfunction, as well as endothelial dysfunction.

In recent years, scientific attention has focused on investigating the physiological functions of hydrogen sulfide and cysteine. These sulfur-containing compounds are known to play an important role in regulating vascular tone and myocardial contractility [11]. At the same time, the question of whether variations in thyroid hormone

levels affect the ability of cysteine and hydrogen sulfide (H_2S) to induce vascular relaxation remains unexplored.

The purpose of the study was to assess the impact of sulfur-containing compounds (H_2S and cysteine) on the contractility of aortic ring segments in control group animals and under the conditions of various thyroid hormone levels (hypothyroidism and hyperthyroidism) in an in vitro study.

Materials and methods. Experimental research, biomaterial processing, and data analysis were conducted at the Scientific Research Clinical Diagnostic Laboratory of National Pirogov Memorial Medical University, Vinnytsya (Accreditation No. 114/21, valid from 03.09.2021 to 02.09.2026).

The experimental tests were performed on 15 sexually mature male outbred white rats (Rattus norvegicus) with a baseline body weight of 160–220 g. All animals were bred in the vivarium of National Pirogov Memorial Medical University, Vinnytsya, and housed under standard laboratory conditions (temperature 21±2 °C, humidity 55–60 %, 12–hour light/dark cycle) with free access to water and food. Before the experiment, all animals underwent a 7-day adaptation period. Euthanasia was performed by inhalation of an ether overdose. Animals were housed in specialized plastic cages (500×320×160 mm) equipped with galvanized wire mesh lids. Pine wood shavings were used as bedding. The animals were maintained on a standard compound feed (pelleted diet) with free access to cooled boiled water ad libitum. The experimental part of the study was conducted from late May to June 2024. Drug administration began on 31 May 2024.

Male animals were selected for this study to mitigate confounding variability in vascular reactivity caused by cyclic estrogen fluctuations in females. It is well established that estrogens modulate nitric oxide synthesis, potassium channel expression, and vascular responsiveness to vasoactive agents, and interact with gasotransmitter systems, notably hydrogen sulfide. Additionally, sex hormones can influence the thyroid-mediated regulation of the cardiovascular system.

Five animals were assigned to each experimental group. This cohort size was selected to adhere to bioethical standards and the 3Rs principle (Replacement, Reduction, and Refinement) while maintaining sufficient statistical power for isolated vessel studies. To mitigate the risk of false-positive or false-negative outcomes, we utilized randomized distribution and matched groups by body weight. All aortic rings were harvested and perfused under standardized conditions, following a uniform protocol for pre-loading and tone stabilization. Data analysis included statistical verification of normal distribution. The high degree of consistency and reproducibility observed across groups confirms the reliability of the findings, notwithstanding the focused cohort size.

All animal experiments were carried out in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (Strasbourg, 1986), in accordance with the rules for keeping experimental animals established by European Parliament and Council Directive (2010/63/EU) and the Order No.134 of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012, No. 249 “On approval of the procedure for conducting tests, experiments on animals by research institutions”, as well as the recommendations of the First National Congress of Ukraine on Bioethics (2001) and the Law of Ukraine No. 3447-IV dated February 21, 2006, “On the Protection of Animals from Cruel Treatment.” The study was considered at a meeting of the Bioethics Commission of National Pirogov Memorial Medical University, Vinnytsya, and a decision was made to publish it under protocol no. 9, dated 5.09.2024.

Laboratory rats were divided into three groups of 5 animals each. Group 1 was a control group receiving 1 % starch solution intragastrically via oral gavage; Group 2 included hyperthyroid animals receiving L-thyroxine (Levothyroxine sodium, Berlin-Chemie AG (Menarini Group), Germany) via oral gavage at the dose of 200 μg/kg dissolved in 1 % starch solution daily for 21 days [6]; Group 3 included hypothyroid animals receiving methimazole (Thiamazole, Pharmaceutical Company “Zdorovye”, Ukraine) via oral gavage at the dose of 10 mg/kg body weight daily for 21 days dissolved in 1 % starch solution [8]. Animal anesthesia and euthanasia, which were carried out in a state of deep sleep by decapitation, were performed using sodium thiopental. 24 hours after the last administration of the test substances.

To confirm hyperthyroid and hypothyroid status, serum levels of free thyroxine (FT_4), free triiodothyronine (FT_3), and thyroid-stimulating hormone (TSH) were measured by enzyme-linked immunosorbent assay using kits from DRG Instruments GmbH (Germany) according to the manufacturer’s instructions.

The effect of sulfur-containing compounds on the contractility of ring segments of the thoracic aorta was assessed in a special-purpose tensometric setup (developed at the State Institution “O.O. Bogomoletz Institute of Physiology of the Academy of Medical Sciences of Ukraine”) under near-isometric conditions, according to a generally accepted methodology [13]. Ring segments of the aorta (2–3 mm wide) were placed in a Teflon chamber, stretched on hooks with a constant force of 0.015–0.2 N (to establish isometric conditions), and perfused with Krebs buffer solution (composition at final

concentrations: 132 mM NaCl, 4.7 mM KCl, 1.4 mM NaH_2PO_4, 1.0 mM $CaCl_2$, 12.5 mM $NaHCO_3$, and 5.6 mM glucose, pH 7.4, t=18–20 °C, saturated with a gas mixture of 95 % O_2 and 5 % CO_2). After 1-hour, tonic contraction of the aortic segments was induced by passing a hyperkalemic Krebs solution (final KCl concentration of 80 mM) through the Teflon chamber for 30 minutes.

To assess the degree of H_2S- or cysteine-induced relaxation, isolated vascular segments were precontracted with phenylephrine at a concentration of 10^{-6} M, then perfused with solutions containing phenylephrine (10^{-6} M) together with NaHS (or cysteine) at various concentrations (from 10^{-2} to 10^{-6} M) for 15 minutes each, while changes in the isometric tension of aortic ring segments were recorded using a mechanotronic sensor and a USB-600-8/6009 analog-to-digital converter (National Instruments, USA).

Statistical analysis of the results was performed using the Statistica 17.0 software package. Results were presented as the arithmetic mean ± standard error of the mean (M±m). The statistical significance of the difference between values was determined using the parametric Student's t-test. Data were considered significant at $p<0.05$.

Results of the study and their discussion. Daily administration of L-thyroxine to animals at the dose of 200 μg/kg for 21 days resulted in sustained hyperthyroidism as confirmed by a significant (2.4-fold) increase in FT_4 concentrations in the blood of rats (from 11.07±0.47 to 26.12±1.85 pmol/L) and a significant (4.25-fold) reduction in TSH levels (from 0.34±0.03 to 0.08±0.01 mIU/L) compared with controls. Under these conditions, the FT_3 concentration only demonstrated an upward trend. However, based on the results of statistical analysis, the changes were found to be statistically insignificant.

Administration of methimazole to animals at the dose of 10 mg/kg daily for 21 days led to the development of hypothyroidism: a significant (almost 3-fold) decrease in FT_4 was observed (from 11.07±0.47 to 4.25±0.42 pmol/L), along with a 6.5-fold increase in TSH levels (from 0.34±0.03 to 2.21±0.16 mIU/L). At the same time, there was a 4-fold decrease in serum FT_3 levels on Day 21 (from 2.58±0.24 to 0.67±0.04 pmol/L) relative to controls.

Investigation of H_2S-stimulated vasodilation in aortic segments showed that significant changes in aortic wall sensitivity to the effect of H_2S were observed only in the setting of thyroid hormone deficiency. The horizontal axis is the decimal logarithm of H_2S concentration (M) in the perfusion solution, the vertical axis is the normalized intensity of relaxation of aortic ring segments in response to increasing H_2S concentrations. The 100 % value represents the level of H_2S-stimulated relaxation of aortic segments corresponding in amplitude to the maximum phenylephrine-induced precontraction. This and the following figures present averaged data from five experiments with corresponding values of standard error of the mean (Fig. 1, Table 1).

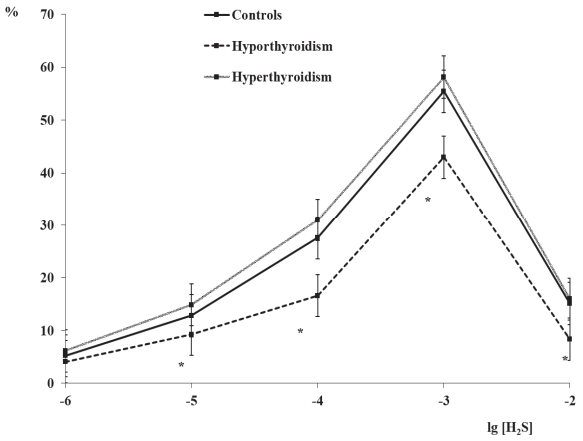

Fig. 1. Dose dependence of H_2S-stimulated relaxation of aortic ring segments in rats of the study groups. Note: * indicates the significance of differences ($p<0.05$) relative to the values for intact animals.

Table 1

Mean effective concentrations of H_2S and cysteine for rat aortas in the study groups

Groups of animals	EC_{50}, μM	
	Hydrogen sulfide	Cysteine
Controls	96.2±6.88	1034±45.9
Hyperthyroidism (Day 21)	85.4±5.52	985±42.4
Hypothyroidism (Day 21)	134±10.5*	1290±58.8*

Note: * indicates the significance of differences ($p<0.05$) relative to the values in the control group.

In the control group of animals, perfusion of aortic ring segments with NaHS solutions within the concentration range of 10^{-3} to 10^{-6} M caused a dose-dependent decrease in isometric tension of the aortic wall: a NaHS solution at the concentration of 10^{-6} M caused a minimal relaxation (5.21±0.74 %), the degree of which increased with escalating NaHS concentrations and reached its peak (55.5±1.05 %) at the concentration of 10^{-3} M. In this case, the NaHS concentration at which aortic wall relaxation reached half of the maximum response ranged from 86 to 106 μM. In rats with hypothyroidism, H2S-stimulated aortic relaxation was reduced, as evidenced by a rightward shift of the dose-response curve. In addition to that, a statistically significant increase in mean effective concentration (EC_{50}) of H_2S was observed (by 39.3 % [$p<0.05$] compared with the control group). Under these conditions, NaHS at the

concentration of 10^{-3} M caused relaxation of the aortic wall by 43.0±0.40 %, which was on average 22.5 % less ($p<0.05$) than in the control group. High concentrations of thyroid hormones had no effect on H_2S-stimulated aortic vasodilation: the dose-response curve approximated the control curve, and mean effective concentration (EC_{50}) of H_2S showed no significant differences relative to the findings in the control group. Such bidirectional regulation highlights the complexity of interactions of thyroid hormones with the vascular system, as also demonstrated by

The effect of thyroid hormones on cysteine-induced vasodilation of aortic ring segments showed similar trends as the effect on aortic relaxation in response to H_2S. The horizontal axis is the decimal logarithm of cysteine concentration (M) in the perfusion solution, the vertical axis is the normalized intensity of relaxation of aortic ring segments in response to increasing cysteine concentrations. The 100 % value represents the level of cysteine-stimulated relaxation of aortic segments corresponding in amplitude to the maximum phenylephrine-induced precontraction. It was found that only insufficiency of thyroid hormones was accompanied by impaired cysteine-stimulated aortic relaxation (Fig. 2).

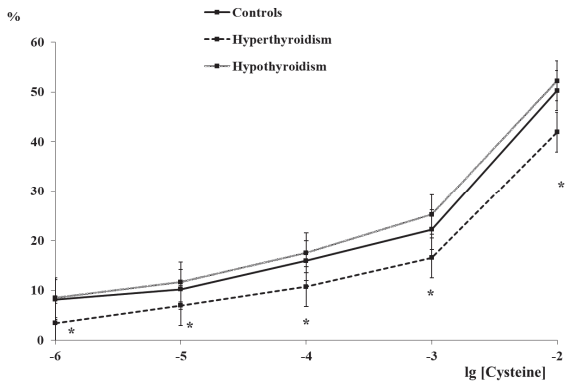

Fig. 2. Dose dependence of cysteine-stimulated relaxation of aortic ring segments in rats of the study groups. Note: * indicates the significance of differences ($p<0.05$) relative to the values in the control group.

In the control group, mean concentrations of cysteine (10^{-3} to 10^{-5} M) caused moderate (by 10.3–22.2 %) relaxation of thoracic aorta segments, whereas the concentration of 10^{-2} M caused maximum vasodilation (50.3±1.29 %). Mean effective concentration of cysteine ranged within 977–1104 μM. At the same time, in the group of hypothyroid animals, cysteine at a concentration of 10^{-2} M caused relaxation of the aortic wall by 42±2.32 % on average, which was 16.5 % lower ($p<0.05$) relative to controls. In addition, the EC_{50} of cysteine was 24.8 % higher relative to controls, and the dose-response curve shifted to the right of the control curve. In animals with high thyroid hormone levels (hyperthyroidism), no significant changes in cysteine-induced vasodilation were observed compared with controls.

Lin et al., 2020 [6] and Tkachenko et al. 2005 [13]. At the same time, these data suggest that elevated thyroid hormone levels may compensate for impairments in the NO protein system and antioxidant defenses, thereby allowing avoidance of excessive vascular spasm.

The experimental studies conducted in an in vitro setting have demonstrated that hypothyroidism is accompanied by a reduction in the capacity of H_2S and cysteine to stimulate relaxation of the aortic walls. In contrast, hyperthyroidism did not significantly affect H_2S- and cysteine-stimulated relaxation of aortic segments.

Reduction of cysteine-stimulated relaxation in the setting of hypothyroidism is apparently due to low activity of cystathionine-γ-lyase, since the vasodilatory effect of cysteine is directly associated with the production of H_2S by the aortic wall mediated by this enzyme [7]. A recent study by Soetedjo et al., 2024 [12] has shown new insights into the mechanisms associated with sulfur-containing compounds, adding to our understanding of the pathogenesis of cardiovascular disorders in thyroid dysfunction. The authors have established that inhibition of genes associated with the synthesis and metabolism of H_2S may act as a key link between thyroid status and vascular dysfunction and offer opportunities for implementation of new therapeutic approaches targeting the modulation of sulfur-containing pathways for correction of vascular tone.

The inhibitory effect of low thyroid hormone levels (hypothyroidism) on H_2S-induced aortic vasodilation can be attributed to the following factors: 1) long-standing deficiency of H_2S in the body of rats [9] accompanied by a reduction of its stimulating effect on vascular tone 2) the development of severe oxidative stress [15], which causes a covalent modification of K^+-ATP channels, the main molecular target of vascular effects of H_2S [5]; 3) reduced activity of the endothelial isoform of NO-synthase [14], which provides an endothelial component of vascular effects of H_2S [3].

Limitations. The primary limitation of this study is the relatively small cohort size (n=5 per group), which, although meeting bioethical standards, may constrain the broader extrapolation of the observed physiological trends. Additionally, the study focused on short-term observations, potentially leaving the long-term effects of the administered substances on vascular remodeling unexplored.

Conclusion

Experimentally induced hypothyroidism caused a statistically significant decrease in H_2S- and cysteine-stimulated relaxation of aortic ring segments, as evidenced by a rightward shift of the dose-response curves and

an increase in mean effective concentrations of H_2S and cysteine in the aorta by 24.8–39.3 % ($p<0.05$) relative to the control group of animals. In contrast, hyperthyroidism did not cause any significant changes of aortic tone in rats in response to H_2S and cysteine.

Prospects of further research. Research into the molecular mechanisms behind the effects of thyroid hormones on the regulation of vascular tone in response to sulfur-containing compounds will enable identification of new pathogenetic links in the development of cardiovascular disorders and justify new approaches to their correction in the setting of different thyroid status.

References

1. Cappola AR, Desai AS, Medici M, Cooper LS, Egan D, Sopko G, et al. Thyroid and Cardiovascular Disease: Research Agenda for Enhancing Knowledge, Prevention, and Treatment. Thyroid. 2019 Jun;29(6):760-777. https://doi.org/10.1089/thy.2018.0416.
2. Di Cesare M, Perel P, Taylor S, Kabudula C, Bixby H, Gaziano TA, et al. The Heart of the World. Glob Heart. 2024 Jan 25;19(1):11. https://doi.org/10.5334/gh.1288.
3. Gomez CB, Sánchez-López A, Carvajal K, Centurión D. Effect of Hydrogen Sulfide on Sympathoinhibition in Obese Pithed Rats and Participation of K+ Channel. Int J Hypertens. 2024 Nov 4;2024:5848352. https://doi.org/10.1155/2024/5848352.
4. Horodynska O, Bobyriova L. Prognostic characteristics of hypothyroidism prevalence in the Poltava region and in Ukraine under conditions of iodine deficiency. Mìžnarodnij Endokrinologìčnij Žurnal, 2016. (2.74), 44–49.
5. Islam KN, Nguyen ID, Islam R, Pirzadah H, Malik H. Roles of Hydrogen Sulfide (H2S) as a Potential Therapeutic Agent in Cardiovascular Diseases: A Narrative Review. Cureus. 2024 Jul 19;16(7):e64913. https://doi.org/10.7759/cureus.64913.
6. Lin YH, Lin KH, Yeh CT. Thyroid Hormone in Hepatocellular Carcinoma: Cancer Risk, Growth Regulation, and Anticancer Drug Resistance. Front Med (Lausanne). 2020 May 22;7:174. https://doi.org/10.3389/fmed.2020.00174.
7. Mendiola PJ, Naik JS, Gonzalez Bosc LV, Gardiner AS, Birg A, Kanagy NL. Hydrogen Sulfide Actions in the Vasculature. Compr Physiol. 2021 Sep 23;11(4):2467-2488. https://doi.org/10.1002/cphy.c200036.
8. Mohamadizadeh E, Arabi MS, Hojati V, Vaezi G, Hosseini SM. Comparison of antithyroid effects and hepatic complications of methimazole with catechin and its nanoencapsulation form in adult male rats. J Complement Integr Med. 2022 Aug 2;19(3):591-598. https://doi.org/10.1515/jcim-2022-0099.
9. Nechiporuk VM, Zaichko NV, Melnik AV, Strutyska EB, Korda MM. Effect of thyroid hormones on the sulfur-containing amino acids remethylation and transsulfuration pathways in rat organs. Medical and Clinical Chemistry, 2019. (1), 103–112. https://doi.org/10.11603/mcch.2410-681X.2019.v0.i1.10028.
10. Pravesh K, Sethi P, Kamalanathan SK, Manavalan M. An Analytical Study to Determine Dose-Volume Threshold for Radiation Induced Hypothyroidism. Asian Pac J Cancer Prev. 2023 Nov 1;24(11):3859-3866. https://doi.org/10.31557/APJCP.2023.24.11.3859.
11. Roorda M, Miljkovic JL, van Goor H, Henning RH, Bouma HR. Spatiotemporal regulation of hydrogen sulfide signaling in the kidney. Redox Biol. 2021 Jul;43:101961. https://doi.org/10.1016/j.redox.2021.101961.
12. Soetedjo NNM, Agustini D, Permana H. The impact of thyroid disorder on cardiovascular disease: Unraveling the connection and implications for patient care. Int J Cardiol Heart Vasc. 2024 Oct 23;55:101536. https://doi.org/10.1016/j.ijcha.2024.101536.
13. Tkachenko MM, Sahach VF, Baziliuk OV, Kotsiuruba AV, Popereka, HM, Stepanenko LH, Seniuk OF. Age-related characteristics of contractile vascular reactions and the content of oxygen free radicals and nitric oxide metabolites in BALB/c mice in conditions of alienation zone. Fiziol Zh, 2005. 51 (3), 32-41.
14. Yousefzadeh N, Jeddi S, Ghasemi A. Impaired Cardiovascular Function in Male Rats with Hypo- and Hyperthyroidism: Involvement of Imbalanced Nitric Oxide Synthase Levels. Endocr Metab Immune Disord Drug Targets. 2021;21(3):526-533. https://doi.org/10.2174/1871530320666200508115543.
15. Zeber-Lubecka N, Kulecka M, Suchta K, Dąbrowska M, Ciebiera M, Hennig EE. Association of Mitochondrial Variants with the Joint Occurrence of Polycystic Ovary Syndrome and Hashimoto's Thyroiditis. Antioxidants (Basel). 2023 Nov 8;12(11):1983. https://doi.org/10.3390/antiox12111983.

Conflict of interest. The authors have no conflicts of interest to declare.

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Article received: 17.01.2025.