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**M. O. Tsebenko[1], M. V. Bilets[1], O. E. Omelchenko[1], M. Ya. Spivak[2], K. S. Neporada[1]**
*[1]POLTAVA STATE MEDICAL UNIVERSITY*
*[2]ZABOLOTNY INSTITUTE OF MICROBIOLOGY AND VIROLOGY NAS OF UKRAINE*

# EFFECT OF NANOCERIUM ON STRESS SYNDROME SEVERITY AND ANTHROPOMETRY IN RATS WITH CHRONIC STRESS AND OBESITY

***Introduction.*** *Nanotechnology has opened new promising directions in modern biology and medicine. Nanocrystalline materials, such as cerium dioxide-based nanomaterials, play a leading role in these developments. Significant experimental data have been accumulated, demonstrating the high biological activity of nanocrystalline cerium and revealing its potential use in the development and application of nanocomposites capable of activating cellular and humoral immune defense systems.*

***The aim of the study*** *– effect of nanocerium on stress syndrome severity and anthropometry in rats with chronic stress and obesity.*

***Research Methods.*** *Experimental studies were conducted on 103 white non-linear rats of both sexes, modeling obesity induced by glutamate, chronic stress, both separately and in combination. Obesity was induced by postnatal subcutaneous injection of sodium glutamate. The animals were maintained on a standard vivarium diet for 4 months. Body weight, BMI, and Lee index were monitored weekly throughout the experiment. Chronic stress was modeled using Hans Selye's method by immobilizing the animals on their backs for 5 hours. The severity of stress was assessed by Selye's triad. Starting from 1 month of age and for the next three months, the experimental rats were orally administered nanocrystalline cerium dioxide using a probe. Somatometric measurements showed that in obese animals, growth was stunted, and body weight did not significantly differ from intact animals.*

***Results and Discussion.*** *For example, the body weight of intact animals was 165,2 ± 5,1 g, while rats with glutamate-induced obesity weighed 213,7 ± 9 g. Despite the increase in body weight, the body length of obese rats was shorter compared to intact animals. It has been shown that nanocrystalline cerium has high antioxidant activity and can be considered a candidate for the creation of a therapeutic agent for the treatment of obesity. Intravenous administration of nanocrystalline cerium to animals with modeled obesity and chronic stress associated with obesity significantly reduced BMI compared to respective control animals.*

***Conclusions.*** *Nanocerium effectively prevented the development of visceral obesity in animals exposed to isolated and combined effects of obesity and stress, as evidenced by a significant reduction in fat mass in the visceral fat depot of the animals. Thus, nanocrystalline cerium dioxide is a potential agent for correcting experimental obesity under chronic stress conditions, as confirmed by other studies.*

KEY WORDS: **periodontal tissues; rats; monosodium glutamate; visceral fat; cerium oxide; thymus; adrenal glands.**



INTRODUCTION. Over the past three decades, obesity has increased worldwide, particularly in low- and middle-income countries due to uncontrolled urbanization and the change-over to a "western-style" diet [1]. Obesity is a rapidly growing pandemic with serious health consequences. In recent years, there is increasing evidence that stress, and in particular increases in the glucocorticoid stress hormone – cortisol, plays a role in the development of obesity [2] .

It is known that in our modern society, the obesity pandemic coincides with an increase in factors that enhance cortisol production, such as chronic stress, consumption of high-glycemic index foods, and sleep deprivation.

This indicates a vicious circle where increased glucocorticoid action, obesity and stress interact and enhance each other [3]. Although it is known that the main cause of the obesity epidemic is an imbalance between energy intake and expenditure, however, when taking account of the effects of chronic exposure to cortisol, there is increasing evidence that cortisol is also a major factor in this pandemic. It has been proven that, on average, obese people have higher levels of cortisol in their hair. A meta-analysis by Stalder et al. also found evidence of a relationship between cortisol and BMI: cortisol in the hair of obese patients increased by 9,8% for every 2,5-point increase in BMI [4].

Nanotechnology is a branch of science that studies particles in the 1–100 nm range. These particles are called nanoparticles, and they exhibit unique electronic, optical, magnetic, and mechanical properties that distinguish them from the basic material. Nanotechnology

has opened up new and promising directions in modern biology and medicine. The leading role in them is occupied by nanocrystalline materials, which include nanomaterials based on cerium dioxide. Currently, considerable experimental material has been accumulated, which testifies to the high biological activity of nanocrystalline cerium and reveals the potential possibility of its use for the development and application of nanocompositions capable of activating the systems of cellular and humoral immune protection, prevention and therapy of viral diseases, as well as for increasing the effectiveness of the treatment of malignant neoplasms [5].

It has been proven that nanocrystalline cerium has high antioxidant activity, it exerts a neurotrophic and neuroprotective effect, increases the viability of brain cells, has antimicrobial properties [6], and can be considered as a candidate for the creation of a drug in the treatment of obesity [7].

RESEARCH METHODS. Experimental studies were conducted on 103 white nonlinear rats of both sexes, modeling glutamate-induced obesity, chronic stress, both separately and in combination.

Obesity was induced by postnatal subcutaneous administration of monosodium glutamate (MSG) to newborn rats [8] at a dose of 4 mg/g on days 2, 4, 6, 8, and 10 after birth. Control group rats received subcutaneous injections of physiological saline at a dose of 8 µl/g. The animals were maintained on a standard vivarium diet for 4 months.

Body weight, BMI, and Lee index were monitored weekly throughout the experiment. Chronic stress was modeled according to Hans Selye's method by immobilizing the animals on their backs for 5 hours during the last week before slaughter, which was performed 2 hours after stress under thiopental anesthesia by exsanguination. Starting from 1 month of age and continuing for the next three months, the experimental rats were administered nanocrystalline cerium dioxide orally via a probe at a dose of 1 mg/kg dissolved in injection-grade water, with a volume of 2,9 ml/kg according to the following schedule: daily administration for 2 weeks, followed by a 2-week break, repeated for 3 months.

The work was carried out within the framework of the research project of the Department of Biochemistry at Poltava State Medical University, titled "Development of means to correct pathological changes in the organs of the digestive system under conditions of civilization diseases" (0124U001922). The obtained results were subjected to statistical processing using the IBM SPSS Statistics 26 software package (2019). The Kruskal – Wallis test was used to determine statistically significant differences between groups. Differences were considered statistically significant at $p < 0,05$.

RESULTS AND DISCUSSION. The experimental efficacy of nanocerium was demonstrated based on the analysis of indicators reflecting the severity of stress, specifically the development of gastric mucosal ulcerations. The results showed a complete absence of ulcers in obese rats treated with nanocerium and a reduction in the frequency, severity, and multiplicity of ulcers in stressed animals and obese animals under stress when treated with nanocerium, compared to the respective control groups (Table 1).

In rats that received (MSG) at a dose of 4 mg/g, visceral obesity developed after 4 months after birth, primarily due to MSG-induced excitotoxicity [9].

Somatometry measurements indicate that growth was slowed in obese animals, while body weight did not significantly differ from intact animals. For example, the body weight of intact animals was 165,2 ± 5,1 g, whereas rats with glutamate-induced obesity weighed 213,7 ± 9 g. Despite the increased body weight, the body length of obese rats was shorter compared to intact animals. The shorter, more rounded body shape of MSG-treated rats explains the lack of difference in body weight. Hence, in this model of glutamate-induced visceral obesity, there is a simultaneous slow-down in development and growth with an accumulation of excess adipose tissue. The most objective anthropometric indicators are body mass index (BMI) and the Lee index.

We found that BMI increased by 22,9% in rats that received neonatal MSG, confirming obesity development in this group. Another crucial index for assessing obesity severity is the Lee index. Using this criterion allows for more precise assessment of differences between groups and the identification of significant differences not apparent with other criterion due to less variance in values.

It was found that glutamate-induced obesity significantly increased the Lee index. In rats with modeled obesity and chronic stress on top of obesity, this index significantly increased compared to intact animals. The combined effect of obesity and chronic stress led to a

Table 1 – **Indicators of severity of stress syndrome in animals according to Selye's triad (x ± SE, n = 103)**

| Animal groups | Rate of gastric mucous ulcers, % | Multiplicity (Number of ulcers for 1 rat per group) | Severity, scores | Relative weight of the thymus, mg/g | Relative weight of the adrenal gland, mg/g |
|---|---|---|---|---|---|
| 1. Control, n = 10 | – | – | – | 1,08 ± 0,17 | 0,23 ± 0,02 |
| 2. Obesity, n = 14 | – | – | – | 1,10 ± 0,07 | 0,16 ± 0,02 |
| 3. Obesity + stress, n = 17 | 60 | 0.93 | 5 | 0,99 ± 0,07[a] | 0,17 ± 0,02 |
| 4. Stress, n = 10 | 75 | 1.79 | 6 | 0,77 ± 0,16 | 0,30 ± 0,04 |
| 5. Nanocerium control, n = 10 | – | – | – | 0,89 ± 0,13 | 0,20 ± 0,04 |
| 6. Obesity + Nanocerium, n = 16 | – | – | – | 1,15 ± 0,08 | 0,23 ± 0,02 |
| 7. Obesity + stress + Nanocerium, n = 16 | 9 | 0.09 | 1 | 1,27 ± 0,10[b] | 0,31 ± 0,06 |
| 8. Stress + Nanocerium, n = 10 | 10 | 0.4 | 2 | 0,79 ± 0,06 | 0,29 ± 0,04 |

Note: In this and following tables, n is the number of animals ($p < 0,05$).

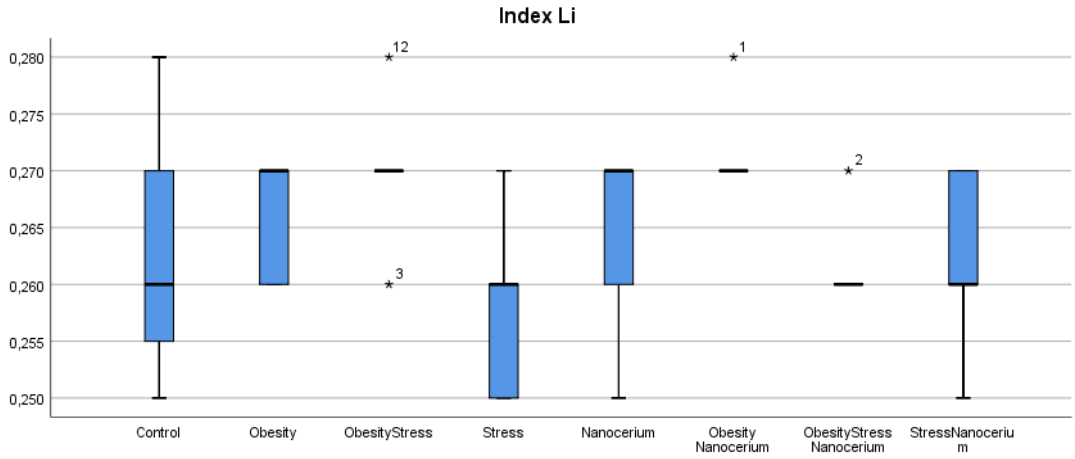


Fig. 1. Weekly Monitoring of the Lee Index ($\sqrt[3]{}$g/cm) in rats with glutamate-induced obesity, chronic stress, and nanocerium correction

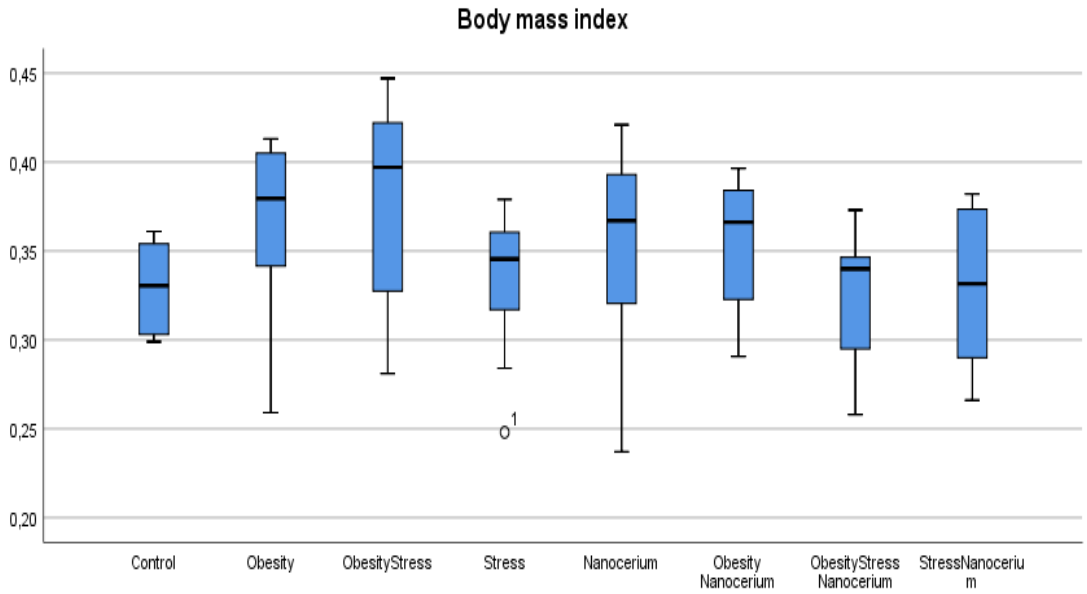


Fig. 2. Weekly Monitoring of Body Mass Index (g/cm$^2$) in rats with glutamate-induced obesity, chronic stress, and nanocerium correction

Table 2 – **Anthropometric indicators in 4-month-old rats with glutamate-induced obesity, chronic stress, and nanocerium correction (x ± SE, n = 103)**

| Animal groups | Lee index, g1/3/cm | Visceral fat mass, g | BMI, g/cm$^2$ |
|---|---|---|---|
| 1. Control, n = 10 | 0,254 ± 0,003$^a$ | 0,50 ± 0,26$^a$ | 0,35 ± 0,01$^a$ |
| 2. Obesity, n = 14 | 0,268 ± 0,003$^b$ | 11,42 ± 1,30$^b$ | 0,43 ± 0,01$^b$ |
| 3. Obesity + stress, n = 17 | 0,263 ± 0,002$^b$ | 12,90 ± 0,74$^b$ | 0,39 ± 0,01$^a$ |
| 4. Stress, n = 10 | 0,258 ± 0,002 | 0,09 ± 0,09$^c$ | 0,37 ± 0,01 |
| 5. Nanocerium control, n = 10 | 0,257 ± 0,002 | 0,00 ± 0,00 | 0,39 ± 0,01 |
| 6. Obesity + Nanocerium, n = 16 | 0,258 ± 0,002$^c$ | 5,05 ± 1,08$^c$ | 0,37 ± 0,01$^b$ |
| 7. Obesity + stress + Nanocerium, n = 16 | 0,251 ± 0,003$^c$ | 1,59 ± 0,47$^c$ | 0,33 ± 0,01$^b$ |
| 8. Stress + Nanocerium, n = 10 | 0,255 ± 0,003$^c$ | 0,71 ± 0,38$^c$ | 0,36 ± 0,01 |

Note: different letters indicate values that differed one from another significantly within one line of the table according to the results of comparison using nonparametric Anova by Kruskal-Wallis.

statistically reliable increase in the Lee index and visceral fat mass compared to those in the glutamate-induced obesity group and intact group (table 2).

CONCLUSIONS. The administration of endogastrally nano-crystalline cerium to animals modeled with obesity and chronic stress contributed to a significant reduction in bmi compared to respective control animals (table 2). In rats with glutamate-induced obesity and chronic stress, nano-cerium administration led to a significant decrease in lee index, visceral fat mass, and bmi by 1.1-time, 8-times, and 1.2-time, respectively, compared to obese animals under chronic stress without correction (table 2). Nano-cerium effectively prevented the development of visceral obesity in animals exposed to isolated and combined effects of obesity and stress, as evidenced by the probable reduction in fat mass in the visceral compartment of the animals' fat depots. Thus, nano-crystalline cerium dioxide is a potential means for correcting experimental obesity under conditions of chronic stress, supported by other researches [7] . El-seidy ama et al demonstrated the effectiveness of nano-cerium based on its positive impact in preventing oxidative stress, adipocyte hormone imbalance, and insulin resistance in obese rats [10].

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Адреса для листування:** *М. О. Цебенко, Полтавський державний медичний університет, вул. Шевченка, 23, Полтава, 36011, Україна, e-mail: tsebenkomari@gmail.com.*

***М. О. Цебенко*[1], *М. В. Білець*[1], О. Є. Омельченко[1], М. Я. Співак[2], К. С. Непорада[1]**
*[1]ПОЛТАВСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ*
*[2]ІНСТИТУТ МІКРОБІОЛОГІЇ ТА ВІРУСОЛОГІЇ ІМЕНІ Д. К. ЗАБОЛОТНОГО НАН УКРАЇНИ*

## ВПЛИВ НАНОЦЕРІЮ НА ВАЖКІСТЬ СТРЕСОВОГО СИНДРОМУ ТА АНТРОПОМЕТРІЮ В ЩУРІВ ІЗ ХРОНІЧНИМ СТРЕСОМ ТА ОЖИРІННЯМ

***Вступ.*** *Нанотехнології відкрили нові перспективні напрями в сучасній біології та медицині. Нанокристалічні матеріали, як-от наноматеріали на основі діоксиду церію, відіграють провідну роль у цих розробках. Накопичено значні експериментальні дані, які демонструють високу біологічну активність нанокристалічного церію та його потенційне використання в розробленні та застосуванні нанокомпозитів, здатних активувати клітинний і гуморальний імунний захист.*

***Мета дослідження*** *– вивчити вплив нанокристалічного церію на важкість стресового синдрому й антропометричні показники в щурів із хронічним стресом і ожирінням.*

***Методи дослідження.*** *Експериментальні дослідження проводилися на 103 білих нелінійних щурах обох статей, моделювали ожиріння, індуковане глутаматом, хронічний стрес як окремо, так і в поєднанні. Ожиріння індукували шляхом постнатальних підшкірних ін'єкцій глутамату натрію. Тварин утримували на стандартній віварній дієті протягом 4 місяців. Протягом усього експерименту щотижня контролювали масу тіла, індекс маси тіла (ІМТ) та індекс Лі. Хронічний стрес моделювали за методом Ганса Сельє шляхом фіксації тварин на спині на 5 годин. Важкість стресу оцінювали за тріадою Г. Сельє. Починаючи з 1-місячного віку та протягом наступних трьох місяців експериментальним щурам уводили нанокристалічний діоксид церію перорально за допомогою зонда. Соматометричні вимірювання показали, що у тварин з ожирінням ріст був уповільнений, а маса тіла не відрізнялася значно від маси інтактних тварин.*

***Результати й обговорення.*** *Наприклад, маса тіла інтактних тварин становила 165,2 ± 5,1 г, тоді як щури з ожирінням, індукованим глутаматом, важили 213,7 ± 9 г. Незважаючи на збільшення маси тіла, довжина тіла щурів з ожирінням була меншою порівняно з інтактними тваринами. Показано, що нанокристалічний церій має високу антиоксидантну активність і може розглядатися як кандидат на створення терапевтичного засобу для лікування ожиріння. Внутрішньовенне введення нанокристалічного церію тваринам зі змодельованим ожирінням і хронічним стресом, пов'язаним з ожирінням, значно знижувало індекс маси тіла порівняно з відповідними контрольними тваринами.*

***Висновки.*** *Нанокристалічний церій ефективно запобігає розвитку вісцерального ожиріння у тварин, підданих ізольованій і комбінованій дії ожиріння тастресу, що підтверджується значним зменшенням жирової маси у вісцеральному жировому депо тварин. Отже, нанокристалічний діоксид церію є потенційним засобом для корекції експериментального ожиріння в умовах хронічного стресу, що підтверджується іншими дослідженнями.*

КЛЮЧОВІ СЛОВА: **пародонтальні тканини; щури; мононатрієвий глутамат; вісцеральний жир; діоксид церію; тимус; надниркові залози.**