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EXPERIMENTAL BISMUTH NANOPARTICLES: ANTIMICROBIAL EFFECTIVENESS AND BIOSAFETY LEVEL

The article presents results of antimicrobial effectiveness estimation in vitro and biosafety level control in vitro and in vivo for experimental substance of spherical 40 nm bismuth nanoparticles. High bactericidal effectiveness against wide spectra of pathogen microorganisms – potential causative agents of livestock and poultry infectious diseases has been revealed for the analyzed nanoparticles as well as biosafety according to the analyzed parameters of genotoxicity, cytotoxicity, mutagenicity and acute toxicity LD₅₀.

Keywords: Bismuth nanoparticles, antimicrobial activity, pathogen test-strains, effectiveness, biosafety level.

Introduction. The problem of making competitive biologically safe products of livestock and poultry industry are very important as for EU-members countries as for other countries in the world.

The main risk of using livestock and poultry farming products for human is contamination by opportunistic and pathogenic microorganisms (such as *Salmonella*, *Campylobacter*, enterotoxigenic *E. coli* etc.) and their metabolites [1, 2]. In many cases these pathogens can be hosted in the animal intestinal tract asymptotically and provoke food-borne diseases after transmission through the food chain to humans.

Opportunistic and pathogen microorganisms are also main cause of morbidity and mortality and the main source of economic losses in the livestock and poultry industry in wide world [3–5].

Therefore, the development of new high effective biosafe substances with bactericidal properties for ensuring reduction of morbidity and mortality in young farm animals and poultry as well as decrease of antibiotic-resistant pathogens' widespread is actual. Solving of this problem gives opportunity to reduce economic loss in the livestock and poultry industry and to decrease the food contamination by opportunistic and pathogen microorganisms especially antibiotic-resistant ones.

According to our previous studies, bismuth nanoparticles are possessed by high potential in this area [6].

The goal of the work was estimation *in vitro* of bismuth nanoparticles antimicrobial activity against wide spectrum of pathogenic test strains of microorganisms – potential causative agents of livestock and poultry diseases with analysis of the nanomaterial biosafety level *in vitro* and *in vivo* according to the parameters of genotoxicity, cytotoxicity, mutagenicity and LD₅₀.

Materials and methods of research. Spherical Bismuth nanoparticles (BiNP) with average particle size 40 ± 2.0 nm have been synthesized by the method of chemical condensation in water medium according to the original protocol developed in F.D. Ovcharenko Institute of biocolloidal chemistry. The concentration of obtained BiNP was 77.5 mg/ml. 100% of Bi content is determined in the BiNP according to the data of X-ray microanalysis [6]. Figure 1 shows transmission electron microscope (TEM) image of the synthesized spherical BiNP used in presented investigations.

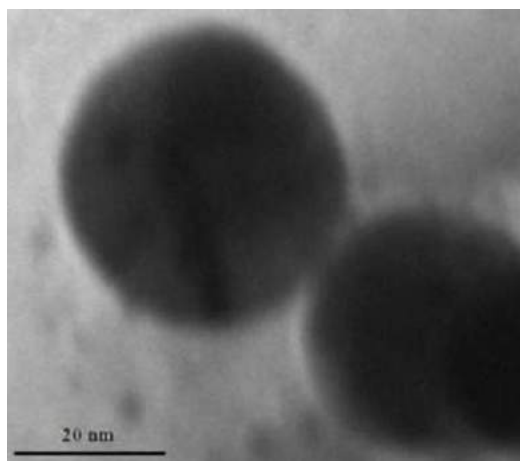


Fig. 1. TEM image of the synthesized spherical 40 nm Bismuth nanoparticles.

BiNP antimicrobial activity was estimated by the «Method of serial dilutions in agar» according to the Guidelines for Susceptibility Testing of Microorganisms to Antibacterial Agents (4.2.1890-04) [7]. Muller-Hinton agar has been used as determination medium. Certain concentrations of sterile BiNP dispersion has been added to sterile Muller-Hinton agar in certain concentration and mixed. Muller-Hinton agar with BiNP has been poured on Petri dishes.

Salmonella typhimurium 16, *Salmonella enteritidis* 34, *Escherichia coli* 25, *Pasteurella multocida* 877, *Campylobacter jejuni* Pl – 09.c, *Listeria monocytogenes* ATCC 19112, *Yersinia enterocolitica* 12/15-08 pathogen test strains from the Collection of The State Scientific Control Institute of Biotechnology and Strains of Microorganisms (Kyiv, Ukraine) have been used for BiNP antimicrobial activity estimation. The effectiveness of BiNP antimicrobial activity has been estimated with terminal seed-dose of each test-microorganism on the Petri dishes 10^3 , 10^4 and 10^5 CFU/cm³.

The nanoparticles' biosafety level *in vitro* has been estimated using parameters of cytotoxicity, genotoxicity and mutagenicity according to the Guidelines «Safety assessment of medical nanopreparations» approved by the Scientific Expert Council

of the State Expert Centre of the Ministry of Health of Ukraine (protocol №8, 26.09.2013) [8].

Biosafety level *in vivo* of the BiNP was assessed using acute toxicity (LD50) parameters under peroral and intravenous routes of administration. Animals used in the experiment were kept under standard vivarium conditions.

Animal studies were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (18.03.1986), Council Directive 86/609/EEC on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (24.11.1986), the Order of the Ministry of Health of Ukraine №66 (13.02.2006), the Law of Ukraine «On the Protection of Animals from Cruelty» (2006).

Acute toxicity of BiNP was studied on white BALB/c mice (females and males) with average weight 20 ± 2 g and 2–2.5 months old. Control animals received the placebo (water for injections). Registration and counting of dead animals in each experimental dose levels were carried out for 14 days. Based on the obtained data probit analysis of dose-effect correlation was performed according to D.J. Finney using computer software BioStat 2009 for Windows (v5.8.4) [9, 10].

Results of research and discussion. Estimation of 40 nm BiNP antimicrobial activity against wide spectra of test strains – causative agents of the widespread infectious diseases: *Salmonella typhimurium* 16, *Salmonella enteritidis* 34, *Escherichia coli* 25, *Pasteurella multocida* 877, *Campylobacter jejuni* Pl – 09.c, *Listeria monocytogenes* ATCC 19112, *Yersinia enterocolitica* 12/15-08 shown high bactericidal action of the BiNP against all investigated pathogen strains (table 1).

High biosafety level is necessary condition in the case of potential bio-medical application of any substances including metal nanoparticles. Taking into account this point of view BiNP biosafety level has been estimated *in vitro* using wide spectrum of parameters according to the protocols and conditions of the Guidelines «Safety assessment of medical nanopreparations» [8].

Table 1

BiNP antimicrobial activity against wide spectra of test strains – causative agents of the widespread infectious diseases in livestock and poultry

Test pathogen	Seed-dose of test strain, CFU/cm ³	Bismuth nanoparticles (C=77,5 mg/ml by metal)		Control of test strain growth
		Terminal concentration of bismuth nanoparticles in determination medium, mg/ml by metal		
		6,50	12,90	
<i>Salmonella enteritidis</i> 34	10 ³	I	I	++++
	10 ⁴	I	I	++++
	10 ⁵	I	I	++++
	10 ⁶	I	I	++++
<i>Salmonella typhimurium</i> 16	10 ³	I	I	++++
	10 ⁴	I	I	++++
	10 ⁵	I	I	++++
	10 ⁶	I	I	++++
<i>Escherichia coli</i> 25	10 ³	I	I	++++
	10 ⁴	I	I	++++
	10 ⁵	I	I	++++
	10 ⁶	I	I	++++
<i>Pasteurella multocida</i> 877	10 ³	I	I	++++
	10 ⁴	I	I	++++
	10 ⁵	I	I	++++
	10 ⁶	I	I	++++
<i>Campylobacter jejuni</i> Pl – 09.c	10 ³	I	I	++++
	10 ⁴	I	I	++++
	10 ⁵	I	I	++++
	10 ⁶	I	I	++++
<i>Listeria monocytogenes</i> ATCC 19112	10 ³	I	I	++++
	10 ⁴	I	I	++++
	10 ⁵	I	I	++++
	10 ⁶	I	I	++++
<i>Yersinia enterocolitica</i> 12/15-08	10 ³	I	I	++++
	10 ⁴	I	I	++++
	10 ⁵	I	I	++++
	10 ⁶	I	I	++++

Note: «I» – total inhibition of microorganisms growth;
 «++++» – intensive growth of microorganisms.
 CFU – colony-forming unit.

The data of BiNP biosafety analysis *in vitro* are presented in the table 2.

Table 2

Biosafety level of the synthesized 40 nm spherical BiNP: *in vitro* tests

Parameters of biosafety	Test-method	Biosafety characteristic
Cytotoxicity	Crystal violet staining assay	not cytotoxic
Cytotoxicity	MTT assay	not cytotoxic
Genotoxicity	Comet-assay	not genotoxic
Mutagenicity	Allium-test	not mutagenic

Obtained results define the analyzed BiNP substance as biosafe one according to the all investigated parameters of biosafety level estimation *in vitro*.

Analysis of LD₅₀ parameters for the experimental BiNP substance under peroral and intravenous routs of administration to BALB/c mice revealed, that BiNP can be attributed to the class of low-toxic materials (toxicity class IV):

- LD₅₀ parameter for BiNP under peroral rout of administration was 1810±267 mg/kg for males and 1169±237 mg/kg for females of BALB/c mice;
- LD₅₀ parameter for BiNP under intravenous rout of administration exceeded 800 mg/kg for males and 2000 mg/kg for females of BALB/c mice.

Conclusions and prospects for further research. It has been revealed that experimental substance of BiNP (spherical 40 nm particles) is possessed by high bactericidal effectiveness against wide spectrum of pathogens - causative agents of the widespread infectious diseases of livestock and poultry: *Salmonella*, *Escherichia*, *Pasteurella*, *Campylobacter*, *Listeria*, *Yersinia*.

Along with high bactericidal action of the investigated BiNP substance it has been characterized as biosafe one according to the parameters of cytotoxicity, genotoxicity, mutagenicity.

LD₅₀ indexes for the experimental BiNP substance under peroral and intravenous routs of administration to BALB/c mice attributed BiNP to the toxicity class IV – low-toxic materials.

Obtained results open new perspectives in the area of development and creation of novel biosafe antimicrobial preparations and feed additives for livestock and poultry industry with high bactericidal effectiveness against wide spectrum of pathogen microorganisms.

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ЭКСПЕРИМЕНТАЛЬНЫЕ НАНОЧАСТИЦЫ ВИСМУТА: АНТИМИКРОБНАЯ ЭФФЕКТИВНОСТЬ И УРОВЕНЬ БИОБЕЗОПАСНОСТИ / Резниченко Л.С., Дыбкова С.Н., Рыженко Г.Ф., Горбатюк О.И., Андрияшук В.А., Жовнир А.М., Грузина Т.Г.

В статье представлены результаты оценки антимикробной эффективности in vitro и контроля уровня биобезопасности in vitro и in vivo экспериментальной субстанции сферических наночастиц висмута 40 нм. Показана высокая бактерицидная эффективность анализируемых наночастиц против широкого спектра патогенных микроорганизмов – потенциальных возбудителей инфекционных заболеваний животных и птицы, а также их биобезопасность согласно проанализированным параметрам генотоксичности, цитотоксичности, мутагенности и острой токсичности LD50.

Ключевые слова: наночастицы висмута, антимикробная активность, патогенные тест-штаммы, уровень биобезопасности, эффективность.

ЕКСПЕРИМЕНТАЛЬНІ НАНОЧАСТИНКИ ВІСМУТУ: АНТИМІКРОБНА ЕФЕКТИВНІСТЬ ТА РІВЕНЬ БІОБЕЗПЕКИ / Резніченко Л.С., Дибкова С.М., Риженко Г.Ф., Горбатюк О.І., Андріяшук В.А., Жовнір О.М., Грузина Т.Г.

Вступ. Розробка нових вискоєфективних біобезпечних субстанцій з бактерицидними властивостями з метою забезпечення зниження захворюваності і смертності молодняка сільськогосподарських тварин і птиці, а також боротьби із поширенням стійких до антибіотиків патогенів, є актуальною.

Мета роботи. Оцінка in vitro антимікробної активності наночастинок вісмуту відносно тест-штамів, а також аналіз рівня біобезпечності наночастинок in vitro та in vivo за параметрами генотоксичності, цитотоксичності, мутагенності та LD50.

Матеріали та методи досліджень. В роботі використана субстанція сферичних наночастинок вісмуту (BiNP) із середнім розміром частинок 40±2 нм та 100% вмістом вісмуту у складі частинки.

Антимікробну активність in vitro оцінювали методом серійних розведень в агарі.

Біобезпечність субстанції BiNP in vitro визначали за показниками цитотоксичності, генотоксичності та мутагенності, in vivo – за показниками LD50 при пероральному та внутрішньовенному шляхах введення наночастинок білим мишам лінії BALB/c обох статей.

Результати досліджень та їх обговорення. В дослідженнях in vitro встановлено, що експериментальна субстанція BiNP володіє високою бактерицидною активністю проти всього дослідженого спектру збудників.

Субстанція BiNP охарактеризована як біобезпечна за параметрами цитотоксичності, генотоксичності і мутагенності та відноситься до малотоксичних речовин згідно визначених показників LD50.

Висновки та перспективи подальших досліджень. Встановлено, що досліджена експериментальна субстанція BiNP є біобезпечною та володіє високою бактерицидною

активністю відносно широкого спектру патогенних штамів: *Salmonella*, *Escherichia*, *Pasteurella*, *Campylobacter*, *Listeria*, *Yersinia*.

Отримані результати відкривають нові перспективи у галузі розробки та створення інноваційних біобезпечних антимікробних препаратів і харчових добавок для тваринництва та птахівництва.

Ключові слова: наночастинки вісмуту, антимікробна активність, патогенні тести, рівень біобезпеки, ефективність.

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