2- Bogomoletz National Medical University, Kyiv, Ukraine

EFFECT OF GOLD NANOCOMPOSITES AND QUERCETIN TREATMENT ON MALE REPRODUCTIVE FUNCTION

Kaleynikova O.N.[1], Ukrainska S.I.[1], Muzychenko A.S.[2], Blashkiv O.T.[2], Sribna V.A.[1], Velykiy V.Y.[1], Vinogradova-Anyk A.A.[2], Tarasova K.V.[2], Lagodich T.S.[2], Karvatskiy I.M.[2], Kuziv Y.I.[3], Voznesenska T.Y.[1], Kutsevol N.V.[3]

1-Bogomoletz Institute of Physiology, NAS of Ukraine, Kyiv, Ukraine
2 - Bogomoletz Medical University, Kyiv, Ukraine
3 - Taras Shevchenko National University of Kyiv, Kyiv, Ukraine
E-mail: tblashkiv@gmail.com

Table 2 **Functional state of sperm under conditions of EChKD and treatment with gold nanoparticles in the polymer matrices and Quercetin**

	The concentration of sperm, (1×10^6/ml)	Number of abnormal sperm, %	
		Total	Primary anomalies
Control	35,10±1,70	17,40±1,70	5,70±0,57
D-*g*-PAA(PE)	34,80±1,29	20,60±1,70	6,20±0,95
D-*g*-PAA(PE)/AuNPs	35,00±1,25	20,40±1,91	6,80±0,95
QUE	35,30±1,70	17,10±1,70	5,00±0,57
D-*g*-PAA(PE)+QUE	35,30±1,29	18,60±1,70	6,00±0,95
D-*g*-PAA(PE)/AuNPs+QUE	34,70±1,25	18,40±1,21	6,80±0,95
EChKD	30,40±1,29	38,90±1,25 *	17,50±1,00 *
EChKD+D-*g*-PAA(PE)	30,30±1,25	37,70±1,63 *	17,70±0,81 *
EChKD+D-*g*-PAA(PE)/AuNPs	31,60±0,81	27,60±0,95 * #	15,60±1,50 *
EChKD+QUE	31,40±0,81	24,30±0,95 * #	16,50±1,50 *
EChKD+D-*g*-PAA(PE)+QUE	32,10±0,81	23,20±0,95 * #	16,30±1,50 *
EChKD+D-*g*-PAA(PE)/AuNPs+QUE	31,30±0,81	20,60±0,65 # &	15,90±1,50 *

Notes: * - p <0.05 - probability differences in the average group data with respect to these variables in the control group animals; # - p <0.05 - to these variables in the group animals under conditions of EChKD; & - p <0.05 - to these variables in the group animals under conditions of treatment with EChKD+D-*g*-PAA(PE)/AuNPs; M±σ

Table 3 **The number (%) of testicular spermatogenic cells under conditions of EChKD and treatment with gold nanoparticles in the polymer matrices and Quercetin**

	%			
	Spermatogonia	Spermatocytes	Spermatides	Sertoli
Control	10,20±1,29	16,05±1,25	54,10±2,21	4,30±0,57
D-*g*-PAA(PE)	9,25±0,81	14,20±1,29	50,80±1,70	4,70±0,81
D-*g*-PAA(PE)/AuNPs	9,66±1,29	15,30±1,29	52,00±1,89	4,20±0,81
QUE	10,00±1,29	15,05±1,25	53,50±2,21	4,30±0,57
D-*g*-PAA(PE)+QUE	10,00±1,29	15,05±1,25	53,50±2,21	4,30±0,57
D-*g*-PAA(PE)/AuNPs+QUE	9,91±1,29	16,30±1,29	53,70±1,89	4,20±0,81
EChKD	8,80±0,57	12,80±0,95	41,40±1,73 *	4,60±0,95
EChKD+D-*g*-PAA(PE)	9,40±0,5	12,80±1,25	41,20±0,95*	4,60±0,57
► EChKD+D-*g*-PAA(PE)/AuNPs	9,90±0,81	15,80±1,50	44,40±1,29 *	4,60±0,57
EChKD+QUE	9,70±0,5	15,10±1,25	48,20±0,95 * #	4,50±0,57
EChKD+D-*g*-PAA(PE)+QUE	9,20±0,5	14,90±1,25	47,70±0,95 * #	4,40±0,57
EChKD+D-*g*-PAA(PE)/AuNPs+QUE	10,10±0,81	15,40±1,50	48,40±1,29 * #	4,60±0,57

Примітки: * - p<0.05 – вірогідність відмінностей середніх груп даних у порівнянні з такими величинами у контролі; # - p<0.05 – відносно величин в групі EXXH; M±σ.

Notes: * - p <0.05 - probability differences in the average group data with respect to these variables in the control group animals; # - p <0.05 - to these variables in the group animals under conditions of EChKD; M±σ

Table 4 **The number of testicular cells (primary spermatocytes) with morphological signs of apoptosis and necrosis under conditions of EChKD and treatment with gold nanoparticles in the polymer matrices and Quercetin**

	Spermatocytes, %		
	living	apoptotic	necrotic
Control	87,40±0,90	10,20±1,30	2,60±0,89
D-*g*-PAA(PE)	81,10±3,40	12,50±1,95	6,40±1,52
D-*g*-PAA(PE)/AuNPs	85,00±1,30	11,20±2,17	3,80±1,30
QUE	86,50±1,30	10,20±2,17	3,50±1,30
D-*g*-PAA(PE)+QUE	85,50±3,40	11,50±1,95	3,00±1,52
D-*g*-PAA(PE)/AuNPs+QUE	86,90±1,30	10,00±2,17	3,10±1,30
EChKD	72,60±1,10 *	16,00±1,22 *	10,60±1,14 *
EChKD+D-*g*-PAA(PE)	71,20±2,50 *	18,20±1,64 *	10,60±1,52 *
EChKD+D-*g*-PAA(PE)/AuNPs	76,20±1,30 *	17,60±1,14 *	6,20 ±1,30 #
EChKD+QUE	75,20±2,50 *	15,20±1,64 *	9,60±1,52 *
EChKD+D-*g*-PAA(PE)+QUE	76,00±2,50 *	15,40±1,64 *	8,60±1,52 *
EChKD+D-*g*-PAA(PE)/AuNPs+QUE	79,20±2,50 *	14,20±1,64	6,60±1,52 #

Notes: * - p <0.05 - probability differences in the average group data with respect to these variables in the control group animals; # - p <0.05 - to these variables in the group animals under conditions of EChKD; M±σ.

Due to the peculiarities of structure and control of intramolecular structure, branched polymer systems are interesting objects of basic research, as well as promising functional materials of the new generation. Polymers with a dextran core and grafted polyacrylamide chains dextran-polyacrylamide (D-PAA) in the anionic form of D-*g*-PAA(PE), as a polymer matrix carrier, in particular gold nanoparticles (AuNPs) - are being actively studied.

The aim is to evaluate the effect of five treatment of gold nanosystems (D-*g*-PAA(PE)/AuNPs) and Quercetin on male reproductive function in mice in experimental chronic kidney disease (EChKD).

The study was performed in two series of experiments on male and female mice with EChKD, a model of which was created by immunizing animals with kidney homogenate.

Estimated: sperm viability; the number of sperm (sperm concentration (thousand/ml)) and the number of abnormal forms of sperm (%); the ratio of cells of different generations of spermatogenic epithelium; pathways of cell death of testicular cells (spermatocytes (primary)) and sperm cells of testicular appendages (epididymis); embryonic mortality in mice; the number of live pups per female.

Polymer nanocarrier. Copolymer dextran-graft-polyacrylamide (D-PAA) in nonionic and anionic form D-*g*-PAA and D-*g*-PAA(PE), respectively, was used as a polymer matrix for in situ synthesis of gold nanoparticles (AuNPs) which consists of dextran core (M_w=70·10^5 g/mol) and five grafted PAA chains. The molecular parameters of copolymers were measured by Size exclusion chromatography with Light scattering and Refractometer detectors. The average molecular weight (M_w) for D-*g*-PAA copolymer was 2.15·10^6 g/mol, a radius of gyration (R_g) was 85 nm, and copolymer polydispersity (M_w/M_n) was 1.75. The copolymer in anionic form was prepared by alkaline hydrolysis. The number of carboxylate groups for D-*g*-PAA(PE) was approximately 37% (Bezuglyi et al. 2012; Kutsevol et al. 2012, 2014).

Introduction of substances: according to TEM, AuNPs loaded (synthesized, retained) in D-*g*-PA (PE) are spherical in shape, size 4-11 nm. D-*g*-PAA(PE) (2.00 mg/kg), D-*g*-PAA(PE)/AuNPs (1.96 mg/kg), saline was administered intravenously (in a tail vein of 0.3 ml) once a day, five times according to the immunization schedule after the fourth immunization (the last, 3 weeks after the start of the experiment). Quercetin (Quercetin, Sigma, USA) (50 mg/kg) was administered intraperitoneally once daily, five times according to the immunization schedule after the fourth immunization (last, 3 weeks after the start of the experiment) and after the introduction of gold in the group where they were injected together.

Table 5 **The number of epididymis cells (spermatozoa) with morphological signs of apoptosis and necrosis under conditions of EChKD and treatment with gold nanoparticles in the polymer matrices and Quercetin**

	Spermatozoa, %		
	living	apoptotic	necrotic
Control	88,40±2,40	6,50±1,58	5,10±1,64
D-*g*-PAA(PE)	84,80±2,40	8,80±2,17	6,40±1,41
D-*g*-PAA(PE)/AuNPs	86,20±2,10	7,50±1,87	6,30±1,22
QUE	87,20±2,00	7,00±1,37	5,80±1,00
D-*g*-PAA(PE)+QUE	85,80±2,40	7,70±2,17	6,50±1,41
D-*g*-PAA(PE)/AuNPs+QUE	88,80±2,00	8,75±1,37	2,45±1,00
EChKD	71,80±3,10 *	15,20±2,59 *	13,00±1,41 *
EChKD+D-*g*-PAA(PE)	71,40±3,10 *	14,80±1,30 *	13,80±1,95 *
EChKD+D-*g*-PAA(PE)/AuNPs	79,91±2,40 *#	11,38±1,92 *	7,71±0,89 #
EChKD+QUE	79,70±2,40 * #	9,5±1,92	10,80±0,89 *
EChKD+D-*g*-PAA(PE)+QUE	78,20±2,40 * #	10,1±1,92	11,30±0,89 *
EChKD+D-*g*-PAA(PE)/AuNPs+QUE	84,40±2,40 #	8,6±1,92	7,00±0,89 #

Notes: * - p <0.05 - probability differences in the average group data with respect to these variables in the control group animals; # - p <0.05 - to these variables in the group animals under conditions of EChKD; M±σ.

Table 6 **Embryonic mortality under conditions of EChKD and treatment with gold nanoparticles in the polymer matrices and Quercetin**

	Preimplantation mortality	Postimplantation mortality
Control	8,16±0,73	5,25±0,57
D-*g*-PAA(PE)	9,25±1,70	7,80±1,85
D-*g*-PAA(PE)/AuNPs	8,75±2,05	6,75±1,75
QUE	8,00±0,77	5,15±0,52
D-*g*-PAA(PE)+QUE	8,20±0,33	5,00±0,47
D-*g*-PAA(PE)/AuNPs+QUE	7,16±0,43	5,20±0,37
EChKD	14,76±1,07 *	13,70±1,03 *
EChKD+D-*g*-PAA(PE)	14,77±1,51 *	10,41±1,01 *
EChKD+D-*g*-PAA(PE)/AuNPs	9,14±1,31 #	12,62±1,81 *
EChKD+QUE	9,85±1,31	10,12±1,81 *
EChKD+D-*g*-PAA(PE)+QUE	9,14±1,31	11,62±1,81 *
EChKD+D-*g*-PAA(PE)/AuNPs+QUE	8,04±1,31 #	11,02±1,81 *

Notes: * - p <0.05 - probability differences in the average group data with respect to these variables in the control group animals; # - p <0.05 - to these variables in the group animals under conditions of EChKD; M±σ.

Table 7
The number of live newborns (pups) under the conditions of ECHN treatment with nanoparticles in the polymer matrix and Quercetin

Control	7,64±0,47
D-*g*-PAA(PE)	5,66±0,47
D-*g*-PAA(PE)/AuNPs	6,55±0,50
QUE	7,34±0,47
D-*g*-PAA(PE)+QUE	7,14±0,47
D-*g*-PAA(PE)/AuNPs+QUE	7,55±0,50
EChKD	4,25±0,50 *
EChKD+D-*g*-PAA(PE)	4,00±0,40 *
EChKD+D-*g*-PAA(PE)/AuNPs	5,79±0,50
EChKD+QUE	
EChKD+D-*g*-PAA(PE)+QUE	6,25±0,50
EChKD+D-*g*-PAA(PE)/AuNPs+QUE	6,15±0,40
Control	6,95±0,50 #

Notes: * - p <0.05 - probability differences in the average group data with respect to these variables in the control group animals; # - p <0.05 - to these variables in the group animals under conditions of EChKD; M±σ.

Animals. **Experiments (two series) have been conducted on 120 male and 120 females Albino white laboratory mice (weighing 25-30 g) in compliance with all requirements for work with laboratory animals (International European Convention for the Protection of Vertebrate Animals, Strasbourg, 1986). After the experiments, anesthetized by Nembutal animals were exterminated by cutting the spinal cord.**

In the first series of experiments, **animals (males) were divided into groups treated with: I – physiological solution – control (N=5); II – D-*g*-PAA(PE) (N=5); III - D-*g*-PAA/AuNPs (N=5); IV – QUE (N=5); V - D-*g*-PAA(PE)+QUE**
VI - D-*g*-PAA(PE)/AuNPs+ QUE; VII – EChKD (N=5); VIII - EChKD+D-*g*-PAA(PE) (N=5); IX - EChKD+D-*g*-PAA(PE)/AuNPs (N=5); X - EChKD+QUE (N=5); XI - EChKD+ D-*g*-PAA(PE)+QUE (N=5); XII - EChKD+ D-*g*-PAA(PE)/AuNPs+QUE (N=5); N is the number of animals in the group. On the third day after the last (fifth) injection of substances under ether anesthesia, the experimental material (testes and epididymis) was collected. And evaluated: 1) the number of sperm (sperm concentration (thousand / ml)) and the number of abnormal forms of sperm (%); 2) the ratio of cells of different generations of spermatogenic epithelium (%; 3) pathways of cell death of testicular cells (spermatocytes (primary)) and sperm cells of testicular appendages (epididymis).

In the second series of experiments, **animals were divided in to the same groups treated with: I – physiological solution – control (N=5); II – D-*g*-PAA(PE) (N=5); III - D-*g*-PAA/AuNPs (N=5); IV – QUE (N=5); V - D-*g*-PAA(PE)+QUE**
VI - D-*g*-PAA(PE)/AuNPs+ QUE; VII – EChKD (N=5); VIII - EChKD+D-*g*-PAA(PE) (N=5); IX - EChKD+D-*g*-PAA(PE)/AuNPs (N=5); X - EChKD+QUE (N=5); XI - EChKD+ D-*g*-PAA(PE)+QUE (N=5); XII - EChKD+ D-*g*-PAA(PE)/AuNPs+QUE (N=5); N is the number of animals in the group. On the third day after the treatment, such males were planted to the intact females in a ratio of 1:2 (male/females). Coupling and subsequent manipulation of embryos were performed according to the Mank method (1990). Sampling of experimental material (ovaries, tubes, and uterus) was performed under anesthetic anesthesia for 10/11 days after replanting. The experiment was completed on 24th day after replanting the male with birth in control and experimental animal live newborns (pups). And evaluated/assessed: 1) embryonic mortality in mice; 2) calculated the average number of live newborns (pups) per female.

CONCLUSIONS

Substances used in the work (D-*g*-PAA(PE), D-*g*-PAA(PE)/AuNPs and QUE) did not cause changes in the number of: 1) sperm; 2) spermatogenic cells and Sertoli cells on smears of testicular homogenate; 3) living and dead by apoptosis and necrosis of testicular cells (primary spermatocytes) and epididymis cells (sperm); 4) in the values of pre- and post-implantation embryonic mortality; 5) live newborns (pups) in comparison with such values in control.

Under conditions of EChKD and treatment with D-*g*-PAA(PE)/AuNPs were found: 1) decrease (1.41 times) in the number of abnormal sperm (%); 2) decrease (1.71 times) in the number of necrotic testicular cells (primary spermatocytes); 3) decrease (1.69 times) the number of necrotic cells of the epididymis (sperm); 4) decrease (1.61 times) of the value of preimplantation mortality of embryos in comparison with such values under conditions of EChKD.

Under conditions of EChKD and treatment with QUE were found: 1) decrease (1.60 times) the number of abnormal sperm (%); 2) increase (1.16 times) in the number of spermatids in comparison with such values under conditions of EChKD.

Under conditions of EChKD and treatment with D-*g*-PAA(PE)/AuNPs and QUE, the following were found: 1) decrease (1.88 times) in the number of abnormal sperm (%); 2) increase (1.17 times) the number of spermatids; 3) decrease in the number (1.6 times) of necrotic testicular cells (primary spermatocytes); 4) increase (1.18 times) of living cells and decrease (2.43 times) of necrotic cells of the epididymis (sperm); 5) decrease (1.84 times) of the value of preimplantation mortality of embryos; 6) increase (1.64 times) in the number of live newborns (pups) in comparison with such values under conditions of EChKD.