Article

Study of Wound Healing and Local Irritant Effects of Antioxidant Prophylactic Gel

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Abstract: The effect of dental gel with astaxanthin and polyprenol on the detection of toxicity and local irritant effect on laboratory animals was evaluated. The local inflammatory reaction, wound healing effect, hematological and biochemical parameters of laboratory animals were studied when using a gel with astaxanthin and polyprenol. When conducting a study on laboratory animals, dental gel with astaxanthin and polyprenol showed wound healing effect, absence of toxicity and local irritant effect. The antioxidant gel with astaxanthin and polyprenol showed wound healing properties, as well as the absence of toxicity and local irritant effects on laboratory animals.

Keywords: astaxanthin, polyprenol, dental gel, toxicity.

1. Introduction

The search for new promising dental products with anti-inflammatory, wound-healing, antibacterial and immunomodulatory effects and at the same time showing minimal side effect is quite an urgent task of modern scientific and practical medicine [6,8,9].

Astaxanthin, which is part of the dental gel under study, belongs to the class of carotenoids by its chemical structure and is a natural antioxidant present in various amounts in living organisms [1,3]. Currently, astaxanthin is successfully used in medical practice [2,10]. One of the main tasks in dental practice is to "place" the effective dose of IFN precisely in the focus of inflammation, thus reducing the dosage and the possibility of developing adverse reactions [4,5]. Polyprenols are obtained from the greens of Siberian fir, they play an important function, acting as

natural bioregulators and occurring in small quantities in various plant tissues [4,20]. Polyprenols interact perfectly with antioxidants, increasing the efficiency of their work [3,7].

The gel sample we made contained natural astaxanthin and polyprenol.

2. Materials and methods

The study of wound healing was carried out on mature rats (males, females) with a course of daily intragastric administration of the studied drug and the comparison drug in dosage form for 1 month at the maximum possible dose / volume, which is 1 month at a dose of 0.02 g (100 mg / kg). Rats are the main standard objects in the study of wound healing and local irritating effects of pharmacological substances. The recommended condition for evaluating the studied drug is the use of standard animals of both sexes in the experiment. [1].

The study of the local irritant effect, peripheral blood parameters, biochemical blood parameters on enzyme activity was carried out on rats of both sexes.

The initial body weight (at the first administration of drugs) is 180-200 g, the spread over the initial mass should not exceed ± 10%. Adjustment of the dose of the drug for administration is carried out a week after the start of the experiment based on the latest results of measuring the body weight of animals.

Conditions for keeping animals: standard conditions of the vivarium, placement in groups in marked cages with regularly replaced bedding, with free access to water and food, in conditions of standardized light and temperature conditions.

Each experimental group consists of 10 individuals. The control group consists of 10 individuals. The choice of doses was carried out according to the already available data on the therapeutic dose and the requirements described in the OESD regulatory documentation No. 407. [7,8].

Rats were kept in polycarbonate cages for rats, type R-1, manufactured by Proflab Group of companies, S= 1025 cm2 in groups of 10 individuals of the same sex, on a litter. The floor area in the holding cage for one animal will be 102.5 cm2 (the minimum allowable area is 77 cm2).

Marking of rats is carried out by applying a color mark on the wool (0.5% gentian violet solution).

Clinically healthy animals are included in the experiment after 14 days of quarantine. Conditions for keeping animals: standard conditions of the vivarium, placement in groups in marked cages with regularly replaced bedding, with free access to water and food, in conditions of standardized light and temperature conditions.

Each experimental group consists of 5 individuals of each sex (males, females). The control group consists of 5 individuals of each sex (males, females).

During the experiment, examinations of the animal's condition are carried out in dynamics according to integral indicators; according to functional indicators of the main organs and systems, including physiological, hematological, biochemical indicators with an assessment of histological changes in organs and tissues; as well as with an assessment of local irritant effects (gastrointestinal tract departments).

Statistical data processing was carried out in the GraphPad Prism 8 program (Software GraphPad Software, USA). After determining the normality of the distribution by the Shapiro-Wilk criterion, in the case of a normal distribution, a t-test was used for pairwise comparison or oneway ANOVA (analysis of variance) with the post-hoc Turkey test for multiple comparison. In the case of an abnormal distribution, the Mann-Whitney criterion was used for pairwise

comparison, and for multiple – the Kraskel-Wallis criterion with the Dunn post-hos test. Single-tail tests and a 95% confidence interval were used for the analysis. The differences were considered statistically significant at $p < 0.05$.

3. Results

Effect on body weight, feed and water intake.

During the experiment, the test animals were observed, changes in body weight were recorded weekly. The dynamics of changes in the weight of rats during 30 days of drug use and 14 days after discontinuation of their administration are shown in Table 1.

Table 1. Body weight change in rats during the course of intragastric administration of the studied gel with astaxanthin and polyprenol and placebo, g

	Background	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	Hungry mass
Antioxidant gel with astaxanthin and polyprenols								
N	6	6	6	6	6	3	3	6
M±m	183±80,02	197±63,79	205±75,09	217±69,41	236±65,39	250±54,01	260±37,42	252±80,02
t	-1,07	-1,24	-1,39	-1,77	-1,13	-3,76	-1,13	-1,65
p	0,31	0,24	0,20	0,11	0,31	0,01	0,31	0,13
Placebo								
N	6	6	6	6	6	3	3	6
M±m	186±62,04	195±62,06	2055±63,78	216±20,54	225,6±59,84	245±68,19	255±50,99	240±62,04
t	0,10	-0,08	0,08	0,08	-0,03	1,80	1,74	-0,19
p	0,92	0,94	0,94	0,94	0,98	0,13	0,14	0,86
Control								
N	3	3	3	3	3	1	1	3
M±m	187±93,57	200±88,06	218±67,99	239±43,33	255±49,05	260±18,09	256±12,11	243±93,57
W	0,95	0,95	0,97	0,98	0,97	0,98	0,94	0,89
p	0,41	0,31	0,84	0,94	84,96	0,95	0,55	92,56

*At the significance level α=5% and n=15, the tabular value of the Shapiro-Wilk criterion (W Table)=0,905.

The calculated values of the criterion (Rr.) for the parameters of the rat body mass parameter exceed the tabular value. Thus, when W = > Table. The distribution is normal, the hypothesis H_0 is accepted.

The body weight gain at 5 weeks in experimental animals treated with asntioxidant gel with astaxanthin and polyprenol and placebo differed slightly from the body weight gain of control animals - body weight significantly increased by 4% and 6%, respectively, due to a decrease in feed intake, but, in the recovery period, the growth rates in the study groups leveled off and they did not significantly differ from the mass of control animals, which allows us to conclude about the equitoxicity of the studied drugs.

The daily intake of feed and water was calculated weekly. The amount of food and water consumed by rats receiving the above preparations did not statistically differ from the indicators of animals in the control groups (Table 2.3).

Table 2. Daily feed intake in rats when using antioxidant gel with astaxanthin and polyprenol and placebo, g

	Background	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Antioxidant gel with astaxanthin and polyprenols							
N	6	6	6	6	6	3	3
M±m	22,5±4,52	19,37±3,75	26,5±1,67	29±2,41	21,75±2,69	18,67±6,41	23,33±4,08
t	0,00	-0,99	1,18	1,06	1,13	-1,17	-0,04
p	1,00	0,34	0,27	0,31	0,29	0,30	0,97
Placebo							
N	6	6	6	6	6	3	3
M±m	21,5±2,54	27,5±4,74	19,5±2,66	25,5±1,77	22,6±3,41	17,0±3,54	22,3±4,81
t	-0,22	0,32	-0,66	0,15	0,57	-2,31	-0,15
p	0,83	0,75	0,52	0,88	0,58	0,07	0,89
Control							
N	3	3	3	3	3	1	1
M±m	25,5±5,00	25,0±7,45	21,75±5,74	23,75±6,40	23,75±8,62	20±6,32	20±6,25
W	0,94	0,92	0,88	0,90	0,91	0,88	0,92
p	0,26	0,10	0,01	0,03	0,07	0,14	0,40

*At the significance level α=5% and n=15, the tabular value of the Shapiro-Wilk criterion (W Table)=0,905.

The calculated values of the criterion (Rr.) for the parameters of the rat feed consumption parameter exceed the tabular value. Thus, when W = › Table. The distribution is normal, the hypothesis H_0 is accepted (see Appendix). The obtained empirical values of the Student's T-test are in the zone of insignificance.

Table 3. Daily water intake in rats when using the studied drug and placebo, ml

	Background	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Антиоксидантный гель с астаксантином и полипренолом							
N	6	6	6	6	6	3	3
M±m	34,0±4,05	32,5±4,29	35,0±5,25	25,0±2,86	32,8±4,46	31,7±5,40	31,7±8,90
t	-0,35	-2,04	-1,03	-0,52	-0,21	4,08	1,72
p	0,73	0,07	0,33	0,62	0,84	0,01	0,15
Placebo							
N	6	6	6	6	6	3	3
M±m	31,5±2,67	32,1±2,46	30,6±2,93	31,2±1,86	32,9±3,97	31,3±11,37	32,3±4,81
t	0,39	-1,78	-0,43	-0,20	-0,52	1,34	1,88
p	0,71	0,11	0,67	0,85	0,61	0,24	0,12
Control							
N	3	3	3	3	3	1	1
M±m	30,0±2,44	32,0±3,33	32,5±2,87	31,5±5,0	31,2±6,21	30±10,12	31±11,31
W	0,91	0,88	0,94	0,90	0,92	0,95	0,89
p	0,06	0,02	0,29	0,05	0,11	0,61	0,16

*At the significance level α=5% and n=15, the tabular value of the Shapiro-Wilk criterion (W Table)=0,905.

The calculated values of the criterion (Rr.) for the parameters of the rat feed consumption parameter exceed the tabular value. Thus, when W = › Table. The distribution is normal, the hypothesis H_0 is accepted (see Appendix). The obtained empirical values of the Student's T-test are in the zone of insignificance.

The data of daily water consumption differed slightly at the period of 5 weeks - significantly increased by 6% in experimental animals treated with antioxidant gel with astaxanthin and polyprenol, due to the effect of the drug on the physiological functions of the body, namely, increased diuresis, which is consistent with stress effects, but, in the recovery period, daily water consumption in experimental animals animals receiving the drug and experimental animals

receiving placebo differed slightly from the consumption rate in control animals- significantly decreased by 4% and 3%, respectively, due to the stabilization of physiological functions of the body after discontinuation of the drug, which allows us to conclude about the equitoxicity of the studied drugs.

Effect on peripheral blood parameters

Peripheral blood parameters in animals treated with astaxanthin gel and polyprenols and placebo are presented in Table 4,5,6.

Table 4. The effect of the studied gel with astaxanthin and polyprenols and placebo on the composition of peripheral blood in rats, (M±m) (background)

	RBC, *10^{12}//l (number of red blood cells)	HGB, g/l (hemoglobin)	HCT, % (hematocrit)	MCV, fl (average red blood cell volume)	MCH, pg (average hemoglobin content in erythrocyte)	MCHC, g/l (cf. end of hemoglobin in the erythrocyte)	RDW, mmol/l (width of red blood cell distribution)	WBC, % (number of white blood cells)	Sticks, %	Segments, %	Eosinophils, %	Basophils, %	Monocytes, %	Lymphocytes, %	PLT, % (platelet count)	MPV, % (cf. platelet count indicator)
Antioxidant gel with astaxanthin and polyprenols																
N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
M ± m	4,8 ±0,3	85,7±7,2	24,2±3,9	50,9±1,2	20,2 ±0,9	346,9±18,8	35,6±1,4	17,3±3,9	1±0,2	51,9±4,7	1,6±0,3	3,0±0,3	42,6±4,7	433,1±691	6,4 ±0,4	4,8±0,3
t	-2,72	-0,96	-0,97	-0,30	2,13	0,72	-0,92	1,61	0,78	0,64	0,29	-1,49	0,86	-0,71	-1,89	-0,09
p	0,02	0,36	0,35	0,77	0,06	0,49	0,38	0,14	0,45	0,54	0,78	0,17	0,41	0,50	0,09	0,93
Placebo																
N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
M ± m	6,2 ±0,5	130,6±1(,6	27,6±2,8	52,9±0,6	31,2± 4,3	358,5± 7,9	38,3± 1,4	8,9 ±0,9	1±0,3	46,6±2,9	1,6±0,3	1,6±0,3	49,1± 3,3	346,1±40 6	6,97±0,1	6,2± 0,5
t	0,13	2,27	-0,47	0,36	2,41	1,91	-0,06	0,06	0,59	-0,19	0,25	-1,49	-1,52	0,32	-3,76	1,14
p	0,90	0,05	0,65	0,73	0,04	0,09	0,96	0,95	0,57	0,86	0,81	0,17	0,16	0,76	0,00	0,28
Control																
N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
M ± m	6,1± 0,4	96,2±8,3	29,7±3,8	51,8± 4,9	17±1,3	326,3± 22,5	38,5± 4,2	8,8 ±1,7	0,7±0 3	47,5±4,3	1,5±0,3	2,5±0,7	47,5± 4,5	638,0±94,0	6,45±0,7	6,1± 0,4
W	0,97	0,96	0,98	0,91	0,76	0,97	0,95	0,77	0,77	0,96	0,74	0,24	0,88	0,96	0,98	0,93
p	0,82	0,51	0,93	0,06	0,00	0,83	0,40	0,00	0,00	0,61	0,00	0,00	0,02	0,59	0,93	0,18

*At the significance level α=5% and n=15, the tabular value of the Shapiro-Wilk criterion (W Table)=0,905.

The calculated values of the criterion (Rr.) for the parameters of the rat feed consumption parameter exceed the tabular value. Thus, when W = > Table. The distribution is normal, the hypothesis H0 is accepted (see Appendix). The obtained empirical values of the Student's T-test are in the zone of insignificance.

Table 5. The effect of the studied antioxidant gel with astaxanthin and polyprenols and placebo on the composition of peripheral blood in rats, (M=m) (after 14 days)

	RBC, *10^{12}//l (number of red blood cells)	HGB, g/l (hemoglobin)	HCT, % (hematocrit)	MCV, fl (average red blood cell volume)	MCH, pg (average hemoglobin content in erythrocyte)	MCHC, g/l (cf. end of hemoglobin in the erythrocyte)	RDW, mmol/l (width of red blood cell distribution)	WBC, % (number of white blood cells)	Sticks, %	Segments, %	Eosinophils, %	Basophils, %	Monocytes, %	Lymphocytes, %	PLT, % (platelet count)	MPV, % (cf. platelet count indicator)
Antioxidant gel with astaxanthin and polyprenols																
N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
M ± m	4,1 ±0,6	77,1±12,8	22,2±3,1	54,3±1,6	18,3±1,4	337,6±3,5	38,4±1,4	2,8±4,	0,9±0,2	57,9±4,9	1,6±0,3	2,3±0,3	37,3±4,9	331,4±85,9	6,9±0,2	4,1±0,6
t	-2,08	0,15	-1,41	1,01	0,94	0,00	-0,34	0,46	-0,29	0,48	0,90	-0,51	0,38	-0,52	-0,67	0,85
p	0,06	0,89	0,19	0,34	0,37	1,00	0,74	0,66	0,78	0,64	0,39	0,62	0,71	0,62	0,52	0,42
Placebo																
N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
M ± m	5,5 ±0,6	126,6±13,9	30,1±3,3	54,9 ±0,5	24,9 ±4,9	455,3 ±91,8	40,4±1,4	8,6 ±1,4	0,8 ±0,3	59,5±4,4	1,4±0,3	1,8±0,4	36,5±4,3	346,1±40,6	6,98±0,1	5,5±0,6
t	-0,45	2,52	0,26	1,88	1,30	0,92	0,71	-0,56	-0,55	0,77	0,30	-0,51	-0,33	-0,70	-0,86	1,15
p	0,67	0,03	0,80	0,09	0,22	0,38	0,49	0,59	0,59	0,46	0,77	0,62	0,75	0,50	0,41	0,28
Control																
N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
M ± m	5,8 ±0,3	74,3±14,7	28,9±3,6	51,5±,9	16,1±2,3	337,8±10,9	39,1±0,95	9,95 ±2,5	1,0±0,5	54,5±4,4	1,3±0,3	2,0±0,8	41,0±4,9	872,0±459,8	6,7 ±0,4	5,8±0,3
W	0,92	0,97	0,95	0,84	0,56	0,50	0,84	0,87	0,80	0,95	0,67	0,43	0,81	0,97	0,96	0,98
p	0,11	0,78	0,36	0,00	0,00	0,00	0,00	0,01	0,00	0,34	0,00	0,00	0,00	0,72	0,46	0,86

*At the significance level α=5% and n=15, the tabular value of the Shapiro-Wilk criterion (W Table)=0,905.

The calculated values of the criterion (Rr.) for the parameters of the rat feed consumption parameter exceed the tabular value. Thus, when W = › Table. The distribution is normal, the hypothesis H0 is accepted (see Appendix). The obtained empirical values of the Student's T-test are in the zone of insignificance.

Table 6. The effect of the studied antioxidant gel with astaxanthin and polyprenols and placebo on the composition of peripheral blood in rats, (M=m) (after 14d+2 weeks)

	RBC, *10^{12}//l (number of red blood cells)	HGB, g/l (hemoglobin)	HCT, % (hematocrit)	MCV, fl (average red blood cell volume)	MCH, pg (average hemoglobin content in erythrocyte)	MCHC, g/l (cf. end of hemoglobin in the erythrocyte)	RDW, mmol/l (width of red blood cell distribution)	WBC, % (number of white blood cells)	Sticks, %	Segments, %	Eosinophils, %	Basophils, %	Monocytes, %	Lymphocytes, %	PLT, % (platelet count)	MPV, % (cf. platelet count indicator)
Antioxidant gel with astaxanthin and polyprenols																
N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
M ± m	6,9 ±0,7	129,6±14,6	37,8±3,8	54,4±2,2	18,3±1,1	333,2±15,9	41,1±0,6	20,8±5,4	0,6±0,3	48,1±5,9	1,9±0,4	0,93±0,2	48,5±5,7	579,4±946	6,9 ±0,2	6,9±0,7
t	1,44	1,06	1,53	-0,06	-1,04	-1,45	0,61	0,84	-2,05	-1,07	-0,62		-0,29	1,26	-0,32	1,10
p	0,18	0,31	0,16	0,95	0,32	0,18	0,55	0,42	0,07	0,31	0,55		0,78	0,24	0,76	0,30
Placebo																
N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
M ± m	4,8 ±0,5	97,0±13,0	27,7±3,3	53,0±2,0	18,96±0,9	351,6±14,4	40,4±0,97	7,12±1,4	1,0±0,3	65,6±5,4	1,9±0,4	1,0±0,3	30,5±5,3	339,5±46,2	6,9 ±0,2	4,8±0,5
t	-0,84	-0,63	-0,43	-0,48	-0,71	-0,65	0,03	-2,28	-0,98	1,15	-0,57		0,00	-1,01	-2,42	1,25
p	0,42	0,55	0,67	0,64	0,49	0,53	0,97	0,05	0,35	0,28	0,58		1,00	0,34	0,04	0,24
Control																
N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M ± m	5,4 ±0,3	108,3 ±6,3	29,7±1,6	54,6±3,2	19,95±1,1	364,52±3,5	40,4±1,4	14,3 ±4,3	1,5±0.	57,0±3,4	2,3±0,6	1,0±0,5	38,3±4,5	631,3±17,0	6,6 ±0,2	5,4±0,3
W	0,92	0,94	0,94	0,80	0,96	0,80	0,99	0,84	0,84	0,95	0,81		0,80	0,98	0,92	0,95
p	0,10	0,22	0,24	0,00	0,64	0,00	1,00	0,00	0,00	0,42	0,00		0,00	0,90	0,12	0,42

*At the significance level α=5% and n=15, the tabular value of the Shapiro-Wilk criterion (W Table)=0,905.

The calculated values of the criterion (W calculation) for the parameters of the rat feed consumption parameter exceed the tabular value. Thus, when W is calculated › W is Table. The

distribution is normal, the hypothesis H0 is accepted (see Appendix). The obtained empirical values of the Student's T-test are in the zone of insignificance.

A significant increase in leukocytes by 15% in experimental animals treated with antioxidant gel with astaxanthin and polyprenol and a decrease by 9% in experimental animals treated with placebo in blood plasma compared with control animals indicates the manifestation of effects as a result of 14-day use of the studied drugs. In animals of all experimental groups, after a 14-day course of taking the studied drugs, there are no pathological shifts in the leukocyte nucleus and a significant change in the hematological profile, which allows us to conclude about the equitoxicity of the studied antioxidant gel with astaxanthin and polyprenol and placebo.

After discontinuation of the administration of drugs (recovery period - 2 weeks) and hematological examination, no significant differences were found between the studied gel and placebo. There was a slight significant increase in segmented neutrophils (by 11.3% in the group of experimental animals receiving placebo and by 6% in the group of experimental animals receiving antioxidant gel with astaxanthin and polyprenol compared with the indicators of control animals), which is not associated with a reaction to drugs and indicates the absence of delayed toxic effects. The decrease in hemoglobin content in erythrocytes by 4.7% in the group of experimental animals receiving placebo and by 5% in the group of experimental animals receiving gel with astaxanthin and polyprenol was caused by the stabilization of previously altered hemodynamic parameters when using the studied drug.

Thus, the peripheral blood of rats of all experimental groups after 30 days of administration of the tested antioxidant gel with astaxanthin and polyprenol corresponded to the specific physiological norm in its quantitative and qualitative composition and no significant differences between the studied drug 1 and the placebo drug were revealed.

Influence on blood biochemical parameters

Table 7, 8, 9 presents data on the effect of the studied preparation of an antioxidant gel with astaxanthin and polyprenol and placebo on the main biochemical parameters and on the activity of rat blood enzymes. The study was carried out on a biochemical automatic analyzer ILAB 650 (USA).

Table 7. The effect of the studied preparation Antioxidant gel with astaxanthin and polyprenols and placebo on the main biochemical parameters of the peripheral blood of rats, (background) (M±m)

	Urea, mmol/l	Creatinine, mmol/l	Total bilirubin, mmol/l	AST, units/l	ALT, units/l	SCHF, units/l	Glucose, mmol/l	Total protein, g/l	Direct bilirubin, units/l	LDG, units/l	Amylase, units/l
Antioxidant gel with astaxanthin and polyprenols											
N	6	6	6	6	6	6	6	6	6	6	6
M±m	9,9±0,5	140,6±6,1	5,4±0,6	58,3±3,1	85,1±5,8	65,4±5,0	5,7±0,4	68,3±2,8	1,1±0,1	202,6±13,5	150,4±10,7
t	0,63	4,31	-0,67	-2,10	2,23	-2,78	0,27	1,43	1,51	-0,06	0,69
p	0,54	0,00	0,52	0,06	0,05	0,02	0,79	0,18	0,16	0,95	0,51
Placebo											
N	6	6	6	6	6	6	6	6	6	6	6
M±m	9,6±0,6	126,3±6,4	6,3±0,4	63,6±5,9	71,9±4,6	105,8±10,1	5,97±0,2	68,4±4,2	0,9±0,05	210,9±15,2	145,3±3,4

t	0,19	2,83	0,46	-0,66	1,20	1,25	0,95	1,12	0,46	0,34	0,72
p	0,85	0,02	0,66	0,53	0,26	0,24	0,36	0,29	0,66	0,74	0,49
Control											
N	3	3	3	3	3	3	3	3	3	3	3
M±m	9,4±0,6	96,0±10,89	6,1±0,6	69,3±5,4	59,3±14,7	87,8±7,4	5,6±0,5	61,0±5,8	0,9±0,1	203,8±7,3	139,3±11,8
W	0,95	0,97	0,96	0,98	0,96	0,93	0,89	0,95	0,78	0,96	0,97
p	0,39	0,66	0,63	0,96	0,63	0,12	0,02	0,33	0,00	0,52	0,82

*At the significance level α=5% and n=15, the tabular value of the Shapiro-Wilk criterion (W Table)=0,905.

The calculated values of the criterion (Rr.) for the parameters of the rat feed consumption parameter exceed the tabular value. Thus, when W = › Table. The distribution is normal, the hypothesis H0 is accepted (see Appendix). The obtained empirical values of the Student's T-test are in the zone of insignificance.

Table 8. The effect of the studied antioxidant gel with astaxanthin and polyprenols and placebo on the main biochemical parameters of the peripheral blood of rats, (after 14 days) (M=m)

	Urea, mmol/l	Creatinine, mmol/l	Total bilirubin, mmol/l	AST, units/l	ALT, units/l	SCHF, units/l	Glucose, mmol/l	Total protein, g/l	Direct bilirubin, units/l	LDG, units/l	Amylase, units/l
Antioxidant gel with astaxanthin and polyprenols											
N	6	6	6	6	6	6	6	6	6	6	6
M±m	8,7±0,7	165,8±7,9	6,1±0,3	92,4±16,1	59,4±6,2	54,3±4,8	3,7±0,6	74,4±2,2	1,3±0,1	289,9±29,1	113,4±10,1
t	1,37	0,89	1,79	-0,19	-0,52	-2,15	-2,41	1,54	0,36	0,95	-2,66
p	0,20	0,40	0,11	0,85	0,61	0,06	0,04	0,16	0,73	0,36	0,02
Placebo											
N	6	6	6	6	6	6	6	6	6	6	6
M±m	9,3±0,8	174,4±4,5	5,7±0,4	72,5±10,0	72,5±11,0	69,0±10,3	4,9±0,4	83,3±2,8	1,1±0,1	231,1±20,7	168,6±24,6
t	1,71	1,66	0,58	-1,63	0,14	-0,07	-1,60	2,60	-0,83	-0,63	-0,49
p	0,12	0,13	0,57	0,14	0,88	0,95	0,14	0,03	0,43	0,54	0,64
Control											
N	3	3	3	3	3	3	3	3	3	3	3
M±m	6,9±1,4	151,5±17,9	5,4±0,3	96,8±9,3	69,3±25,2	70,0±4,9	5,9±0,4	63,0±10,1	1,23±0,09	250,5±18,2	189,0±37,1
W	0,92	0,93	0,97	0,96	0,83	0,88	0,94	0,91	0,97	0,98	0,82
p	0,11	0,16	0,74	0,49	0,002	0,015	0,24	0,06	0,78	0,94	0,002

*At the significance level α=5% and n=15, the tabular value of the Shapiro-Wilk criterion (W Table)=0,905.

The calculated values of the criterion (Rr.) for the parameters of the rat feed consumption parameter exceed the tabular value. Thus, when W = › Table. The distribution is normal, the hypothesis H0 is accepted (see Appendix). The obtained empirical values of the Student's T-test are in the zone of insignificance.

There was a significant decrease in glucose and amylase levels in animals treated with the studied drug - antioxidant gel with astaxanthin and polyprenol and placebo (glucose -17%, amylase -32%), as well as a significant increase in protein content in animals treated with placebo by 32% relative to control, indicating a pancreatic reaction, circulatory and hemodynamic changes due to prolonged use (1 month).

Table 9. The effect of the studied antioxidant gel with astaxanthin and polyprenols and placebo on the main biochemical parameters of the peripheral blood of rats, (after 14 days +2 weeks) (M=m)

	Urea, mmol/l	Creatinine, mmol/l	Total bilirubin, mmol/l	AST, units/l	ALT, units/l	SCHF, units/l	Glucose, mmol/l	Total protein, g/l	Direct bilirubin, units/l	LDG, units/l	Amylase, units/l
Antioxidant gel with astaxanthin and polyprenols											
N	3	3	3	3	3	3	3	3	3	3	3
M±m	8,6±1,2	131,3±14,2	5,6±0,3	103,3±33,3	60,0±27,0	48,7±24,1	5,9±0,8	72,0±5,3	1,2±0,1	276,0±34,8	116,0±10,4
t	1,05	-0,47	1,02	0,30	-0,26	-1,54	0,10	0,12	-0,41	0,04	-1,39
p	0,34	0,66	0,35	0,77	0,80	0,18	0,92	0,91	0,70	0,97	0,22
Placebo											
N	3	3	3	3	3	3	3	3	3	3	3
M±m	7,4±0,8	127,7±10,1	5,8±0,4	69,3±26,60	50,7±4,3	43,3±17,0	6,6±0,4	78,0±12,3	1,2±0,3	294,3±15,1	123,0±17,3
t	0,37	-0,65	1,32	-1,20	-0,62	-2,63	1,42	0,61	-0,33	0,89	-1,04
p	0,73	0,55	0,24	0,28	0,56	0,05	0,21	0,57	0,76	0,41	0,34
Control											
N	1	1	1	1	1	1	1	1	1	1	1
M±m	6,95	125	4,7	70	54	73	5,8	72	1,4	307	132
W	0,94	0,85	0,96	0,97	0,72	0,90	0,91	0,94	0,98	0,97	0,84
p	0,55	0,06	0,76	0,93	0,00	0,24	0,29	0,58	0,94	0,89	0,04

*At the significance level α=5% and n=15, the tabular value of the Shapiro-Wilk criterion (W Table)=0,905/

The calculated values of the criterion (Rr.) for the parameters of the rat feed consumption parameter exceed the tabular value. Thus, when W = › Table. The distribution is normal, the hypothesis H0 is accepted (see Appendix). The obtained empirical values of the Student's T-test are in the zone of insignificance.

There was a significant decrease in glucose and amylase levels in animals treated with the studied drug - antioxidant gel with astaxanthin and polyprenol and placebo (glucose -17%, amylase -32%), as well as a significant increase in protein content in animals treated with placebo by 32% relative to control, indicating a pancreatic reaction, circulatory and hemodynamic changes due to prolonged use (14 days).

During the recovery period (14 days after the end of the drug administration), a decrease in the alkaline phosphatase index (by 41%) was observed in animals receiving placebo, in animals receiving the studied drug - an antioxidant gel with astaxanthin and polyprenol, the previously changed indicators (glucose, amylase) did not significantly differ from those in the control animal group, all the indicators have stabilized.

As can be seen from the data presented above, both the studied and placebo drugs in the tested dose do not have a sharply negative effect on the basic biochemical parameters of blood and the activity of plasma enzymes.

Study of possible local irritant action

With intragastric administration of the studied drugs, there were no signs of a local inflammatory reaction of the mucous membrane of the gastrointestinal tract (infiltration, redness), which was confirmed by visual and histological examination.

Examination of the gastric mucosa – the stomach wall is represented by mucous, muscular and serous membranes, the villi of the mucosa are high, the pituitary-cervical sections are not

deepened, uniform distribution of glands, pathological changes of the mucous membrane (hyperemia, edema, erosion) are not revealed. *Examination of the intestinal mucosa (12-digit, thin and thick intestine)* – the muscular membrane is represented by bundles of smooth muscle fibers, the serous membrane is formed by connective tissue and covered with mesothelium, without defects, folding is preserved, the mucous membrane of the small intestine is represented by a single-layer cylindrical epithelium, a wide brush border and multiple goblet cells, crypts are preserved. Intestinal villi without atrophy, all layers of the intestine (own mucosal plate, muscle membrane, serous membrane) without pathological changes (hyperemia, edema, erosion). The serous membrane is formed by a thin connective tissue and is covered with mesothelium. The mucous membrane of the large intestine is without defects, formed by a single-layer cylindrical epithelium, lined with goblet-shaped cells, has parallel crypts. The muscle membrane is formed by the inner circular and outer longitudinal layers of myocytes. The serous membrane is formed by a thin connective tissue and is covered with mesothelium.

No local inflammatory reaction was detected after intragastric administration of drugs (Table 10).

Table 10. Registration of local inflammatory reaction in rats after administration of antioxidant gel with astaxanthin and polyprenol and placebo

Medication	Reaction on time	
	14 days, points (n=3)	14 days+2 weeks, points (n=3)
Antioxidant gel with astaxanthin and polyprenols (n=6)	0	0
Placebo (n=6)	0	0

The histological picture obtained during this study allows us to judge the absence of a local irritant effect in the studied gel with astaxanthin and polyprenol and placebo, which does not exclude the possibility of developing a reaction with individual sensitivity of the body in humans.

4. Conclusion.

In an experimental study on laboratory animals, no local inflammatory reaction was recorded, as well as pathological changes in hematological and biochemical parameters of blood, which indicates the absence of toxicity and local irritating effect of the antioxidant gel with astaxanthin

Application of artificial intelligence: The article is written without the use of artificial intelligence technologies.

Conflicts of Interest: The authors declare no conflict of interest

References

1. Pechinsky SV, Kuregyan AG, Oganesyan ET, Stepanova EF. Synthesis of lutein and astaxanthin esters and prediction of their activity. Journal of General Chemistry. 2019; 89(5):721-5.
2. Capelli B, Talbott S, Ding L. Astaxanthin sources: Suitability for human health and nutrition. Functional Foods in Health and Disease. 2019; 9 (6):430-45.
3. Belov AA, Tsareva TV, Sudarikova NA. Possibilities of local therapy with non-injectable interferon drugs. Russian dentistry. 2016; 9(2):93-94.
4. Rezanka T, Vortuba J. Chromatography of long chain alcohols (polyprenols) from animal and plant sources - J. Chromatogr. 2001; 936 (1-2):95-110.
5. Sharma S, Venkatesan V, Prakhya BM, Bhonde R. Human Mesenchymal Stem Cells as a Novel Platform for Simultaneous Evaluation of Cytotoxicity and Genotoxicity of Pharmaceuticals. Mutagenesis. 2015; 30:391–399.

6. Chibirova TT, Islaev AA. Experience in the use of mesenchymal stromal cells of various origins in the healing of skin wounds. Modern problems of science and education. 2022; 6-2.
7. Rybtsov SA, Lagarkova MA. The development of hematopoietic stem cells in the early mammalian embryo. 2019; 84:297-313.
8. Bigildeev AE, Pilunov AM, Sats NV, Surin VL, Shipunova IN, et. al. Clonal composition of multipotent human mesenchymal stromal cells: application of genetic barcodes for research. 2019; 84:365-379.
9. Lebedeva OS. Creation of a model system for studying the function of genes associated with Parkinson's disease using genetic reprogramming technology: dis. ... cand. biol. sciences. 2016; 158.
10. Dementieva EV, Medvedev SP, Kovalenko VR, Vyatkin YuV, Kretov EI, et. al. The use of patient-specific induced pluripotent stem cells to create a model of hypertrophic cardiomyopathy. 2019; 84:413-422.