Ministry of Health of Ukraine

National University of Pharmacy

Problem Laboratory of Morphofunctional Research

"APPROVE"

Pro-rector on Scientific and Research Work
of the National University of Pharmacy
Prof. S.M. Kovalenko

Signature

2009

Stamp of the NUPh

REPORT:

ON THE STUDY OF SPECIFIC ACTIVITY AND TOXIC ACTION OF WATER SOLUTION OF HYDRATED C_{60} FULLERENE (C_{60}FWS).

(English Version of Report after Ukrainian Manuscript Draft Translation)

Kharkiv – 2009 - Ukraine

Міністерство охорони здоров'я України

Національний фармацевтичний університет

Проблемна лабораторія морфофункціональних досліджень

«ЗАТВЕРДЖУЮ»

Проектор з НДР НФаУ проф. Коваленко С.М.

«____» _____ **2009 р.**

ЗВІТ
ВИВЧЕННЯ СПЕЦИФІЧНОЇ АКТИВНОСТІ ТА ТОКСИЧНОЇ ДІЇ ВОДНОГО РОЗЧИНА ГІДРАТОВАННОГО ФУЛЕРЕНУ C_{60} (ВРГФ).

Харків-2009

THE LIST OF EXECUTORS

1	Head, the Head of Problem Laboratory of Morphofunctional Research (PLMR), the Head of the Department of biology, physiology and anatomy, professor, Doctor of Biological science	L.M. Maloshtan
2	Associate professor, candidate of Biological science	O.M. Shapoval
3	Associate professor, candidate of Pharmaceutical science	O.V. Dolzhikova
4	Assistant lecturer, candidate of Medical science	O.M. Shatalova
5	Assistant lecturer, candidate of Pharmaceutical science	I.M. Mudryk
6	Senior laboratory assistant	K.O. Stepanova

CONTENTS

1. DETERMINATION OF TOXICITY OF WATER SOLUTION OF C_{60} HYDRATED FULLERENE (C_{60}FWS)

1.1. Determination of acute toxicity of C_{60}FWS

In addition to characteristic of pharmacological properties, it is necessary to estimate the level of toxic influence of C_{60}FWS on the organism of experimental animals. Conducted research as for safety study of the active substance – fullerene C_{60} – allowed to determine some characteristics of C_{60}FWS [1].

Materials and methods.

Acute toxicity of concentrated C_{60}FWS, provided for the study by the customer (with fullerene C_{60} concentration 1 mg/l), was studied by the method of G.V. Pastushenko on nonlinear mature white rats of both sexes with body weight of 180-220g at single intragastric and intra-abdominal routes of introduction [1, 3]. The estimation of toxicity was done by generally accepted classification of K.K. Sidorov [2].

At the first stage of the study of toxicological characteristics of C_{60}FWS there was determined acute toxicity in white nonlinear rats of both sexes with body weight of 180-220g at intragastric introduction. The animals were divided into 4 groups: groups 1 and 2 of females, who were introduced C_{60}FWS as a single dose and during the day; groups 3 and 4 of males, who were introduced C_{60}FWS as a single dose and during the day. As concentrated C_{60}FWS, provided for the study, is a liquid, containing 1 mg/l of active substance, it was administrated in rats at doses of 4 ml per animal (maximal permitted quantity of intragastric introduction of liquid substance in rats with body weight of about 200 g [1]) as a single dose and during the day once every hour for 6 times [1, 3].

Observation of the general state and behaviour of the animals was carried out for 14 days. There was considered: appearance of the experimental animals, peculiarities of their behaviour, intensity and character of movements, state of the fur and other indexes, which can be used in estimation of toxic effect.

Results of the study.

The results of the experiment are given in Table 1.1.

Table 1.1

Determination of acute toxicity of concentrated C_{60}FWS in rats at intragastric introduction (n=5)

Group of animals (rats) males ♂ females ♀	Dose, ml/1 animals	Survived intragastric ♂/♀	Died intragastric ♂/♀	Mortality intragastric ♂/♀
1/3	4	5/5	0/0	0/0
2/4	24	5/5	0/0	0/0

Studies in rats demonstrated, that in animals of the first and the third test groups, who received concentrated C_{60}FWS as a single intragastric dose, the state of animals, their behaviour, appetite, locomotor activity, and state of the fur were within the norm, i.e. there were no signs of intoxication.

In animals of the second and the fourth study groups, who received concentrated C_{60}FWS, on the 4^{th} hour there were observed the signs of inhibited locomotion, in some animals – raised fur. Indicated phenomena disappeared the following day. Other signs of intoxication were not seen. All animals survived.

The obtained data proves the absence of toxicity of concentrated C_{60}FWS, provided for the study (1 mg/l fullerene C_{60}) at introduction of 4 ml as a single dose and 24 ml during the day intragastrically.

Therefore, LD_{50} of the studied substance at intragastric introduction was not determined.

For further study of acute toxicity of concentrated C_{60}FWS there was chosen parenteral (intra-abdominal) route of introduction.

At intra-abdominal introduction the rats were divided into 2 groups: group 1 – males, group 2 – females, who received concentrated C_{60}FWS at the dose of

5 ml per animal (maximal permitted quantity of intra-abdominal introduction of liquid substance in rats with body weight of about 200 g [1]) as a single dose.

Observation of the general state and behaviour of the animals was carried out for 14 days. There was considered: appearance of the experimental animals, peculiarities of their behaviour, intensity and character of movements, state of the fur and other indexes, which can be used in estimation of toxic effect.

The results of the experiment are given in Table 1.2.

Table 1.2

Determination of acute toxicity of concentrated C_{60}FWS in rats at intra-abdominal introduction, (n=5)

Group of animals (rats)	Dose, ml/ 1 animals	Survived intra-abdominal	Died intra-abdominal	Mortality intra-abdominal
1 ♂	5	5	0	0
2 ♀	5	5	0	0

Single intra-abdominal introduction of concentrated C_{60}FWS in rats did not cause changes in appearance and behaviour of rats during the observation period, the state of the animals corresponded to the initial physiological state; animal mortality was not observed.

The research demonstrated that introduction of concentrated C_{60}FWS in rats did not cause the signs of intoxication or decrease in locomotor activity. None of the animals died at intragastric (single and during the day) and intra-abdominal introduction of concentrated C_{60}FWS. At the end of the observation period all rats were active, cheerful, had good appetite and bright fur.

Summarizing the obtained results, it is possible to conclude, that the studied concentrated C_{60}FWS did not cause mortality of animals at intra-abdominal and oral routes of introduction. LD_{50} was not determined. Therefore, concentrated C_{60}FWS, provided for the study,

belongs to rather harmless substances according to the classification of K.K. Sidorov [2].

Literature.

1. Preclinical trials of medicinal preparations: guidelines / Edited by O.V. Stefanov – K.: Avicenna, 2001. – 528 p.

2. Sidorov K.K. On classification of poison toxicity at parenteral routes of introduction / K.K. Sidorov // Toxicology of new industrial chemical substances. – M.: Medicina, 1973. – Issue 13. – P. 47-57.

3. Express method of determination of median lethal doses of chemical substances / G.V. Pastushenko, L.B. Marushny, A.A. Zhukov, et al // Hygiene and Sanitation. – 1995. – No 6. – P 46-48.

1.2. Study of chronic toxicity of C_{60}FWS.

The main information as for interaction of the organism and the substance for most studied medicinal preparations is possible to reveal only in chronic and subchronic experiments.

Materials and methods.

Minimal duration of the introduction of the medicinal preparation in chronic toxicological experiment was established due to the purpose and duration of its use in clinical practice. The selected route of introduction of the medicinal preparation to experimental animals also corresponded to the possible way of use in clinics (intragastric).

General toxic influence was studied in dynamics for 30 days in accordance with the Guidelines [3].

The research was conducted on mature rats of both sexes with body weight of 190-220g [5].

During the whole experiment the animals were kept in the same conditions on adequate water and dietary intake.

The influence of C_{60}FWS (with fullerene C_{60} concentration $2x10^{-3}$ mg/l) on the organs and systems at long-term use was estimated on the basis of general state of animals, dynamics of body weight, indexes of cardiovascular system, peripheral blood pattern (number of erythrocytes, leukocytes, haemoglobin, leukogram) functional state of the CNS, liver, kidneys, weight coefficients of internal organs.

Morphological composition of blood and leukogram of the animals were studied according to generally accepted methods of clinical investigation [7, 10].

The influence of long-term action of C_{60}FWS on the liver state of the animals was estimated on the basis of the content of: whole protein, creatinine, serum activity of transminases and glucose [2]. To reveal significant disturbances of membrane cell integrity of the liver and myocardium under the influence of C_{60}FWS, the activity of alanine aminotransferase (AlAT) and aspartate aminotransferase (AsAT) was studied.

Considering the fact, that synthesis of the factors providing coagulation of

blood takes place in the liver, we considered coagulation time as an integral index of hemocoagulation [2].

Function of the pancreas was estimated on the basis of blood glucose level, determined by colour reaction with orthotoluidine [7, 10].

Functional state of kidneys was studied by the complex of methods that allow to estimate filtration, reabsorbtion and nitrogen-excretory functions. In chronic experiment, diuresis and urea content in urine were considered [7].

At the end of the study period the animals were removed from the experiment by euthanasia, and weight of internal organs, such as liver, kidneys, heart, spleen and adrenal glands, was determined.

1.2.1. Influence of C_{60}FWS at long-term use on the dynamics of body weight, weight coefficients of internal organs and blood biochemical indexes of rats.

For this study there were selected experimental groups of female and male rats in order to determine significant toxic influence of C_{60}FWS due to the sex. The rats were divided into the following groups: 2 control groups (females and males), 2 groups (females and males), which received C_{60}FWS at dose of 1.8 ml/kg for 30 days.

The results of observation as for general state and behaviour of the animals demonstrated satisfactory tolerance of daily use of C_{60}FWS for one month. Activity, need in food and water, appearance, reaction to exogenous irritants of the test groups' rats did not differ from those of control ones. There was no animal mortality in any of the groups.

For estimation of significant toxic influence of C_{60}FWS in the organism of rats at long-term use, there was studied the dynamics of body weight and weight coefficients of internal organs (Table 1.3-1.4).

The analysis of the indicated indexes demonstrated that during the experiment under the same nutritional conditions in the group of female animals there was observed more evident increase in body weight at the action of C_{60}FWS

against females of the control groups. Under the influence of C_{60}FWS there was established probable weight gain of adrenal glands in females against control group on average by 1.3 times (Table 1.3). (*As for positive influence on adrenal gland at long-term use of C_{60}FWS see Section 3* **"Study of adaptogenic action of C_{60}FWS on the model of forced swimming in rats"**)

Table 1.3

Influence of C_{60}FWS on the weight of internal organs at long-term use (M±m, n=5)

Indexes	Sex of the animals	Group of the study	
		Control	C_{60}FWS in 30 days of use
Weight of the liver, g	females	5.56±0.43	6.63±0.39
	males	7.17±0.22	6.58±0.29
Weight of the kidney, g	females	1.29±0.07	1.36±0.07
	males	1.69±0.05	1.64±0.08
Weight of the spleen, g	females	0.57±0.06	0.63±0.03
	males	0.84±0.08	0.77±0.05
Weight of the heart, g	females	0.65±0.05	0.74±0.05
	males	0.85±0.05	0.79±0.05
Weight of adrenal glands, mg	females	70.6±7.82	91.4±5.63
	males	55.0±5.03	50.2±4.34

Note.

* – deviation is significant with respect to intact control ($p<0.05$).

But in males at the action of C_{60}FWS variation in dynamics of body weight, weight of internal organs and, in particular, of adrenal glands, did not differ significantly from analogous indexes of control group.

Obtained data allow to assume that the action of C_{60}FWS depends on the sex, which can be stipulated by its influence on sex hormones. So, further it is

important to study the influence of the studied C_{60}FWS on the level of sex hormones, in particular, in females and males.

Table 1.4

Influence of C_{60}FWS on the dynamics of body weight at long-term use (M±m, n=5)

Indexes	Sex of the animals	Group of the study			
		Initial data		Data in 30 days	
		Control	C_{60}FWS	Control	C_{60}FWS
Body weight, g	females	158.0±5.15	178.0±6.04	168,00±3,74	217,0±7,35*
	males	232.0±8.0	216.0±6.21	253,0±9,95	270,0±5,47
Gain of body weight against initial data, g			females	10.0±2.45	39.0±2.45
			males	38.0±3.73*	37.0±3.74

Note.

* – deviation is significant with respect to intact control ($p<0.05$).

The aim of further research into significant negative influence of C_{60}FWS at long-term intragastric introduction was studying its influence on peripheral blood content. With this purpose there were studied in dynamics such hematological indexes as: total hemoglobin, number of erythrocytes, leukocytes; leukogram was also counted.

As a result of conducted studies, it was found that C_{60}FWS did not cause statistically admissible deviations in leukocyte number (Table 1.5), as well as in number of basophiles, eosinophils, neutrophils, lymphocytes and monocytes in control and test groups during the whole period of observation, both in males and females.

In test groups, receiving C_{60}FWS, the deviation in the number of erythrocytes and hemoglobin against control and initial data was not observed.

Table 1.5

Influence of C_{60}FWS on the indexes of peripheral blood in rats at long-term use (M±m, n=5)

Indexes	Sex of the animals	Group of the study		
		Initial data	Control	C_{60}FWS
Coagulation time, min	females	141.60±2.93	141.2±3.39	146.40±3.20
	males	141.60±2.66	149.8±7.64	140.80±2.13
Erythrocytes, 10^{12}/l	females	3.45±0.37	3.66±0.28	3.91±0.21
	males	3.50±0.14	3.78±0.16	3.67±0.26
Hemoglobin, g/l	females	98.31±4.32	152.26±15.43	162.65±14.64
	males	126.99±5.39	169.69±5.34	162.07±9.88
Leukocytes, 10^9/l	females	10.80±0.80	10.40±0.93	11.00±1.05
	males	10.20±0.97	10.60±0.68	10.0±0.71
Basophils, %	females	0.00±0.00	0.00±0.00	0.00±0.00
	males	0.00±0.00	0.00±0.00	0.00±0.00
Eosinophils, %	females	0.60±0.25	0.40±0.25	0.40±0.25
	males	0.60±0.25	0.60±0.25	0.20±0.20
Stab neutrophils, %	females	4.80±0.58	4.80±0.37	5.0±0.45
	males	4.40±0.51	4.40±0.51	5.4±0.40
Segmented neutrophils, %	females	12.40±1.21	10.00±0.78	12.6±1.08
	males	10.80±1.36	10.20±1.16	11.8±1.49
Lymphocytes, %	females	78.80±1.02	81.20±1.43	76.6±0.75
	males	80.80±1.08	80.60±1.50	78.4±2.06
Monocytes, %	females	3.60±0.51	3.60±0.60	4.4±0.51
	males	3.406±0.75	4.20±0.58	4.2±0.58

Note.

* – deviation is significant with respect to intact control ($p<0.05$).

Functional state of the liver in rats, receiving C_{60}FWS for a long time, was estimated by studying indexes that characterize enzyme-synthetic and protein-synthetic functions of the liver. Enzyme-synthetic function of the liver was estimated on the basis of alanine and aspartate aminotransferase activity.

The results of the studies are given in Table 1.6.

Table 1.6

Influence of C_{60}FWS on biochemical indexes of blood in rats at long-term use 30 days (M±m, n=5)

Indexes	Group of the study	Sex	
		males	females
Whole protein, g/l	Control	67.78±2.19	74.62±2.93
Albumin, g/l		32.70±2.21	29.9±1.54
Glucose, mmole/l		5.61±0.33	5.84±0.52
AsAT, mmole/h ml		0.74±0.03	0.68±0.02
AlAT, mmole/h ml		0.59±0.06	0.591±0.05
Whole protein, g/l	Initial data	71.76±2.73	75.86±3.15
Albumin, g/l		30.20±0.77	28.93±1.40
Glucose, mmole/l		5.33±0.36	5.69±0.35
AsAT, mmole/h ml		0.68±0.03	0.73±0.02
AlAT, mmole/h ml		0.56±0.03	0.52±0.05
Whole protein, g/l	C_{60}FWS in 30 days	68.0±3.28	73.1±3.45
Albumin, g/l		28.74±1.29	30.65±0.61
Glucose, mmole/l		5.03±0.19	5.4±0.26
AsAT, mmole/h ml		0.71±0.03	0.75±0.03
AlAT, mmole/h ml		0.49±0.04	0.53±0.05

Note.

* – deviation is significant with respect to intact control ($p<0.05$).

It was determined, that C_{60}FWS in the studied dose at long-term daily use in rats did not cause toxic influence on enzyme-synthetic function of the liver: the activity of indicator enzymes – AlAT and AsAT (Table. 1.6) did not exceed the

indexes of control group rats and was normal [8]. On this basis it is possible to conclude that C_{60}FWS in indicated doses does not influence enzyme-synthetic function of the liver and does not have cytolytic action.

Significant changes in the content of serum whole protein and albumin correspond to disorders in protein-synthetic function due to lesions of liver parenchyma. The estimation of C_{60}FWS influence at long-term use on protein-synthetic function of the liver was done on the basis of the number of serum whole protein and albumin.

Analysis of quantitative content of whole protein and albumin in test groups revealed statistically insignificant differences against intact control (Table.1.6); it indicates the absence of toxic influence of C_{60}FWS on protein-synthetic function of the liver.

The index of blood sugar content is also of great importance in the studies of liver functions. The study of this index did not reveal statistically significant differences in indexes of control and test groups, which proves the absence of negative influence on carbohydrate metabolism in animals.

Table 1.7

Influence of C_{60}FWS on the indexes of the functional state of kidneys in rats at long-term use for 30 days (M±m, n=5)

Group, dose	Sex	Diurnal diuresis, ml	Urine pH	Relative density, g/cm^3
Control	males	3.7±0.5	6.7±0.12	1.005±0.001
	females	3.1±0.23	6.8±0.17	1.006±0.001
Initial data	males	3.9±0.3	6.8±0.17	1.009±0.001
	females	4.1±0.3	6.6±0.21	1.011±0.003
C_{60}FWS	males	1.35±0.20	7.1±0.14	1.005±0.001
	females	4.30±0.38	6.9±0.12	1.007±0.001

Note.

* – deviation is significant with respect to intact control ($p<0.05$).

For estimation of significant unfavourable influence of C_{60}FWS at long-term use on the functional state of kidneys there was used a scheme that provides for determination in the dynamics of diuresis, indexes that characterize functional state of nitrogen-excreting function of kidneys (urinary and serum urea and creatinine). Data indexes characterize filtration capacity of kidneys and reabsorbtion of liquid via renal tubules. The results of the experiment are given in Tables 1.7-1.8.

The given results demonstrate that C_{60}FWS in males and females did not cause statistically significant deviations in the studied indexes. Sugar, ketones and protein in the urine of the experimental animals were not found.

Table 1.8

Indexes of functional state of kidneys in rats after long-term use of C_{60}FWS (M±m, n=5)

Group, dose	Sex	Urinary urea, mmole/l	Serum urea, mmole/l	Serum creatinine, μmole/l
Control	males	256.0±15.4	6.72±0.34	63.0±5.2
	females	237.4±12.7	7.50±0.54	69.4±3.1
Initial data	males	250.2±11.6	8.37±0.42	67.5±6.3
	females	226.3±9.8	7.37±0.46	71.8±4.9
C_{60}FWS	males	240.6±14.7	7.74±0.57	68.9±4.1
	females	232.9±8.7	7.66±0.54	77.3±5.4

Note.

* – deviation is significant with respect to intact control ($p<0.05$).

Therefore, analyzing everything described above, it is possible to conclude that C_{60}FWS at long-term use does not cause toxic influence on vital organs and tissues of the experimental rats.

Literature:

1. Gatsura V.V. Sernov L.N. Elements of experimental pharmacology // M. Medicina. – 2000. – 325 p.
2. Gromashevskaya L.M. Biochemical research at liver diseases // Journal of pract. doctor, 1999. – No1. – P.24-28.
3. Preclinical trials of medicinal preparations (guidelines) / Edited by O.V. Stefanov – K.: Avicenna, 2001. – P. 74-97, 292-306.
4. Doshchitsin V.L. Clinical electrocardiography. – M.: Medical information agency, 1999. – 373p.
5. Zapadnyuk M.P., Zapadnyuk V.I., Zahariy Ye.A. Laboratory animals. Use in the experiment. - K.: Higher school, 1983.-878 p.
6. Lapach S.M., Chubenko A.V., Babych P.M. Statistical methods in medical and biological research with the use of Excel // K.: Morion. 2001.-408 p.
7. Menshikov V.V., Delektorskaya L.N., Zolotnitskaya R.P. et al. Laboratory methods of research in clinic: Reference book. – M.: Medicina, 1987. – P. 106-125, 122, 179-180.
8. The problem of the norm in toxicology (modern concepts and methodological approaches, main parameters and constants) / Trachtenberg I.M., Sova R.Ye., Sheftel V.O., Onikiyenko F.A. Edited by I.M Trachtenberg. – M.: Medicina, 1991. – 208 p.
9. Rybolovlyev Yu.R., Rybolovlyev R.S. Dosing of substances for mammals on constants of biological activity // Reports of the AS of the USSR. – 1979. – Vol. 247. – No 6. – P. 1513-1516.
10. Kolb V.G., Kamyshnikov V.S. Reference book on clinical chemistry. – Minsk: Belarus, 1982. – 366 p.
11. Sidorov K.K. Toxicology of new industrial chemical substances. – M.: Medicina, 1973. – 47 p.

2. Study of cardioprotective action of C_{60}FWS on the model of doxorubicine-induced cardiomyopathy in rats.

From numerous literary sources it is known, that the term “cardioprotectors” refers to medicinal preparations that optimise work and function of the heart, both under normal conditions and in different pathological states, preventing the action of different irritating actions [9]. Cardioprotective activity is an integral index that reflects functional state of the phospholipids, cellular metabolism, ion homeostasis, significant structural and functional changes of cardiac hystiocyte membranes.

Long-term experience testifies to the effect that at use of antitumor anthracycline antibiotic – doxorubicine, especially exceeding its cumulative dose by more than 450-550 mg/kg, there develops a disease identical by its clinical and morphological picture of dilated cardiomyopathy [2].

In the mechanism of specific cardiotoxic doxorubicine action, there is separately distinguished inhibition of nucleic acid and protein synthesis due to the ability to inhibit topoisomerase II and interaction with DNA molecule [3, 12]. Study of doxorubicine-induced cardiomyopathy pathogenesis also indicates two main hypotheses as for reasons of myocardium lesion. The grounds for the first hypothesis are increased relation of doxorubicine to cardiolipine – one of the most spread phospholipids of myocardium membranes, especially nucleus and mitochondria [13]. The grounds for the second one are the ability of doxorubicine to interfere directly oxidising metabolism of myocardium with increased process of generation of active oxygen metabolites and lesion of cardiac hystiocyte membranes [3, 5, 7]. That is why preventive protection of myocardium against destructive influence of toxic compounds provides for the use of preparations with protective action on myocardium.

Materials and methods.

Doxorubicine-induced cardiomyopathy (DCMP) was induced in white nonlinear rats by repeated with 3-day interval intravenous (into caudal vein) introduction of doxorubicine produced by KMP (Ukraine) at doses of 7 mg/kg. In the experiment there were used 36 white nonlinear rats. Cardiomyopathy developed on day 4. Studied C_{60}FWS was introduced at doses of 1.8 ml/kg intragastrically for 7 days before and 5 days after doxorubicine introduction [5].

The group of animals of intact control received equivalent amount of water, which was introduced intragastrically with cannula. On day 6 of the experiment in groups of animals the percentage of survival was counted, ECG was registered, for which the rats were narcotized with intra-abdominal introduction of 1% Barbamyl solution at the dose of 0.8 ml/100 g of body weight.

As a reference preparation there was selected quercetin [4, 5]. The reference preparation was introduced intra-abdominally at the dose of 5 mg/kg. In experimental studies there was used quercetin as granules with apple pectin (BCPP, Kyiv). As for its chemical structure, quercetin is 3',4',3,5,7-pentaoxiflavone. It is one of the most powerful antioxidants. It has membrane-stabilizing action, prevents displacement of oxidative homeostasis of the organism.

The doses of the tested substances for the rats were calculated with specific sensitivity coefficient by Yu.R. Rybolovlyev on the basis of average daily human dose [10].

Determination of metabolic and dystrophic disorders of myocardium tissue was carried out after euthanasia on the basis of aspartate aminotransferase (AsAT) and creatine phosphokinase (CPK) activity with diagnostic test-sets “Lachema”. Aspartate aminotransferase (AsAT) activity in serum, as the indicator of cardiac hystiocyte cytolysis, was determined with Reitman’s method [6] with diagnostic test-sets “Lachema” (Czech).

The estimation of the intensity of oxidative catabolic transformations of structural lipids in the organism of white rats in case of doxorubicine-induced cardiomyopathy was carried out on the basis of biochemical blood analysis and

heart homogenate. In blood and heart tissue there was estimated the content of diene conjugants (DC), reduced glutathione (RG) and malonic dialdehyde (MDA) [11].

Statistical processing of the results of the conducted research was carried out by methods of variation statistics with Student's coefficient (t), X^2 and nonparametric methods of mathematical statistics by White, Fisher and Wilcoxon-Mann-Whitney U test. The calculations were done with special programme Statistica 5.0 for Windows on PC Pentium 2000.

Results of the study.

Data as for the change in body weight and weight of internal organs are given in Table 2.1.

Table 2.1

Influence of C_{60}FWS on body weight and weight of internal organs of rats under conditions of doxorubicine-induced cardiomyopathy (M±m), n=8.

Study conditions	Loss in body weight of rats, g	Weight of internal organs, g			
		Liver	Heart	Kidney	Spleen
Intact control	+ 8.3±0.3	7.20±0.22	0.60±0.02	1.41±0.05	0.60±0.03
Untreated DCMP	-56.5±2.4*	6.82±0.39	0.70±0.03*	1.50±0.09	0.19±0.02*
DCMP, treated with C_{60}FWS	-38.7±1.9 */**/***	7.06±0.25	0.62±0.01 **/***	1.34±0.04	0.28±0.03 */**/***
DCMP, treated with quercetin	-53.6±2.6*	6.91±0.34	0.66±0.01*	1.59±0.06	0.18±0.014*

Note.

* - p≤0.05 deviation is significant with respect to intact control,

** p≤0.05 deviation is significant with respect to untreated DCMP,

*** p≤0.05 deviation is significant with respect to animals, treated with quercetin.

The conducted study revealed that after doxorubicine introduction on day 6 of the experiment in animals of the control pathology group (DCMP without treatment) there were 2 dead animals. Medicinal and preventive introduction of C_{60}FWS at the dose of 1.8 ml/kg in experimental rats first of all increased survival up to 87.5% (against 80% in the group of control pathology), when use of quercetin did not prevent high lethality of animals in the study group (25%, similar to the group of untreated DCMP).

Changes of dystrophic character caused by doxorubicine use were accompanied with significant loss of body weight in untreated rats in comparison with initial data (see Table 2.1). At the same time, rats receiving C_{60}FWS, lost weight less evidently, and body weight of the rats receiving reference preparation, decreased similarly to the changes in the groups of untreated animals.

Lesions of cardiac hystiocyte membrane apparatus due to disorders of cellular metabolism, DNA structure, initiation of peroxidative mechanisms under the influence of doxorubicine caused disorder of contractile myocardium function, which was registered with ECG-examination (Table. 2.2).

Table 2.2

Influence of C_{60}FWS on ECG indexes in rats against the background of doxorubicine-induced lesion of the heart muscle.

Index	Study conditions			
	Intact control	Untreated DCMP	DCMP, treated with C_{60}FWS	DCMP, treated with quercetin
Heart rate, beats per minute	420.1±24.6	276±10.5*	398.3±14.9**	300.7±23.7*
PQ, sec	0.040±0.002	0.053±0.004*	0.043±0.002**	0.048±0.004*
QRS, sec	0.020±0.002	0.014±0.004*	0.016±0.002	0.018±0.002
Q-T, sec	0.062±0.004	0.086±0.009*	0.064±0.006	0.076±0.004*
R, mB	0.66±0.07	0.45±0.05*	0.64±0.06	0.49±0.05
P, mB	0.10±0.02	0.15±0.02	0.10±0.02	0.10±0.02
T, mB	0.15±0.01	0.21±0.02*	0.15±0.02	0.18±0.04
ST shift from isoline, mm	0	2.0±0.6	0.4±0.1**	0.6±0.2**

Note.

* - $p \leq 0.05$ deviation is significant with respect to intact control,

** $p \leq 0.05$ deviation is significant with respect to untreated DCMP.

Doxorubicine-induced cardiotoxicity in rats of control pathology group was manifested by decrease in heart rate in comparison with analogous index of intact control, i.e. was typical for bradyarrhythmia.

The emaciation of functional capacity of the heart muscle was also proved by significant prolongation of PQ interval, increase in amplitude of T and P waves, prolongation of ventricular complex duration (Q-T), significant decreases in residual fragment of ventricular complex – ST segment and low-amplitude QRS complex (low R wave) (see Table 2.2).

On the selected model it was determined that the studied C_{60}FWS causes some changes in the functional activity of the heart, which was registered with ECG. The use of C_{60}FWS in medicinal and preventive regimen had an impact on normalization of heart rate index, which nearly approached the limits of intact

animals. Authentically as for control pathology there were changes in amplitude of T and P waves, decrease in duration of PQ interval (see Table 2.2). There were expressively, though not significantly, reduced ECG-indexes, which characterise the functional state of ventricular apparatus of the heart – QRS complex and Q-T interval, amplitude of R wave.

During ECG-examination of the rats, receiving quercetin, there was registered bradyarrhythmia character of the heart rate practically at the level of untreated animals and with significant difference from intact animals (Table 2.2). Other ECG-indexes had tendency towards intact control indexes with insignificant difference from control pathology.

Reorientation of oxidizing metabolism in myocardium on anaerobic ways due to intoxication and lesion of the heart muscle, induced with doxorubicin is reflected by increased CPC activity and AsAT hyperenzymemia in the serum of rats, observed in animals of control pathology group (Table 2.3). Doxorubicine introduction causes cardiodystrophic changes, characterised by abrupt increase in the activity of creatine phosphokinase (CPC), lactate dehydrogenase (LDG) and aspartate aminotransferase (AsAT) in comparison with indexes of intact animals. AsAT hyperenzymemia can be estimated as the manifestation of cardiomyocytolysis, as well as compensatory synthesis of the indicated key enzyme of transamination reaction, necessary for restoration of nonessential amino acid pool under conditions of proteinaceous degeneration.

Use of C_{60}FWS promotes preserving CPC and LDG activity practically at the level of intact control. The reference preparation also promotes decrease of AsAT and CPC activity, but the action of C_{60}FWS on indicated indexes is more expressed, though the difference of the influence is not significant.

Table 2.3

Influence of C_{60}FWS on serum enzymological indexes in rats on the model of doxorubicine-induced cardiomyopathy

Study conditions	Enzyme activity		
	CPC, μkat/L	LDG, μmole/L	AsAT, Kmole/L
Intact control	0.42± 0.03	7.1±0.6	0.64±0.034
Untreated DCMP	0.92±0.09*	11.2±1.2*	1.36±0.026*
DCMP, treated with C_{60}FWS	0.54±0.04**/***	7.4±0.4**	0.96±0.03**/***
DCMP, treated with quercetin	0.61±0.05*/**	8.3±0.5	1.12±0.027*

Note.

* - p≤0.05 deviation is significant with respect to intact control,

** p≤0.05 deviation is significant with respect to untreated DCMP,

*** p≤0.05 deviation is significant with respect to animals, treated with quercetin.

The intensification of free radical transformation of membrane lipids of cardiomyocytes under conditions of model pathology is proved by significant increase in DC and MDA content, both in serum and in myocardium homogenate (Table 3.4). The intensification of free radical transformation of membrane lipids of cardiomyocytes is proved by high content of MDA and DC reactants, both in myocardium homogenate and serum of untreated rats. Against the background of WSHF use there were registered decreased levels of MDA, RG and DC in heart muscle homogenate and serum.

As for the indexes of antioxidant system there was noticed an increase of RG concentration in blood, and in myocardium homogenate, RG content, on the contrary, significantly decreased in comparison with indexes of intact animals, which reflects emaciation of glutathione protection at organ level. At C_{60}FWS action RG content with significant difference from control pathology approached

intact indexes in blood and myocardium homogenate, at the same time C_{60}FWS activity was at the level of the reference preparation.

Table 2.4

Influence of C_{60}FWS on LPO/AOS state in case of doxorubicine-induced cardiomyopathy

Index	Intact control	Untreated DCMP	DCMP, treated with C_{60}FWS	DCMP, treated with quercetin
	Serum			
MDA, μmole/L	2.0±0.12	5.4±0.26*	3.13±0.23**	4.01±0.20*
DC, μmole/L	1.4±0.01	2.0±0.02*	1.8±0.01	1.9±0.02
RG mg%	16.2±1.2*	36.9±2.3**	21.4±1.8**	27.7 ±2.2*
	Heart homogenate			
MDA, μmole/g	34.7±1.25	160.4±4.4*	130.4±4.9**	120.2±7.0**
DC, μmole/g	3.4±0.5	12.3±0.74*	44.4±1.7**	36.1±1.62**
RG mg%	70.41±12.8*	35.2±6.41*	74.2±10.5**	77.8±11.6

Note.

* - $p \leq 0.05$ deviation is significant with respect to intact control,

** $p \leq 0.05$ deviation is significant with respect to untreated DCMP.

Therefore, it was established experimentally that the studied C_{60}FWS inhibits the formation of doxorubicine intoxication of myocardium in white rats, having expressed normalizing action on the functional state of heart muscle, not yielding to quercetin as for the effect and as for some indexes – even exceeding it. Corrective action of the studied agent was manifested in normalization of electrocardiographic and most typical for this pathology biochemical indexes.

The described changes testify to its cardioprotective properties. Taking into consideration the mechanisms of doxorubicine-induced myocardium lesion and the change dynamics of indexes of lipid peroxidation/antioxidative system (LPO/AOS systems) at the action of C_{60}FWS against the background of experimental pathology, it is possible to assume that therapeutic effectiveness of the studied

agent on the selected model is conditioned by direct dependency of the expressiveness of cardioprotective action on the power of antioxidant effect. Possibly, it is due to indicated mechanisms the capacity of C_{60}FWS to prevent shifting of oxidizing metabolism and provide the integrity and functional ability of cardiomyocytes is realized.

Literature:

1. Balykova L.A. Experimental and clinical study of the effectiveness of metabolic therapy of heart rate disorders: Abstract of a thesis .... Doct. of Med. Sciences-Kazan – 1999.
2. Vasylenko V.K., Feldman S.B., Khitrov N.V. Myocardiodystrophy. – M.: Medicina. – 1989. – 272 p.
3. Vatutin N.T., Kalinkina N.V., Keting Ye.V. Anthracene cardiomyopathy. Donetsk: DonSIAI, 2001
4. Vladimirov Yu.A. Free radicals and antioxidants // Herald of the RAMS. - M., 1998. – No7. – P.43-51.
5. Derymedved L.V. Antioxidants in cardiology: characteristic of most used agents // Provisor.-1998.-No13.-P.42.
6. Doshchytsyn V.L. Clinical electrocardiography. – M.: Medical information agency, 1999. – 373p.
7. Kamyshnikov V.S. Reference book on clinical and biochemical diagnostics / V.S. Kamyshnikov. – M.: MEDpress-inform, 2004. – 920 p.
8. Kapelko V.I., Popovych M.I. Metabolic and functional bases of experimental cardiomyopathy. – Kishinev: “Shtiintsa”, 1990. – 22p.
9. Kapelko V.I. Functional and structural changes of myocardium in the early stage of adriamycin action/ V.I. Kapelko, V.L. Lakomkin, V.G. Tsyplenkova // Cardiological Herald. – 2006. – Vol.1, No 2. – P.182.
10. Cardioprotectors – clinical and pharmacological aspects / I.S. Chekman, N.A. Gorchakova, S.B. Frantsuzova, V.O. Mintser // Ukrainian medical journal. – 2003 - No 6 (38). – P. 18-25.

11. Rybolovlyev Yu.R., Rybolovlyev R.S. Dosing of substances for mammals on constants of biological activity // Reports of the AS of the USSR. – 1979. – Vol. 247. – No 6. – P. 1513-1516.

12. Stalnaya I.D. Method for determination malonic dialdehyde with thiobarbituric acid / I.D. Stalnaya, T.G. Garyshvili // Modern methods in biochemistry. – M.: Medicina, 1977. – P.63–64, 66–68.

13. Dudnakova T.V. Alterations in myocardial cytoskeleton and regulatory protein expression following a single doxorubicin injection / T.V. Dudnakova, V.L. Lakomkin, V.G. Tsyplenkova // J. Cardiovasc. Pharmacol. – 2003. – Vol.41, No5. – P.789-794.

14. Wallace K.B. Doxorubicin-induced cardiac mitochondrionopathy / K.B. Wallace // Pharmacol. Toxicol. – 2003. – Vol.93, No3. – P.105–115.

15. Wold L.E. Doxorubicin induces cardiomyocyte dysfunction via a p38 MAP kinase-dependent oxidative stress mechanism / L.E. Wold, N.S. Aberle, J. Ren // Cancer Detect. Prev. – 2005. – Vol.29, No3. – P.294–299.

3. Study of adaptogenic action of C_{60}FWS on the model of forced swimming in rats.

Abrupt decrease of adaptative ability and functional reserves is the reality of modern human state of the organism. Ecological pressing, highly complicated social medium, psychoemotional overstrain head the list of risk factors for level of health.

For the majority of really existing combinations of pathogenetic factors, the result of long-term influence on the organism is unknown and this complicates its prevention. That is why one of the most promising approaches of disease prevention via increase of the organism's resistance is the use of adaptogens, nomenclature of which in Ukraine is rather limited.

The group of adaptogens includes pharmacological agents that accelerate and improve adaptation of the organism to different factors of the environment [13]. They act non-specifically, but provide expressive regulatory properties, which promote decrease of fatigability, increase in working capacity [3, 5, 14].

Materials and methods.

The determination of adaptogen action of C_{60}FWS was carried out on the model of forced swimming with a load [13]. Swimming of animals is used in pharmacological studies as a model of muscle work. Under the influence of systematic muscle activity in skeletal muscles there is enhanced susceptibility of specific protein-receptors of target organs to metabolic action of their own hormones, activated polyamine synthesis, increased level of proteins that bind fatty acids for most effective supply of energy [4, 5, 12]. Therefore, short-term, adequate as for its intensity, physical activity is a physiological anabolic stimulus. Under conditions of muscle work with use of anabolic agents there is accelerated directivity of the metabolism, increased protein synthesis, muscle growth, total body weight and weight of internal organs [8].

In the experiment there were used mature white rat males with initial weight of 150-170 g. All animals were weighed before the initiation of the experiment and every time before swimming procedure, according to these data there was

calculated the weight of the load (7.5% of the animal body weight). Swimming activity was carried out in the bath with water layer not less then 60 cm at termoneutral water temperature +32°±2°C. The load was attached to the tail of the animal with elastic rubber ring. The observation was carried out until total fatigability (the criterion was 10-second stay of the rat under water). At the stage of group forming there was carried out 3-day adaptation of the animals to swimming and there were selected the rats capable to swim with corresponding load. As the result of selection there were formed 4 groups (8 rats in each): group 1 – control (intact animals that were forced to swim without training on day 15, 30 and 45 of the study), 2 – animals against the background of forced trained swimming, 3 – animals against the background of trained swimming, receiving the studied C_{60}FWS, 4 – against the background of trained swimming, receiving the reference preparation – apilac.

C_{60}FWS was introduced intragastrically at the dose of 1.8 ml/kg daily, as a single dose, for 45 days. The reference preparation, apilac, was introduced on the analogous scheme at the dose of 1.8 mg/kg. Apilac belongs to the group of preparations of apiculture-based products, which influence metabolic processes. It is used in order to increase working capacity, for correction of dystrophic changes.

The estimation of physical endurance was carried out in the dynamics of the capacity to decrease the time of fatigability. The data were registered at the beginning of the study (initial data) and in 15, 30 and 45 days.

Against the background of C_{60}FWS use, there was estimated body weight, weight of internal organs, content of whole protein in muscles (heart and gastrocnemius muscles), content of blood and urine urea of the experimental animals, as well as serum glucose. Biochemical indexes of blood and urine were estimated with diagnostic sets [6]. The study of glucose content was done with glucose oxidative method.

All studied indexes were compared with analogous indexes of intact animals, kept under standard vivarium conditions.

Results of the study.

Data as for the influence of C_{60}FWS on the body weight of the rats against the background of their forced training by swimming are given in Table 3.1.

Table 3.1

Influence of 45-day intragastric introduction of C_{60}FWS on body weight of the rats against the background of forced swimming with a load (M±m), n=8

Study conditions	Body weight, g		Body weight gain vs. data at the beginning of the study, g
	At the beginning of the study	At the end of the study	
Control	166.0±2.60	200.0±2.50	34.0
Trained swimming	161.4±2.94	216.7±5.50*	55.3
Swimming + C_{60}FWS	163.4±2.20	221.0±3.40*	57.6
Swimming + apilac	157.8±3.2	209.0±4.20	51.2

Note.

* - $p \leq 0.05$ deviation is significant with respect to intact control.

As it is seen from Table 3.1, initiated swimming in rats causes significant gain in body weight. It corresponds to the literary data that adequate physical exercise is an anabolic stimulus that lead to acceleration of protein synthesis processes in the organism, which in due course have an impact on increased working ability and endurance [9, 12, 14]. Intragastric introduction of C_{60}FWS against the background of forced swimming also promotes gain in body weight, but insignificant in comparison with animals trained by swimming.

Analysis of the weight of internal organs of the rats against the background of forced swimming with a load demonstrated that the weight of the liver, heart and kidneys in all study groups did not differ significantly from control data (Table 3.2). Taking into consideration the fact that swimming leads to more expressed gain in body weight against intact animals, as well as given data on the weight of internal organs, it is possible to assume that growth of the organism occurs mainly

due to muscle tissue. It is the muscles that gain tension as a result of exercise by swimming.

It is necessary to indicate that in rats against the background of 45-day trained swimming there was observed adaptive gain in the weight of adrenal glands, which can indicate stress tension of the organism by long-term, maybe, inadequate physical exercise.

Table 3.2

Influence of 45-day daily intragastric introduction of C_{60}FWS on the weight of internal organs of rats against the background of forced swimming with a load (M±m), n=8

Study conditions	Absolute weight of internal organs, g			
	Liver	Heart	Kidney	Adrenal glands
Control	5.36±0.09	0.522±0.011	0.615±0.009	0.702±0.003
Trained swimming	5.40±0.15	0.524±0.012	0.608±0.019	0.730±0.004*
Swimming + C_{60}FWS	5.43±0.12	0.523±0.014	0.595±0.013	0.696±0.002
Swimming + apilac	5.42±0.08*	0.511±0.012*	0.620±0.014*	0.715±0.004*

* - $p \leq 0.05$ deviation is significant with respect to intact control.

Use of C_{60}FWS inhibits stress tension at physical exercise and prevents development of adrenal gland hypertrophy (their weights do not differ significantly from intact control data). At the same time, the use of the reference preparation, apilac, has less expressed stress-protective effect on adrenal glands.

Moderate increase of the activity of protein synthesis processes, manifested by increased whole protein in internal organs, can be accompanied by increase of physical abilities of the organism [11].

Obtained experimental data testify to the fact that physical exercise leads to activation of metabolic processes in the organism. The use of the studied C_{60}FWS promotes increase of whole protein in heart and gastrocnemius muscles, not yielding to the reference preparation, apilac (Table 3.3).

Possibly, C_{60}FWS is able to regulate the intensity of intracellular muscle metabolism in the period of their functional activity, optimising conditions both for the work itself and for plastic processes in the recovery period [14].

Table 3.3

Influence of fullerene on whole protein content in the tissues of rats against the background of forced swimming with a load (M±m), n=8

Study conditions	Whole protein content, mg/100mg	
	Gastrocnemius muscle	Heart muscle
Control	16.5±0.40	16.2±0.6
Trained swimming	17.8±0.40	17.7±0.4
Swimming + C_{60}FWS	18.0±1.10*/**	18.2±0.8*/**
Swimming + apilac	18.1±0.6*/**	18.1±0.3*/**

Note.

* - p≤0.05 deviation is significant with respect to intact control,

** p≤0.05 deviation is significant with respect to the group against the background of swimming.

Changes of the indexes of whole protein in muscles supports formation of structural trace, which can provide stable adaptation to possible stress factor.

The results of obtained data (Table 3.4) prove that against the background of trained swimming physical endurance in rats increases: on day 15 – by 1.78 times, on day 30 – by 2.84 times and on day 45 – by 2.9 times.

Use of C_{60}FWS promoted more expressed increase of physical endurance of animals against the background of forced swimming. So, on day 15 of the experiment the studied index exceeded the data of the animals against the background of only trained swimming on day 15 of the experiment by 20.2 %, on day 30 – by 21.0% and on day 45 – by 31.4%.

Table 3.4

Influence of C_{60}FWS on physical endurance of rats on the model of forced swimming with a load (M±m) n=8

Study conditions	Initial data, min.	Physical endurance, min		
		Day 15	Day 30	Day 45
Control	5.1±0.2	4.7± 0.3	4.5±0.5	4.8 ± 0.7
Trained swimming	4.4±0.1	8.4±0.4*	12.8±1.3*	14.0±0.5*
Swimming + C_{60}FWS	4.6±0.3	10.1±0.7*	15.5 ± 0.9	18.4 ± 2.6*
Swimming + apilac	5.0±0.3	9.8 ± 0.5*	14.7± 2.1*	16.5 ± 1.8*

Note.

* - $p \leq 0.05$ deviation is significant with respect to intact control,

** $p \leq 0.05$ deviation is significant with respect to the reference preparation group.

It is necessary to indicate, that the studied C_{60}FWS as for its influence on physical endurance of the rats did not yield to the reference preparation, apilac, exceeding its action approximately by 13.5 % only on day 45 of the experiment.

Therefore, it is possible to conclude, that C_{60}FWS at the dose of 1.8 ml/kg, probably increasing the time of physical endurance, has adaptogenic activity.

It is known, that nitrogen, formed as a result of aminoacid deaminization, is excreted from the organism owing to urea cycle. Adaptogenic action of the preparations can be realized due to formation of the “structural trace” of protein exchange indexes, in particular, be reflected by the changes of the functional state of kidneys.

Possible increase of protein synthesis processes is manifested by decreased serum and urine urea content, which indicates nitrogen retention in tissues. Dystrophic processes that occur under the influence of irrigative agents, on the contrary, are accompanied by increased serum and urine urea content of the experimental animals.

Table 3.5

Influence of C_{60}FWS on serum and urine urea content of the rats on the model of forced swimming with a load (M±m), n=8

Study conditions	Serum urea content, mmole/l	Urine urea content, mmole/l
Intact control	3.96±0.19	45.8±6.3
Trained swimming	4.14±0.20	51.8±5.9
Swimming + C_{60}FWS	3.87±0.21	43.6±6.1
Swimming + apilac	4.28±0.25	50.0±4.3

Note.

* - p≤0.05 deviation is significant with respect to intact control,

** p≤0.05 deviation is significant with respect to the group against the background of swimming.

Additional determination of serum and urine urea content against the background of initiated swimming in rats did not demonstrate significant changes of this index.

Table 3.6

Blood glucose content in healthy rats under the influence of the studied substances after exercise with swimming (M±m) n=8

Study conditions	Blood glucose level, mmole/l			
	Initiation of the study	Day 15	Day 30	Day 45
Intact control	5.53±0.12	5.64±0.21	5.42±0.19	5.21±0.14
Trained swimming	5.40±0.13	4.39±0.19	5.69±0.20	6.09±0.20*
Swimming + C_{60}FWS	5.50±0.15	5.82±0.15	5.37±0.16	5.30±0.14
Swimming + apilac	5.2±0.11	5.35±0.11	5.94±0.15*	6.12±0.12*

Note.

* - p≤0.05 deviation is significant with respect to control.

The main source of energy in the organism at muscle activity is carbohydrates, and hypoglycemia, as well as low level of steroid hormones, decreases significantly the intensity of skeletal muscle contraction. In this connection there was determined blood glucose level in experimental animals.

Data given in Table 4.6 testifies that in the process of exercise by swimming in the rats of experimental groups blood glucose content changed. But in the animals of intact control group no changes of this index was registered. It should be noted that increased blood glucose content under conditions of physical exercise was within the limit of physiological norm.

Basing on the results of experimental data it was estimated that the period of intense muscle endurance in rats under the influence of swimming is reflected first by decrease and then – by gradual increase of glucose level (from 5.40±0.13 to 6.09±0.20 on day 45 of the experiment).

Endurance by swimming against the background of C_{60}FWS introduction promotes preserving of glucose content at the level of intact control during the whole study. In comparison with the data of the group of animals, only trained by swimming, there was noted some decrease in blood glucose level, especially on day 30 and day 45 of the experiment. The reference preparation, apilac, did not influence glucose level, proved by the data, which did not differ from those against the background of exercise only by swimming. Indicated changes of blood glucose content can be explained by activation of glucose capture by tissues (including those by working muscles) in presence of the substance with adaptogenic action.

The obtained experimental data allow to conclude, that the studied C_{60}FWS have adaptogenic action on the chosen model. The spectrum of the given results testifies to the multiple action of the studied agent, owing to which its mild normalizing, probably protective, action on the functions of the organism is realized.

Literature:

1. Alekseyeva S.N. Influence of adaptogens on immune and flood-forming systems under conditions of rational and cytostatic impact: abstract of a thesis…..of Cand. of Sciences. – Novosibirsk, 1996. – 1996. – 16 p.
2. Borisova I.G., Seyfulla R.D., Zhuravlev A.I. Action of antioxidants on physical efficiency and lipid peroxidation in the organism // Pharmacology and toxicology, 1989. – No 4. – P. 89-92.
3. Brekhman I.I. Man and biologically active substances. – L.: Nauka, 1980. – 120 p.
4. Viru A.A., Kyrge P.K. Hormones and sport efficiency. – M.: FiS, 1983. – 158 p.
5. Denisenko P.P. Increase of workability with pharmacological agents of mediator and non-mediator action under usual and experimental conditions // Pharmacological regulation of physical and psychical efficiency. – M., 1980. P. 5.
6. Kamyshnikov V.S. Reference book on clinical and biochemical diagnostics / V.S. Kamyshnikov. – M.: MEDpress-inform, 2004. – 920 p.
7. Meyerson M.G. Adaptation to stress situations and physical overload/ M.G. Meyerson, P.I. Pshennikova. – M.: Medicina, 1988. – 254 p.
8. Mokhan R., Glesson M., Grinhaff P.L. Biochemistry of muscle activity. K.: Olympic literature, 2001. – 295 p.
9. Pchelenko L.D. Adaptogenic effect of ecdysteroid-containing fraction of Serratula Coronata L. / L.D. Pchelenko, L.G. Metelkina, S.O. Volodina // Chemistry of vegetable raw material. – 2002. – No 1. – P. 69–80.
10. Vegetable adaptogens: physiological significance of the analogues of neurotransmitters and hormones of в vegetable origin, their role in prevention of aging diseases / Composite authors. – Odessa: Astroprint, 2000. – 52 p.
11. Seyfulla R.D. Adaptogens and physical efficiency. – M.: OK RF, 1997. – 62 p.

12. Turkhanova L.V. Synthesis of polyamines in skeletal muscles of rats under physical endurance and introduction of 17 – methyl testosterone: abstract of a thesis…..of cand. of biolog. sciences. / L.V. Turkhanova. – SPB., 1998. – 24 p.
13. Pharmacologic correction of fatigue/ Yu. G. Bobkov, V.M. Vinogradov, V.F. Katkov et al. – M.: Medicina, 1984. – 207 p.
14. Yaremenko K.V. Adaptogens as means of preventive medicine. – Tomsk: Tomsk university press, 1990. – 90 p.

4. Study of membrane stabilizing properties of C_{60}FWS by JAGER F.C. method.

It is known, that recovery of permeability of the cell wall that depends on the state of cell membranes, is one of the major factors for treatment of inflammatory pathologies [1, 3].

Disturbance of the integrity of cell membranes, caused by activation of lipid peroxidation processes, leads to the changes of cell functional activity and, as a result, of the organ as a whole [1, 2, 3]. The expressiveness of destructive and inflammatory changes depends on the extent of cell membrane apparatus lesion [4, 5].

To prove membrane stabilizing action of C_{60}FWS at the dose of 1.8 ml/kg we have studied its influence on the process of spontaneous peroxide destruction of erythrocyte membranes on the model of spontaneous haemolysis by Jager F.C. [6].

Materials and methods.

The method of Jager F.C. is based on estimation of the degree of spontaneous peroxide destruction of erythrocyte membranes. For this purpose there was estimated the extinction of ectoglobular hemoglobin that gets into the blood as a result of spontaneous lysis of erythrocyte membranes caused by lipid peroxidation with air oxygen by means of spectrophotometry on SPh-46 at 540 nm [6].

The study was carried out on white outbred rats of both sexes with body weight of 200-220 g. The animals of the test group received C_{60}FWS at the dose of 1.8 ml/kg as intragastric single dose for three days. The reference preparation was vitamin E, which, according to literature [7], has antioxidant properties. It was introduced to the animals at the dose of UA_{50} (unit of activity) 50 mg/kg intragastrically as an emulsion. Control rats received water [4, 5].

On day 4 the animals underwent blood analysis from the caudal vein for determination of erythrocyte haemolysis degree in the test and control groups according to the following formula:

$$X = \frac{(E_1 + E_2)}{2\,E_3} \times 100\ \%,$$

Where

X – haemolysis degree, %;

E_1, E_2 – extinctions of the 1st and 2nd test with working solution;

E_3 – extinction of the test with distilled water.

Statistic processing of the results of the conducted study was carried out with the use of the methods of variation statistics with Student's coefficient (t).

The results of the conducted study are given in Table 4.1.

Table 4.1

The study of membrane stabilizing action of C_{60}FWS (n=5)

Study conditions	Dose,	Erythrocyte haemolysis degree, %	Membrane stabilizing activity, %
Intact control	-	33.80±1.49	-
C_{60}FWS	1.8 ml/kg	18.20±2.99*	46
Vitamin E	50 mg/kg	18.60±2.62*	45

Notes:

* – deviation is significant with respect to intact control, $p \leq 0.05$;

It was estimated that C_{60}FWS at the dose of 1.8 ml/kg had expressed membrane stabilizing action, which is confirmed by significant inhibition of erythrocyte haemolysis degree by 1.9 times against intact control. The analysis of membrane stabilizing influence of C_{60}FWS against the reference preparation, vitamin E, proved that as for its activity C_{60}FWS (46 %) does not yield to vitamin E (45%).

Therefore, the conducted study revealed expressed membrane stabilizing properties of C_{60}FWS, manifested by decreased degree of spontaneous haemolysis as a result of peroxide destruction of erythrocyte membranes, and also proved its anticytolytic action. The revealed properties can be realized due to antioxidant properties of C_{60}FWS, which are proved in literature [8].

Literature

1. Belotskiy S.M. Inflammation. Cell mobilization and clinical effects / S.M. Belotskiy, R.R. Avtalion. – M.: BINOM Press, 2008. – 240 p.
2. Degtyaryova I.I. Diseases of digestive organs / I.I. Degtyaryova – K.: Demos, 2000. – 321 p.
3. Medicinal therapy of the inflammatory process. Experimental and clinical pharmacology of anti-inflammatory preparations / A.Ya. Sigidin, G.Ya. Shvarts, A.P. Arzamastsev et al. – M.: Medicina, 1988.–240 p.
4. Karbusheva I.V. The study of membrane-protective properties of altan / I.V. Karbusheva // Pharmacy Herald. – 2001. – No 3 (27). – P. 174.
5. Karbusheva I.V. Experimental study of pharmacological effectiveness of the novel polyphenol preparation, altan, for ulcer colitis: abstract of a thesis…..of cand. of biolog. sciences: spec. 14.03.05. / I.V. Karbusheva. – O., 2003. – 18 p.
6. Textbook for laboratory and seminar lessons on Biological chemistry: methodological textbook for higher educational establishments/ L.M. Voronina, V.F. Desenko, V.M Kravchenko et al. – Kh.: Osnova, 1996. – 432 p.
7. Mashkovskiy M.D. Medicinal agents: in 2 vol. – 14th ed., revised, corrected and completed. – M.: "Novaya Volna Press" Ltd., 2000. – Vol. 2. – P. 90-91.
8. Hydrated C_{60} fullerenes (C_{60}HyFn) protect liver and brain of rats against oxidising stress, induced by ethyl alcohol and aluminium ions / Tykhomirov A.A., Korsa V.V., Sogina T.M., Klochkov V.K., Andriyevskiy G.V. // Nano-Toxicology: Biomedical aspects: Materials of the 1st International Symposium "NANOTOX", Miami, Florida, Jan., 29 – Febr., 1 2006.- Annotated translation.

5. Study of anti-inflammatory action of C_{60}FWS.

Fullerenes are the third after diamond and graphite allotropic form of carbon, which commonly exists in nature and dissolves only in organic solvents. In order to understand biological and medical properties of fullerene in 1993 G.A. Andriyevsky obtained water solution of fullerenes via "incorporating" them in natural water structure. In particular, there was developed a solution that concomitantly combines the properties of both true solutions and colloidal systems – C_{60}FWS.

Considering the lack of gene- and cytotoxic properties of C_{60}FWS and concomitant presence of powerful antioxidant, anti-atherosclerotic, antitumor and neuroprotective (anti-amyloid) action, further study of pharmacological properties of C_{60}FWS is reasonable.

Due to the fact that inflammatory processes accompany the development of most pathologies, the assessment of anti-inflammatory activity of C_{60}FWS is one of the major tasks of pharmacological studies.

During recent years there can be observed the increase in the number of people with different inflammatory diseases, that is why inflammation is one of the most important problems in general pathology and clinics [1, 2, 5, 9, 11].

Inflammation is the process that develops in any tissue and organ as a reaction of live tissues for local injury, which originated in the course of evolution; it consists of complex stage-by-stage changes of microcirculatory bloodstream, system of blood and connective tissue, directed towards isolation and elimination of disturbing agent and restoration (or substitution) of disturbed tissues [2, 5, 11, 15, 16, 17, 19].

Tissue disruption at the development of inflammatory process is accompanied by structural membrane change and causes getting of its mediators – histamine and serotonin in the centre of inflammation, increased intensity of free radical oxidation, as well as activation of proteases and kinin system [1, 2, 15, 16, 18, 19].

There is increased activity of phospholipase A and transformation of arachidonic acid with formation of prostaglandins (PG) and thromboxanes that act as medullar system, which is tightly connected with the function of cyclic nucleotides. There occurs activation of other eicosanoids, which further leads to antibody formation [10, 11, 16, 19]. There occurs antigen-antibody reaction that activates complement system. As the result of the complex of reactions there occur vascular and cellular reactions, these processes accompany acute inflammation [2, 9, 11, 15].

Disturbance of microcirculation is stipulated by the increased permeability of capillary network, which led to the development if tissue edema and hyperaemia [1, 2, 10]. Further there occurs disturbance of capillary walls, capillary and venular stasis, and increase in intravascular concentration of different cells with aggregation of erythrocytes and thrombocytes, as well as plasma coagulability factors. Exudative link of inflammation is connected with active migration of leukocytes and macrophages from the bloodstream in the centre of inflammation, accumulation blood cell elements in it, as well as local mast cells and fibroblasts [1, 2, 10, 19]. As a result in the centre of inflammation there activates phagocytosis with release of liposomal enzymes into intercellular main substance of the connective tissue, which carry out degradation of tissue decay products, and in case of their excessive activation cause tissue destruction. At the destruction of pathogenetic agent there occurs the final stage of the inflammatory process – reparation that end with healing of tissue defects and discontinuation of the reaction. If the irritation source is still in the organism, acute inflammation transforms into chronic one [2, 11].

Any inflammatory process, irrespectively to its causative factor, progresses similar to chain reaction, which consists of stereotype links and specific elements, typical for this particular inflammation. It starts as inflammatory reaction for cellular disturbance with vascular reaction [1, 2, 5, 8]. At the same time there is increased excretion of biologically active substances – histamine,

serotonin, dopamine and proteolytic enzymes into interzone, which induces activation of the processes of lipid peroxidation (LPO) [10]. Disintegration of lysosomes and release of their enzymes into cytoplasm in case of different lesions activate hydrolytic processes, whose products promote accumulation of hydrogen ions and increase of cytoplasm acid reaction. There develops acidosis, which is an important general sign of cellular damage [1, 2, 9, 10, 11, 19].

Damage of lipid components of cellular and subcellular membranes at inflammation occurs via rather different ways. One of them is peroxidation of unsaturated fatty acids and phospholipids (the process is activated by iron compounds) [1, 11, 18].

The most important cellular reaction at inflammation is the reaction of the immune system [1, 2, 5, 11].

It is considered, that in the regulation of immune inflammation the main role belongs to PG, cyclic nucleotides, adrenergic, cholinergic substances and disorders of glycolytic processes [1, 2, 5, 9, 1, 14, 15, 16, 18, 19].

In complex study of the developed biologically active supplement (BAS) – C_{60}FWS – the significant role belongs to experimental investigations, which include determination of its antiinflammatory activity. The ground for conducted research was powerful antioxidative effect of C_{60}FWS. In its turn, inhibition of the processes of peroxidation of biological molecules, as it is generally known, correlates with inhibition of inflammatory processes, and anti-inflammatory therapy is rather urgent in treatment of wide spectrum pathological states.

Materials and methods.

Determination of anti-inflammatory activity of the preparation was carried out on its antiexudative action, which was studied on the model of carrageenan-induced and zymozan-induced paw edema in rats [3, 4, 6].

At the first stage of the investigation anti-inflammatory activity of C_{60}FWS was studied on the model of carrageenan-induced edema [4, 6, 14, 19] with use of

the dose of 1.8 ml/kg (the dose is calculated by re-calculation from human therapeutic dose with re-calculation coefficient after Yu.R. Rybolovlyev) [12].

The study was conducted on 18 white nonlinear rats of both sexes with weight of 200-220 g. The animals were divided into 3 groups (6 rats in each). One hour before carrageenan introduction the animals of the 1st group received C_{60}FWS intragastrically at the dose of 1.8 ml/kg; animals of the 2nd group received the reference preparation – tablets "Voltaren" ("Novartis", Switzerland), introduced at UA_{50} 8 mg/kg; animals of the 3rd group were not treated (control group), they received equivalent amount of water. Anti-inflammatory activity was assessed by the degree of inhibition of paw edema in animals on day 3 of the study against control animals, in % [6, 3, 4, 18, 19].

One of the most modern approaches in conducting pharmacological screening is the search of inhibiting substances for lipoxygenase pathway of arachidonic acid transformation. Considering the fact, that one of the reasons of inflammation is leukotrienes (LT), it was reasonable to study antiexudative activity of WSHF in case of inflammation induced by zymozan [6, 3, 19, 20].

Zymozan is a structural liposaccharide, located in the cells of mould wall, specifically promotes formation and excretion of LT and provokes local acute inflammatory reaction [6]. At the early stage lipoxygenase inhibitors prevent zymozan-induced edema [7, 20].

Zymozan was introduced in rats subplantarly at 0.1 ml per animals as 2% suspension. Maximal edema developed in 30 min after phlogogen introduction [6].

On the model of zymozan-induced edema in rats as a reference preparation there was used vegetative anti-inflammatory preparation – lipoxygenase inhibitor – Quercetin granules (SIC "Borshchahivskiy CPP", Kyiv) at conditioned therapeutic dose of 5 mg/kg.

Division of animals into experimental groups and introduction of the studied substances was done according to the scheme, as for the model of carrageenan-induced edema.

Anti-inflammatory activity (on the models of carrageenan-induced and zymozan-induced edema) was calculated by the formula:

$$A = 100\% - \frac{(M_{ept} - M_{ht}) \times 100}{M_{epc} - M_{hc}}, \text{ where}$$

A – antiexudative activity, %;

M_{ept} – weight of the edematic paw in the test;

M_{ht} – weight of the healthy paw in the test;

M_{epc} – weight of the edematic paw in control;

M_{hc} – weight of the healthy paw in control.

In all cases the weight of the paw was expressed in mg [6].

The results of the experiments were processed by the methods of mathematical parametric and non-parametric statistics. Statistically significant were considered the data at the level of significance $P \leq 0.05$. To receive statistical conclusions at comparison of corresponding variable samples, after single-factor analysis of variance (or Kruskal-Wallis test for the data not subject to normal distribution law) revealed the difference between experimental groups, there was used Newman-Keuls test for numerical comparison (or Mann-Whitney test). Standard program package "Statistica 6.0" was used.

5.1. Study of antiexudative action of C_{60}FWS on the model of carrageenan-induced edema in rats.

Results of the study.

At the first stage of the research there was conducted a study of anti-inflammatory (antiexudative) action of C_{60}FWS on the model of acute inflammatory edema, which was induced by subplantar introduction of 0,1ml 1 % water solution of carrageenan into hind leg of the rat [5, 6, 15, 16]. It is widely known, that in pathogenesis of carrageenan-induced inflammation in 1.5-5.5 hours after phlogogen introduction, the main role belongs to PG, which enables to draw a

conclusion about the influence of the studied extract on cyclooxygenase system [1, 16].

The results of the experiment are given in Table 5.1.

Table 5.1

The results of determination of antiexudative activity of C_{60}FWS on the model of carrageenan-induced paw edema in rats (n=6)

Study conditions	Gain in paw size after carrageenan introduction (stand. unit)	Antiexudative activity (%)
C_{60}FWS 1.8 ml/kg	16.83±0.40	19.2*/**
Voltaren 8 mg/kg	11.00±0.52	47.2*
Control pathology	20.83±0.60	–

Notes:

1. * – deviation is significant with respect to control pathology, $p \leq 0.05$;
2. ** – deviation is significant with respect to the reference preparation, Voltaren, $p \leq 0.05$.

Obtained data indicate, that C_{60}FWS manifests its anti-inflammatory (antiexudative) activity in 3 hours after phlogogen introduction (this time corresponds to maximal development of carrageenan-induced edema).

So, at the dose of 1.8 ml/kg antiexudative action of C_{60}FWS was 19.2%, it indicates moderate influence of this preparation on inhibition of cyclooxygenase (COG) activity.

Though C_{60}FWS had anti-inflammatory effect, it yielded as for the level of activity to Voltaren. Anti-inflammatory capacity of Voltaren at the dose of 8 mg/kg constituted 47.2%. Voltaren exceeded antiexudative action of C_{60}FWS nearly by 2.5 times. This can be explained by the fact, that Voltaren is a classical synthetic preparation of NSAID group, which, according to literature, is a powerful non-specific COG inhibitor, which leads to decrease of PG production and decreases the expressiveness of inflammation. In addition, Voltaren UA_{50} nearly by 5 orders

exceeds the dose of fullerene C_{60}, provided as C_{60}FWS, which was chosen for the study.

Therefore, there was experimentally determined presence of moderate antiexudative activity of C_{60}FWS at the dose of 1.8 ml/kg on the model of carrageenan-induced edema in rats. Obtained data indicated the ability of the studied agent to influence prostaglandin phase of carrageenan-induced inflammation and inhibit COG.

5.2. Study of antiexudative action of C_{60}FWS on the model of zymozan-induced edema in rats.

In the development of exudation the main role belongs to biologically active derivatives of arachidonic acid. There are two alternative ways of arachidonic acid transformation from cell membrane phospholipids into biologically active compounds: cyclooxygenase way – PG formation with COG participation and lipoxygenase way – LT formation with lipoxygenase (LOG) participation [6, 20]. Activation of both ways is of great significance for the development of the inflammatory reaction, so, the efficiency of anti-inflammatory action of the preparation will depend on its ability to inhibit both COG and LOG activity [6].

In order to determine the ability of C_{60}FWS to inhibit the activity of key enzymes of arachidonic acid transformation we used the model of zymozan-induced edema, which has LT formation in its mechanism of development (for 0.5 hour).

As the reference preparation there was selected quercetin – preparation of polyphenol origin, able to inhibit LOG activity.

Zymozan introduction led to the development of edema in the control group of animals in 0.5 h. Established dynamics of edema development is typical for this model. Abrupt its increase in 0.5 h, probably, is connected with intensive LT formation at the site of inflammation.

Preliminary C_{60}FWS introduction to the animals inhibited the development of zymozan-induced edema by 27.8% (Table 5.2). So, in 0.5 h after zymozan

introduction to the animals, in rats, treated with C_{60}FWS, the edema was significantly lower, by 1.4 times, than in the group of control pathology. So, it is possible to state that C_{60}FWS inhibits the action of LT. However, as for the level of its antiexudative activity, C_{60}FWS somewhat yields to the anti-inflammatory action of quercetin.

Table 5.2

The results of determination of antiexudative activity of C_{60}FWS on the model of zymozan-induced paw edema in rats (n=6)

Study conditions	Gain in paw size after zymozan introduction (stand. unit)	Antiexudative activity (%)
C_{60}FWS 1.8 ml/kg	15.7±0.49	27.8*/**
Quercetin 5 mg/kg	12.3±0.42	43.9*
Control pathology	22.0±0.68	–

Notes:

1 * – deviation is significant with respect to control pathology, $p \leq 0.05$;

2 ** – deviation is significant with respect to the reference preparation quercetin, $p \leq 0.05$.

Preliminary quercetin introduction to the animals also inhibited the development of zymozan-induced edema. As it is seen from the data give n in Table 5.2, in 0.5 h after zymozan introduction the size of edema was 1.8 times less, than in the group of control pathology. Antiexudative activity of quercetin within this term was 43.9%. The latter can be explained by the fact, that quercetin sufficiently influences LT activity.

Therefore, obtained data indicate antiexudative activity of C_{60}FWS. The ability of C_{60}FWS to inhibit the development of zymozan-induced edema at early terms, possibly, is connected with the ability to inhibit LOG activity [9]. On the basis of the obtained data it is possible to draw a conclusion about anti-lipoxygenase activity of C_{60}FWS, which only by 3.4% yields to the action of quercetin. The ability of C_{60}FWS to inhibit the development of zymozan-induced

edema in 0.5 h after inflammation induction also enables to draw a conclusion about its moderate anti-lipoxygenase activity.

Therefore, conducted studies demonstrated that on the model of zymozan-induced edema in rats C_{60}FWS showed sufficient level of its antiexudative activity (27.8%), and on the model of carrageenan-induced edema its anti-inflammatory action was 19.2%. It enables to assume, that at the early stages of inflammatory reaction development C_{60}FWS more actively inhibits the formation of LT, and in 3 hours – moderately PG.

Literature:

1. Belotskiy S.M. Inflammation. Cell mobilization and clinical effects / S.M. Belotskiy, R.R. Avtalion. – M.: BINOM press, 2008. – 240 p.
2. Inflammation: guidelines for doctors / Ed. by V.V. Serov, V.S. Paukov. – M.: Medicina, 1995. – 640 p.
3. Galuzinska L.V. Experimental study of the anti-inflammatory activity of polyphenol extract of the aboveground part of Lotus corniculatus: abstract of a thesis…..of cand. of pharm. sciences: spec. 14.03.05. / L.V. Galuzinska. – Kh., 2008. – 21 p.
4. Jehad Ibrahim. Anti-inflammatory properties of doxycycline and glucosamine hydrochloride combination/ Jehad Ibrahim, E.F. Grintsov, I.A. Zupanets // Clinical pharmacy. – 2004. – Vol. 8, No 1. – P. 52-55.
5. John Hopkins. Big encyclopaedia if medical diagnostics / John Hopkins; ed. by S. Margolis. – M.: AST Astrel, 2007. – 325 p.
6. Preclinical trials of medicinal preparations: guidelines / Edited by O.V. Stefanov – K.: Avicenna, 2001. – 528 p.
7. Karbusheva I.V. Experimental study of pharmacological effectiveness of the novel polyphenol preparation, altan, for ulcer colitis: abstract of a thesis…..of cand. of biolog. sciences: spec. 14.03.05. / I.V. Karbusheva. – O., 2003. – 18 p.
9. Klimenko N.A. Clinical aspects of the study of general inflammation pathology problems / N.A. Klimenko // Medical practice. – 1999. – No 6. – P. 5-10.
10. Klimenko N.A. Mediators of inflammation and principles of anti-inflammatory therapy / N.A. Klimenko // Medical practice. – 1997. – No 5-6. – P. 3-9.
11. Medicinal therapy of the inflammatory process. Experimental and clinical pharmacology of anti-inflammatory preparations / A.Ya. Sigidin, G.Ya. Shvarts, A.P. Arzamastsev et al. – M.: Medicina, 1988.–240 p.

12. Rybolovlyev Yu.R. Dosing of substances for mammals on constants of biological activity / Yu.R. Rybolovlyev, R.S. Rybolovlyev // Reports of the AS of the USSR. – 1979. – Vol. 247. – No 6. – P. 1513-1516.
13. Chernov Yu.N. Physiological role and pharmacological correction of prostanoids and leukotrienes effect / Yu.N. Chernov, G.A. Batishcheva, M.V. Vasin // Pharmacology and toxicology. – 1990. – Vol. 53, No 6. – P. 71.
14. Shubich M.G. Mediator aspects of the inflammatory process / M.G. Shubich, M.G. Avdeyeva // Pathology archive. – 1997. – Vol. 59, No 2. – P. 3-8.
15. Adorini L. Pathogenesis and immunotherapy of autoimmune disease / L. Adorini, F. Sinigaglia // Trenda Immunol. – 1997. – Vol. 18, No 4. – P. 209-211.
16. Babak Mohajer. Eicosanoids and the small intestine / Babak Mohajer, Y. M. Thomas // Prostaglandins and Lipid Mediators. – 2000. – Vol. 61, No 2. – P. 125-143.
17. Brune K. In Pharmacology of inflammation / K. Brune, R. Zauz. – Amsterdam; New York; Oxford: Mosby, 1985. – P. 413-419.
18. Brune K. Modulation by drugs of leukotriene and prostaglandin production from mous peritoheae macrophages / K. Brune, B.A. Peskar // Int. Tissue React. – 1985. – Vol. 7, No 2. – P. 97-103.
19. Di Rosa M. Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine / M. Di Rosa, J.P. Giround, D.A. Willoughby // J.Patol. – 1971. – Vol.104, No 15. – P. 29.
20. Gado K. Zymozan inflammation. A new method suitable for evaluating new anti-inflammatory drugs / K. Gado, G. Gigler // Agents and Actions. – 1991. – Vol. 32, No 1-2. – P. 119-121.

6. Study of antiulcerous action of C_{60}FWS on the model of acetylsalicylic gastric ulcer.

Gastric ulcer (GU) is a chronic disease that has polycyclic course and is characterized by defect formation in the mucous coat of the stomach [1, 10, 11].

In addition to neurogenic, alimentary and endocrine factors, according to modern views, the processes of free radical oxidation are one of the most significant links of GU pathogenesis [2, 9, 10, 11, 12, 13]. It is proved, that activation of free radical oxidation in covering epithelial layer of the mucous coat of stomach is one of the factors that inhibit resistance of the mucous coat of the stomach (MCS) of gastroduodenal zone. It is unconditionally proved, that one of possible reasons of decreased regeneratory abilities at GU is accumulation of cell membrane lipids in the tissues of intermediate products of free radical oxidation (FRO), which inhibit proliferating and secretory processes in MCS and act as inhibitors of cellular distribution [4, 8, 9, 12, 15, 16, 17]. There is data on significant activation of FRO processes in plasma and erythrocyte membranes, as well as directly in MCS of GU patients [4, 10, 11, 12, 15, 16, 17]. In some works there are indications as for a significant role of free radicals in pathogenesis of experimental ulcers [4, 15, 16, 20, 24, 26].

One of FRO activation factors may be antioxidant insufficiency of MCS [4, 15, 16, 27, 28].

MCS destruction as a consequence of activation of lipid peroxidation (LPO) is also connected with abrupt decrease of antioxidant activity, presence of hypoxia, ischemia, disorders of microcirculation, changes in rheological properties of mucus, which protects MCS cells against damages of aggressive level of fatty acids hydroperoxides [4, 15, 16, 17].

Therefore, GU develops against the background of LPO activation and devastation of endogenous antioxidative system (AOS) reserves. Chain increase of LPO and this beyond AOS control process cause disorders of tissue metabolism, (membrane disintegration, secondary damaging action of lysosomal hydrolases, DNA mutations, disorganization of enzyme activity, inhibition of formation of cell

division regulators, etc.), which causes trophic changes. In MCS the essence of the destructive influence of FRO processes comes to different microscopic and histological changes: vessel damage, bleedings, haemorrhages, epithelium desquamation, stasis development in microvessels, which lead to necrosis and development of gastroduodenal ulcers [2, 4, 8, 9, 15, 16].

The main task in GU treatment is fast elimination of acute condition and decreased quantity of disease recurrences. Considering intensification of LPO processes and decreased provision of the organism with antioxidants, many researches admit the appropriateness of the use of antioxidant preparations at GU treatment. It is determined, that introduction of antioxidants is pathogenically grounded and improves therapy results, promoting more efficient healing of ulcerous defect [4, 12, 13, 15, 16, 17, 18, 19, 23, 26].

The objective of our investigation was pharmacological study of antiulcerous action of C_{60}FWS on the model of acetylsalicylic gastric ulcer in rats. The grounds for conducted studies were availability of powerful antioxidant effect in C_{60}FWS proved earlier. In its turn, intensification of LPO processes is one of the most significant reasons of ulcerogenesis. Inhibition of the processes of peroxidation of biological molecules, as it is known, correlates with inhibition of inflammatory processes, which is actual in GU therapy.

Materials and methods.

The study of antiulcerous action of C_{60}FWS was conducted on the model of acetylsalicylic gastric ulcer in rats, which was reconstituted in accordance with Guidelines of Pharmacological Centre of the MH of Ukraine [3].

In the process of modelling of this pathology experimental animals received acetylsalicylic acid (ASA) intragastrically at the dose of 150 mg/kg 5-fold for 3 days [3, 15, 22, 25]

As a reference preparation there was used a known antiulcerous preparation, Altan, at its conditionally therapeutic dose of 1 mg/kg [6, 7, 17, 18, 19].

The study was carried out on 24 mature white non-linear rats with body weight of 200 – 220 g. The animals were divided into 4 groups: group 1– intact

animals; group 2 – animals with untreated MCS pathology; group 3 – animals, receiving C_{60}FWS at the dose of 1.8 ml/kg (the dose is calculated by re-calculation from human therapeutic dose with re-calculation coefficient after Yu.R. Rybolovlyev [14]); group 4 – animals, receiving reference preparation, Altan.

C_{60}FWS and reference antiulcerous preparation Altan were introduced to experimental groups of animals intragastrically in preventive and medicinal regimen (concomitantly with pathology formation) [3, 15].

Control group animals received the equivalent amount of water.

On day 3 of the study the animals were removed from the experiment and there was conducted the estimation of microscopic changes in order to determine the degree of protective action of the studied agents under conditions of experimental GU.

Medicinal and preventive action of C_{60}FWS and the reference preparation was estimated on their influence on the course of the disease in rats against group of the animals of control pathology [3].

The index of the severity of the lesion is presence of hyperaemia, edema, disorders of rugosity, haemorrhage of MCS. In addition, there was assessed the degree of MCS ulceration in each group of animals on such generally accepted indexes as average area of ulcers in the group (expressed in points [5]), % of animals with ulcer in the group and ulcerous index (UI).

Gradation of the ulcerous surface was carried with 5–point scale due to the area of each ulcerous defect [5].

UI value was calculated by the formula [3]:

$$UI = \frac{S_u \times T_u}{100}, \text{ where:}$$

S_u – area of the ulcer, mm

T_u – % of animals with ulcer in the group.

All experimental studies were conducted in accordance with “General ethic principles of experiments on animals» (Ukraine, 2001), which conforms to the statements of “European Convention for the Protection of Vertebrate Animals Used for Experimentation and other Scientific Purposes” (Strasbourg, 1985), as well as “Guidelines on removal of laboratory animals from the experiment” [3].

The results of the experiments were processed by the methods of mathematical parametric and non-parametric statistics. Statistically significant were considered the data at the level of significance $P \leq 0.05$. To receive statistical conclusions there was used standard program package “Statistica 6.0”.

Results of the study.

The model of MCS lesion with ASA is characterised by subchronic course and erosion hemorrhagic lesions of MCS. It is thought, that the mechanism of damaging influence of ASA on the mucous coat of the stomach occurs via direct local irritative influence on MCS and is accompanied by focal necrosis, which promotes the loss of protective barrier properties of the mucous, epithelium desquamation and development of massive areas of haemorrhagic erosions and ulcers [3, 4, 15, 16].

The results of modelling of acetylsalicylic ulcer are given in Table 6.1.

The analysis of results of the experiment indicated, that all animals of intact control group as for their general state were normal, and at macroscopic study of their stomachs no changes in MCS or ulcerous defects were observed (Fig.6.1).

Fig. 6.1. State of the mucous coat of the stomach in rats of intact control group.

Table 6.1

Antiulcerous activity of C_{60}FWS on the model of acetylsalicylic ulcer in rats (n=6)

Study conditions	Number of animals with ulcer in the group, %	Average area, points	Ulcerous index (UI), stand. unit	Antiulcerous activity, %
Control pathology	100%	18.67±0.88	1.12	–
C_{60}FWS, 1,8 ml/kg	83%	5.33±1.15*	0.27	76.3
Altan, 1mg/kg	67%	3.67±1.23*	0.15	86.7

Note:

* – deviation is significant with respect to control pathology, $p \leq 0.05$;

By the results of the study it was estimated, that intragastric introduction of ASA to the animals for 3 days led to worsening of their general and development of MCS lesion. In control pathology group the general state of the animals was bad against intact control group: there was observed loss of appetite, decreased

locomotor activity, weak reaction to external irritations, the animals drank much water.

At examining the stomachs of the animals of control pathology group it was noted that ASA introduction led to development of a number of deep, both dotted and massive, MCS ulcerations. Edema, significant hyperaemia, a great number of MCS haemorrhages, disorders of its rugosity (Fig.6.2) were observed.

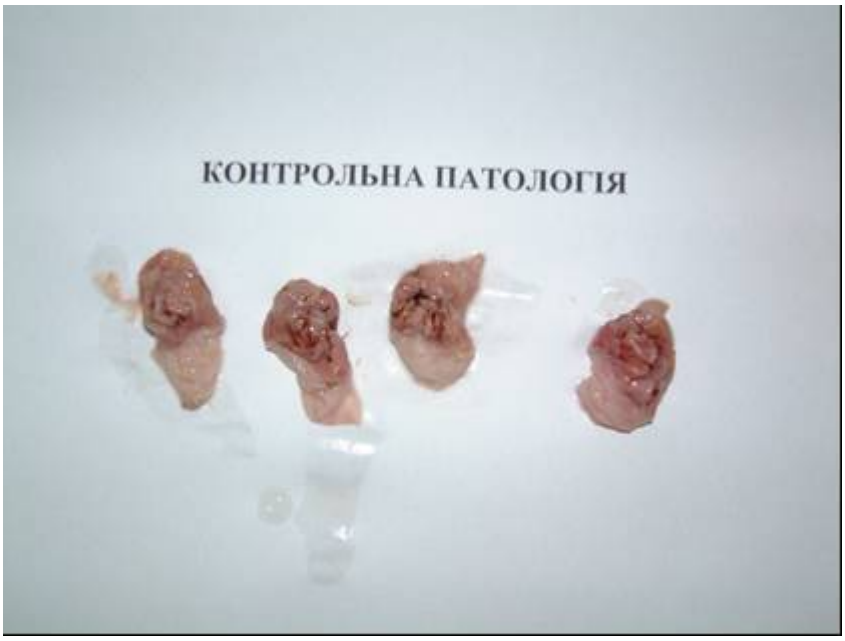

Fig. 6.2. State of the mucous coat of the stomach in rats of control pathology group (hyperaemia, disorders of rugosity, numerous haemorrhages, ulcerous defects).

Abdominal distension was observed in all animals. Ulcerous defects were observed in all animals of this group (100%), the degree of MCS ulceration was 18.67±0.88 points, UI value – 1.12 (Table. 6.1).

The introduction of C_{60}FWS at the dose of 1.8 ml/kg and the reference preparation, Altan, at the dose of 1 mg/kg led to significant improvement of the animals' state. Animals' appearance was satisfactory. There were no signs of anxiety, abdominal distension, loss of appetite and decreased locomotor activity. At macroscopic examination of the stomachs of the rats of these groups the rugosity and colour of the mucous coat of the stomach nearly did not differ from the characteristics of the group of intact animals (Fig.6.3 and Fig.6.4).

Under the influence of C_{60}FWS at the dose of 1.8 ml/kg, at macroscopic examination of the MCS of the rats, there was observed significantly less number of ulcerations, the haemorrhages were not numerous and dotted, the colour and rugosity of the MCS were close to from the characteristics of the intact control group (Fig.6.3). The number of animals with ulcers in the group decreased to 83%. In comparison with the group of control pathology there was observed significant decrease of average area of ulcers and UI at the action of C_{60}FWS at the dose of 1.8 ml/kg – by 3.5 and 4.1 times, correspondingly (Table.6.1). Analysing the obtained results, it is possible to say, that C_{60}FWS introduction in medicinal and preventive regimen led to inhibition of the ulcerous process course.

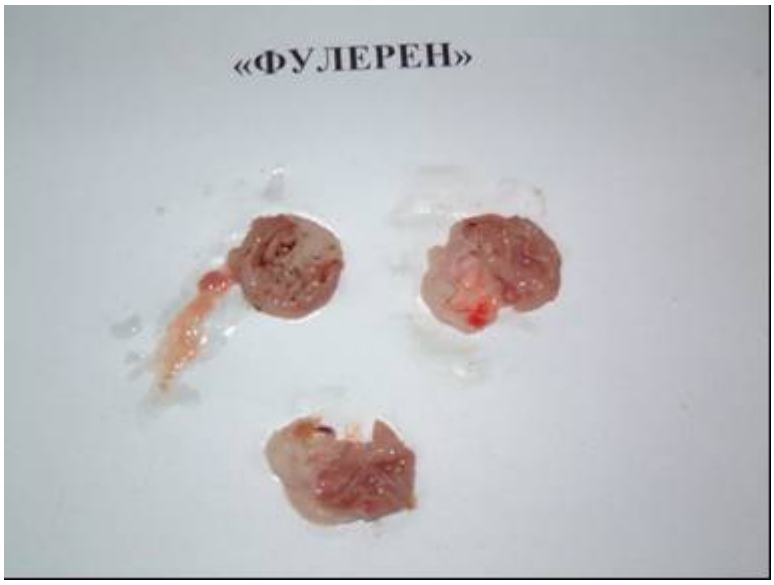

Fig. 6.3. State of the mucous coat of the stomach in rats under the influence of C_{60}FWS.

Introduction of the reference preparation, Altan, also caused decreased expressiveness of the ulcerous process course in MCS of rats (Fig.6.4). Ulcers were observed in 67% of the study group animals. Under the influence of the reference preparation, Altan, average area of ulcers decreased by 5 times, and UI value – by 7.5 times against the group of control pathology (Table.6.1).

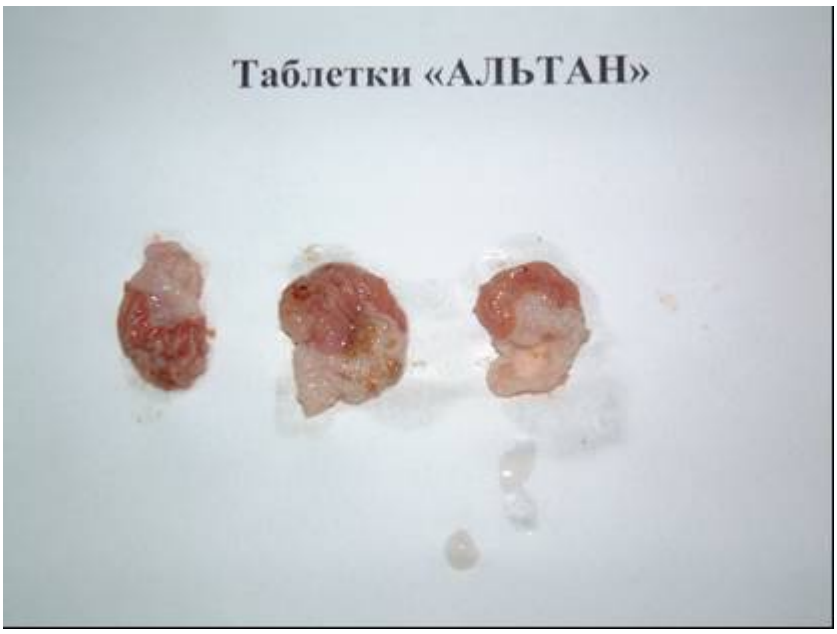

Fig. 6.4. State of the mucous coat of the stomach in rats under the influence of Altan.

As the result of the experiment it was determined, that on the model of subchronic lesion of MCS in rats, induced by ASA, there was an expressed anti-ulcerous action of C_{60}FWS, which constituted 76.3% and did not yield significantly as for its expressiveness to the reference preparation, Altan, (86.7%), it indicates expressed medicinal and preventive influence of C_{60}FWS at the dose of 1.8 ml/kg and shows its ability to restore damaged tissues of the mucous coat of the stomach in rats.

Therefore, model of subchronic lesion of MCS, induced by ASA, there was determined an expressed anti-ulcerous action of C_{60}FWS at the level of 76.3%, which indicates its expressed medicinal and preventive influence on MCS. Under conditions of experimental acetylsalicylic gastric ulcer in white rats it was proved, that, for its expressiveness of anti-ulcerous effect C_{60}FWS at the dose of 1.8 ml/kg does not yield to the reference preparation, Altan, at the dose of 1 mg/kg. Obtained experimental data enable to draw a conclusion about prospects of further deeper study of pharmacological properties of C_{60}FWS.

Literature:

1. Bereznyakova A.I. Physiopathology / A.I. Bereznyakova. – Kh.: NUPh press, 2000. – P. 129-154.
2. Vladimirov Yu.A. Lipid peroxidation in biological membranes / Yu.A. Vladimirov, A.I. Archakov. – M.: Nauka, 1972.–252 p.
3. Preclinical trials of medicinal preparations: guidelines / Edited by O.V. Stefanov – K.: Avicenna, 2001. – 528 p.
4. Drogovoz S.M. Comparative study of different antioxidants on functional indexes of the work of gastro-intestinal tract / S.M. Drogovoz, T.O. Kutsenko, I.M. Kartashevska, N.D. Bunyatyan // Liky. – 2007. – No 1–2. – P. 46 – 49.
5. Zupanets I.A. Grounds for the use of the combination of ethanol derivatives in the screening of gastroprotectors / I.A. Zupanets, L.V. Yakovleva, V.V. Prypysnova // Clinical Pharmacy. – 1998. – Vol. 2, No 3. – P. 29 – 33.
6. Karbusheva I.V. Study of the influence of Altan and Salazopyridazinum on functional activity of gastro-intestinal tract / I.V. Karbusheva, E.V. Gladukh // Clinical Pharmacy. – 2002. – Vol. 6, No 2. – P. 66 – 69.
7. Karbusheva I.V. Experimental study of pharmacological effectiveness of the novel polyphenol preparation, altan, for ulcer colitis: abstract of a thesis…..of cand. of biolog. sciences: spec. 14.03.05. / I.V. Karbusheva. – O., 2003. – 18 p.
8. Maloshtan L.M. Study of regenerative and anti-ulcerous activity of Circassian walnut / L.M. Maloshtan, O.G. Bashura, T.M. Kovalyova // Clinical Pharmacy – Vol. 3, No 2. – P. 153 – 156.

9. Menshikova Ye.B. Oxidative stress anti-inflammation / Ye.B.Menshikova, N.K. Zenkov // Success of modern biology. – 1997. – No 2. – P. 155-171.
10. Moksimova O.G. Digestive diseases in children: textbook / O.G. Moksimova, I.I. Petrukhina. – Rostov-na-Donu.: Phenix, 2006. – 144 p.
11. Noskov N.A. Gastroenterology. Actual medical therapy: reference book/N.A. Noskov. – Rostov-na-Donu.: Phenix, 2008.–395 p.
12. Piter R. McNely Secrets of Gastroenterology // Piter R. McNely; ed. by prof. Z.A. Aprosina. – M.: BINOM press, 2005. – 928 p.
13. Polushkina N.N. Diagnostic reference book of gastroenterologist / N.N. Polushkina. – M.: AST, 2007. – 670 p.
14. Rybolovlyev Yu.R. Dosing of substances for mammals on constants of biological activity / Yu.R. Rybolovlyev, R.S. Rybolovlyev // Reports of the AS of the USSR. – 1979. – Vol. 247. – No 6. – P. 1513-1516.
15. Seyfeddin M.S. Comparison of the activity of modern and prospective antioxidants on the model of acetylsalicylic ulcer / M.S. Seyfeddin, T.O. Kutsenko, K.G. Shchokina // Pharmacy Herald. – 2004. – No 3 (39). – P. 72– 75.
16. Seyfiddin M.S. Pathogenetic grounds of the necessity to use antioxidant preparations at ulcer disease and their characteristics / M.S. Seyfiddin, S.M. Drogovoz, T.O. Kutsenko, K.G. Shchokina // Clinical Pharmacy. – 2004. – Vol. 8, No 2. – P. 27 – 30.
17. Comparison of antioxidant properties of new preparations, derivatives of bioflavonoids and tanning agents / L.V. Yakovleva, O.A. Gerasimova, I.V. Karbusheva, N.D. Bunyatyan, T.S. Sakharova // Experimental and clinical pharmacology. – 2001. – Vol. 64, No 2. – P. 55 – 59.
18. Yakovleva L.V. Analysis of the results of clinical trials of the medicinal preparations, developed in NUPh / L.V. Yakovleva // Clinical Pharmacy. – 2000. – Vol. 4, No 2. – P. 63-65.

19. Yakovleva L.V. Altan, a new preparation for treating ulcer disease of gastro-intestinal tract / L.V. Yakovleva, O.S. Yevdokimova // Pharmacy Herald. – 1993. – No 1–2. – P. 96 – 103.
20. Bandyopadhyay S.K., Pakrashi S.C., Pakrashi A.J. // Ethnopharmacol. 2000. – Vol. 70, No2. - P. 171-176.
21. Bast A. Oxidants and antioxidants: State of the art / A. Bast, R. M. M. Guido, C. I. A. Doclam // Americ. T. Med. – 1991. – Vol. 91, No 3. – P. 12–13.
22. Bigby M. Cutaneous reactions to nonsteroidal anti-inflammatory drugs. A review / M. Bigby, R. Stern // J. Amer. Acad. of Dermatol. – 1985. – Vol. 12, No 5. – P. 866-876.
23. Brune K. In Pharmacology of inflammation / K. Brune, R. Zauz. – Amsterdam; New York; Oxford: Mosby, 1985. – P. 413-419.
24. Klein H., Cremer G. // Rheumamediz. – 1991. – Vol. 4. – P. 136-141.
25. Loeb D.S. Management of gastroduo-denopathy associated with use of nonsteroidal anti-inflammatory drugs / D.S. Loeb, D.A. Ahlquist, N.J. Talley // Mayo Clin. Proc. – 1992. – Vol. 29, No 67. – P. 354-364.
26. Martin M., La-Cosa C., Alarkon-de- la Lastra C. et al. //Z. Naturforsch. [C] – 1998. – Vol. 53, No1-2. – P.82-88.
27. Suzuki Y., Ishihara M., Segami T., Ito M. // Jpn. J. Pharmacol. – 1998. Vol. 78, No4. – P. 435-441.
28. Yamaguchi F., Saito M., Ariga T. et al. // Fgric. Food Cytm. 2000. – Vol.48, No6. – p. 2320-2325.

7. Study of the influence of C_{60}FWS on the processes of cytolysis, lipid peroxidation and antioxidant protection in rats under conditions of subchronic liver lesion, induced by ethanol and tetrachlormethane.

In accordance to modern views on pathogenesis of pathology development, one of the starting mechanisms is initiation of the processes of lipid peroxidation (LPO) of cell membranes, which is accompanied by their destruction, formation of free radicals, lipid peroxides and activation of inflammation factors – biogenic amines, kinins, prostaglandins and leukotrienes. The excess of lipid peroxides disturbs physicochemical structure of cell membranes and inhibits their enzyme systems, inactivates cytoplasmic enzymes, depolymerises DNA strand, splits ATP and amino acids, decreases the activity of tolite enzymes, which leads to the development of alterative and exudative processes in tissues. Considering this, for correction of such pathologies there used antioxidants, which due to its antioxidant, membrane stabilizing, vessel-strengthening and anti-inflammatory properties normalize the activity of LPO processes and restore the functions of antioxidative system. Antioxidants are biologically active substance (BAS) of synthetic, natural or vegetative origin. Basing on the literature facts, that fullerene has antioxidant properties, it is reasonable to study fullerene's influence on the processes of cytolysis, LPO and antioxidant protection in rats under conditions of subchronic liver lesion, induced with ethanol and tetrachlormethane (CCl_4). The latter inhibit microsomal liver enzymes, activate LPO in hepatocytes and are classical membrane toxins. In addition, tetrachlormethane intoxication is a classical model of subcellular hepatocyte membrane lesion, and the use of ethanol enhances the pathology via microsomal liver enzymes inhibition, which leads to organism' intoxication with decay products of hepatocyte membranes. And as a consequence of tetrachlormethane metabolism products of free radical origin are formed in the organism, which are LPO inductors, as a result, there are disturbed structures of liver cells and their main functions.

As reference preparation there was selected classical hepatoprotectors "Carsil", which has as its active substance a complex of flavonoids under general

name “Silymarin”, obtained from milk thistle fruits, which stipulates for anti-cytolytic, antioxidant and anti-inflammatory properties, providing hepatoprotective action of carsil.

Materials and methods.

The study was carried out on white outbred rats with body weight of 180-200 g. The animals were divided into 4 groups: 1 – intact control; 2 – control pathology; 3 – treated with C_{60}FWS at the dose of 1.8 ml/kg; 4 – treated with carsil at the dose of 25.2 mg/kg [1]. According to the guidelines [1] subchronic hepatitis was induced via intragastric introduction of ethanol and tetrachlorethane in rats according to the accepted scheme for 6 days: first there was introduced 50% oil tetrachlorethane solution at the dose of 0.4 ml/100g of body weight, in 2 h – 40% water solution of ethanol at the dose of 1.3 ml/100g of body weight. The study objects, C_{60}FWS and carsil, were introduced to the animals in the medicinal regimen daily for 6 days in 2 h after ethanol introduction [1]. C_{60}FWS was introduced in conditionally therapeutic dose of 1.8 ml/kg, established in previous studies. The reference preparation, carsil, was used at the dose of 25.2 mg/kg, which was calculated for the rats according to Yu.R.Rybolovlyev et al. [2] with the use of re-calculation coefficient from human daily dose of carsil, which is 210 mg (2 dragée 3 times a day). On day 7 the rats were weight and removed from the experiment by the method of decapitation under conditions of barbamyl narcosis. The functional state of the liver in rats was assessed with the following indexes: animal survival rate, body weight dynamics, mass liver coefficient (MLC), biochemical indexes, determined in liver homogenate and serum [1]. In the serum there was determined the activity of cytolytic enzymes of alanine aminotransferase (AlAT) and aspartate aminotransferase (AsAT) [1]. The intensity of LPO processes and the state of antioxidant protection were assessed by the content of diene conjugates (DC), TBA (thiobarbituric acid) reactants and reduced glutathione (G-SH) in liver homogenate [1]. The results of the study are processed statistically with the methods of mathematical statistics [3,4] and are given in Tables 7.1-7.3.

Results of the study.

Table 7.1

Dynamics of body weight, survival rate and liver weight under conditions of subchronic hepatitis, induced by tetrachlormethane and ethanol

Index	Study conditions			
	Intact control	Control pathology	C_{60}FWS 1,8 ml/kg	Carsil, 25.2 mg/kg
Initial body weight, g	193.13±2.66	210.00±1.29*	208.33±3.80*	186.88±2.66
Body weight at the end of the study, g	207.50±5.17	200.00±2.89	205.83±6.25	182.50±3.54*
Dynamics of body weight, %	+7.5%	-5.0%	0	0
Survival rate, %	100	75	75	100
Weight of the liver, g	6.48±0.12	10.16±0.48*	9.73±0.24*	7.74±0.29*/**
MLC,	3.13±0.08	5.08±0.21*	4.74±0.15*	4.25±0.18*/**

Notes:

* – index deviation is significant with respect to intact control, p≤0.05;

** – index deviation is significant with respect to the control pathology group, p≤0.05.

Table 7.2

Activity indexes of cytolytic enzymes in serum of rats under conditions of subchronic hepatitis, induced by tetrachlormethane and ethanol

Index	Study conditions			
	Intact control	Control pathology	C_{60}FWS, 1,8 ml/kg	Carsil, 25.2 mg/kg
AlAT, mmole/g×l	0.37±0.02	1.05±0.06*	0.82±0.04*/**	0.80±0.04*/**
AsAT, mmole/g×l	0.38±0.02	0.82±0.06*	0.54±0.06*/**	0.54±0.04**

Notes:

* – index deviation is significant with respect to intact control, p≤0.05;

** – index deviation is significant with respect to the control pathology group, p≤0.05.

Table 7.3

LPO-AOS system indexes in liver homogenate in rats under conditions of subchronic hepatitis, induced by tetrachlormethane and ethanol

Index	Study conditions			
	Intact control	Control pathology	C_{60}FWS, 1,8 ml/kg	Carsil, 25,2 mg/kg
DC, μmole/g	3.93±0.84	7.92±0.0.62*	5.34±1.17	5.79±0.88
TBA-reactants, μmole/g	92.96±10.49	181.63±36.91*	119.66±21.11**	201.94±29.24*
G-SH, stand. unit	26.97±1.06	12.93±1.45*	44.49±9.00*/**/***	18.14±5.09

Notes:

* – index deviation is significant with respect to intact control, p≤0.05;

** – index deviation is significant with respect to the control pathology group, p≤0.05.

*** – index deviation is significant with respect to the Carsil group, p≤0.05.

Given in Tables 7.1-7.3 results of the study testify to the fact, that development of toxic liver lesion in the animals of the control pathology group, stipulated for pro-oxidant and membrane damaging action of tetrachlorethane and ethanol, is manifested by significant (against intact control) disturbance of geodynamics and trophic processes in the liver (lethal rate in rats of the group is at the level of 25%, decrease in body weight by 5%, significant (against intact control) increase of liver weight and MLC) (Table 7.1), cytolysis development (in the serum significant (against intact control) increase of AlAT and AsAT activity by 2.9 and 2.2 times, correspondingly) (Table 7.2), activation of the processes of lipid peroxidation (in liver homogenate significant (against intact control) increase of DC levels by 2.02 times and TBA-reactants – 1.96 times), connected with it devastation of the function of antioxidant system of the animal organism (significant (against intact control) devastation of G-SH reserves in liver homogenate by 2.1 times) (Table 7.3).

C_{60}FWS at the dose of 1.8 ml/kg and the reference preparation, carsil, at the dose of 25.2 mg/kg promote restoration of geodynamics and trophic processes in liver of rats against the background of subchronic hepatitis, induced by

tetrachlormethane and ethanol. So, values of body weight of the animals on day 7 of the study corresponded to their initial level, when body weight of the rats of the control pathology group decreased by 5%. C_{60}FWS, unlike carsil, insignificantly decreased liver weight, which was reflected in MLC index (Table 7.1) and indicates insignificant anti-inflammatory properties of C_{60}FWS.

Expressed similar anticytolytic properties of C_{60}FWS and carsil are reflected in significant against control pathology group equil decrease of the levels of AlAT – by 1.3 times, and AsAT – by 1.5 times (Table 7.2).

On this model, unlike carsil, which had significant antioxidant effect, C_{60}FWS significantly against control pathology group decreased by 1.5 times the level of LPO TBA-reactants finished products in liver homogenate to intact values, which indicates its expressed antioxidant properties and advantage over the reference preparation, carsil (Table. 7.3). Under the influence of C_{60}FWS and carsil there was also observed insignificant against control pathology group decrease of LPO DC intermediate products' concentration in liver homogenate of the rats – by 1.5 and 1.3 times, correspondingly (Table 7.3).

Unlike carsil, under the influence of C_{60}FWS at the dose of 1.8 ml/kg there was observed a stimulation of the function of antioxidant protection of the animal organism, which is proved by significantly increased level of the main enzyme of their system – reduced glutathione (G-SH) in liver homogenate against intact control – by 1.65 times, and against control pathology – by 3.44 times. The described changes testify to the ability of C_{60}FWS to limit LPO processes at different levels and, therefore, limit not only the processes of free radical oxidation, but also to delay a number of cytolytic processes.

The results, given above, testify to the fact that under conditions of subchronic toxic hepatitis C_{60}FWS at the dose of 1.8 ml/kg, as well as the reference preparation, carsil, at the dose of 25.2 mg/kg, have cytoprotective action, stipulated by anti-cytolytic, antioxidant and membrane stabilizing properties. But the obtained results reflect the advantage of C_{60}FWS over carsil, manifested in much more expressed antioxidant properties, and enables to assume the presence

of direct antioxidant action of C_{60}FWS, when carsil, the active substance of which is a sum of silymarin polyphenols, is an indirect antioxidant.

Therefore, against the background of experimental subchronic toxic hepatitis, induced by tetrachlormethane and ethanol, C_{60}FWS at the dose of 1.8 ml/kg causes expressed antioxidant, anticytolytic and membrane stabilizing action, improving functional activity of hepatocytes, decreasing the expressiveness of cytodestructive processes, inhibiting the processes of lipid peroxidation and restoring antioxidant protection of the organism of the experimental animals.

Literature

1. Preclinical trials of medicinal preparations: (guidelines) / Edited by corresponding member of the AMS of Ukraine O.V. Stefanov – K.: Avicenna, 2001. – P. 334-349.
2. Rybolovlyev Yu.R., Rybolovlyev R.S. (1979) Dosing of substances for mammals on constants of biological activity. Reports of the AS of the USSR. – 1979. – Vol. 247. – No 6. – P. 1513-1516.
3. The main methods of statistical processing of the results of pharmacological experiments / In the book: Guidelines for experimental (preclinical) study of new pharmacological substances. – M.: Remedium. – 2000. – P. 349-454.
4. Lapach S. N., Chubenko A. V., Babych P. N. Statistical methods in microbiological investigations with use of Excel. – 2001. – 320 p.